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June 23 - 26, 2012
Nürnberg, Germany

Abstracts

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European Human Genetics Conference

In conjunction with the

European Meeting on Psychosocial Aspects of Genetics

and the

German Society of Human Genetics

June 23 - 26, 2012
NCC Ost, Nürnberg, Germany

Abstracts

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Milano, Italy
June 21 - 24, 2014

ESHG Spoken Presentations

Plenary Lectures .... PL1.1 – PL5.1 4
Concurrent Symposia .... S01.1 – S16.3 7
Educational Sessions .... ES1.1 – ES8.2 14
Concurrent Sessions .... C01.1 – C18.6 16

ESHG Posters

P01. Genetic counseling, including Psychosocial aspects, Genetics education, Genetic services, and Public policy. .... P01.01 – P01.37 43
P02. Clinical genetics and Dysmorphology .... P02.001 – P02.265 50
P03. Cytogenetics .... P03.001 – P03.145 105
P04. Reproductive genetics .... P04.01 – P04.58 133
P05. Prenatal and perinatal genetics .... P05.01 – P05.70 143
P06. Cancer genetics .... P06.001 – P06.218 157
P07. Cancer cytogenetics .... P07.01 – P07.34 201
P08. Statistical genetics, includes Mapping, linkage and association methods .... P08.01 – P08.45 208
P09. Complex traits and polygenic disorders .... P09.001 – P09.142 218
P10. Evolutionary and population genetics, and Genetic epidemiology .... P10.01 – P10.59 248
P11. Genomics, Genomic technology including bioinformatics methods, gene structure and gene product function and Epigenetics .... P11.001 – P11.142 261
P12. Molecular basis of Mendelian disorders .... P12.001 – P12.265 289
P13. Metabolic disorders .... P13.01 – P13.50 342
P14. Therapy for genetic disorders .... P14.01 – P14.23 352
P15. Laboratory and quality management .... P15.01 – P15.15 357

ESHG Published Abstracts 360

EMPAG Abstracts

Spoken presentations

EMPAG Plenary Lectures .... EPL1.1 – EPL7.6 417
EMPAG Workshops .... EW1 - EW2 426

Posters

EP1 Psychosocial issues in neurodegenerative diseases EP1.01-03 427
EP2 Communicating genetic information EP2.01-07 427
EP3 Reproductive decision making EP3.01-02 429
EP4 Living with genetic disease EP4.01-03 429
EP5 Psychosocial issues in cancer genetics EP5.01-12 430
EP6 Education and professional development EP6.01-08 433
EP7 Access to genetic services EP7.01-03 434
EP8 Ethical issues EP8.01-06 435

Indices

Keyword Index 437
Author Index 452

Abstracts with a presentation number highlighted in gray are talks of Young investigator Candidates
Abstracts with a presentation number highlighted in blue are ESHG Poster Award Candidates
Abstracts with a presentation number highlighted in green are within the Best Scored Posters

ESHG Plenary Lectures

PL1.1
The Regulome - the Next Frontier in Human Genetics

***S. Mundlos**;*
Berlin, Germany.

No abstract received as per date of publication. Please check the programme planner at http://www.eshg.org/abstracts2012.0.html for possible updates.

PL1.2
Oncogenomics of pediatric brain tumors: From molecular profiling towards clinical Translation

***P. Lichter**;*
Heidelberg, Germany.

No abstract received as per date of publication. Please check the programme planner at http://www.eshg.org/abstracts2012.0.html for possible updates.

PL1.3
Myocardial infarction: common disease, common variants, common mechanisms

***H. Schunkert**;*
Lübeck, Germany.

The primary manifestation of coronary disease occurs often suddenly and unexpectedly in form of myocardial infarction. Thus, the prediction of silent atherosclerotic alterations in coronary arteries is a highly relevant medical need. Recent genomic research identified numerous genetic variants that associate with a higher prevalence of coronary disease. At present, association with coronary artery disease has been demonstrated at more than 40 chromosomal locations with risk alleles increasing relative risk by 8-25 % per allele. Moreover, genetic variants primarily affecting cardiovascular risk factors such as hypertension or LDL cholesterol were shown to affect the risk of coronary disease as well.

This enormous progress has been facilitated by genome-wide association studies. By nature, these studies focus on frequent alleles. Thus, the alleles that have been identified to increase the risk of coronary disease are also relatively frequent in our population, i.e. allele frequencies range between roughly 10-90%. As a consequence, virtually all individuals of our population carry a variable degree of genetic predisposition. More recently, the focus turned to rare variants with more profound effects. In this regard, the domain of human genetics, i.e. family based research and counseling, received more attention - once again. The presentation will address how this information can be utilized for a better understanding of disease mechanisms as well as for genomic prediction of coronary artery disease.

PL2.1
Genome sequencing of childhood medulloblastoma brain tumors links chromothripsis with TP53 mutations - a discovery with clinical implications

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Somatic structural variations typically occur progressively during tumor development. Recent findings, however, suggest an alternative mechanism, involving chromosome shattering and reshuffling ('chromothripsis'), the underlying mechanistic basis of which is unknown. In the context of the International Cancer Genome Consortium (ICGC) Pediatric Brain Tumor Project (www.pedbraintumor.org), whole-genome sequencing of a Sonic-Hedgehog medulloblastoma (SHH-MB) brain tumor from a patient with a germline TP53 mutation (Li-Fraumeni syndrome) revealed massive, complex rearrangements resulting from chromothripsis. Integrating TP53 status with genomic rearrangement data in additional medulloblastomas revealed a striking association between TP53 mutation and chromothripsis in SHH-MBs. Unexpectedly, five seemingly sporadic SHH-MB patients with chromothripsis harbored TP53 germline mutation. Our analysis of additional tumor entities substantiated a link between TP53 mutation and chromothripsis, beyond general genomic instability. Among these, we observed a strong association between somatic TP53 mutations and chromothripsis in acute myeloid leukemia, and an increased occurrence of chromothripsis in Li Fraumeni Syndrome-associated malignancies other than medulloblastoma. Our findings implicate p53 in the initiation of, or cellular reaction to, chromothripsis - a novel role for the 'guardian of the genome'. In addition, our findings are of relevance for clinical management and personalized medicine, since TP53 germline mutations represent an "actionable" genetic variant. Regular cancer screening in TP53 germline mutation carriers can lead to a survival benefit. Furthermore, in patients with known Li-Fraumeni Syndrome, administration of high-dose radiotherapy or DNA-damaging chemotherapy has to be thoroughly weighed against the potential of these modalities of readily inducing secondary malignancies.

PL2.2
KLHL3 and Cullin-3 mutations cause Familial Hyperkalemic Hypertension by impairing ion transport in the distal nephron

***H. Louis dit Picard**[1,2,3], J. Barc[4], D. Trujillano[5], S. Miserey-Lenkei[6], N. Bouatia-Naji[1,2], O. Pylypenko[6], G. Beaurain[1], A. Bonnefond[7], C. Simian[3], E. Vidal-Petiot[1,2], C. Soukaseum[1,2], P. Bruneval[1], X. Estivill[5], P. Froguel[7], J. Hadchouel[1,2], J. Schott[4], X. Jeunemaitre[1,2,3];*
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Familial Hyperkalemic Hypertension (FHHt), also known as Pseudohypoaldosteronism type 2, is a rare inherited disease which associates net positive Na^+ balance with renal K^+ retention. Only a minority of cases are caused by mutations in the genes encoding With No lysine (K) kinases 1 and 4.

We carried out linkage analysis combined with whole exome sequencing in two informative French families and identified mutations in the *KLHL3* gene. This gene encodes for an actin-binding protein that recruit substrates for the Cullin3-based ubiquitin-ligase complex. Direct sequencing of 47 additional cases revealed 11 inherited missense *KLHL3* mutations in 16 families with dominant or recessive transmission. Analysis of the *CUL3* gene revealed de novo splice-site mutations clustered around exon 9 and observed in younger and more severe cases. Three-dimensional structural modeling showed that the mutated KLHL3 residues are located within conserved motifs at the surface of the molecule, whereas all *CUL3* mutations lead to a loss of 57 residues corresponding to a segment linking BTB and RING-binding domains of the protein. KLHL3 is highly expressed in the distal nephron like the Na^+-Cl^- cotransporter (NCC) and its inhibition by RNA interference leads to an increase of NCC expression at the cell membrane. We further showed that KLHL3 and NCC co-immunoprecipitate in HEK293T cells, suggesting that KLHL3 directly mediates a negative regulation of NCC expression, probably through ubiquitination.

In conclusion, we identifies KLHL3 and CUL3 as members of a new pathway regulating ion transport in the distal nephron and thus blood pressure.

PL2.3
Duplications of *BHLHA9* are associated with ectrodactyly and tibia hemimelia inherited in non-Mendelian fashion

*S. Lohan[1,2], S. C. Doelken[1], S. Stricker[2], C. W. Ockeloen[3], R. Soares Thiele de Aguiar[4], K. Lezirovitz[4,5], R. C. Mingroni Netto[4], A. Jamsheer[6,7], H. Shah[8], I. Kurth[9], R. Habenicht[10], M. Warman[11], K. Devriendt[12], U. Kordaß[13], M. Hempel[14,15], A. Rajab[16], O. Mäkitie[17], M. Naveed[18], U. Radhakrishna[19], S. E. Antonarakis[19,20], D. Horn[1], S. Mundlos[1,2], **E. Klopocki**[1,2];*
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Split-hand/foot malformation (SHFM) is a congenital disorder characterized by severe malformations affecting the central rays of hands and/or feet. The combination of SHFM and long bone deficiency represents a distinct entity termed SHFLD. Although six different loci/mutations (SHFM1-6) have been associated with SHFM the underlying cause in a large number of cases is still unresolved.

We performed array CGH in a SHFM/SHFLD cohort which detected microduplications on chromosome 17p13.3; a locus previously associated with SHFLD. Detailed analysis of 17 families revealed that this CNV serves as a susceptibility factor for a highly variable phenotype with reduced penetrance, particularly in females. Compared to other known causes 17p duplications appear to be the most frequent cause of SHFLD. A ~11.8kb minimal critical region was identified encompassing a single gene, *BHLHA9*, a putative basic-helix-loop-helix transcription factor. Whole mount in situ hybridization showed expression restricted to the limb bud mesenchyme underlying the apical ectodermal ridge (AER) in mouse and zebrafish embryos. Mouse models suggest that a defect of the central AER leads to the SHFM phenotype. Knock-down of *bhlha9* in zebrafish resulted in shortening of the pectoral fins indicating a role of this gene in limb development. In summary, we demonstrate microduplications encompassing *BHLHA9* associated with SHFLD and non-Mendelian inheritance characterized by a high degree of non-penetrance with gender-bias. Our finding shows that rare CNVs can serve as a susceptibility factor for congenital disease, a mechanism which may explain increased recurrence risk in conditions otherwise considered to be sporadic.

PL2.4

A novel molecular and functional mechanism predisposing to ototoxicity

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While our knowledge about molecular mechanisms underlying Mendelian forms of hearing loss tremendously increased over the last years, the genetic basis and pathogenesis for drug induced hearing impairment remains unclear. Aminoglycosides are the most commonly used antibiotics worldwide. Although highly effective, their use is restricted by side effects such as ototoxicity in a significant subset of patients. However, underlying pathogenesis and pharmacogenetic risk variants are largely unknown. Here we show that dysfunction of an actin remodeling protein (named here ARP) can result in a drug-inducible disturbance of actin dynamics and an irreversible hearing impairment in humans. By positional cloning, we identified a homozygous missense variant, p.L329P, in ARP as a cause of aminoglycoside-induced hearing impairment in a large consanguineous family from Turkey with 4 affected individuals. Complete ARP loss in knock out mice leads to hearing loss associated with shortened stereocilia. We demonstrate that the protein is a component of the tip complex that regulates stereocilia length and that it interacts with whirlin. The mutation severely impairs this interaction in vitro. Extensive biochemical studies showed that myosinXVa can stabilize the ARP-whirlin interaction complex, and we show for the first time that kanamycin has a negative effect on this complex formation, which is even more pronounced in mutant complexes, thereby explaining the development of hearing loss in affected individuals after aminoglycoside treatment. Taken together, we link ototoxicity after aminoglycoside treatment to actin dynamics and this finding will help in devising strategies to counteract this severe side-effect of aminoglycosides.

PL2.5

Genome-wide association and functional studies identify the DOT1L gene to be involved in cartilage thickness and hip osteoarthritis

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Hip osteoarthritis (HOA) is one of the most disabling and common joint disorders with a large genetic component which is, however, still ill defined. To date, genome-wide association studies (GWAS) in osteoarthritis (OA) and specifically in HOA have yielded only few loci, which is partly explained by the heterogeneity in OA definition. Therefore, we here focused on radiographically measured joint space width (JSW), a proxy for cartilage thickness and an important underlying intermediate trait for HOA. In a GWAS of 6,523 individuals on JSW of the hip, we identified the G-allele of rs12982744 on chromosome 19p13.3 to be associated with a 5% larger joint space width ($P= 4.8\times10^{-10}$). The association was replicated in 4,442 individuals from 3 UK-cohorts with an overall meta-analysis P value of 1.1×10^{-11}. The SNP was also strongly associated with a 12% reduced risk for HOA ($P =1\times10^{-4}$). The SNP is located in the *DOT1L* gene, which is an evolutionarily conserved histone methyltransferase. Immunohistochemical staining of DOT1L protein during mouse limb bud development supports a role for DOT1L in chondrogenic differentiation and adult articular cartilage. Silencing of *Dot1l* inhibited chondrogenesis. *Dot1l* knock-down reduces proteoglycan and collagen content, and mineralization during chondrogenesis. In the *in vitro* ATDC5 chondrogenesis model system, DOT1L interacts with TCF and Wnt signalling. These data are a further step to understand the role of Wnt-signaling during chondrogenesis and cartilage homeostasis. DOT1L may represent a therapeutic target for osteoarthritis.

PL2.6

Insights into tissue-specific mechanisms of gene regulation involving genetic variants and DNA methylation

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Multiple studies have demonstrated the importance of genetic variants affecting gene expression (eQTLs) and initial limited studies have identified genetic variants affecting local DNA methylation levels (mQTLs). Moreover, DNA methylation is known to be associated with gene silencing, and more recently with active gene expression when present within the gene body. Yet the mechanisms by which genetic variation and DNA methylation affect gene expression and tissue specificity are not well understood. In this study, we use a cohort of 210 newborn Caucasian individuals who we genotyped (2.5 million SNPs) and for whom we measured mRNA levels through RNA-seq in three cell-types derived from cord blood and umbilical cord: lymphoblastoid cell lines, T-cells and fibroblasts. For ~110 samples of each of the three cell-types, we have assayed DNA methylation levels in more than 400,000 CpG sites through bisulfite conversion and bead chips. We characterized the tissue-specificity of eQTLs, allele-specific expression, mQTLs and eQTMs (methylation-expression associations). We identified genetic variants and a modest number of methylation sites affecting alternative splicing. Additionally, we tested the contribution of different mechanistic models. We provide evidence for synergistic interactions between SNPs and methylation sites. We found a significant number of cases in which the SNP effect on gene expression is mediated through methylation. Finally, we describe the mechanistic differences between patterns of methylation associated with increase and decrease of gene expression. Overall, our results provide insights into the genetics and epigenetics involved in gene regulation and tissue specificity, which is important for better understanding complex traits.

PL3.1

Found in Translation: New Insights into the Pathogenesis and Treatment of Marfan syndrome and related disorders

H. Dietz;

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Dysregulation of TGF signaling has been implicated in many disease states including Marfan syndrome (MFS), a condition caused by deficiency of the extracellular matrix protein fibrillin-1. Many manifestations of MFS can be attenuated in mouse models using interventions that antagonize TGF signaling. TGF can initiate both canonical (Smad-dependent) and noncanonical (prominently including the MAPKs ERK, JNK and p38) signaling cascades. Multiple lines of evidence will be presented that implicate ERK signaling as the primary TGF-dependent event that drives disease including the ability of ERK antagonists to achieve phenotypic rescue. Despite this progress, our understanding of how fibrillin-1 deficiency initiates altered TGF activity remains incomplete, as does knowledge regarding events that culminate in tissue failure or that that account for the wide intrafamilial variability in the severity of vascular disease. In attempt to refine our mechanistic understanding of disease pathogenesis (with a focus on aortic aneurysm), we have launched initiatives to identify environmental and genetic modifiers of MFS. Use of calcium channel blockers to mitigate hemodynamic stress resulted in a marked acceleration of aortic growth and rupture in mice with MFS and correlated with accentuation of ERK signaling. The deleterious consequences of this gene-by-environment interaction were abrogated using ERK antagonists. In a separate study, we identified a single major protective modifying locus for MFS coincident with the map position for MAP3K4 (a major JNK and p38 kinase). Taken together, these data reinforce the concept that noncanonical TGF signaling is central to disease progression. Insights regarding initiating events derived from our demonstration that a congenital presentation of skin fibrosis (scleroderma) is caused by mutations in fibrillin-1 that specifically impair integrin binding to its RGD sequence. Mice harboring a RGD to RGE mutation in fibrillin-1 (causing an obligate loss of integrin binding) show dense dermal fibrosis in association with increased expression of an integrin subtype (v3) known to activate TGFbeta and ERK, and are protected from fibrosis by manipulations that mimic the interaction between fibrillin-1 and other integrins (e.g. 51). When stimulated with TGF, scleroderma fibroblasts show unique and marked activation of ERK when compared to control cells. This effect is prevented by treatment with an integrin 1-activating or 3-blocking antibody. These data suggest that 3 integrin not only augments TGF signaling, but also specifically influences the choice between the Smad and ERK cascades (favoring ERK), perhaps through a direct potentiating interaction between αvβ3 and TβRII. RGE mice also develop aortic aneurysm, providing an ideal system to test the hypothesis that loss of matricellular integrin-ligand interaction is an inciting event in the MFS aorta and to test integrin-targeted therapies.

PL3.2

Molecularly Targeted Treatments in Tuberous Sclerosis Complex (TSC)

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Until recently, no targeted treatments were available to individuals with genetic syndromes such as TSC. The evidence emerging from an increasing number of genetic disorders has, however, begun to challenge this irreversibility assumption, and is showing how an understanding of the neurobiological mechanisms underlying a syndrome can lead to biologically- or molecularly-targeted treatments.

Advances in the molecular biology of tuberous sclerosis have shown that TSC is an mTOR (mammalian Target Of Rapamycin) overactivation syndrome, and phase III trials are currently underway for physical phenotypes of the disorder. The neuropsychiatric phenotype of TSC was presumed to be caused by the structural brain abnormalities and/or seizures seen in the disorder. However, over the last few years research has shown that there are also direct molecular pathways from genetic mutation to neurocognitive phenotypes.

Molecularly-targeted treatments using mTOR inhibitors are showing great promise for the physical and neurological features of the disorder. Intriguingly, pre-clinical and early-phase clinical studies of neuropsychiatric phenotypes are suggesting that specific aspects of cognition or neurodevelopment might also be reversible, even in adults with the disorder.

In this talk, we will follow the history of tuberous sclerosis complex from first description to molecularly targeted treatments.

PL3.3

Targeted treatments in Fragile X syndrome

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Fragile X syndrome (FXS) is caused by expansion of a CGG repeat in the 5‘ untranslated region of the fragile X mental retardation 1 (FMR1) gene. This mutation is associated with hypermethylation at the FMR1 promoter and subsequent transcriptional silencing. The absence of FMRP (FMR1 protein) at the synapse has many consequences, including up-regulation of metabotropic glutamate receptor 5 (mGluR5)-mediated signaling. It has been postulated that this increased mGluR5 signal may be responsible for many of the clinical manifestations observed in fragile X syndrome. mGluR5 receptor antagonists have repeatedly been shown to rescue many phenotypes and endophenotypes in animal models of the fragile X syndrome. Comprehensive phenotype correction also occurs when treatment is administered later in the adult KO mice. We examined whether a receptor subtype-selective inhibitor of mGluR5, AFQ056, improves the behavioral symptoms of FXS in a randomized, double-blind, two-treatment, two-period, crossover study of 30 male FXS patients aged 18 to 35 years. We detected no significant effects of treatment on the primary outcome measure, the Aberrant Behavior Checklist-Community Edition (ABC-C) score, at day 19 or 20 of treatment. In an exploratory analysis, however the patients with full FMR1 promoter methylation and no detectable FMR1 messenger RNA improved, as measured with the ABC-C, significantly more after AFQ056 treatment than with placebo ($P < 0.001$). If confirmed in larger and longer-term studies, these results suggest that blockade of the mGluR5 receptor in patients with full methylation at the FMR1 promoter may show improvement in the behavioral attributes of FXS.

PL4.1

Mendel Lecture

E. Eichler*;*
Seattle, WA, United States.

No abstract received as per date of publication. Please check the programme planner at http://www.eshg.org/abstracts2012.0.html for possible updates.

PL5.1

ESHG Award Lecture

P. Lichter*;*
Heidelberg, Germany.

No abstract received as per date of publication. Please check the programme planner at http://www.eshg.org/abstracts2012.0.html for possible updates.

ESHG Concurrent Symposia

S01.1

Combined tests for rare variants

***B. Neale**;*

Boston, MA, United States.

No abstract received as per date of publication. Please check the programme planner at http://www.eshg.org/abstracts2012.0.html for possible updates.

S01.2

Statistical analysis of rare variants in genome-wide association studies of complex traits

***A. Morris**;*

Oxford, United Kingdom.

No abstract received as per date of publication. Please check the programme planner at http://www.eshg.org/abstracts2012.0.html for possible updates.

S01.3

Homing in on causative genes in GWAS-identified risk loci using eQTL information

***Y. Momozawa**, V. Deffontaine, B. Charloteaux, F. Crins, A. Gori, C. Lecut, M. Mni, C. Oury, C. Reenaers, E. Théâtre, E. Louis, M. Georges;*

University of Liege, Liege, Belgium.

GWAS have mapped hundreds of risk loci for tens of complex diseases, including inflammatory bowel disease (IBD). However, for most risk loci the causative genes amongst positional candidates remain unknown.

To aid in the identification of causative genes, we have generated transcriptome data for nine IBD-relevant cell-types in more than 200 healthy individuals. We have mapped cis- and trans-eQTL in all cell types. We first quantified tissular overlap between eQTL as well as their tissue-specific degree of coincidence with IBD risk loci.

We then used the ensuing eQTL information to pinpoint likely causative genes in known risk loci as follows. It is becoming increasingly apparent that a substantial proportion of inherited risk is due to regulatory variants that alter the expression profile of the causative genes. In such cases, and independently of the number of risk variants involved, disease association profile (i.e. the combination of p-values exhibited by all variants in a risk locus) and eQTL association profile are bound to be highly correlated in the disease-relevant tissue. We have devised and characterized the behavior of metrics that measure such correlations. These metrics are made independent of known disease-associated coding SNPs mapping to the risk loci of interest. We have used these metrics to prioritize candidate genes in the 118 known risk loci associated with Crohn's disease and/or Ulcerative Colitis. eQTL-informed high-throughput resequencing of candidates in large case-control cohorts to confirm their causality is in progress. Latest results will be presented.

S02.1

Copy number variation and selection during reprogramming

***A. Nagy**;*

Toronto, ON, Canada.

No abstract received as per date of publication. Please check the programme planner at http://www.eshg.org/abstracts2012.0.html for possible updates.

S02.2

Investigating Genomic Heterogeneity in Breast Cancer by Single-Cell Sequencing

***N. Navin**;*

Houston, TX, United States.

As a tumor evolves from a single cell, it acquires complex somatic mutations and diverges to form distinct lineages of clones. This intratumor heterogeneity confounds basic research and clinical diagnosis, because tools do not exist to resolve it. To address this problem, we developed a single-cell sequencing method to profile genomic copy number in individual tumor cells. We used this method to profile hundreds of single cells from two triple-negative (ER-, PR- and Her2-) breast cancer patients. Analysis of 100 single cells from a heterogeneous tumor revealed three distinct clonal subpopulations that represent sequential clonal expansions. Additional analysis of 100 single cells from a homogeneous primary tumor and its liver metastasis indicated that a single clonal expansion formed the primary tumor and seeded the metastasis. In both primary tumors, we also identified an unexpectedly abundant subpopulation of genetically diverse ‚pseudodiploid' cells that do not travel to the metastatic site. In contrast to the prevailing models of gradual tumor progression, our data suggest that these tumors grew by punctuated clonal expansions, in which hundreds of chromosomal rearrangements were acquired in short bursts of evolution. Recently, we have also developed a method to perform whole-genome sequencing of single cells. From this data we can detection many classes of chromosomal mutations (point mutations, indels and structural variants) at base-pair resolution in single cells. We are using this tool to investigate several major areas of cancer biology including invasion, metastasis and response to chemotherapy.

S02.3

Genomic instability in early stages of cancer development

*A. C. Bester, E. Ozeri-Galai, **B. Kerem**;*

Department of Genetics, The Life Sciences Institute, The Hebrew University, Jerusalem, Israel.

Chromosomal instability in early cancer stages is caused by stress on DNA replication. The molecular basis for this replication perturbation was unknown. We showed the replication dynamics in cells in which a regulator of S-the effect of other oncogenes and of micronutrients on the replication dynamics and genomic instability. These results will be presented and discussed.

The perturbed DNA replication in early stages of cancer development induces chromosomal instability preferentially at fragile sites. However, the molecular basis for this instability was unknown. We showed that already under normal conditions, replication fork progression along the fragile site, FRA16C, is slow and forks frequently stall at AT-rich sequences, leading to activation of additional origins. Under mild replication stress, the frequency of stalling at AT-rich sequences is further increased. Strikingly, unlike in the entire genome, in FRA16C additional origins are not activated, suggesting that all potential origins are already activated under normal conditions. We further studied the replication dynamics of another fragile site FRA16D. The results of this analysis will be presented and discussed. Altogether, our results provide a mechanism explaining the replication stress sensitivity of fragile sites and thus, the basis for genomic instability during early stages of cancer.

S03.1

Epigenetic regulation of the circadian clock

***P. Sassone Corsi**;*

Irvine, CA, United States.

No abstract received as per date of publication. Please check the programme planner at http://www.eshg.org/abstracts2012.0.html for possible updates.

S03.2

Epigenetics in diabetes

***A. El-Osta**;*

Baker IDI Heart and Diabetes Institute, Melbourne, Australia.

No abstract received as per date of publication. Please check the programme planner at http://www.eshg.org/abstracts2012.0.html for possible updates.

S03.3

Epigenetics of the impact of early trauma on behavior across generations

***J. Bohacek**;*

Brain Research Institute, University/ETH Zürich, Zurich, Switzerland.

The development and expression of behaviors in mammals are strongly influenced by environmental factors. When favorable and positive, these factors can facilitate appropriate responses and normal behaviors, but when aversive and stressful, they can lead to behavioral pathologies. Adverse and traumatic events early in life are particularly strong risk factors for behavioral and psychiatric conditions such as depression, personality and conduct disorders, and antisocial behaviors. Such disorders can not only affect the individuals directly exposed to trauma, but can also be transmitted and similarly expressed in the following generations[1]. The mechanisms underlying the etiology and inheritance of behavioral symptoms induced by traumatic stress early in life have been proposed to involve epigenetic processes, but remain undefined. This talk will discuss novel developments in transgenerational epigenetics, and introduce an experimental model of early traumatic stress in mice that provides initial evidence for the contribution of epigenetic processes to the inherited impact of stress on behavior. This model shows that chronic and unpredictable maternal separation causes depressive- and impulsive-like behaviors, social withdrawal and cognitive defects in adult mice, and that these symptoms are transmitted to the following offspring. It provides initial evidence that these alterations are associated with persistent changes in DNA methylation in the promoter-associated CpG island of several genes, in the brain of the offspring and in the germline of their stressed fathers[2-4]. These findings suggest that epigenetic mechanisms contribute to the impact of negative environmental conditions early in life on adult behavior and its inheritance[5-8]. Novel molecular candidate mechanisms thought to contribute to transgenerational epigenetic inheritance will also be discussed.

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S04.1

Integrating clinical and genomic approaches to identify causative genetic loci for nonsyndromic orofacial clefting

***E. Mangold*;**
Institute of Human Genetics, University of Bonn, Bonn, Germany.

Formal genetic and epidemiological studies have indicated that the etiology of nonsyndromic orofacial clefts is multifactorial, with both genetic and environmental factors contributing to the phenotype. Improved understanding of the etiology of clefting may facilitate development of new preventative measures and therapeutic approaches, and could improve genetic counselling for families at risk.

In this talk the latest genetic findings for nonsyndromic orofacial clefts will be discussed as well as their biological and functional implications.

Most epidemiological and molecular genetic studies of nonsyndromic orofacial clefts have been performed in patients with nonsyndromic cleft lip with or without cleft palate (nsCL/P), which is the most common form of orofacial clefting. Nonsyndromic cleft palate only (nsCPO) is the second most common subtype, and only limited research has been conducted into the genetic factors underlying nsCPO.

For nsCL/P linkage and candidate gene studies have attempted to elucidate the underlying genetic architecture, however, only the interferon regulatory factor 6 (*IRF6*) gene has been identified as causative. Recent genome-wide association studies (GWAS) have substantially extended our knowledge of the underlying risk factors. Four GWAS of nsCL/P have been conducted, and these have identified five new chromosomal loci. One locus, located in an intergenic region of chromosome 8q24, has been implicated in all GWAS and constitutes a major susceptibility locus. Although there is considerable overlap between studies in terms of the regions identified (particularly for the loci at 8q24 and 10q25), the causative variants still await identification. With the exception of 8q24, each of the identified loci harbors candidate genes. However, proof of causality is still lacking in all cases.

S04.2

Dissecting Treacher Collins syndrome using the mouse as a model organism

***M. J. Dixon*[1], *P. A. Trainor*[2], *J. Dixon*[1];**
[1]University of Manchester, Manchester, United Kingdom, [2]Stowers Institute for Medical Research, Kansas City, MO, United States.

Treacher Collins syndrome (TCS) is an autosomal dominant disorder of craniofacial development that occurs with an estimated incidence of 1 in 50,000 live births. TCS is characterized by a combination of: bilateral downward slanting of the palpebral fissures; colobomas of the lower eyelids with a paucity of eyelashes medial to the defect; hypoplasia of the facial bones; cleft palate; malformation of the external ears; atresia of the external auditory canals; and bilateral conductive hearing loss. A high degree of inter- and intra-familial variation in the clinical phenotype is observed. The majority of TCS patients are heterozygous for a mutation in *TCOF1* which introduces a premature termination codon into the encoded protein Treacle. Treacle is a nucleolar phosphoprotein which co-localizes with upstream binding factor (Ubf) and RNA polymerase 1 in nucleolar organizing regions where it functions in ribosomal DNA (rDNA) gene transcription.

To investigate the developmental basis of TCS, we have generated a mouse model through germline mutation of *Tcof1*. Haploinsufficiency of *Tcof1* results in marked apoptosis in the neuroepithelium leading to a deficiency of migrating neural crest cells and resulting in severe craniofacial malformations. We have further demonstrated that Treacle is required cell autonomously for the formation and proliferation of neural crest cells and that Treacle elicits its role through regulating the production of mature ribosomes. Subsequently, we have demonstrated that haploinsufficiency of *Tcof1* induces nucleolar stress and p53 checkpoint activation which results in p53 stabilization and cyclin G1-mediated, cell-cycle arrest. Collectively, these perturbations underpin the tissue specificity of neuroepithelial apoptosis and neural crest cell hypoplasia characteristic of TCS. Importantly, chemical or genetic inhibition of p53 prevents cyclin G1-driven apoptotic elimination of neural crest cells and rescues the craniofacial abnormalities associated with mutations in *Tcof1*. Notably, recent evidence has indicated that a subset of TCS cases arise from mutations in two genes encoding subunits of RNA polymerases I and III, *POLR1D* and *POLR1C*, providing further evidence that TCS is a ribosomopathy.

S04.3

Human Facial Dysostoses

***D. Wieczorek*;**
Institut für Humangenetik, Universitätsklinikum Essen, Essen, Germany.

Human facial dysostoses can be subdivided into mandibulofacial (MFD) and acrofacial dysostoses (AFD). Both are characterized by hypoplasia of facial structures derived from the first and second branchial arches. In addition to facial findings AFDs include anomalies of the extremities.

The best known MFD is the Treacher Collins syndrome, a monogenic disease caused by mutations of the *TCOF1*, *POLR1D* or *POLR1C* genes (Dauwerse *et al.*, 2011), which are involved in rRNA transcription. MFD with microcephaly is another important form of MFD. Very recently, haploinsufficiency of a spliceosomal GTPase encoded by the *EFTUD2* gene was identified in these patients (Lines *et al.*, 2012). Other MFDs such as MFD type Toriello or Burn-McKeown syndrome are more rare and their molecular etiology has not been resolved so far.

AFDs fall into two groups: i) AFDs with postaxial malformations of extremities, e.g. Miller syndrome, and ii) AFDs with preaxial involvement, e.g. Nager syndrome. Miller syndrome is caused by mutations within the *DHODH* gene, involved in the pyrimidine biosynthesis pathway (Ng *et al.*, 2010). Haploinsufficiency of *SF3B4*, a component of the pre-mRNA spliceosomal complex, leads to Nager syndrome (Bernier *et al.*, 2012). In other, much rarer AFDs the molecular etiology is still unknown.

S05.1

Alzheimer's disease

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S05.2

Schizophrenia Genetics

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It has long been known that schizophrenia has high heritability, but as for other complex disorders, identifying the specific genes responsible has been a major challenge. However, through the technological revolution in genomics, and by the deployment of very large samples through international collaboration, highly significant evidence for the involvement of around 15 common genetic variants has emerged, each making a small contribution to risk of the disorder. Replication studies suggest that of 81 independent variants surpassing p<10-5 in the largest schizophrenia GWAS, the vast majority are likely to be true risk variants but it is also clear from multi-locus tests that around a thousand or more risk variants exist. There is clear evidence that these common variants substantially overlap with risk variants for bipolar disorder, and emerging evidence that they also overlap with those conferring risk of other disorders, in particular ADHD. The same developments that have allowed GWAS have also identified a number of abnormalities of chromosome structure with clear evidence for their involvement in schizophrenia and that these occur as de novo mutations in about 5% of cases. Although rare, the CNVs confer a substantial increase in risk of schizophrenia to carriers as well as to other neurodevelopmental phenotypes including ADHD, Autism and Intellectual Disability. Although only a small amount of the total genetic variation that contributes to schizophrenia and bipolar disorder has been allocated to specific DNA variants, recent findings have strongly implicated specific aspects of neuronal function that are of importance in the pathogenesis of psychosis.

S05.3

Bipolar affective disorder

S. Cichon*;*
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S06.1

Tailoring and implementing aCGH for prenatal diagnosis: experience from Hong Kong

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Chinese University of Hong Kong, Hong Kong, China.

Microarray analysis has been recognized as a powerful diagnostic tool for detection of chromosome copy number aberrations in infants and children with congenital malformations and neurodevelopmental disabilities. Although microarray-based comparative genomic hybridization (aCGH) was made available at the same time as the postnatal setting, in the prenatal setting it has had a slower adoption rate. Based on the CNVs abnormality rate detected from the recent NICHD sponsored multicentre trial and meta-analysis reported, aCGH is a more sensitive diagnostic test and adds incremental value to conventional karyotyping. There were also emerging evidences suggested that a targeted or lower resolution array appeared to be more appropriate for the detection of majority of clinically relevant chromosome copy number abnormalities while maintaining a low rate of results with unknown clinical significant. Our laboratory has been offering „one-stop" prenatal screening and diagnostic service using low resolution targeted array (aCGH; 4x44K) since 2009. In this study, we present a prospective follow-up survey of 500 pregnancies after prenatal screening and aCGH analysis with subsequent antenatal care in our unit. This is a prospective follow-up study, using pregnancy outcome as the end point to investigate the accuracy, efficacy, clinical advantages and shortcoming of prenatal diagnosis using targeted aCGH as compared to standard conventional chromosome analysis using microscopy and QF-PCR. A few cases neglected by aCGH but subsequently identified to have chromosomal or genetic abnormalities by other methods will be discussed.

S06.2

Prenatal diagnosis: Challenges in the interpretation of high resolution genetic screening tests

K. Devriendt*;*
Center for Human Genetics, UZ Leuven, Leuven, Belgium.

High resolution genetic screening tests based on array-CGH are being introduced in the prenatal setting. They significantly increase the detection of genetic variants with clinical implications. Several flow-charts exist for the interpretation of CNV's in postnatal setting. However, the clinical interpretation of CNV's in a prenatal setting is more challenging compared to a postnatal setting, since the clinical information needed to interpret CNV's is often incomplete, time is pressing and decisions taken are irreversible. In high risk pregnancies, e.g. (multiple) malformations detected on ultrasound, the added value of array-CGH often is limited, since decisions with regard to pregnancy management are already taken based on the detected anomalies. In contrast, array-CGH can have a major added value as a screening tool in low risk pregnancies, this has to be outweighed against the difficult issues such as CNV's with reduced penetrance, variable expressivity and pleiotropic effects, unclassified CNV's, incidental findings etc. The huge demand for pre-test counseling raised by these difficulties cannot be met by most genetic centers. Also, one can question to what extent parents can take truly informed decisions, not only on taking a prenatal array-CGH test, but also taking decisions regarding pregnancy management if a variant is detected.

S06.3

Get ready for the flood of fetal gene screening

H. T. Greely*;*
Stanford, CA, United States.

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S07.1

Pharmacogenomic biomarkers: growing promise and hype

W. Sadee*;*
The Ohio State University, Columbus, OH, United States.

Genomic sciences supported by ultra-high throughput technologies begin to change the landscape of medicine, disease prevention, and therapy. Development of genomic biomarkers guiding drug therapies has moved to the forefront of translation into clinical practice. The US FDA's Table of Pharmacogenomic Biomarkers in Drug Labels (1) now contains >120 drug entries where evidence of a genetic effect has been described. However, confidence in the presented evidence and effect size with regards to drug response or toxicity vary over a wide range - from definitive clinical recommendations to 'for information only'. While strong genetic factors predictive of adverse drug reactions move into clinical practice as biomarker tests, drug efficacy is typically multifactorial, with few tests capable of predicting outcomes - companion biomarker tests for targeted cancer chemotherapy a possible exception. Yet accurate prediction of positive response to therapy has the potential to improve drug therapy substantially, while much of the genetic variability in drug response genes has yet to be discovered. Our research focuses on expression genetics, based on the premise that regulatory variants (affecting transcription, RNA processing, and translation) are more prevalent than nonsynonymous SNPs that alter protein function directly. I will present specific examples and highlight emerging opportunities and hurdles to translation of genetics/genomics into therapeutic advances. Supported by NIH U01 GM092655.

1. http://www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378.htm

S07.2

Clinical pharmagenomics: perspectives and limitations

M. Schwab[1,2]*;*
[1]*Dr Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, Germany,*
[2]*Department of Clinical Pharmacology, University Hospital, Tuebingen, Germany.*

Variation in drug disposition and response among patients is a major concern associated with many therapeutical agents used in all disciplines of medicine. The clinical relevance of variability is most evident with drugs that have a narrow therapeutic window (i.e., the dose used is close to the dose probably resulting in drug-related toxicity in most individuals). With increasingly information available from the Human Genome Project and the HapMap Project, pharmacogenomics aims to elucidate the genomic determinants of drug efficacy and toxicity. For instance, variants in genes that are relevant for ADME processes such as drug metabolizing enzymes, drug transporters and nuclear receptors have profound effects on patient outcome. Recent clinically important examples are pharmacogenomics of tamoxifen, a well established drug for treatment of postmenopausal breast cancer, and pharmacogenomics of clopidogrel, an antiplatelet drug. However, it is unlikely that one single gene will affect exclusively disease or treatment outcome, and therefore a more comprehensive approach will be to consider genetic polymorphisms in entire biological/ pharmacological pathways. Recently developed '-omics' appoaches (e.g. genomics, transcriptomics, proteomics, metabonomics) will be helpful to identify further putative targets for better prediction of drug response and will complement each other. Array technologies (e.g. cDNA arrays, GWA), next generation sequencing and metabonomics have shown to be helpful for identifying novel genes, redefining disease diagnosis and predicting therapy response to specific drugs. Finally, non-genetic factors as well as epigenetics (e.g. methylation, miRNA) have to be considered more intensively in the future. Experimental as well as computational approaches are required to obtain holistic, mechanistic information on disease networks and drug response. Thus, only systems pharmacology allows the integration of the systems-level understanding of drug response with genome medicine to promote the idea of personalized medicine.

S07.3

Pharmacogenomics - Is it a hype really?

***H. J. Guchelaar**;*
Leiden, Netherlands.

No abstract received as per date of publication. Please check the programme planner at http://www.eshg.org/abstracts2012.0.html for possible updates.

S08.1

Research about psychological issues related to genetic counselling and genetic testing: a personal view on past, present and future

***G. Evers-Kiebooms**;*
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Notwithstanding the complex psychological risks faced by individuals at increased risk for developing genetic disease or for having offspring with genetic disease, psychological issues related to genetic risk, genetic counselling and genetic testing were hardly studied before 1980. Thereafter the attention for the psychological dimension increased rather slowly and moreover most studies were descriptive and did not use adequate control groups . In several countries the success of the human genome project also stimulated research on other than technical or medical aspects of the new genetics. Unfortunately more attention and funding went to ELSI (Ethical, Legal, Social issues) than to Psychological issues. ELSI instead of PELSI…

The fact that the "teaching model" of genetic counselling dominated the "counselling model" for many years, resulted initially in psychological research that was mainly focused on cognitive aspects of genetic counseling (understanding of probabilistic information, recall of risks). Seymour Kessler played an important role in drawing the attention on genetic counseling as a complex communication process and on the factors involved in processing emotionally laden information, coping with genetic disease and communication about genetic issues within the family. The first part of the presentation is dedicated to a personal selection of research findings about the impact of genetic counselling as well as about directiveness versus non-directiveness.

The progress in diagnostic possibilities allowed the detection of more and more genetic diseases in adults or children, prenatally and even in the embryo. The second and major part of the presentation consists of a personal looking back on research about psychological aspects of prenatal testing, carrier testing and predictive testing. For each of the three types of genetic testing two major dimensions will be considered: decision making about testing and the psychological impact of testing. Where applicable in the context of predictive testing attention will also be paid to a third dimension: psychological issues involved in preventive health behavior or life style changes that may be induced by a genetic test result. The focus in this part of the presentation will be on predictive testing, prenatal testing and preimplantation genetic diagnosis for Huntington's disease, with special attention for longitudinal studies evaluating the impact of the test result on the psychological wellbeing of the tested person and his or her partner and on subsequent family planning.

So far psychological research to delineate the challenges as well as the pitfalls of whole genome sequencing for predicting future health problems is very limited. Based on relevant research in psychological decision making and health psychology, mainly on risk perception, the consequences of information overload and differences in coping style, a few comments will be formulated in the closing part of the presentation.

S08.2

Risk Communication and Behaviour Change: Exploring the Chasm

***T. Marteau**;*
London, United Kingdom.

There is a strong belief held by the public, health care practitioners and science funders that using biomarkers and in particular genotypes, will motivate behavior change to reduce the identified risks. This paper will present the result of a recently updated Cochrane Review which supports the conclusions of the original review (Marteau et al 2010) that communicating genetic risk information is unlikely to make much discernible impact upon the change in behaviour needed to reduce the high and rising rates of non-communicable diseases attributable to smoking, diet and physical inactivity. This is in keeping with recent evidence from neuroscience and psychology that highlights the finding that much behaviour taking place outside of awareness.

While the communication of genetic risk information is unlikely to form part of broader public health strategies to change health related behavior in order to improve population health, genetic and other biomarker-based risk information will continue to be given in some clinical contexts. The next generation of research in this latter context could usefully take as its starting points first, that there is nothing particularly motivating about biomarker risk information and second, that engagement in some programmes can change health-related behaviour.

Key references

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Evans JP, Meslin EM, Marteau TM, Caulfied T Deflating the genomic bubble. Science 2011 33: 861-2.

S08.3

Psycho-onco-genetics: historical background and future challenges

***E. M. A. Bleiker**;*
The Netherlands Cancer Institute, Amsterdam, Netherlands.

'Psycho-onco-genetics' is the domain where psychology, oncology and genetics meet. A brief historical background of the three sciences is presented, followed by suggestions for future research.

Cancer is as old as mankind. In Western countries, first successful treatments for cancer were given between 1900 and 1950. In the 1960s, the taboo decreased and a cancer diagnosis was more openly communicated to the patient. At that time, psychology and psychiatry entered oncology. By the mid-seventies, the first psycho-oncological investigators studied issues like anxiety and depression. Questionnaires assessing 'quality of life' were developed in the 1980s and 1990s, to measure physical, social, and emotional wellbeing. In the 21st century, the screening of cancer patients for distress ('the 6th vital sign') is being advocated. Parallel to the developments in cancer treatment and psycho-oncology, the field of genetics developed. The hypothesis that a disease like breast cancer could be inherited was for the first time reported by Paul Broca in 1866. In 1953, Watson and Crick were among the first to report on the structure of DNA, and more important discoveries followed in the next decades. In the 1960s the first papers on genetic counseling for cancer appeared. A literature search on this topic revealed over 10.000 papers published since 1967, with a strong increase in the 1990s, when a number of genes associated with cancer syndromes were identified.

Concerns were raised about the possible negative psychosocial impact of genetic testing and preventive surgery for (breast) cancer. In general, results did not support this concern. However, a number of questions still need to be answered. For example: are the currently used distress-questionnaires sufficiently sensitive to assess the specific problems encountered by the counselees? Are those who do not request counseling psychologically more vulnerable? To what extent should counselors play an active role in the communication of genetic test results to distant relatives of mutation carriers? What will be the best use of SNP's in clinical practice, and how can we support counselees with coping with these small elevated risks for cancer? How can we improve psycho-education about reproductive decision making such as prenatal and pre-implantation genetic diagnosis? In addition, a number of questions need to be answered when commercial genetic testing will arrive in Europe, making genetic testing accessible without the intervention of a trained genetic counselor.

S09.1

Opposing roles for IL-23 and IL-12 in maintaining occult cancer in an equilibrium state

M. W. Teng[1,2], *M. D. Vessely*[3], *H. Duret*[1], *N. McLaughlin*[1], *J. E. Towne*[4], *R. D. Schreiber*[3], *M. J. Smyth*[1,2];

[1]Peter MacCallum Cancer Centre, Melbourne, Australia, [2]Sir Peter MacCallum Department of Oncology, University of Melbourne, Melbourne, Australia, [3]Department of Pathology and Immunology, Washington University School of Medicine, St Louis, MO, United States, [4]Inflammation Research, AMGEN Incorporated, Seattle, WA, United States.

The detailed mechanism controlling the equilibrium phase of cancer immunoediting that results in immune-mediated dormancy of cancer remains to be delineated. Here, we investigate the length of the equilibrium phase during immune control of methycholanthrene (MCA) induced cancers and extend these observations to aging, cancer prone p53 mutant mice. We also demonstrate, for the first time, the critical and opposing roles of IL-23 and IL-12 in maintaining cancer cells in a state of immune-mediated dormancy. Over a series of experiments, inhibition of IL-23p19 was shown to reduce the malignant potential of lesions established by MCA inoculation, while inhibition of IL-12/23p40 enhanced tumor outgrowth. Furthermore, agonistic anti-CD40 antibody treatment mimicked the effects of anti-IL-23p19 mAb treatment. Other cytokines such as IL-4, IL-17, TNF, and IFNαβ, which are known to play important roles either in MCA tumorigenesis or in the elimination phase of cancer immunoediting did not play critical roles in maintaining the equilibrium phase. Taken together, these data indicate opposing roles for IL-23 and IL-12 in determining the outgrowth versus dormancy of occult neoplasia and suggest a potential long-term danger in using IL-12/23p40 antibodies for treating human autoimmune inflammatory disorders.

S09.2

Paracrine and Autocrine Signals Induce and Maintain Mesenchymal and Stem-Cell States in the Breast

C. Scheel;

Helmholtz Center Munich, German Research Center, for Environmental Health, Neuherberg, Germany.

The epithelial-mesenchymal transition (EMT) has been associated with the acquisition of motility, invasiveness, and self- renewal traits. During both normal development and tumor pathogenesis, this change in cell phenotype is induced by contextual signals that epithelial cells receive from their microenvironment. The signals that are responsible for inducing an EMT and maintaining the resulting cellular state have been unclear.

We describe three signaling pathways, involving transforming growth factor (TGF)-beta and canonical and noncanonical Wnt signaling, that collaborate to induce activation of the EMT program and thereafter function in an autocrine fashion to maintain the resulting mesenchymal and stem cell-like state. Importantly, the downregulation of endogenously synthesized inhibitors of autocrine signals by epithelial cells enables the induction of the EMT program. Conversely, disruption of autocrine signaling by added inhibitors of these pathways inhibits migration and self-renewal in primary human mammary epithelial cells and reduces tumorigenicity and metastasis by their transformed derivatives.

Our results indicate that ongoing autocrine signaling is required for maintenance of mesenchymal and stem cell traits both in primary and transformed mammary epithelial cells. At the same time, given the appropriate signaling context, these factors act in a paracrine manner that allow the derivation of mesenchymal and stem cell-like cells in both primary and transformed populations of mammary epithelial cells that do not display these attributes.

In the longer term, our observations may provide the basis for efficiently inducing differentiated epithelial cells to pass through an EMT and enter into a SC state without relying on genetic alteration. Such an approach may eventually be of great utility in the area of regenerative medicine. Acting in the opposing direction, the tumor-initiating cells of certain carcinomas may be forced to exit the mesenchymal, stem cell-like state by therapeutically interrupting multiple autocrine signaling pathways required for their continued residence in this state.

S09.3

Microsatelite instability and cancers: From biology to clinics

A. Collura, *A. Duval*;

Inserm, Team ‚Microsatellite Instability and Cancer', Paris, France.

Recently, we identified a mutated form of HSP110 (HSP110DE9) in a subset of colorectal cancer (CRC), i.e. CRC displaying microsatellite instability (MSI) (Dorard et al., Nat. Med. 2011). The human tumour phenotype referred to as MSI is frequent, being associated with inherited neoplasms (Lynch syndrome) and with 10-15% of sporadic colon, gastric and endometrial cancers. Unpublished results we recently obtained show that HSP110 is frequently mutated in human MSI neoplasms regardless of their primary location.

Heat shock proteins (HSPs) are necessary for cancer cell survival. The HSP110DE9 mutant is the first HSP inhibitor produced endogenously by the cancer cell. It is generated from an aberrantly spliced mRNA and lacks the HSP110 substrate binding domain. HSP110DE9 expression is observed at variable levels in MSI cancer cells and tightly correlates with the size of allelic deletions in a T17 DNA repeat located in HSP110 intron 8. HSP110DE9 impaired both the normal cellular localization of HSP110 and its interaction with other HSPs, thus abrogating the chaperone activity and anti-apoptotic function of HSP110 in a dominant negative manner. Forced overexpression of HSP110ΔE9 causes the sensitization of cells to anticancer agents such as oxaliplatin and 5-fluorouracil regardless of their microsatellite status. Importantly, these in vitro results have clinical significance, since MSI CRC highly expressing HSP110DE9 due to large T17 deletions show significantly longer relapse-free survival and response to chemotherapy compared to those with a low ratio (Collura et al., Submitted). More generally, we suspect HSP110 mutation to constitute a first step towards understanding of the clinical behaviour of colorectal, gastric and endometrial MSI tumours that have been reported to show an improved prognosis and possibly a different response to chemotherapeutic agents.

S10.1

Estimation of the human mutation rate by whole-genome sequencing

L. Jorde[1], *C. D. Huff*[2];

[1]Department of Human Genetics, University of Utah School of Medicine, Salt Lake City, UT, United States, [2]Anderson Cancer Research Center, Houston, TX, United States.

Whole-genome sequences of related individuals in large pedigrees provide new opportunities and challenges for disease-gene discovery. They also permit direct estimates of sex-specific human mutation rates. We have analyzed whole-genome sequence data from 21 individuals in a 5-generation pedigree that was ascertained because of autosomal dominant transmission of cardiac septal defects. We used our VAAST software package to identify the disease-causing mutation. VAAST incorporates pedigree information and the observed inheritance pattern with information about genetic variation in a control population and amino acid substitution severity under a unified likelihood analysis framework The variant responsible for septal defects in the family is a known missense mutation in the *GATA4* gene that had been previously identified through traditional linkage analysis. In our whole-genome analysis of the septal defect phenotype, the GATA4 gene was highly significant (Bonferroni corrected p-value = $8.9x10^{-5}$), and no other gene reached statistical significance. To estimate the male-specific intergenerational mutation rate, we identified novel single nucleotide variants (SNVs) that were absent in a father but were present on the paternal chromosomes of one of the father's offspring. We identified 12 de novo mutations in approximately 600 Mb of sequence data, with estimated false-positive and false-negative rates of less than $1x10^{-3}$. We estimated a male-specific, intergenerational mutation rate that is approximately five times greater than the female-specific mutation rate. This result agrees well with estimates based on phylogenetic comparisons. It is also consistent with our mutation rate estimates based on a three-generation kindred in which two members of the third generation have Miller syndrome.

S10.2

Fragile genomes generate more de novo mutations

E. Eichler;

Seattle, WA, United States.

No abstract received as per date of publication. Please check the programme planner at http://www.eshg.org/abstracts2012.0.html for possible updates.

S10.3

De Novo Mutations in Neurodevelopmental disorders

G. A. Rouleau, *J. Michaud*;

Centre of Excellence in Neurosciences of Université de Montréal, Centre Hospitalier de l'Université de Montréal, Faculty of Medicine, Université of Montréal, Montreal, QC, Canada.

INTRODUCTION: Schizophrenia (SCZ), autism spectrum disorders (ASD) and intellectual deficiency (ID) are common, devastating and poorly treated neurodevelopmental brain disorders. The wide spectrum of symptoms and clinical variability in these disorders suggest a complex genetic etiology, which is consistent with the numerous loci thus far identified by linkage, copy number variation and association studies. Although heritability in all three disorders may be as high as ~80%, the genes responsible for much of this heritability remain to be identified. Based on the observed reduced reproductive fitness, the relatively uniform world wide incidence, the increased risk of disease with increasing paternal age and the phenotypic complexity of each disease, we, and others, hypothesized that a fraction of this missing heritability may be the result of the occurrence of *de novo* mutations affecting any of a large number of genes. In order to test this hypothesis we first sequenced over 400 synaptic genes in 148 subjects with SCZ, 148 subjects with ASD and 96 subjects with ID. Many likely de novo mutations were identified - these plus relevant functional studies will be presented. Next we sequenced the exomes of SCZ, ASD and ID probands, plus their parents, identifying numerous additional *de novo* mutations (DNMs). In addition, 1/4 of identified DNMs are nonsense mutations, which is more than what is expected by chance. Interestingly, some of the identified genes, such as *SHANK3,* show deleterious *de novo* mutations in patients from the three disease cohorts, suggesting close biological overlap in these disorders. Our study supports the notion that DNMs may account for some of the missing heritability SCZ, ASD and ID while providing a list of genes possibly involved in disease pathogenesis.

S11.1
Genetic basis of primary microcephaly

C. G. Woods;
Cambridge, United Kingdom.

Primary Microcephaly can be inherited as a dominant and recessive disorder. Dominant genes are KIF11, and others to be reported. For KIF11 hemizygosity appears to be the mutational mechanism. Recessive genes are MICROCEPHALIN, WDR62, CDK5RAP2, CEP152, ASPM, CPAP, STIL and CEP63, with others to be reported. The mutational mechanism is null mutations - non-sense, splicing and frame-shifting INDELS. The exception being WDR62, where mis-sense mutations are found, but non-sense mutations additionally cause cerebral dysplasia.
All the Primary Microcephaly genes encode proteins involved in mitosis. One is involved in the timing of entry into mitosis, others in the mitotic spindle and the remainder in centrosome and centriole function during mitosis. These processes are ubiquitous, but it remains unexplained why it is only the brain that is affected. Furthermore, no unifying mechanism has yet emerged to explain how these particular mitotic apparatus proteins interact to modulate brain growth.

S11.2
Clinical aspects of primary microcephaly

A. Verloes;
Paris, France.

No abstract received as per date of publication. Please check the programme planner at http://www.eshg.org/abstracts2012.0.html for possible updates.

S11.3
DNA repair and microcephaly

B. Wollnik;
Köln, Germany.

No abstract received as per date of publication. Please check the programme planner at http://www.eshg.org/abstracts2012.0.html for possible updates.

S12.1
Molecular classification of primary lymphedema

P. Ostergaard;
St. George's University of London, London, United Kingdom.

We have demonstrated that stringent phenotyping can be helpful in gene identification. Building on 12 years of experience in our Primary Lymphoedema Clinic at St George's Hospital, London, an updated classification of this condition has been proposed by Connell *et al* (2012, Clin Genet). This new tool has been useful in our research department and we have had success in identifying genes for Primary Lymphoedema using this rigorous phenotyping combined with linkage analysis, Sanger sequencing and/or Whole Exome Sequencing. In this talk, the classification pathway for Primary Lymphoedema will be presented together with the latest genes we have discovered such as *GJC2*, *GATA2* and *KIF11*.

S12.2
Mouse models of lymphedema

T. Petrova;
Epalinges, Switzerland.

No abstract received as per date of publication. Please check the programme planner at http://www.eshg.org/abstracts2012.0.html for possible updates.

S12.3
Therapeutic trials in lymphedema

K. Alitalo;
Helsinki, Finland.

No abstract received as per date of publication. Please check the programme planner at http://www.eshg.org/abstracts2012.0.html for possible updates.

S13.1
Exome sequencing in sporadic autism spectrum disorders

J. Shendure;
Seattle, WA, United States.

No abstract received as per date of publication. Please check the programme planner at http://www.eshg.org/abstracts2012.0.html for possible updates.

S13.2
Autism genetics: at the crossroads of genomics and cognitive neuroscience

D. H. Geschwind;
Los Angeles, CA, United States.

No abstract received as per date of publication. Please check the programme planner at http://www.eshg.org/abstracts2012.0.html for possible updates.

S13.3
Large-scale dissection of molecular networks and mechanisms underlying Intellectual Disability Disorders

A. Schenck;
Nijmegen, Netherlands.

No abstract received as per date of publication. Please check the programme planner at http://www.eshg.org/abstracts2012.0.html for possible updates.

S14.1
Identification of cis- and trans-regulatory variation modulating microRNA expression levels

S. Antonarakis;
Geneva, Switzerland.

No abstract received as per date of publication. Please check the programme planner at http://www.eshg.org/abstracts2012.0.html for possible updates.

S14.2
Interrogating the RNA heterogeneity within cellular compartmens

R. Guigo;
Center for Genomic Regulation, Barcelona, Spain.

The unfolding of the instructions encoded in the genome is triggered by the transcription of DNA into RNA, and the subsequent processing of the resulting primary RNA transcripts into functional mature RNAs. RNA is thus the first phenotype of the genome, mediating all other phenotypic changes at the organism level caused by changes in the DNA sequence. While current technology is too primitive to provide accurate measurements of the RNA content of the cell, the recent development of Massively Parallel Sequencing Instruments has dramatically increased the resolution with which we can monitor cellular RNA. Using these instruments, the ENCODE project has surveyed the RNA content of multiple cell lines and subcellular compartments. The results of these surveys underscore pervasive transription, as well as great RNA heterogeneity between and within cells. Comparison of RNA surveys with other genome wide epigenetic surveys_such as those of binding sites for Transcription Factors, or of Histone modifications_reveals a very tightly coupling between the different pathways involved in RNA processing, transcription and splicing in particular.

S14.3

Transcribed dark matter: meaning or myth?

C. Ponting;
Oxford, United Kingdom.

No abstract received as per date of publication. Please check the programme planner at http://www.eshg.org/abstracts2012.0.html for possible updates.

S15.1

LRP5 in bone

M. Warman;
Boston, MA, United States.

No abstract received as per date of publication. Please check the programme planner at http://www.eshg.org/abstracts2012.0.html for possible updates.

S15.2

Acromelic dysplasia and TGFbeta signaling

V. Cormier-Daire;
Paris, France.

The acromelic dysplasia group includes four disorders namely Weill-Marchesani Syndrome (WMS), Geleophysic Dysplasia (GD), Acromicric dysplasia (AD) and Myhre syndrome (MS), all characterized by severe short stature, short extremities, restricted joint mobility, thick skin and pseudomuscular build. They are distinct by additional features and their pattern inheritance. WMS is characterized by the presence of dislocation of microspherophakia and has autosomal dominant or recessive mode of inheritance. GD is the more severe one, resembling a storage disorder with a progressive cardiac valvular thickening, tip toe walking, tracheal stenosis, bronchopulmonary insufficiency and often an early death. AD has an autosomal dominant mode of inheritance, distinct facial and skeleton features (a hoarse voice and internal notch of the femoral head). Finally, MS is sporadic, characterized by prognathism, deafness, developmental delay, thickened calvarium, and large vertebrae with short and large pedicles.

We first identified mutations in Fibrillin1(*FBN1*) in the dominant form of WMS and then mutations in A Disintegrin-like And Metalloproteinase domain with ThromboSpondin type 1 repeats 10 (*ADAMTS10*) in the recessive form of WMS. The function of ADAMTS10 is unknown but these findings support a direct interaction between ADAMTS10 and FBN1.

We then identified mutations in *ADAMTSL2* in the recessive form of GD and a hotspot of mutations in *FBN1* in the dominant form of GD and in AD (exon 41-42, encoding TGFβ binding protein-like domain 5 (TB5) of FBN1). The function of ADAMTSL2 is unknown. Using a yeast double hybrid screen, we identified Latent TGFβ Binding Protein 1 as a partner of ADAMTSL2. We found an increased level of active TGFβ in the fibroblast medium from patients with *FBN1* or *ADAMTSL2* mutations and an enhanced phoshorylated SMAD2 level, allowing us to conclude at an enhanced TGFβ signaling in GD and AD. Finally, a direct interaction between ADAMTSL2 and FBN1 was demonstrated suggesting a dysregulation of FBN1/ADAMTSL2/ TGFβ interrelationship as the underlying mechanism of the short stature phenotypes.

To identify the gene responsible for MS, we performed exome sequencing in 2 MS and selected *SMAD4* as a candidate gene. We identified *de novo* missense mutations, all involving Isoleucine residue at position 500, in the MH2 domain of SMAD4 in a total of 20 MS patients. In MS fibroblasts, we found decreased ubiquitination level of SMAD4 and increased level of SMAD4 supporting a stabilization of SMAD4 protein. Functional SMAD4 is required for canonical signal transduction through the oligomerization with phosphorylated SMAD2/3 and SMAD1/5/8. We therefore studied the nuclear localization of mutant SMAD complexes and found that the complexes translocate to the nucleus. We finally observed a decreased expression of downstream TGFβ target genes supporting impaired TGFβ driven transcriptional control in MS.

All together, our findings support a direct link between the short stature phenotypes and the TGFβ signalling. However, the finding of enhanced TGFβ signaling in Marfan phenotypes suggest the existence of yet unknown mechanisms regulating TGFβ action, possibly including tissue specific modulations. Finally, remembering the severity of GD, our ultimate goal is the design of drugs that can selectively inhibit this pathway.

S15.3

Osteogenesis imperfecta

B. Lee;
Baylor College of Medicine and Howard Hughes Medical Institute, Houston, TX, United States.

Over the past several years, a discovery of new genes causing osteogenesis imperfecta (OI) has provided exciting new insights into bone biology and the pathogenesis of brittle bone disease. At the same time, therapeutic studies in the area of osteoporosis have led to study of osteoporosis drugs in osteogenesis imperfecta. Most have focused on the use of anti-resorptive approaches such as intravenous bisphosphonates. More recently studies focused on anabolic therapies such as intermittent parathyroid hormone have been performed in adults with OI. The different clinical and diagnostic endpoints for these studies will be reviewed and potential new therapies informed by pathophysiological discoveries on these new OI genes will be discussed.

S16.1

To Tell or Not to Tell - How should we handle incidental findings obtained in the course of genome sequencing?

C. Netzer;
Universität Köln, Medizinische Fakultät - Uniklinik Köln, Institut für Humangenetik, Köln, Germany.

Next-generation sequencing (NGS) technologies have revolutionized genetic research within a few years and may very soon become part of routine clinical testing. There is a growing debate about the question whether incidental genetic findings about disease susceptibilities should be reported to the patients or research participants in all cases, in special cases, or not at all. The answer to this question is crucial for the informed-consent procedure and for the work-up of NGS data-sets. In this talk, some real-life examples of incidental genetic findings will be presented to illustrate the multiple dimensions of the problem. Possible solutions will be discussed with the audience.

S16.2

Biobanks: should individuals be informed of findings from biobank studies? Can informed consent be realized?

A. Cambon-Thomsen;
Toulouse, France.

No abstract received as per date of publication. Please check the programme planner at http://www.eshg.org/abstracts2012.0.html for possible updates.

S16.3

Informed consent in non-invasive prenatal diagnosis

H. Strange, *Z. Deans, A. Newson;*
ESRC Centre for Economic and Social Aspects of Genomics (Cesagen), Cardiff, United Kingdom.

Non-invasive prenatal diagnosis (NIPD) offers the opportunity for exciting changes to prenatal screening and diagnosis. It can give definitive results reliably at an early stage of pregnancy and without the miscarriage risk associated with invasive testing. NIPD is already used within the UK for fetal sexing for women at high risk of sex-linked disorders, and in routine screening for fetal RHD in D negative pregnant women. It is also available in the USA and China as advanced screening test for trisomy 21.

While the technology advances quickly, there is a need to examine carefully how the introduction of NIPD into public clinics and the private sphere might affect women and couple's capacity for making informed decisions.

The presentation starts by outlining reasons to safeguard a fully informed decision-making process, as far as is possible. This is followed by highlighting three key areas in which the process of informed decision-making might be affected by the introduction of NIPD. These are: prenatal screening programmes; direct-to-consumer testing; possible expansion to genome-wide sequencing.

Prenatal screening programmes: Against the background concern that prenatal screening and testing programmes have become 'routinised', evidence suggests that, with the apparent ease of NIPD and without any associated risk of miscarriage, healthcare professionals may regard informed decision-making as less important for NIPD.

Direct-to-consumer testing: Any test that is partially carried out at home raises concerns about consent, pressure, and possible coercion by individuals and companies. Direct-to-consumer testing also presents logistical challenges for making sure the results are communicated accurately and are properly understood and interpreted by the recipient.

Possible expansion to genome-wide sequencing: Although not yet clinically available, if NIPD were to be offered for whole genome sequencing, this would present a significant challenge to traditional models of informed consent procedures.

ESHG Educational Sessions

ES1.1
Molecular Genetic Analysis in Complex Diseases
***M. Nöthen**;*
Bonn, Germany.

No abstract received as per date of publication. Please check the programme planner at http://www.eshg.org/abstracts2012.0.html for possible updates.

ES1.2
Turning discovery into prediction
***C. van Duijn**;*
Rotterdam, Netherlands.

No abstract received as per date of publication. Please check the programme planner at http://www.eshg.org/abstracts2012.0.html for possible updates.

ES2.1
Blistering Diseases
***L. Bruckner-Tuderman**;*
Freiburg, Germany.

No abstract received as per date of publication. Please check the programme planner at http://www.eshg.org/abstracts2012.0.html for possible updates.

ES2.2
Ichtyosis
***J. Fischer**;*
Institut für Humangenetik, Universitätsklinikum Freiburg, Freiburg, Germany.

The epidermis forms the outermost, protective layer of the skin and functions as the essential barrier of the body against dehydration, mechanical insults, and the intrusion of microbes, toxins, and allergens. Ichthyoses comprise a clinically and genetically heterogeneous group of disorders of keratinization/cornification characterized mainly by abnormal skin scaling over the whole body; some patients present with severe symptoms, including a collodion membrane at birth. The main skin phenotypes are lamellar ichthyosis and congenital ichthyosiform erythroderma. Most ichthyoses are inherited genetic disorders, in which gene defects (mutations) lead to an impaired epidermal permeability barrier. Genetic analyses have elucidated numerous associations between gene mutations and the presence of an ichthyosis phenotype. Here we will present an update on the main genetic forms of ichthyoses.

ES3.1
How to get published in the European Journal of Human Genetics
***G. B. van Ommen**;*
Leiden, Netherlands.

No abstract received as per date of publication. Please check the programme planner at http://www.eshg.org/abstracts2012.0.html for possible updates.

ES4.1
The Family's Experience of a Genetic Disorder
***S. McDaniel**;*
Rochester, NY, United States.

No abstract received as per date of publication. Please check the programme planner at http://www.eshg.org/abstracts2012.0.html for possible updates.

ES4.2
Using systemic ideas in Genetic Counsellors' group supervision
***T. O'Neill**;*
North Manchester CAMHS, Central Manchester University Hospitals NHS Foundation Trust, Manchester, United Kingdom.

The presenter, a Consultant Family Therapist working in child and Adolescent Mental Health, will describe the use of a Reflecting Team format in the supervision of Genetic Counsellors. Reflecting Teams have been used in family therapy since Tom Andersen and his colleagues in Norway introduced them in the 1980's. Giving clients the opportunity to see and hear the therapy team talk about clients' dilemmas remains a widely used practice in contemporary family therapy.

The method has also been applied to other teaching, training and supervision situations because of the potential advantages such as presenting feedback in a non-threatening manner and offering a multiverse of perspectives and new ideas and it is for these reasons that the presenter has applied the approach to supervision with Genetic Counsellors. The presenter will explain some of the background to this approach and the practicalities of its application illustrated by reference to anonymised cases discussed in the supervision.

ES5.1
Array CGH and applications
***L. Feuk**;*
Uppsala, Sweden.

No abstract received as per date of publication. Please check the programme planner at http://www.eshg.org/abstracts2012.0.html for possible updates.

ES5.2
Next Generation Sequencing and Applications
***I. G. Gut**;*
Centro Nacional de Analisis Genomico, Barcelona, Spain.

Nucleic acid sequencing has been the workhorse of genome research from the very beginning in the late 80's. Classical Sanger sequencing was used for the Human Genome Sequencing project and was successively refined and finally used with automated capillary gel electrophoresis separation. The first sequence of the human genome was generated using exclusively this technology (Lander et al. Nature 2001, Venter et al. Science 2001). In 2005 nucleic acid sequencing saw a paradigm shift with the introduction of the Genome Sequencer from Roche and was shortly followed by other 2nd generation sequencers from Illumina, LifeTechnologies and Helicos. 2nd generation sequencers rely on the preparation of random physically separated arrangements of individual fragments of the input nucleic acid, followed by cyclic base additions to the random array and high resolution imaging. 2nd generation sequencers are combinations of high-resolution imaging instruments and microfluidic devices. 2nd generation sequencers are the main tool used in large-scale projects such as the 1000 Genomes, the International Cancer Genome Consortium (ICGC), the International Human Epigenome Consortium (IHEC) and will play an important role in the International Rare Disorder Research Consortium (IRDiRC).
However, development of sequencing methods has not stopped at 2nd generation. Several instruments have been introduced, that move beyond in their characteristics. Detection systems are shifting from optical detection to electrical detection. I would characterise true 3rd generation as a method that does not rely on a replication method, such as primer extension or oligonucleotide ligation, for sequence determination, and delivers long clonal reads. Methods such as the GridION System from Oxford Nanopore Technologies fall into this category. Even 4th generation sequencing methods are already showing on the horizon with developments of the EU-funded FP7 project READNA (www.cng.fr/READNA). A 4th generation sequencing method would allow the determination of nucleic sequences cell-by-cell within a histological section.

ES6.1
Clinical and genetic heterogeneity of amyotrophic lateral sclerosis
***M. Sabatelli**;*
Department of Neurology, Pol. ‚A. Gemelli', Università Cattolica del Sacro Cuore, Rome, Italy.

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disorder involving upper and lower motor neurons. Within definite ALS, variants are recognized based on the age of onset, site of localization of first signs, rate of progression of the disease, relative mix of upper and lower motor neuron deficits and the presence of fronto tempora dementia (FTD). The question of whether ALS is a single disease with variable expression or different diseases with heterogeneous causes still remains unsolved.
Most ALS cases are sporadic (SALS) while familial ALS (FALS) account for about 5% of total cases. Over the last 20 years the pathogenic role of genes such as SOD1 , TARDBP, FUS, C9ORF72, OPTN, ATXN2 , VCP , ANG and UBQLN-2 has emerged and mutation in these genes have been identified in

about 50-60% of FALS. A large hexanucleotide (GGGGCC) repeat expansion in the first intron of C9ORF72, represent the most common mutation in FALS being respomsible of about 40% of cases. Near 50% of patients harbouring C9ORF72mutation have FTD, compared to 30% of patients with TARDBP mutation and 9% of cases with unknown gene. Patients with FUS and C9ORF mutations have shorter survival with respect to patients with different mutations. .

Discoveries in the genetics of FALS are changing the scenario of sporadic ALS as well, making the distinction between familial and apparently sporadic ALS less clear than previously assumed. In fact mutations in the same genes involved in FALS may be identified also in a considerable proportion of patients with apparent sporadic disease. The same genes may act as either Mendelian genes in FALS or low-penetrance risk alleles in SALS. This challenges the current indication for DNA analysis only in cases with a known family history of ALS.

ES6.2
Hereditery neuropathies

V. Timmermann;
Antwerpen, Belgium.

No abstract received as per date of publication. Please check the programme planner at http://www.eshg.org/abstracts2012.0.html for possible updates.

ES7.1
Next generation sequencing goes diagnostic: First experiences

L. Biesecker;
National Human Genome Research Institute, NIH, Bethesda, MD, United States.

Medicine is being challenged by the DNA sequencing revolution. Next generation (NGS) has led to a precipitous drop in costs, making clinical sequencing of the genome or exome comparable in cost to other diagnostic tests. The question before us is how we can harness this technology to benefit patients. As well, we do not know which medical scenarios are appropriate for NGS, nor how we should work with patients and their families to communicate the results. To address these questions, research is needed to develop an evidence base upon which we can create practice standards. We developed the ClinSeq™ pilot project to explore these questions. The goals of this project are to enroll 1,000 subjects, initially focusing on cardiovascular disease, apply NGS technologies, and pilot approaches to consent, data analysis, and return of results.

The initial analyses of ClinSeq™ have shown that subjects have good abilities to understand the sequencing research through informed consent. They are eager to undergo sequencing and receive results, both for their own benefit as well as to benefit science. They are highly motivated, willing to engage in follow-up research to correlate genotype and phenotype. We began our analyses by screening 572 exomes for highly penetrant mutations for serious medical conditions. These included familial hypercholesterolemia (n=9 identified), cancer susceptibility (n=8), cardiomyopathies (n=4), arrhythmias (n=4), malignant hyperthermia (n=2), and hereditary liability to nerve and pressure palsies (n=3). Most of these have been returned to the subjects with medical and genetic counseling. In total, we have detected and returned genetic test results for 30 patients. Most of these diagnoses were not suspected to be present by the patient or clinician before the sequencing. While this highly educated and affluent cohort of patients is not representative, there is a substantial desire for NGS results. Further analyses of additional categories of genes (e.g., recessive carrier status) will yield many more clinically relevant results. I will give examples of some of the results we have returned to illustrate the range of the disorders and the reactions of the subjects. These data should be useful to others in planning how to deploy MPS in clinical settings.

ES7.2
BRCA1 and 2 diagnostics

G. Matthijs;
Leuven, Belgium.

No abstract received as per date of publication. Please check the programme planner at http://www.eshg.org/abstracts2012.0.html for possible updates.

ES8.1
Huntington

A. Durr;
Paris, France.

No abstract received as per date of publication. Please check the programme planner at http://www.eshg.org/abstracts2012.0.html for possible updates.

ES8.2
Myotonic dystrophy: complex repeats in a complex disorder

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Myotonic dystrophy is an autosomal dominant disorder presenting a wide range of symptoms frequently including cataracts, heart conduction defects, insulin insensitivity and hypersomnia, along with muscular atrophy and myotonia. Disease severity is extremely variable and the disorder displays striking anticipation progressing from the mild late onset form to congenital disease in as little as three generations. The most common form of myotonic dystrophy, type 1, is caused by the expansion of a CTG repeat in the 3'-untranslated region of the DMPK gene. The CTG tract ranges from 5 to ~40 repeats in the general population. Patients inherit from 50 to 1,000+ repeats, with longer alleles associated with earlier age at onset. The expanded repeat is highly unstable and nearly always increases when transmitted from one generation to the next, explaining the anticipation observed. The expanded repeat is also unstable in the soma in a process that is age-dependent, tissue-specific and expansion-biased, with particularly large expansions in the affected tissues. Although the majority of DM1 patients present with a pure expanded CTG repeat array, recent evidence has revealed that a subset of patients carry alleles with variant repeats. These variant repeats stabilise the array and are associated with milder symptoms. Pathogenesis appears to be primarily caused by the gain of function of the DMPK transcript containing a large CUG tract that remains trapped within foci in the nucleus and disturbs the function of two families of RNA splicing factors that leads to genome-wide dysregulation of alternative splicing. In particular, mis-splicing of the CLC1 chloride channel gene appears to be associated directly with myotonia and mis-splicing of the insulin receptor gene with insulin insensitivity. Exciting recent developments suggest that the RNA gain of function defect may be alleviated using antisense oligonucleotides paving the way for new treatments in this devastating disorder.

ESHG Concurrent Sessions

C01.1

Mutations in the chromatin modifier gene *KANSL1* cause the 17q21.31 microdeletion syndrome

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The 17q21.31 microdeletion syndrome is characterized by intellectual disability (ID), hypotonia, facial dysmorphism, epilepsy, and congenital malformations. The recurrent deletion encompasses five known genes, *CRHR1*, *IMP5*, *MAPT*, *STH,* and *KANSL1*, in addition to four putative genes.

We identified two atypical *de novo* deletions encompassing only part of *MAPT* and *KANSL1* in two children with ID and typical features of 17q21.31 microdeletion syndrome. Next, we selected 16 individuals with core features of the 17q21.31 microdeletion syndrome. Sanger sequencing revealed no pathogenic changes in *MAPT*. However, sequence analysis of *KANSL1* did reveal two heterozygous mutations, a nonsense mutation and a splice site mutation, both predicted to cause loss-of-function.

KANSL1 is a widely expressed gene encoding a member of the highly conserved NSL complex. This complex contains, among others, the H4K16 acetyltransferase KAT8.

To explore the effect of haploinsufficiency of *KANSL1* on gene expression levels, we performed whole transcriptome (mRNA) sequencing using EBV-transformed cell lines of the individual with the *KANSL1* splice site mutation and three individuals with the classical 17q21.31 deletion. Functional annotation clustering of genes that were differentially expressed in all samples compared to controls revealed enrichment of genes involved in neuronal processes. Further evidence that *KANSL1* has a function in neurons is provided by our studies in *Drosophila*. Tissue-specific knockdown of wah (fly ortholog of *KANSL1*) in the mushroom bodies was sufficient to cause a 25% reduction in learning ability ($P<0.05$).

In conclusion, our findings demonstrate that haploinsufficiency of *KANSL1* is sufficient to cause the classical 17q21.31 microdeletion syndrome phenotype.

C01.2

Mutations in the *KIAA1267* gene cause the 17q21.31 deletion syndrome phenotype

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The chromosome 17q21.31 deletion syndrome is a genomic disorder usually associated to a recurrent chromosome deletion, recently restricted to a 160-274 kb segment on 17q21.31, including only three genes, *MAPT, STH* and *KIAA1267*. A question to be still addressed is whether this condition is a contiguous gene syndrome or a monogenic disorder. *MAPT* has been considered the major candidate gene, however no productive mutations have been identified so far. Starting from the hypothesis that a single gene mutation in undeleted patients could affect either one gene residing within the deletion interval, or another gene participating in a shared molecular pathway, we performed exome sequencing of one undeleted patient and both parents. A *de novo* heterozygous nonsense mutation within exon 6 of the *KIAA1267* gene (OMIM *612452) was identified and validated by Sanger sequencing: c.C1816T, p.R606X. This result prompted us to sequence *KIAA1267* in a second undeleted patient, in which a *de novo* heterozygous frameshift mutation introducing a premature stop codon was detected within exon 13: c.2785_2786 delAG, p.R929G fsX44. Both patients presented with a full chromosome 17q21.31 deletion syndrome phenotype, including intellectual disability, highly distinctive facial features, failure to thrive in infancy, hypotonia, motor delay, and a friendly behavior. We consider that chromosome 17q21.31 deletion syndrome is a single gene disorder, caused by haploinsufficiency of *KIAA1267*. Knowledge of the major causal gene will broaden the diagnostic spectrum of the 17q21.31 deletion syndrome, and will accelerate the understanding of its molecular pathogenesis.

C01.3

Loss of function mutations in *TGFB2* cause Loeys-Dietz syndrome

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Loeys-Dietz syndrome (LDS) is an autosomal dominant connective tissue disorder with both features that overlap with and distinguish it from Marfan syndrome (MFS). In its most typical presentation, LDS is characterized by the triad of hypertelorism, bifid uvula/cleft palate and widespread aortic/arterial aneurysms and tortuosity. LDS is most often caused by heterozygous loss-of-function mutations in genes encoding positive effectors of transforming growth factor beta (TGFβ) signaling including either subunit of the TGFβ receptor (TGFβR1/2) and Smad3. Nevertheless, in aneurysmal aortic tissues a signature compatible with increased TGFβ signaling is consequently observed, engendering controversy regarding the mechanism of aortic aneurysmal disease. TGFβs comprise three multipotential cytokines, encoded by three separate genes, that regulate multiple aspects of cellular performance including, proliferation, differentiation, migration and synthetic repertoire. Here, we report heterozygous deletions or loss-of-function mutations of the gene encoding the transforming growth factor beta 2 (TGFβ2) ligand in eight families characterized by a phenotype within the MFS/LDS spectrum and demonstrate upregulation of TGFβ signaling in aortic tissue from affected individuals. Furthermore, haploinsufficient Tgfb2-/+ mice demonstrate aortic root aneurysm by 8 month of age and biochemical evidence of increased canonical and noncanonical TGFβ signaling. Mice that harbor a mutant MFS allele (*Fbn1*$^{C1039G/+}$) in the context of *Tgfb2* haploinsufficiency show a pronounced increase in TGFβ signaling and phenotypic worsening in association with normalization of TGFβ2 expression and excessive expression of TGFβ1. Taken together, these data implicate compensatory autocrine and/or paracrine events and excessive TGFβ signaling in the pathogenesis of TGFβ vasculopathies.

C01.4

Identification of the cause of Blue Rubber Bleb Nevus Syndrome

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Blue rubber bleb nevus syndrome (BRBN) is a rare sporadic congenital disorder (OMIM # 112200) characterized by multiple venous malformations all over the skin, often on hands and feet. Patients can present with a few to hundreds of cutaneous and pathognomonic gastrointestinal lesions. These are most commonly in the small intestine, documented by endoscopy, colonoscopy, or magnetic resonance imaging (MRI). Although several case reports have been published, the etiopathology of BRBN is still unknown.

Since inherited venous malformations (VMCMs) are caused by germline activating TIE2 mutations and common sporadic venous malformations (VMs)

are due to strongly hyperphosphorylating somatic TIE2 mutations, we hypothesized that BRBN may also be part of the spectrum of TIE2-mediated phenotypes.
To test this, we screened the coding region of TIE2 by direct sequencing of genomic blood DNA and cDNA from the resected lesions of 14 patients. In 16 tissues from 10 patients, we identified mutations leading to amino acid changes, absent in the blood DNA from patients as well as in cDNA from control tissues. These changes occur at highly conserved residues, and are not found in dbSNP. In contrast to VMCMs and VMs, BRBNs predominantly show double (*cis*) mutations, suggesting a phenotype-genotype correlation. They cause ligand-independent receptor hyperphosphorylation *in vitro*. These results unequivocally demonstrate that BRBNs are caused by post-zygotic activating TIE2 mutations.

C01.5

Serin diet relieves symptoms of Hereditary Sensory and Autonomous Neuropathy type 1A caused by a c.992 C>T, p.(Ser331Phe), mutation in SPTLC1

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Hereditary sensory and autonomic neuropathies (HSAN) are a genetically and clinically heterogeneous group of disorders associated with sensory dysfunction. HSAN1 is a dominantly inherited sensorimotor axonal neuropathy. The patient has 3 healthy siblings and healthy parents. Symptoms: Coinciding with the start of ambulation motor disabilities; generalized hypotrophy; reduced walking distance; unstable gait; multiple falls; no mental retardation; MRI studies of the brain were normal; reduced pain sensitivity: recurrent traumatic and thermal injuries of feet, knees and hands; disturbed wound healing; ulcerated fingertips; unnoticed fracture of a metatarsal bone; at the age of 9 years: bilateral lensectomy due to juvenile cataract; cataract surgery: repetitive ulcerations of the cornea, poor healing tendency; complete retinal detachment (right eye); nerve biopsy: marked wasting of myelinated fibres, axonal damage; axonal motor and sensory neuropathy. Finally a de novo c.992 C>T, p.(Ser331Phe), mutation in the SPTLC1 gene was identified. This mutation turns the sphingolipid synthesis to neurotoxic lipids in this patient as well as in cell cultures. A serin diet (400mg/kg bodyweight) developed in an animal model (SPTLC1 mutation p.(Cys133Trp)) resulted after 3 months in an overall improvement: ameliorated growth of nails and hair; wounds of the skin show faster healing; gain of weight; coughassist could be reduced to only one treatment during the winter period; sweating is reconstituted; ability to an extended period of upright standing. This HSAN patient is an excellent example for effective translational medicine and the first individual in Germany receiving a causal treatment for a Hereditary Sensory and Autonomous Neuropathy.

C01.6

SMA patients show concordant responses to valproic acid from blood to neurons while nonresponsivness is facilitated by CD36

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Proximal spinal muscular atrophy (SMA) is the number one genetic killer during early infancy. SMA is caused by functional absence of SMN1 leading to progressive degeneration of spinal α-motoneurons. Currently, no cure for SMA is available.
Therapeutic approaches are focusing on SMN2, since its copy number mainly modifies disease severity. Previously, we have been able to show that the anti-epileptic drug valproic acid (VPA) increases SMN levels in vitro, ex vivo as well as in VPA-treated SMA patients. A pilot clinical trial with VPA revealed that 1/3 of the patients responded positively to VPA treatment, while for 2/3 either no response or even the opposite effect was detected. To elucidate mechanisms underlying VPA-nonresponsiveness, we collected fibroblasts lines from >30 SMA-patients undergoing VPA-treatment. We demonstrated that response to VPA was concordant in about 65% between blood and fibroblasts. Furthermore, by generating GABAergic neurons from fibroblast-derived iPS cells, we showed that similar response to VPA is retained even in the CNS neurons. This is the first prove that response to a potential SMA drug is concordant between blood, fibroblasts and neurons. Moreover, by transcriptome-wide μ-array we identified increased expression of CD36, a known LCFA-translocase, as the pivotal factor suppressing positive response to VPA.
Our data provide first evidence that monitoring VPA response in fibroblasts is indeed feasible to infer response in CNS neurons. Furthermore, CD36 was identified as the crucial protein suppressing response to VPA. This is of major implication also for other diseases treated with VPA such as epilepsy or migraine.

C02.1

KIAA1797/FOCAD encodes a novel focal adhesion protein with tumor suppressor function in gliomas

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In a strategy to identify novel genes involved in glioma pathogenesis by molecular characterization of chromosomal translocation breakpoints, we identified the *KIAA1797* gene, encoding a protein with an as yet undefined function, to be disrupted by a 7;9-translocation in a primary glioblastoma culture. Array-based comparative genomic hybridization detected deletions involving *KIAA1797* in around half of glioblastoma cell lines and glioblastomas investigated. Quantification of mRNA levels in human tissues demonstrated highest *KIAA1797* expression in brain, reduced levels in all glioblastoma cell lines and most glioblastomas, and similar levels in glial and neuronal cells by analysis of different hippocampal regions from murine brain. Antibodies against KIAA1797 were generated and showed similar protein levels in cortex and subcortical white matter of human brain, while levels were significantly reduced in glioblastomas with *KIAA1797* deletion. By immunofluorescence of astrocytoma cells, KIAA1797 co-localized with vinculin in focal adhesions. Physical interaction between KIAA1797 and vinculin was demonstrated via co-immunoprecipitation. Functional *in vitro* assays demonstrated a significant decrease in colony formation, migration and invasion capacity of LN18 and U87MG glioma cells carrying a homozygous *KIAA1797* deletion ectopically expressing *KIAA1797* compared to mock-transduced cells. In an *in vivo* orthotopic xenograft mouse model, U87MG tumor lesions expressing *KIAA1797* had a significantly reduced volume compared to tumors not expressing *KIAA1797*. In summary, the frequently deleted *KIAA1797* gene encodes a novel focal adhesion complex protein with tumor suppressor function in gliomas, which we name “focadhesin”. Because *KIAA1797* genetic variation has been implicated in Alzheimer’s disease, our data is also relevant for neurodegeneration.

C02.2

Exome sequencing of late recurrence T-cell acute lymphoblastic leukemia in children confirms second leukemia and exposes predisposition candidate genes

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Second hematologic malignancies in non-syndromic children without family history for cancer may be mistaken for relapses or therapy-related malignancies. Recently, we identified 8 T-cell acute lymphoblastic leukemia (T-ALL) patients with two fully discordant consecutive leukemias based on TCR-rearrangements and DNA copy number aberrations, strongly suggesting predisposition (J.Clin.Oncol.2011). Here, we performed exome sequencing on leukemic and complete remission samples from four of these patients in order to identify predisposing mutations.
Per exome, we found ~25,000 variants. Known, synonymous, and intronic variants as well as variants called in <20% of the reads were excluded. We identified and validated between one and six somatic variants per leukemic sample, the majority of which affected known T-ALL genes, such as *PTEN*, *FBXW7* and *PHF6*. None of these were shared between two consecutive leu-

kemic samples, which confirms that samples are clonally unrelated and thus represent independent second leukemias.

With respect to genetic predisposition, we focused on recurrently affected and known T-ALL associated genes. In three patients we identified highly conserved missense variants in *TYK2, RANBP17,* and *TIAL1*, respectively. *TYK2* belongs to the family of Janus kinases, which play a role in the pathogenesis of several hematologic malignancies. The TYK2 variant G761V is located in a highly conserved region of the pseudokinase-like domain, which is frequently affected in the homologous JAK2 kinase in precursor B-cell leukemias.

In conclusion, we confirmed that consecutive leukemic presentations in patients with late T-ALL recurrences may be fully discordant and, thus, represent independent leukemia occurrences, most likely caused by predisposing germline mutations.

C02.3

Somatic GATA2 zinc finger 1 mutations are exclusively associated with bi-allelic CEBPA mutations in acute myeloid leukemia (AML) and disrupt the capacity of GATA2 to enhance CEBPA-mediated activation of transcription

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Cytogenetically normal acute myeloid leukemia (CN-AML) with biallelic CEBPA gene mutations (biCEPBA) represents a distinct disease entity with a favourable clinical outcome. So far, it is not known if other genetic alterations cooperate with biCEBPA mutations during leukemogenesis. To identify additional mutations, we performed whole exome sequencing of five biCEBPA patients and detected somatic GATA2 zinc finger 1 (ZF1) mutations in 2 out of 5 cases. Both GATA2 and CEBPA are transcription factors crucial for hematopoietic development. Inherited or acquired mutations in both genes have been associated with leukemogenesis. Further mutational screening detected novel GATA2 ZF1 mutations in 13 of 33 biCEBPA positive CN-AML patients (13/33: 39.4%). No GATA2 mutations were found in 38 CN-AML patients with a monoallelic CEBPA mutation and in 89 CN-AML patients with wild-type CEBPA status. In Kaplan Meier survival analysis, the presence of GATA2 mutations did not negatively impact on the favourable overall survival and event free survival of biCEBPA patients. In reporter gene assays, all tested GATA2 ZF1 mutants showed reduced capacity to enhance CEBPA-mediated activation of transcription, suggesting that the GATA2 ZF1 mutations may collaborate with biCEPBA mutations to deregulate target genes during malignant transformation. We thus provide evidence for a genetically distinct subgroup of CN-AML. The specific association of mutations affecting two interacting regulators of hematopoiesis suggests a novel concept for leukemogenesis: The simultaneous mutational targeting of two transcription factors that function in the same differentiation pathway in AML.

(P.A.G. and A.D. contributed equally to this work)

C02.4

Integrated genomic and epigenomic profiling of TP53 and non-TP53 Li-Fraumeni syndrome (LFS) tumors reveals multiple and shared hits in the p53 network

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Li-Fraumeni Syndrome (LFS) is a prototypic, clinically and genetically heterogeneous inherited cancer syndrome. Most cases (>70%) are due to dominant, variably penetrant, germline mutations in the tumor suppressor gene *TP53*. In *TP53* and non-*TP53* LFS, there is evidence for risk heterogeneity within and between families. While *TP53* mutations predispose LFS patients, they do not appear to be sufficient and additional genetic and epigenetic "hits" are necessary for tumorigenesis. To identify second somatic hits as downstream drivers of p53-mediated tumorigenesis, we performed genomic and epigenomic profiling of primary soft tissue sarcomas, osteosarcomas and matching constitutive samples of 10 LFS patients (6 with, 4 without *TP53* mutations). We also performed whole genome sequencing of a subset of tumor/normal pairs with an inherited *TP53* mutation. Although we observed chromothripsis in a subset of tumor samples, it was independent of *TP53* mutation status. Integration of the observed genetic genomic and epigenomic alterations into the extended p53 and p16/RB pathways revealed multiple and shared hits (irrespective of *p53* status or tumor type), in numerous p53 transcriptional targets and interacting proteins (e.g., *ATM*, *CDKN1A, CDKN2A, CHK1, PTEN, MDM2/4*). Identification of recurrent somatic alterations in p53-network genes in independent LFS tumors is remarkable. This indicates that p53 defects alone (due to inherited mutations) are not sufficient and that additional hits in genes with p53-associated functions are not redundant but rather are a necessary part of LFS tumorigenesis. Recurrent somatic alterations cooperating with p53 in LFS tumors appear to cluster in a limited number of cellular pathways.

C02.5

Leupaxin mediates cytoskeleton remodeling in prostate cancer cells

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The focal adhesion protein leupaxin (LPXN) is overexpressed in prostate cancer (PCa). Recently, we showed that LPXN is involved in the progression of PCa via deregulation of p120CTN. In the present study we analyzed the LPXN-mediated adhesive and cytoskeletal changes during PCa progression. After downregulation of LPXN expression we could show an unambiguous reduced adhesiveness of PCa cells PC-3 and DU 145. LPXN knockdown resulted in a reduced cell surface area and reduced formation of focal adhesion sites in these cells. Interestingly, we found decreased expression of small GTPase RhoA and increased expression of Rac1. Furthermore, the expression pattern of several integrins was deregulated after LPXN knockdown, specifically β1-integrin expression was downregulated.

To identify a candidate protein that mediates the cytoskeletal changes after LPXN knockdown, we performed a Yeast-two-Hybrid screen. The actin binding protein caldesmon (CaD) was identified as a putative interaction partner of LPXN. Co-immunoprecipitation and a proximity ligation assays confirmed the interaction of LPXN with CaD. Furthermore, we demonstrated that CaD expression is upregulated in PCa cells and that knockdown of CaD by RNA interference leads to an increased migration and invasion, whereas no changes in proliferation were detected. Interestingly, we found that knockdown of LPXN lead to a decrease in phosphorylated, inactive CaD (pCaD) but total CaD levels remained unaffected. Subsequently, low levels of pCaD resulted in reduced migration of PCa cells.

Taken together our present results indicate that LPXN mediates cytoskeletal changes during PCa progression through the regulation CaD phosphorylation.

C02.6

Clinical Application of Next Generation Sequencing Technology for the Detection of Clinically Actionable Mutations

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Next-generation sequencing (NGS) technologies have significantly accelerated the identification of cancer-causing mutations. However, the clinical application of NGS technologies to detect cancer gene mutations has been extremely limited. We have assessed the performance of a novel NGS technology that merges multiplex PCR with ion semiconductor sequencing in our clinical diagnostic laboratory. The test interrogates 739 common mutations in 46 cancer genes including many clinically actionable mutations concurrently. First, we studied 12 tumor samples including 4 archived FFPE, 4 blood/bone marrow, and 4 cell line samples with known mutations to evaluate the sensitivity and specificity of the test. We then studied 34 de-identified, archived FFPE tumor samples of unknown genotype to further evaluate the efficacy of the test. Using the technology, we successfully identified all known mutations previously detected by Pyposequencing or Sanger sequencing technologies. Multiple serial dilution studies showed that the test could detect mutations at frequencies as low as 5% with 99% confidence. For the samples of unknown genotype, we detected 29 COSMIC mutations in 22 samples. Analysis of the variant call data showed that a minimum of 100X coverage is required in order to detect mutations at 10% frequency or above; a minimum 300K final library reads are necessary in order to minimize/ eliminate amplicon dropout. Our experience demonstrated that this targeted NGS test can effectively detect hundreds of cancer gene mutations with input DNA as low as ten nanograms, turn around time as short as two days, and significantly lower cost compared to traditional Sanger sequencing.

C03.1

Arm to Leg Transformation in Humans associated with CNVs at the PITX1 locus

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Here we report three non-related families in which affected individuals show features of an arm-to-leg transformation. On X-ray examination, the distal humerus was broadened and the olecranon was missing thus resembling the shape of the femur. The hands were medially deviated and in the wrist a fusion of the triquetral and pisiform formed a structure similar in shape to the calcaneus of the ankle. Furthermore, attachments of the tendons were abnormal. We mapped the condition to a 5 Mb region on the long arm of chromosome 5 (5q31.1) but were not able to identify a coding mutation. Next we screened the linkage interval for CNVs by custom high-resolution array CGH analysis which detected a microdeletion 400 kb 5' of PITX1. A similar deletion was identified in a second family. A third family did not show the deletion but, suspecting a balanced structural variation, we performed whole genome sequencing in one individual. Using a bioinformatic approach focused on the PITX1 genomic region we identified a translocation with the breakpoint located 3' of the deletions detected in the other cases. PITX1 is a transcription factor known to determine hindlimb identity. Based on previous mouse work, the genomic rearrangements are likely to result in a misexpression of PITX1 in the forelimb thus causing a partial arm-to-leg transformation. The structural variations identified are likely to remove active PITX1 forelimb suppressor or insulator elements and relocate forelimb enhancer elements into the gene desert neighbouring the PITX1 gene.

C03.2

Microduplications Upstream of *MSX2* are Associated with a Phenocopy of Cleidocranial Dysplasia

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Cleidocranial Dysplasia (CCD) is an autosomal dominant skeletal disorder characterized by hypoplastic or absent clavicles, increased head circumference, large fontanels, dental anomalies, and short stature. Although CCD is usually caused by mutations leading to haploinsufficiency of *RUNX2*, the underlying genetic cause remains unresolved in about 25% of cases. Besides RUNX2, MSX2 is another transcription factor known to play important roles during many developmental processes including tissue organogenesis, craniofacial and limb development.

Here we describe two unrelated individuals with microduplications upstream of *MSX2* on chromosome 5q35.2. One of the affected individuals presented with a phenocopy of CCD. In addition to a classical CCD phenotype the other affected individual had a complex synpolydactyly of the hands and postaxial polydactyly of the feet which have so far never been reported in association with CCD or copy number variations (CNVs) on 5q35.2. The microduplications overlap in a ~219 kb region that contains several highly conserved non-coding elements which are likely to be involved in *MSX2* gene regulation. Functional analyses using viral overexpression in chicken cells demonstrated that the inhibitory effect of *Msx2* overexpression on mineralization can not be ameliorated by forced *Runx2* expression.

Our results indicate that CNVs affecting non-coding regions upstream of a gene can cause developmental defects, and that the resulting phenotype can be distinct from those caused by point mutations or CNVs encompassing the corresponding gene. Taken together, our findings reveal an additional mechanism for the pathogenesis of CCD, particularly with regard to the spatiotemporal regulation of *MSX2*.

C03.3

Mutations in distinct domains of BMP1 cause Osteogenesis Imperfecta with variable bone phenotypes

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Using an innovative exome sequencing strategy, in affected individuals from a consanguineous Turkish family with autosomal recessive Osteogenesis Imperfecta (OI) associated with an increased bone mineralization density, we identified a causative homozygous missense mutation, p.Gly12Arg, in the novel OI gene BMP1. The mutation is located within the signal peptide and we provide evidence for an impaired secretion and alteration in post-translational modification of the mutant protein. To determine the underlying molecular pathogenesis, we show that hypomorphic bmp1 zebrafish mutants present with delayed osteogenesis, defects in bone formation, recurrent fractures in fin rays, and osteopenia in vertebrae, which during larval stages develops into a significant high bone mass phenotype in these mutants. Further screening of patients with OI identified a second homozygous mutation, p.Asp284Val, in BMP1. Interestingly, the index patient of this family presented with a classical severe form of OI with drastically reduced bone density. The mutation is located nearby the proteolytic domain of BMP1 suggesting a different pathogenic mechanism. Ongoing functional studies of both mutations will offer novel insights into the underlying pathogenesis and will show how different functional effects of BMP1 mutations lead to variable bone phenotypes in patients with OI.

Taken together, we present a novel genetic cause for a high mineralization OI phenotype, describe the functional mechanism, and provide evidence for a genotype-phenotype correlation in patients with BMP1 mutations.

C03.4

Increased sensitivity to DNA damage in a recessive form of Weaver syndrome caused by functional loss of an E3 ubiquitin ligase

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Weaver syndrome is a rare congenital disorder mainly characterized by pre- and postnatal overgrowth, marked macrocephaly, learning disability and a typical facial appearance. Dominant mutations in EZH2 encoding a histone-lysine N-methyltransferase of the polycomb repressive complex have been recently reported to cause Weaver syndrome. We now present an autosomal recessive form of Weaver-like syndrome in two affected siblings from a consanguineous Turkish family. Using a whole-exome sequencing strategy combined with determination of homozygous stretches of identified variants, we found a homozygous nonsense mutation in a novel gene encoding an E3 ubiquitin ligase. The mutation is located within the N-terminal region of the protein leading to a complete loss of the ubiquitin ligase activity. To determine the underlying molecular pathogenesis, we analyzed patients' fibroblasts and could show that cells lacking this ubiquitin ligase activity have an increased sensitivity to DNA-damage and responded with prolonged activation of checkpoint kinase 1 and increased level of apoptosis. Our data link the molecular pathogenesis of overgrowth syndromes to altered DNA-damage response and increased cancer risk. Additional sequencing of a cohort of patients with Weaver syndrome for mutations identified novel de novo mutations in EZH2 and provided evidence for further genetic heterogeneity in Weaver syndrome. Exome sequencing is currently performed in one large dominant pedigree with Weaver syndrome as well as in unsolved sporadic cases aiming to identify additional genes involved in altered control of growth and DNA-damage response in Weaver syndrome.

C03.5

Mutations at a single codon in Mad Homology 2 domain of SMAD4 cause Myhre syndrome

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Myhre syndrome (OMIM #139210, MS) is a developmental disorder characterized by pre and postnatal short stature, brachydactyly, facial dysmorphism (short palpebral fissures, maxillary hypoplasia, mandibular prognathism, short philtrum), muscular hypertrophy, joint limitation and deafness. Other features include intellelectual disability behavioral disturbance, cardiac defects, and cryptorchidism. Skeletal manifestations include thickened calvarium, cone-shaped epiphyses, shortened tubular bones, hypoplastic iliac wings, broad ribs and large vertebrae with short and large pedicles. All reported MS cases are sporadic supporting de novo dominant mutations. Using exome sequencing in 2 MS cases, we selected mothers against DPP homolog 4 (SMAD4) as a candidate gene based on its pivotal role in BMP and TGFβ signalling. SMAD4 mutations were subsequently found in additional 18 affected individuals. We identified only
3 distinct heterozygous missense SMAD4 mutations all affecting Isoleucine 500 which in located in the Mad Homology 2 (MH2) domain, near the monoubiquitinated site Lys519. We further demonstrated a defect in SMAD4 ubiquitination in patient fibroblasts and increased level of SMAD4 suggesting a stabilization of SMAD4 protein in Myhre syndrome. These results were in contrast of those observed with SMAD4 loss of function mutations, identified juvenile polyposis syndrome and associated with SMAD4 instability. We further study the nuclear localization of mutant SMAD complexes and found that the complexes translocate to the nucleus. We finally observed a decreased expression of downstream TGFβ target genes supporting impaired TGFβ driven transcriptional control in MS.

C03.6

Homozygossity mapping and whole exome sequencing identifies *MAP4* mutations in short stature

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Shortness of stature is one of the most common paediatric concerns. 3 % of the general population present with a body height below -2 SD score. However, only in a small number of cases there is a genetic diagnosis. To uncover further underlying genetic mechanism we performed genome wide homozygossity mapping using an Affymetrix SNP 6.0 array in 8 patients with idiopathic short stature of consanguineous families. We identified runs of homozygossity with an average size between 2.7 and 300.1 Mb not reported in European populations. As the gene content averaged 874 genes we carried out whole exome sequencing after Agilent sure select enrichment v3 with 50 Mb. Runs were performed on a Life Tech5500xl platform. After Mapping and SNP calling with LifeScope variants were annotated with ANNOVAR. This identified the homozygous missense mutation p.A391T in the *MAP4* gene not present in 300 controls or the 1000genomes project. MAP4 is a major protein for microtubuli assembly during mitosis. The patient's phenotype showed significant overlap with those reported for Seckel syndrome and Microcephalic Osteodysplastic Dwarfism type Majewski which are also caused by defects in centrosomal proteins. The *MAP4* mutation creates a novel phosphorylation site in the KDM domain of the MAP4 protein which is predicted to destabilise the microtubuli architecture. Immunofluorescence analysis on a fibroblast cell line of the affected patient showed a higher rate of centrosome duplications and confirmed this hypothesis. This result illustrates the feasibility of our approach using exome sequencing to identify recessive genes for short stature.

C04.1

High frequency of indels at the breakpoint junctions of *MECP2* duplication rearrangements strongly support replicative-based mechanisms

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Copy number gain in Xq28 including *MECP2* is the most commonly identified subtelomeric CNV in patients with developmental delay and associated clinical findings. To date, we have collected a cohort of 65 patients with CNV including *MECP2*. Previous analyses derived from high-resolution comparative genomic hybridization arrays (aCGH) revealed the frequent occurrence of complex rearrangements within our patient cohort in 27% of cases. Here we studied 31 patients carrying duplications including *MECP2* in whom we were able to accomplish DNA sequencing for each of the rearrangement breakpoint junction. Surprisingly, DNA sequencing unveiled the presence of complexities in up to 50% of the rearrangements. All complex alterations have at least one breakpoint within or flanking the low copy repeats (LCRs) supporting our hypothesis that such LCRs stimulate those rearrangements. The most striking observation, however, was the high frequency (42%) of small insertions and deletions (indels) observed at/or flanking the breakpoint junctions, none of which were found present in dbSNP (built 135) suggesting that they were generated concomitant with the rearrangement. This observation strongly supports a role for replication-based mechanisms underlying such rearrangements as break-induced replication (BIR) was recently shown to increase the rate of frameshift mutations in yeast. In addition, SNP genotyping revealed absence of heterozygosity (AOH) within the altered genomic region strongly suggesting that the *MECP2* duplication is mainly an intrachromosomal event. In summary, our results add to a growing body of data documenting a role for a DNA replication mechanism in complex genomic rearrangements associated with genomic disorders.

C04.2

How to deal with genomic imbalances in the imprinted region 11p15.5: Insights in the complex regulation of two imprinting domains

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Duplications or deletions affecting the imprinting control regions 1 and/or 2 (ICR1/2) in 11p15.5 have been reported for both growth retardation and overgrowth. However, due to the complexity of the 11p15.5 imprinting regions, the interpretation of copy number variations (CNVs) is difficult. The clinical outcome in 11p15.5 CNV carriers is influenced by the size, the breakpoint positions, their parental origin and the imprinting status of the affected genes. We report on three carriers of different CNVs (two duplications and one deletion) restricted to the telomeric ICR1: two patients were referred with the diagnosis of Silver-Russell syndrome (SRS), in the third case the clinical features were probably not associated with the imbalance. Summarising our results and those from the literature, a central role of the *IGF2* gene and its telomeric enhancer elements for the clinical course of 11p15 disturbance carriers is obvious: whereas duplications or deletions of H19 do apparently not affect the phenotype, imbalances of both IGF2 as well as its enhancer can separately cause abnormal phenotypes depending on the parental origin. As a result, carriers of ICR1 duplication can even show a normal phenotype. A similar pattern can be delineated for the centromeric ICR2: here the type of imbalances affecting the CDKN1C and the KCNQ1OT1 genes and their differentially methylated region influences the clinical outcome. As a result, CNVs in 11p15.5 require an extensive workup to delineate risk figures for 11p15.5 associated syndromes. Additionally they allow profound insights in the complex regulation of the imprinted factors localised in 11p15.5.

C04.3

Nonlinear and nonrandom genome organization of SNRPN, UBE3A, and GABRB3 in the normal human nucleus by three-color 3D-fluorescence in situ hybridization

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Higher-order chromatin organization and spatial arrangement of genomic region within the nuclear space seems to play an important role in genome function via epigenetic mechanisms in the human nucleus. The aim of this study was to search for the new evidence related to genomic organization and function. We investigated the spatial positioning of three target regions containing the SNRPN, UBE3A, and GABRB3 genes mapped on chromosome 15q11.2-q12 by three-color 3D-fluorescence in situ hybridization on the interphase nuclei of normal human cells. The three target regions were not arranged linearly in most of the cells analyzed, and GABRB3 was positioned closer to SNRPN than UBE3A at a high proportion differently from genomic map. In addition, the distances from SNRPN to UBE3A (SU) and from UBE3A to GABRB3 (UG) between the alleles in each cell were different in both distances, and the SU ratio (longer/shorter SU distance between alleles) was larger than the UG ratio (longer/shorter UG distance between alleles). Moreover, the distances between the regions were different between the SU and UG regions on each chromosome in each nucleus. Thus, our results indicated that SNRPN, UBE3A, and GABRB3 have a nonlinear and nonrandom curved spatial positioning in principle, but there were some differences between the alleles and between the regions in the nucleus. These observations of structural differences in normal human cells might be reflected the status of gene as the SNRPN gene is known to have paternal-only expression.

C04.4

Age-related somatic structural changes in the nuclear genome of human blood cells

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Structural variations are among the most frequent interindividual genetic differences in the human genome. The frequency and distribution of de novo somatic structural variants in normal cells is poorly explored. Using age-stratified cohorts of 318 monozygotic twins (MZ) and 296 single-born subjects, we describe age-related accumulation of copy-number variation in the nuclear genomes in vivo and frequency changes for both megabase- and kilobase-range variants. Megabase-range aberrations were found in 3.4% of subjects ≥60 years old; these subjects included 78 MZ twin pairs and 108 single-born individuals. No such findings were observed in 81 MZ pairs or 180 single-born subjects who were ≤55 years old. Recurrent region- and gene-specific mutations were observed. Longitudinal analyses of 43 subjects whose data were collected 7-19 years apart suggest considerable variation in the rate of accumulation of clones carrying structural changes. Furthermore, the longitudinal analysis of individuals with structural aberrations suggests that there is a natural self-removal of aberrant cell clones from peripheral blood. In three healthy subjects, we detected somatic aberrations characteristic of patients with myelodysplastic syndrome. The recurrent rearrangements uncovered here are candidates for common age-related defects in human blood cells. We anticipate that extension of these results will allow determination of the genetic age of different somatic-cell lineages and estimation of possible individual differences between genetic and chronological age. Our work might also help to explain the cause of an age-related reduction in the number of cell clones in the blood; such a reduction is one of the hallmarks of immunosenescence.

C04.5

Clinically relevant mosaic findings in a total of 8,374 patients and parents in constitutional genome diagnostics using genome wide high resolution SNP array analysis

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We routinely perform genome wide SNP array analysis as the first-line diagnostic test for patients with intellectual disability and/or congenital anomalies and in prenatal diagnosis in case of structural ultrasound anomalies. In addition to *de novo* (6.5%), rare inherited (9.1%) or X-linked (0.8%) copy number variations (CNVs), we observed a significantly increased percentage of homozygosity in patients (6.1%) which subsequently led to the identification of pathogenic mutations in recessive disease genes, uniparental disomies, or low-mosaic aneuploidies in several patients. A mosaic finding was detected in 22 of 6,500 patients and in seven mothers of a total of 1,874 parents. In November 2011, we switched from the Affymetrix 250K SNP array to the CytoScan HD array platform which further enhanced the resolution, improved the detection of mosaic imbalances and also enabled us to detect clinically relevant, mosaic, copy neutral allelic imbalances in an additional three patients. The percentage of mosaicism (CNV, aneuploidy or allelic imbalance) often differed between tissue samples of mesodermal or ectodermal origin from each of these individuals. In two patients such tissue-dependent differences were shown to change over time.

Genome wide SNP array analysis is a suitable and highly effective method to reliably identify mosaic abnormalities that appear to be relatively common in both patients (1 in 300) and parents (1 in 270). Because of the significantly increased recurrence risk, it is crucial to know whether an apparently *de novo* imbalance in an affected child is in fact due to a mosaic aberration in the (unaffected) parent.

C04.6

Modelling neurogenesis impairment in Down syndrome using induced pluripotent stem cells from monozygotic twins discordant for trisomy 21

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Down syndrome (DS), caused by trisomy 21, is the most common chromosomal disorder, with an incidence of 1 in 800 live births. Its phenotypic characteristics include intellectual impairment and several other developmental abnormalities, for the majority of which the pathogenetic mechanisms remain unknown. Here, we report the generation and the characterization of induced pluripotent stem cells (iPSCs) derived from monozygotic twins discordant for trisomy 21: Twin-N-iPSCs for the normal and Twin-DS-iPSCs for the DS-affected iPSCs. We hypothesize that these samples were ideal to study the effect of the supernumerary chromosome 21, since the rest of the genome is identical between the two samples. Karyotype and high-resolution array-based comparative genomic hybridization analysis, confirmed the chromosomal constitution of these iPSCs. Transcriptome analysis by mRNA-Seq showed alterations in the expression of genes that impact on DS features. *In vivo* differentiation of Twin-DS-iPSCs revealed an abnormal teratoma formation in NOD-SCID mice. *In vitro*, Twin-DS-iPSC-derived neurospheres showed a reduced number of neuroprogenitor cells (NPCs). When NPCs were further induced to mature into neurons, we found structural changes in the architecture and density of neural populations together with alterations in the expression of genes involved in lineage specification in neurogenesis and brain development. Furthermore, we provide novel evidence that the increased expression and activity of the dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A protein underlie these defects. In conclusion, these findings establish these iPSCs as a unique cellular model to study the detailed mechanisms involved in the pathogenesis of DS and design new therapies.

C05.1

RNAi-based functional profiling of loci from blood lipid genome-wide association studies

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Genome-wide association studies (GWAS) are powerful tools to unravel genomic loci associated with common traits and complex human disease. However, GWAS only rarely reveal information on the exact genetic elements and pathogenic events underlying an association. In order to extract functional information from genomic data, strategies for systematic follow-up studies on a phenotypic level are required. Here we address these limitations by applying RNAi to analyze >100 candidate genes within 55 loci identified by GWAS as associated with blood lipid levels, coronary artery disease and/or myocardial infarction for a function in regulating cholesterol levels in cells. The genes were knocked-down with siRNAs and the consequences on cellular free cholesterol (FC) and the efficiency of LDL-internalization into cells were quantified using automated microscopy and multiparametric image analysis. We will show evidence that loss-of-function of a surprisingly high number of the trait-associated genes affected LDL-uptake, FC, or both. For several genes without previously known lipid-regulatory roles the functional effects upon gene knockdown closely correlated with altered LDL-receptor levels. By providing strong evidence for disease-relevant functions of lipid trait-associated genes our study demonstrates that quantitative, cell-based RNAi is a scalable strategy for a systematic, unbiased detection of functional effectors within GWAS loci.

C05.2

Accumulation of common genetic variants influences lipid levels in patients with T2D and improves prediction of hypercholesterolemia

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A large proportion of type 2 diabetes (T2D) patients have dyslipidemia, an important cause of micro- and macrovascular complications. A recent GWAS in the general population identified 95 common genetic variants associated with lipid levels. To obtain insight into the genetics underlying diabetic dyslipidemia, genotype scores approximating the additive effects of those variants were calculated for each major lipid class (total cholesterol, TC; high-density lipoprotein cholesterol, HDL; low-density lipoprotein cholesterol, LDL; and triglycerides, TG) in individuals with/without T2D in the Rotterdam Study (n=7735) and Erasmus Rucphen Family Study (n=2313). Adjusted for age, sex and bmi, all four genotype scores were significantly associated with lipid levels in both non-diabetics and diabetics. The genotype scores for HDL, LDL, and TG were also used to predict hypercholesterolemia. The area under the ROC curve (AUC) for the genotype scores was 0.68[0.66-0.69] in non-diabetics and 0.72[0.68-0.76] in diabetics, which was better than the predictive ability of age, sex and BMI ($AUC_{\text{non-diabetics}}$=0.65[0.64-0.66], $AUC_{\text{diabetics}}$=0.69[0.65-0.73]). Combining the genetic scores with the non-genetic risk factors improved the AUC in both non-diabetics and diabetics ($AUC_{\text{non-diabetics}}$=0.69[0.68-0.71], $AUC_{\text{diabetics}}$=0.75[0.71-0.79]). The non-genetic, genetic and combined AUCs for prediction of hypercholesterolemia were all significantly higher in diabetics than in non-diabetics ($P_{\text{nongenetic\_AUC}}=0.038$, $P_{\text{genetic\_AUC}}=0.028$, $P_{\text{combined\_AUC}}=0.006$). In conclusion, genotype scores derived from common lipid variants are associated with lipid levels not only in the general population, but also in patients with T2D and can, especially in diabetics, improve prediction of hypercholesterolemia. These data suggest that the role of common variation may be modified in the context of diabetes.

C05.3

Detailed metabolic and genetic characterization of known lipid loci

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The exact functions and causative variants remain largely unknown for the 95 genetic loci that are identified to associate with levels of TC, LDL-C, HDL-C or TG. We identified new metabolic or genetic associations (p<5×10-8) for 30 of the 95 lipid loci by further characterizing the loci utilizing extensive serum metabolite profiles, including a broad panel of lipoprotein subclasses and tens of other serum metabolites obtained via NMR spectroscopy, and a dense set of 440,807 directly genotyped and imputed variants around the previously identified lead SNPs in 8330 Finnish individuals.

In the majority of the loci the more detailed metabolite measures appeared to better describe the underlying biology than the conventional lipids. In four loci, including PLTP and LIPC, the directions of associations to small and large HDL particles were the opposite, pinpointing the diversity of HDL subclasses not captured in the routine measurement of HDL-C. Also, 14 loci had associations beyond the individual lipoprotein measures, including the APOA1 locus where a marker known to associate with CAD was associated with serum lactate (p=3.79×10-13). Additionally, in 27 loci we identified SNPs with a stronger association than the previously reported markers, and twelve loci, including APOB and LIPC, had two or more independent associations across the metabolite measures.

Wide metabolite profiling combined with the dense set of SNPs provided insight into the metabolic and genetic architecture underlying the known lipid loci. Further understanding of these processes may open up new possibilities to understand mechanisms involved in atherosclerosis and other metabolic conditions.

C05.4

Estimating the fraction of established metabolic trait loci with discernible pleiotropic effects

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Genetic variation influences human quantitative trait levels and risk of metabolic disease. The patterns of trait association observed in genome-wide association studies (GWAS) at individual cardio-metabolic risk-loci are highly variable and allelic heterogeneity is often observed for association within and between traits.

The Cross-Consortia Pleiotropy Group was formed to investigate the patterns of multi-trait associations across the genome for cardio-metabolic traits. We aimed to estimate the fraction of established GWAS loci associated with more than one cardio-metabolic trait showing strong (r2>0.8), moderate (r2>0.2) linkage disequilibrium (LD) or evidence of allelic heterogeneity between associated variants.

We evaluated 271 SNPs representing associations from published GWAS meta-analyses (Nov.2010) in Europeans of 20 quantitative and two disease phenotypes from six cardio-metabolic trait consortia. We identified 106 regions associated with multiple traits, defined as sets of adjacent variants located less than 500kb apart. We used LD estimated from 1000 Genomes CEU data.

Across the 106 regions defined by SNPs of interest, we observed 49 (30%) containing the same SNP associated with more than one trait. Of these, 37 contain SNPs associated with highly correlated traits, e.g. lipids and obesity. Of the remaining regions, 38 (23%) contain variants in strong LD and 30 (19%) contain variants in moderate LD, and 46 (28%) regions contain variants in only modest LD (r2<0.2).

Our results highlight that a substantial proportion of metabolic trait loci incorporate complex patterns of multi-trait allelic heterogeneity, suggesting that statistical approaches that model epidemiological correlations between phenotypes may increase power and resolution of gene-mapping efforts.

C05.5

Dysfunctional NO signaling due to a double mutation in GUCY1A3 and CCT7 identified by whole exome sequencing increases risk for myocardial infarction

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Myocardial infarction is a life-threatening disease, which results from sudden atherothrombotic occlusion of a coronary artery. Most cases of MI occur sporadically but in rare cases the disease accumulates in families. Here we show by using exome sequencing in such family the identification of heterozygous mutations (p.Leu163Phefs*24 and p.Ser525Leu) in two functionally related genes, GUCY1A3 and CCT7, in 7 and 11 out of 15 affected family members, respectively. Single-locus linkage-analysis revealed no significant LOD score, however two-locus linkage-analysis considering both mutations revealed a significant maximum LOD score of 5.68. Moreover, a GWAS of 28K MI or CAD cases and 75K controls identified a signal across the GUCY1A3 locus (P<1.74x10-8 for rs7692387). While GUCY1A3 encodes the alpha1 subunit of soluble guanylyl cyclase (sGC), CCT7 a member of the chaperonin containing TCP1 complex (TRiC/CCT), which, among other functions, stabilizes sGC. This enzyme generates cGMP upon stimulation with nitric oxide (NO) and thereby pacifies platelets among other functions. We subsequently demonstrated that in-vitro sGC activity is severely impaired by the GUCY1A3 and CCT7 mutations. Platelets from double mutation carriers contained less sGC protein and consequently a reduced NO-induced cGMP formation. Moreover, in mice deficient for the alpha1 sGC protein subunit thrombus formation in the microcirculation in-vivo upon local trauma was accelerated. In conjunction, we linked mutations in GUCY1A3 and CCT7; encoding two functionally related proteins, to MI, associated a common variant in one of the genes to the same disease, and propose defective sGC dependent NO signaling as a mechanism leading to MI.

C05.6

Gene expression in an extended pedigree

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We have used RNA-sequencing to profile the transcriptome of 17 European-descendent individuals in a three generation pedigree. Each individual has a sequenced genome using Complete Genomics technologies. We assess the de novo mutation rate using this extended pedigree. Furthermore, we report patterns of linkage with expressed transcripts to describe the effects of rare and common variants in this cohort. This provides us with an estimate of the functional de novo rate and further allows us to dissect the impact of structural versus single nucleotide polymorphisms. Using techniques we have previously reported (Montgomery, Nature, 2010), we assess allele specific expression (ASE) and now identify variants identical-by-descendent and the subsequent heritability of ASE.

C06.1

The evolution of African great ape subtelomeric heterochromatin and the fusion of human chromosome 2

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Chimpanzee and gorilla chromosomes differ from human chromosomes by the presence of large blocks of subterminal heterochromatin thought to be composed primarily of arrays of tandem satellite sequence. Due to the high-copy repetitive nature of these sequences, like centromeric and secondary constriction on acrocentric chromosomes, the subtelomeric heterochromatin is not represented in the existing genome assemblies of the chimpanzee and gorilla. We use a combination of molecular cytogenetics, clone-based sequencing, and phylogenetic analysis to explore their sequence composition and organization and show a complex organization composed of specific sets of segmental duplications, which have hyperexpanded in concert with the formation of subterminal satellites. These regions are highly copy number polymorphic between and within species and copy number differences involving hundreds of copies can be accurately estimated by assaying read-depth of next-generation sequencing datasets. Phylogenetic and comparative genomic analyses suggest that the structures have arisen largely independently in the two lineages with the exception of a few seed sequences present in the common ancestor of humans and African apes. We propose a model where an ancestral human-chimpanzee pericentric inversion and the ancestral chromosome 2 fusion both predisposed and protected the chimpanzee and human genomes respectively to the formation of subtelomeric heterochromatin. Our findings highlight the complex interplay between duplicated sequences and chromosomal rearrangements that rapidly alter the cytogenetic landscape in a short period of evolutionary time.

C06.2

Genome-wide search for gender different genetic loci for human anthropometric traits: Methods and results from genome-wide meta-analyses across 270,000 Individuals

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Recently, a sex-specific follow-up of a few genetic variants associated with human anthropometric traits, detected in sex-combined analyses, revealed significant difference between the sexes.

To more systematically detect further sexually dimorphic loci, we conducted sex-specific genome-wide association meta-analyses of 46 studies (60,586 men, 73,137 women) and followed-up the results in 48 independent studies (62,395 men, 74,657 women) within the GIANT consortium. Each study tested for association of 2.8M SNPs and 9 phenotypes: height, weight, body mass index (BMI), waist and hip circumference, waist-hip ratio (WHR), the latter three with and without adjustment for BMI.

Opted to detect signals with association in only one sex, we controlled sex-specific P-Values at 5 % false-discovery-rate (FDR) and as such selected 348 independent signals. A follow-up yielded 7 hits with significant (<5% FDR) replication sex-difference P-values: (a) 6 women-specific loci without any effect in men, including 3 novel (near *MAP3K1, HSD17B4, PPARG*) and 3 previously established (near *GRB14/COBLL1, LYPLAL1/SLC30A10, VEGFA*) loci; and (b) one previously published locus (near *ADAMTS9*) with a less pronounced effect in men. Of particular interest is the *PPARG* region, well-known for its role in type 2 diabetes therapy, which showed a women-specific association with WHR adjusted for BMI. A second approach to search for sex-differences was particularly powered to detect signals with association in both sexes and with opposite effect direction. This method, however, did not yield any signals.

Our results underscore the importance of sex-stratified analyses in order to illustrate a sexually dimorphic genetic underpinning for anthropometric traits.

C06.3

Analysis of structural variation in the Genome of the Netherlands (GoNL) project

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on behalf of Genome of the Netherlands consortium

The Genome of the Netherlands (GoNL) is a national collaboration that aims at characterizing genetic variations in the Dutch population of 250 families. Here we report on the pilot results of the structural variation analysis for 18 families. Our analysis employs several methodologies for detection of different types and size ranges of variants.

Using GATK Unified Genotyper, we identified 1,459,968 small indels of which 23% are novel compared to 1000 Genomes phase 1 data, and 86% overlap with Pindel's short indel calls. In silico functional analysis indicates that 819 are causing premature stop codons and frameshifts in 749 genes.

A combination of 4 approaches, read depth (CNVnator, DWAC-Seq), read pair (123SV, BreakDancer and GenomeSTRiP), split-read (Pindel), and *de novo* assembly (SOAPdenovo, CLC) ensures detection of structural variants of different types and size ranges. For example, we identified a 1.8 kb insertion absent in genome reference, but common in Dutch population (allele frequency=42%), while rare (5%) in 1000 Genomes project.

Homozygous DNA segments were identified using PLINK and VCFtools.

We performed hundreds of PCR/Sequencing assays to determine false-positive rate for each tool and to establish de novo mutation rate of indels and structural variants.

The set of wide size range, multi-type, high-quality SVs calls, together with GoNL SNP set (reported separately) describes common genomic variants in the Dutch genomes. This variation catalogue is essential for understanding population history, interpretation of GWAS and analysis of other studies involving West-European samples.

C06.4

Duplications at PAK7 are a significant risk factor for schizophrenia and bipolar disorder

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To identify novel rare risk copy number variants (CNVs) for schizophrenia we performed a CNV analysis in 1,564 cases and 6,944 controls from Ireland and the UK. Three novel and significant (p<0.01) CNVs from this analysis at chr2cen-q13, chr3p25.1 and chr20p12.2 were confirmed by quantitative real-time PCR and taken forward for replication in a large independent dataset of European ancestry containing 7,123 cases and 62,694 controls. This confirmed association with schizophrenia for rare chr20p12.2 duplications (replication p=0.0008). In the combined sample there are 12 duplications in 8,687 cases and 13 in 69,808 controls (p=4.3x10-6, OR=7.44). All cases had a 132-146.5kb duplication involving the first two exons of the p21 Protein-Activated Kinase 7 (PAK7) gene. Most case carriers of the duplication were severely affected with poor clinical outcome and limited response to conventional antipsychotic therapy. Based on evidence of overlapping genetic etiology we examined 2,686 bipolar disorder cases and identified 4 duplication carriers (p=0.0002). Altogether, these data support PAK7 duplications as a rare but significant risk factor for psychosis (p=2.14x10-7, OR=7.63). PAK7 is one of a family of serine/threonine protein kinases, which are regulated by the Rho family of small G proteins and are involved in multiple intracellular signaling pathways. In mouse cortical neurons, we found that protocols that induce activity-dependent synaptic plasticity also modulated PAK7 expression suggesting that like other p21-activated kinases, PAK7 may play an important role in maintaining synaptic networks. Thus, our novel finding may provide new insight into the pathophysiology of schizophrenia and bipolar disorder.

C06.5

Variation in transcription factor binding among humans

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Differences in gene expression may play a major role in speciation and phenotypic diversity. Although variations in gene expression among individuals have been documented, the origins of these differences are not clear, and studies that directly measure differences in transcription factor binding sites among humans have not been performed. We have examined genome-wide variation in transcription factor binding in different individuals and a chimpanzee using chromatin immunoprecipitation followed by massively-parallel sequencing (ChIP-Seq). The binding sites of RNA Polymerase II (Pol II) as well as a key regulator of immune responses, NFκB, have been mapped in ten lymphoblastoid cell lines derived from individuals of African, European, and Asian ancestry, including a parent-offspring trio. Using a stringent threshold, approximately 7.5% and 25% of the respective NFκB and Pol II binding regions exhibit differences between any two individuals. To understand the underlying basis of the variations, we examined the effect of SNPs and genomic structural variations (SVs) on binding differences among individuals. We find that many binding differences are associated with SNPs and SVs. Comparison of the binding data with gene expression data generated by RNA-Seq revealed that differences in binding often correlate with gene expression differences. Furthermore, comparison of the Pol II human sites with binding sites identified in the chimpanzee suggests a high level of divergence in binding relative to our closest evolutionary neighbor. Our results indicate that many differences in individuals occur at the level of TF binding and provide insight into the genetic events responsible for these differences.

C06.6

Poly(A) binding protein nuclear 1 (PABPN1) levels affect alternative polyadenylation

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The choice for a polyadenylation site (PAS) determines the length of the 3'-untranslated region (3'-UTRs) of an mRNA. Inclusion or exclusion of regulatory sequences in the 3'-UTR may ultimately affect gene expression levels. Poly(A) binding protein nuclear 1 (PABPN1) is involved in polyadenylation of pre-mRNAs. A repeat expansion in PABPN1 causes oculopharyngeal muscular dystrophy (OPMD), a late onset and progressive muscle disorder.

Microarray expression profiling of mice overexpressing expanded-PABPN1 showed alternative PAS usage in 8% of the interrogated genes. We hypothesized that previously observed disturbed gene expression patterns in OPMD muscles may have been the result of an effect of PABPN1 on alternative PAS usage.

To explore PAS usage on a genome-wide level, we developed a single molecule PAS sequencing method. We identified 2,012 transcripts with altered PAS usage. In the far majority, alternative more proximal PAS were used. This resulted in overall shortening of 3'-UTRs and increased expression for most of the transcripts.

We recapitulated these changes in PAS usage in myogenic cells by low overexpression of expanded but not wild-type PABPN1. Since expanded-PABPN1 is known to be trapped in intranuclear inclusions, we investigated the effect of sh-RNA mediated downregulation of PABPN1. We found that reduced Pabpn1 levels also resulted in shortening of 3'-UTRs. Our data suggest that PABPN1 is involved in PAS selection. We propose therefore that reduced availability of functional PABPN1 in OPMD muscles results in use of alternative, proximal PAS, leading to large-scale deregulation of gene expression.

C07.1

Exome sequencing; what are the lessons learned?

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Today, many scientific laboratories apply whole exome sequencing. Considering the number of published articles and abstracts at different meetings, many groups are successful in identifying causative variants for Mendelian disorders. However, at least as many cases currently remain unsolved and exome sequencing comes with several disadvantages. It is labor intensive, it mostly misses variants outside the targeted regions and it generates sequence data with a large variability in coverage across the regions sequenced. Furthermore, to cope with the sheer number of variants encountered, choices have to be made regarding the variants that deserve follow-up work.

We have attempted to uncover the disease causing genes in 16 different cases using exome sequencing. We were able to solve 9 of them (56%). Important factors to success appear to be (1) phenotyping by expert clinical geneticists, (2) an elaborate 'diagnostic' work-up, such as considering SNP array analysis to perform linkage in extended families, (3) a careful selection of sequencing design and (4) a reproducible and robust protocol for exome sequencing and (5) finally, a proper pipeline for data analysis, both with respect to variant calling and annotation.

We consider factors of failure to be (1) suboptimal work-up or study design, (2) hidden causative variant (e.g. non-conserved missense), (3) genetic heterogeneity of the disorder or a (4) non-genetic cause of the disorder.

We will discuss several examples of cases for which we were (un)successful and what attempts were made to prove the pathogenecity of the variants.

C07.2

Exome sequencing in the clinic: diagnostic-driven analysis of exome sequencing data

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Exome sequencing has great potential in genetic diagnostics, particularly if used for strong heterogeneous diseases were diagnostic yield is currently low. The analysis and interpretation of exome data in diagnostics has specific challenges with respect to quality control, the possibility of incidental findings, and allowing for easy and flexible interpretation. We now have implemented a routine diagnostic exome sequencing workflow.

Six heterogeneous diseases are interrogated by exome sequencing: Intellectual Disability ('de novo' strategy), Blindness, Deafness, Movement disorders, Oncogenetics, OXPHOS diseases (disease gene package). The patient and/or legal representative need to sign an IC before exome sequencing can be requested by the clinical geneticist. It includes an agreement for sequencing their exome and subsequent reporting of all medically relevant findings, including possible findings not related to the initial enquiry. Sequence variants detected can be analyzed using a diagnostic-oriented GUI that automatically limits genetic findings to the relevant genomic loci for a disease, and allows interpretation using predefined filter schemes.

The results of a cohort of 300 patients (including 100 ID patients) will be presented. QC, cases, and pitfalls will be discussed. Data-analysis shows a median coverage of 67x per exome. With the analysis still ongoing, disease gene package analysis detects on average 378 variants with 2 to 18 private non-synonymous variants per patient. The 'de novo' approach for ID demonstrates its potential with ~25% of the ID-patients having causal mutations identified in known ID-genes.

This represents the first in-use approach to establish a genetic diagnosis of patients by exome sequencing.

C07.3

Next Generation Sequencing in Mainstream Diagnostic Genetic Testing: Two years experience and over 1400 Patient Reports

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We have established Next Generation Sequencing (NGS) at the core of diagnostic molecular genetic testing in Leeds.

The first service developed was for breast cancer gene screening, and around 1000 reports have been issued since the first in March 2010. Since then, further NGS services have been developed systematically - some were formerly analysed by Sanger sequencing and transformation has brought considerable benefits including reduced costs and improved turnaround time. Enhanced productivity has provided scope to make continued developments and introduce new diagnostic services, widening benefits to new patient groups. The Leeds repertoire of NGS services so far includes breast cancer (BRCA1&2), HNPCC (3 genes), phaeochromocytoma (9 genes), Marfan syndrome (FBN1), Loeys-Dietz syndrome (TGFBR1&2), hypertrophic cardiomyopathy (4 genes), Li Fraumeni syndrome (TP53), Aicardi-Goutieres syndrome (5 genes), and FAP1 (APC). All current services follow standardised protocols which are conducted in parallel and incorporate long-range PCR to target the genes of interest, robotics and automated library construction. Sequencing is on the Illumina platform and results are processed using NextGENe software. Data handling is facilitated by customised spreadsheets which include quality checks and assist with assessment of variants.

Already, around two thirds of our molecular workload is based on NGS technology (measured by UK workload units). But significant transformations in diagnostic services are anticipated to continue, particularly in the fields of molecular cytogenetics and whole exome analysis.

C07.4

Doubly heterozygous LMNA and TTN Mutations Revealed by Exome Sequencing in a Severe Form of Dilated Cardiomyopathy

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Familial dilated cardiomyopathy (DCM) is characterized by cardiac enlargement and heart failure associated with systolic dysfunction and impaired contraction of the left ventricle, and about 30-50% of affected individuals have a familial form of DCM. The genetics of familial DCM are complex, with 31 autosomal and two X-chromosomal disease genes currently known, none of which account for more than about 6-8% of cases. The causes of intrafamilial variability in DCM have remained largely unknown. In this study, whole-exome sequencing (WES) was used to investigate the causes for clinical variability in an extended family with 14 affected subjects, four of whom showed particular severe manifestations of cardiomyopathy requiring heart transplantation in early adulthood. Whole-exome and conventional sequencing identified the mutation p.K219T in the lamin A gene in all 14 affected patients. An additional variant in the gene for titin, p.L4855F, was identified in the severely affected patients. The age to heart transplantation was significantly less for LMNA:p.K219T/TTN:p.L4855F double heterozygotes than for LMNA:p.K219T single heterozygotes by Kaplan-Meier analysis. There was also a clearly different distribution of VO2 and ejection fraction between the two groups. Myocardial specimens of doubly heterozygote individuals showed increased nuclear length and myonuclear clustering compared with samples from single heterozygotes. Our results show how WES can be used to address questions about clinical variability in human genetics. The LMNA and TTN mutational status may be useful in this family for risk stratification in persons at risk for familial DCM.

C07.5

Trio-aware variant calling for accurate genotyping and de novo mutation detection

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Background

Detection of *de novo* mutations and accurate genotyping from pedigree NGS data is challenging, especially at low or intermediate depth of coverage. Both require accurate and quantitative calibration of the evidence supporting the individuals' genotypes using all the available information, including their familial relationship and population allele frequency.

Methods

We present here a new GATK module, Phase By Transmission, which uses a probabilistic model to compute the most likely genotype combination within a trio given individual genotype likelihoods and a mutation probability prior. When analyzing multiple samples from the same population, it considers the evidence across all samples in its calculation. This module directly uses variant calls (VCF) as input to easily integrate in NGS processing pipelines for genotyping, and *de novo* mutation detection.

Results

Genotyping accuracy was evaluated using concordance between whole-genome SNP calls from 47 trios sequenced at 12x coverage and Immunochip data (170k loci). More than 35% of the discordant genotypes and more than 99.98% of the Mendelian violations in the SNP calls (when performed without familial relationship) could be corrected.

De novo mutation detection was assessed using whole exome data in a cohort of 104 trios sequenced at 60x depth for which validation data for 94 *de novo* mutations was available as part of an independent study. We reported all 91 validated *de novo* mutations and none of the 3 false positives from the original study, and suggested an additional 32 candidates.

C07.6

The Diagnostic Mutation Database (DMuDB). Collecting, managing and publicising clinical variant data.

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DMuDB was established by NGRL Manchester to collect and share genetic variants identified by UK molecular diagnostic laboratories. To date over 41,000 variants from 15,000 patient referrals have been submitted. The database's remit has now expanded to accept members and data from European Molecular Quality Network (EMQN) member diagnostic laboratories worldwide on a subscription basis.

Variant data in DMuDB have been generated as part of a clinical diagnosis rather than research. Therefore, access has been limited to those involved in genetic testing for patient diagnosis. However it is recognised that there may be benefits to the clinical community in allowing wider access. For example, the sharing of BRCA1 and BRCA2 data with the ENIGMA consortium could improve the classification of variants of unknown significance. The need to balance these benefits against the need for confidentiality needs to be recognised though.

We have been investigating the use of the Cafe Variome system to publicise diagnostic variants and allow discovery by interested third parties while controlling access. This has helped highlight issues that will need to be resolved for future sharing of variant data. At the same time, the Human Variome Project (HVP) is developing a worldwide model for sharing variant data collected using country nodes and distributing it to gene/disease specific databases and to central databases, e.g., at NCBI and EBI.

We discuss the issues raised in publicising and sharing data and how the DMuDB/Cafe Variome model relates to the HVP country node model of variant data sharing.

C08.1

The ENCODE effort combining RNA-seq and RT-PCR-seq allows to catalog thousands of novel lncRNAs

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Within the ENCODE consortium, GENCODE aimed to accurately annotate all protein-coding genes, pseudogenes and non-coding transcribed loci in the human genome through manual curation and computational methods. Lower confidence transcribed loci were systematically experimentally evaluated by RT-PCR amplification followed by highly multiplexed sequencing readout, a method we coined RT-PCR-seq. 79% of all assessed junctions are confirmed by this evaluation procedure demonstrating the high quality of the annotation reached by the GENCODE gene set. We further took advantage of the deep transcriptome profiling generated by the Illumina "Human Body Map" in 16 human tissues to uncover 5918 novel gene models that do not overlap any loci depicted in GENCODE. They potentially represent new non-coding RNA genes or alternatively unannotated 5' or 3'UTR portions of known genes, as the vast majority of these models was shown to have poor coding potential using comparative genomics and mass spectrometry. We experimentally validated using RT-PCR-seq, 73% of the new HBM models, *de facto* enriching the complexity of the human genome annotation of non-coding RNA genes by more than 4000 novel genes. Our findings demonstrate the effectiveness of unbiased RNA-seq combined with targeted RT-PCR-seq to uncover new genome features. These two technologies were simultaneously similarly paired to unravel expressed pseudogenes by the GENCODE consortium. Our RT-PCR-seq targeted-approach also has the advantage of identifying novel exons of known genes, as we discovered unannotated exons in about 11% of assessed introns. We thus estimate that at least 18% of known loci have yet-unannotated exons.

C08.2

Allele Specific Expression Single cell RNA-Seq Analysis

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Dissecting the relationship between genotype and phenotype is one of the central goals in biology. Cell development is driven and controlled by temporal and spatial changes in gene transcription,. Single cell RNA-Seq analysis can be instructive concerning how individual cells respond to signals at critical stages of cell fate determination, or when they acquire aberrant phenotype. Essentially all cells within an individual organism share a virtually identical genotype, but the individual transcriptomes reflect expression of a subset of genes, which is determined by their epigenetic state, including DNA methylation and histone modifications. If these two copies of a gene are expressed at different levels, the quantities of messenger (mRNA) from individual alleles will differ. This is called allele specific gene expression (ASE). ASE is potentially important for development, and during evolution when a new allele emerges from mutation, which might immediately affect development through changes in expression levels in a heterozygous cell. However, the extent of ASE in individual mammalian cells and, has not so far been examined. Here, we took advantage of recent advances concerning single cell RNA-Seq, to analyze allele specific gene expression within individual early mouse blastomeres. We found that around 50% of distinguishable expressed alleles of individual genes showed differential allele specific expression. This shows that ASE is widespread at the earliest stages of mammalian development. ASE is likely to occur as a result of sequence polymorphisms of cis regulatory elements, or this might occur in response to different local chromatin structures mediated by epigenetic modifications.

C08.3

Systematic assessment of the immune system by genetic mapping of its quantitative dimensions

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Genome-wide association scans (GWAS) have identified hundreds of regions associated with immune diseases, but identification of the specific causal variants and clarification of the underlying functional mechanisms remain a great challenge. Better genetic and phenotypic resolution in GWAS, integrating sequencing data and assessing the immune system's various components, can improve our understanding of such loci and lead to novel discoveries. Here we have used polychromatic flow cytometry to evaluate quantitative variation of 271 immune-related traits, representing levels of the majority of lymphocyte cell populations (T and B cells, Natural Killer cells, regulatory T cells, dendritic cells, and their subsets) as well as T cell maturation, in 1628 volunteers of the SardiNIA project by polychromatic flow cytometry. Heritability estimates showed that the genetic component accounts for >40% of the phenotypic variation for most of the traits. Samples were genotyped with Affymetrix arrays as well as MetaboChip and ImmunoChip, and ~14 Million variants were imputed from a reference panel of 1,656 haplotypes deriving from 828 Sardinian samples sequenced at 3x average coverage. We performed a GWAS for each trait and observed, overall, 101 independent variants at 58 loci (1x10-202<P<5x10-8). Notably, our results include the previously reported association at the Type 1 Diabetes associated IL2RA gene and mean CD25 levels on CD4+ memory T cells (P<10-11). Another five loci have been previously associated with immune and non-immune pathologies, illustrating the relevance of the approach for the identification of immune-related genetic factors in both health and disease.

C08.4

Zooming in on causal variants by eQTL meta-analysis in 5,311 samples

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Genome-wide association studies (GWAS) have linked many genetic variants in the form of single nucleotide polymorphisms (SNPs) with complex traits. However because of linkage disequilibrium, it is often difficult to identify causal variants. Currently, GWAS are sometimes accompanied by limited eQTL (expression quantitative trait locus) data to prioritize the genes within the associated regions, however, a large reference panel is not yet publicly available.

Here, we report on a comprehensive cis-eQTL (distance between SNP and probe mid-point position < 250kb) meta-analysis of 5,311 samples (full-blood) from seven cohorts using imputed GWAS data. We identified cis-effects for 12,500 unique probes (reflecting 8,600 unique genes) and 12,000 independent SNPs, a number that is twice as large as those currently reported in NHGRI GWAS catalog (of which 38% have a cis-effect in our analyses). Genes for which we did not detect a cis-eQTL were generally very weakly expressed in blood.

Since many of the cis-eQTLs p-values were less than 10-100, we hypothesized that the top eQTLs were enriched in causal variants.. By comparing these extreme eSNPs with small-effect eSNPs, we observed highly significant enrichment of SNPs mapping to splice sites, introns, UTRs, miRNA binding sites and especially transcription factor binding sites. Surprisingly, we found that less than 6% of the top eSNPs were coding while only 3% were non-synonymous, indicating that regulation of gene expression is predominantly determined at non-coding regions. These results imply that a stronger focus on investigating non-coding variants in future GWAS fine-mapping studies is justified.

C08.5

Mapping genetic and epigenetic factors influencing human hippocampal gene expression

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Several studies have investigated the effects of genetic variation on gene expression (expression quantitative trait loci, eQTLs) in peripheral tissue, cell lines, or post-mortem brain tissue. EQTL studies from pre-mortem, fresh-frozen brain samples would be highly interesting but are hampered by the restricted accessibility of such samples. At the University of Bonn, we have access to a unique sample of pre-mortem human hippocampus samples originating from surgery of treatment-resistant epilepsy patients. To systematically determine eQTLs in a total of 148 hippocampus samples, we generated whole-genome SNP (Illumina Human660W) and gene expression data (Illumina HumanHT-12v3). In addition to the conventional data analysis, we applied a new "hidden factor" analysis that identifies and corrects for unknown confounding factors in the data and thus diminishes the false-positive and false-negative eQTL rate (PEER, https://github.com/PMBio/peer/wiki). Fifteen hidden factors were identified and used as co-variates for expression analysis. We detected 78 trans-regulating (>1Mb between SNP and probe) eQTLs that withstood Bonferroni correction for multiple testing. Moreover, 1,925 cis-regulating (≤1Mb distance) eQTLs remained significant after permutation-based Westfall-Young correction. In an additional step, we extended our analysis to the systematic investigation of the influence of DNA methylation on gene expression. Genome-wide methylation measurement was performed using Illumina's new HumanMethylation450 array which interrogates more than 485,000 methylation sites. To our knowledge, our study is the first to integrate genotype, expression and methylation data from pre-mortem brain tissue and will provide a valuable resource for the functional interpretation of genetic and epigenetic sites, in particular those associated with brain diseases.

C08.6

A gene co-regulation network based on 80,000 samples allows for accurate prediction of gene function

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High-throughput DNA microarray technology now provides us with a detailed view of the human transcriptome under different biological conditions. The increasing amount of publicly available microarray data helps in identifying and predicting genes that contribute to the same biological processes. To create a gene co-regulatory model of the human transcriptome, enabling the prediction of gene function, we analyzed 55,000 human, 17,000 mouse and 6,000 rat Affymetrix microarrays from the Gene Expression Omnibus. We created an integrated three-species gene network with 20,000 unique human genes and developed a principal component based statistical algorithm to predict function for individual genes.

We benchmarked the algorithm against several pathway databases (including Gene Ontology, KEGG, BioCarta and Reactome) and observed that gene function could be generally predicted very well. For over 75% of all 20,000 genes we could predict at least one significant pathway association, function or protein localization. Furthermore, predictions could be made for over 50% of the 5,004 genes that currently lack any known function.

These results indicate that through the integration of many gene expression arrays biological knowledge can be obtained, even for those genes for which currently nothing is known.

C09.1

The Skeletome project: towards a community-driven knowledge curation platform for skeletal dysplasias

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The generation of a comprehensive description of all human diseases and its interconnections in a universal, computer-readable format („the Phenome") is the next frontier in human genetics. Major hurdles include the enormous amount of information to capture and the non-standardised and often ambiguous nature of current clinical terminology. The Skeletome project aims to develop a comprehensive phenotype database for the skeletal dysplasia domain by tapping into the collective knowledge and patient data available around the world and making it accessible in a standardised format. To this aim, we have transformed the current Nosology of Genetic Skeletal Disorders into an ontology. We then created a collaborative editing platform that allows the scientific community to collate their collective knowledge into an online encyclopaedia of skeletal dysplasias. Finally, we have created an online database that allows clinicians worldwide to submit detailed clinical information on patients with skeletal dysplasias and to share this data in anonymised form and in a standardised manner with the scientific community. The systematic use of ontologies and other semantic web technologies ensures a high level of connectivity within the project and with existing biomedical databases, allowing complex querying and computer-assisted reasoning. For example, we have implemented a diagnostic algorithm that suggests diagnoses based on clinical features. The performance of this early prototype already matches the diagnostic accuracy of non-expert clinicians. We hope that the Skeletome platform will become the prototype of phenotypic databases for other rare diseases.

C09.2

Exome sequencing identifies PDE4D mutations as another cause of acrodysostosis

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Acrodysostosis [MIM 101800)] is a dominantly inherited condition associating 1) a skeletal dysplasia characterized by facial dysostosis, severe brachydactyly with cone-shaped epiphyses, advanced bone maturation and short stature 2) resistance to mutiple hormones 3) moderate to mild intellectual disability. Differential diagnoses include Albright hereditary osteodystrophy and pseudopseudohypoparathyroidism due to loss of function mutations in GNAS (α-stimulary subunit of the G-protein).

Recently, a recurrent mutation in the PRKAR1A has been identified in 3 individuals with acrodysostosis and resistance to multiple hormones (p.Arg368X). Studying ten unrelated acrodysostosis cases, we identified de novo PRKAR1A mutations in 5/10 (p.Arg368X mutation in 4/10 and p.Tyr373His mutation in 1/10). We then performed exome sequencing in 2/5 remaining cases and selected phosphodiesterase 4D (PDE4D) as a candidate gene. PDE4D encodes a class IV cAMP-specific phosphodiesterase, regulating cAMP concentration. We finally identified heterozygous PDE4D mutations in 4/5 cases. All mutations occurred de novo in all 4 cases. Neither PDE4D nor PRKAR1A mutations were found in one adult patient with characteristic skeletal features but no hormone resistance or facial dysostosis.

Splitting our series based on the disease causing gene revealed interesting genotype-phenotype correlations. Indeed, the four patients carrying PDE4D mutations shared characteristic facial features, namely midface hypoplasia with the canonical nasal hypoplasia and moderate intellectual disability. No hormone resistance was observed in 3/4 patients with PDE4D mutations while hormone resistance was consistently observed in the 5 patients carrying PRKAR1A mutations. Finally, our study further supports the key role of cAMP signaling pathway in skeletogenesis.

C09.3

Primary hypertrophic osteoarthropathy and isolated digital clubbing are caused by mutations in the prostaglandin transporter encoding gene *SLCO2A1*

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Primary hypertrophic osteoarthropathy (PHO) is a rare hereditary condition, which is characterized by digital clubbing, periostosis, arthropathy, pachydermia, and hyperhidrosis. Recently, mutations within the gene *HPGD* encoding the prostaglandin E2 (PGE2) catabolizing enzyme 15-hydroxyprostaglandin dehydrogenase were found to cause PHO. Here, we analyzed PHO patients lacking mutations in *HPGD* for mutations in genes involved in prostaglandin metabolism namely: *PTGS1*, *PTGS2*, *PTGES*, *PTGES2*, *PTGES3*, *PTGER1*, *PTGER2*, *PTGER3*, *PTGER4*, *SLCO2A1*, *SLCO3A1*, *SLCO4A1*, *PTGR1*, and *PTGR2*. In three unrelated families we identified hetero- and homozygous mutations within the solute carrier organic anion transporter family 2A1 (*SLCO2A1*) gene (MIM ID *601460, chromosome 3q21) as underlying genetic cause for isolated digital clubbing and PHO, respectively. Mutations in *SLCO2A1* comprise a homozygous insertion c.830_831insT, resulting in a premature stopcodon at p.Phe276fsX18; a homozygous missense mutation c.1670T>C introducing an amino acid substitution p.Phe557Ser and a heterozygous nonsense mutation c.754C>T resulting in p.Arg252X. *SLCO2A1* encodes the prostaglandin transporter (PGT), which is involved in carrier-mediated re-uptake of PGE2 across the plasma membrane for metabolic clearance of prostaglandins. Consequently, in patients with mutations in *SLCO2A1*, elevated PGE2 levels induce the PHO phenotype as well as isolated digital clubbing. By phenotypic correlation of hitherto identified *SLCO2A1* mutations, we found an incomplete penetrance of isolated digital clubbing in heterozygous carriers, suggesting a threshold effect of impaired PGE2 degradation defining disease progression and severity. In summary, our study establishes mutations in *SLCO2A1* as further molecular determinant of PHO and digital clubbing supporting the importance of PGE2 metabolism for bone, joint and skin physiology.

C09.4

Comprehensive genome wide CNV screening in 47 individuals with VATER/VACTERL association

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The term VATER/VACTERL association is used as an acronym for the combination of at least three of the following congenital anomalies: vertebral defects (V), anorectal malformations (A), cardiac defects (C), tracheoesophageal fistula with or without esophageal atresia (TE), renal malformations (R), and limb defects (L). The causes of the VATER/VACTERL association are likely to be heterogeneous, with individual environmental or genetic risk factors still being largely unknown. In the present study we aimed to identify copy number variants (CNVs) that contribute to VATER/VACTERL association. Molecular karyotyping, utilizing 1,134,514 SNPs SNPs (single nucleotide polymorphisms), was performed to screen 47 individuals with VATER/VACTERL association and their parents for causative de novo events. To identify potential CNVs, the SNP fluorescence intensity were analyzed with QuantiSNP using an Objective-Bayes Hidden-Markov model for calling putative CNVs. Genes which were located in regions of rearrangements were prioritized by expression data in mice. Three de novo microduplications were identified involving chromosomal region 1q41, 2q37.3, and 8q24.3. Mice expression data suggest GPR35 and EPPK1 as candidate genes for the VATER/VACTERL association. Currently, both genes are systematically sequenced in the complete sample to identify high-penetrance mutations involving small sequence changes.

C09.5

Comprehensive clinical and molecular analysis of 12 families with type I recessive cutis laxa

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Autosomal recessive cutis laxa type I (ARCL type I) is characterized by generalized cutis laxa with pulmonary emphysema and/or vascular complications. Rarely, mutations can be identified in the *FBLN4* or *FBLN5* genes. Recently, *LTBP4* mutations have been implicated in a similar phenotype. Studying *FBLN4*, *FBLN5* and *LTBP4* in 12 families with ARCL type I, we found bi-allelic *FBLN5* mutations in 2 probands, whereas 9 probands harbored biallelic mutations in *LTBP4*. No mutations were identified in *FBLN4*. *FBLN5* and *LTBP4* mutations cause a very similar phenotype associated with severe pulmonary emphysema, in the absence of vascular tortuosity or aneurysms. Gastro-intestinal and genitourinary tract involvement seems to be more severe in patients with *LTBP4* mutations. Functional studies showed that most premature termination mutations in *LTBP4* result in severely reduced mRNA and protein levels. This correlated with increased transforming growth factor beta (TGFβ) signaling. However, one mutation, c.4127dupC, escaped nonsense-mediated decay. The corresponding mutant protein (p.R1377fsX27) caused altered binding to fibrillin-1 and loss of binding to fibronectin, leading to an abnormal morphology of microfibrils in fibroblast cultures, while retaining normal TGFβ signaling. We conclude that *LTBP4* mutations are more prevalent than *FBLN5* mutations in ARCL type I. *LTBP4* mutations cause disease through both loss of function and gain of function mechanisms.

C09.6

Discriminative features in three cutis laxa syndromes; Geroderma Osteodysplastica, Cutis laxa type IIA, Cutis laxa type IIB

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Geroderma osteodysplastica (GO), cutis laxa type IIA and IIB are autosomal recessive conditions with cutis laxa as the main clinical feature. It has been difficult to differentiate these conditions solely based on clinical features. In the past few years with the identification of genes responsible for these conditions, it is easier to classify the patients with cutis laxa. Some of the patients initially diagnosed with Geroderma osteodysplastica, or wrinkly skin syndrome turned out to have mutations in *PYCR1*, or *ATP6V0A2* gene.

We present13 patients with genetically confirmed mutations in *GORAB*, *ATP6V0A2* and *PYCR1* genes confirming GO, Cutis laxa type IIA and IIB respectively. We elaborate on clinical features that are similar and different in these three conditions, which can be helpful in differentiating between these syndromes.

These three conditions have cutis laxa of trunk, wrinkling of dorsum of hand and feet, hyperlaxity, pes planus and congenital dislocation of hip and developmental delay in common.

Progeroid features, triangular face, pinched nose, loss of adipose tissue are specific for *PYCR1* mutations. Loose redundant folds in trunk seen in the other two forms of cutis laxa, is not a common feature.

Facial features of *ATP6V0A2* have bossing forehead, downslanting palpebral fissures, midface hypoplasia, anteverted nares, short nose and small mouth. Additional features are late closure of fontanels and myopia.

Droopy, jowly face with a degree of malar hypoplasia and mandibular prognathism is specific for GO. Osteoporosis and bone fracture is a feature of this syndrome not a common finding in the other two.

C10.1

Genomic instability in 25,000 cancer samples: A limited number of copy number aberration configurations

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Genomic instability is one of the hallmarks of cancer. However, so far genomic instability has only been systematically investigated in a limited number of individuals.

To overcome this we reanalyzed Affymetrix gene expression data of a heterogeneous set of 25,000 human cancer samples. We observed that the far majority of expression variation among these samples could be attributed to physiological, metabolic and cell-type specific variation. However, by applying principal component analysis we could correct all samples for these differences. After correction we observed that most genes showed very strong dosage-sensitivity to copy number alterations, permitting us to reconstruct aCGH-like copy number aberration profiles for each of the 25,000 cancer samples.

Subsequent analysis of the deletions and duplications revealed that a few combinations of certain deletions and duplications occur very often, irrespective of the particular type of cancer. This indicates the presence of strong selective forces, resulting in the survival of those cancer cells with particular cytogenetic aberrations.

The characteristics of these different cytogenetic aberration configurations are very different: Different classes of genes are affected in different configurations, which suggests that therapeutic intervention might improve by tailoring this to the type of (cyto)genetic aberration configuration that is relevant for each individual cancer sample.

C10.2

Deep intronic APC mutations explain a substantial proportion of patients with familial or early onset adenomatous polyposis

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In up to 50% of patients with colorectal adenomatous polyposis no germline mutation in the currently known genes *APC*, causing Familial Adenomatous Polyposis (FAP) or *MUTYH*, causing *MUTYH*-associated polyposis (MAP), can be identified by routine diagnostics. To uncover aberrant transcripts pointing to pathogenic deep intronic variants, we performed a systematic *APC* mRNA analysis in 125 apparently unrelated mutation negative polyposis patients.

Overall, we identified 11 reproducible aberrant transcripts in 10 patients (8% of whole study cohort; 30% of familial cases; 21% of patients with early onset manifestation). In eight of these patients two different out-of-frame insertions between intact exons (pseudoexons) were found. Sequencing of the aberrant bands revealed a 167 bp insertion from intron 4 in five families with a shared founder haplotype and a 83 bp insertion from intron 10 in three patients, caused by the heterozygous germline mutations c.532-941-G>A, c.1408+731C>T, or c.1408+735A>T, respectively. All mutations are supposed to activate cryptic splice sites. On cDNA level complete skipping of exon 9 was observed in two patients and a complex insertion/deletion rearrangement in another patient.

In conclusion, we identified a few deep intronic hotspots and founder mutations contributing substantially to the *APC* mutation spectrum. cDNA analysis and/or target sequencing of certain intronic regions should be considered as an additional mutation discovery approach in polyposis patients in whom no germline *APC* or *MUTYH* mutation was identified so far, in particular in patients with autosomal dominant pattern of inheritance and/or early onset disease.

C10.3

A common *BIM* deletion polymorphism mediates intrinsic resistance and inferior responses to tyrosine kinase inhibitors in cancer

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Tyrosine kinase inhibitors (TKI) elicit high response rates among individuals with kinase-driven malignancies, including chronic myeloid leukemia (CML) and epidermal growth factor receptor-mutated non-small cell lung cancer (EGFR NSCLC). However, the depth and duration of responses are heterogeneous, suggesting the existence of genetic modifiers of response. Using paired-end DNA sequencing, we discovered a common intronic deletion polymorphism in the gene encoding BCL2-like 11 (BIM). BIM is a pro-apoptotic member of the BCL2 family of proteins, and its upregulation is required for TKIs to induce apoptosis in kinase-driven cancers. The polymorphism switched BIM splicing from exon 4 to exon 3, encoding for BIM isoforms lacking the pro-apoptotic BCL2-homology domain 3 (BH3). The polymorphism was sufficient to confer intrinsic TKI resistance in CML and EGFR NSCLC cell lines, a resistance that could be overcome with BH3-mimetic drugs. Importantly, individuals with CML and EGFR NSCLC harboring the polymorphism experienced significantly inferior TKI responses. Our results offer an explanation for the heterogeneity of TKI responses, and suggest the possibility of personalizing therapy with BH3-mimetics to overcome BIM polymorphism-associated resistance.

C10.4

The impact of a cancer family history on ovarian cancer risk in *BRCA1* and *BRCA2* mutation carriers.

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Purpose: To study the effect of a family history of breast/ovarian cancer on the lifetime risk and age at diagnosis of ovarian cancer in *BRCA1* and *BRCA2* mutation carriers.

Patients and Methods: A prospective single center cohort study including a consecutive series of 1846 women from 367 different *BRCA1/2* families, followed-up between 1996 and 2011. The occurrence and age of diagnosis of breast and ovarian cancer in all available family members was recorded. Cox-regression analysis was applied to assess the correlation between age related penetrance of ovarian cancer and the presence and the age at diagnosis of breast and ovarian cancer within the family.

Results: In total 263 ovarian cancer cases were diagnosed. Among *BRCA2* mutation carriers, the risk of ovarian cancer was significantly higher with relatives with ovarian cancer before age 50: HR=2.34, (95% CI=1.18-4.64, p=0.02) with first-degree affected relatives and HR=2.11 (95% CI=1.12-3.98, p=0.02) with first- or second-degree affected relatives). Family histories with breast cancer reduced ovarian cancer risk, especially in *BRCA2* families: HR=0.47 (95% CI=0.34-0.67) in *BRCA1* and HR=0.29 (95% CI=0.17-0.51) in *BRCA2*, p<0.01).

Conclusion: Our findings indicate that family histories including early age ovarian cancer may double the risk of ovarian cancer in *BRCA2* mutation carriers, while a family history with breast cancer decreases ovarian cancer risk in *BRCA1/2* mutation carriers with more than 50%. These results may be important to apply in cancer risk algorithms used in genetic counselling.

C10.5

Combined whole genomic, exomic and transcriptomic sequencing identifies genes recurrently mutated in Burkitt lymphomas

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Germinal center (GC) derived B-cell lymphomas are the most frequent malignant lymphomas with Burkitt lymphoma (BL) being the predominant subtype in children. Within the German BMBF-funded International Cancer Genome Consortium (ICGC) Network Project ICGC MMML-Seq we analyzed four IG-MYC positive BL from children treated within the BFM-NHL trials. Genome, exome, transcriptome, miRNAome and methylome sequencing was performed using Illumina technology.

We detected between 1751 and 3174 somatic mutations. There was a good concordance between exome and whole genome sequencing. We identified between 59 and 105 potentially protein changing somatic mutations, including previously known changes in MYC and TP53. Sanger sequencing validated 20/25 new mutations.

A total of 16 genes were affected at least twice by potentially protein changing mutations, including known lymphoma-associated genes (e.g. FBXO11). Remarkably, two of the genes recurrently mutated, ID3 and SMARCA4, are part of the BL index recently described by us and are strongly expressed in BL (Hummel et al., NEJM, 2006). Analysis of the RNAome sequences confirmed the high expression of both genes in BL and provided evidence for aberrant splicing. As we previously reported recurrent homozygous loss at the ID3 locus (Scholtysik et al., Haematologica, 2010) we sequenced ID3 in an extended series of 100 molecularly pre-characterized IG-MYC positive GC-derived B-cell lymphomas. We identified ID3 mutations in 36/55 IG-MYC positive BL but only 6/45 IG-MYC positive GC-derived other B-cell lymphoma ($p<0.001$). In the BL, ID3 mutation was associated with a BCL6+/BCL2- phenotype, lower genomic complexity (all $p<0.05$) and a trend for a better prognosis.

C10.6

Complex tumor genomes inferred from plasma-DNA and circulating tumor cells of patients with colorectal cancer

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With the increasing number of available predictive biomarkers the serial accurate monitoring of tumor genotypes, which are prone to changes, becomes more important in the clinical management of human cancer. Here we analyzed peripheral blood of 32 patients with advanced-stage colorectal cancer (CRC) for mutant DNA-fragments in the plasma and circulating tumor cells (CTCs). We demonstrate that a subset of patients had a biphasic size distribution of plasma DNA fragments. This is accompanied by increased numbers of CTCs and elevated plasma-DNA concentrations and has implications for diagnostic tests, because tumor specific copy number changes can be established directly from plasma-DNA. Our CTC analyses included massively parallel sequencing of a panel of 68 CRC associated genes and a comparison with the mutation status in the primary tumors and associated metastases. We demonstrate that CTCs have a tremendous heterogeneity characterized by different copy number changes and mutations in cancer driver genes. Our analyses provide novel insights into tumor evolution and may extend current non-invasive approaches for disease monitoring.

C11.1

High frequency of potentially pathogenic SORL1 mutations in autosomal dominant early-onset Alzheimer disease

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Performing exome sequencing in 14 autosomal dominant early-onset Alzheimer disease (ADEOAD) index cases without mutation on known genes (APP, PSEN1 and PSEN2), we found that in 5 patients the SORL1 gene harbored unknown nonsense ($n=1$) or missense ($n=4$) mutations. These mutations were not retrieved in 1500 controls of same ethnic origin. In a replication sample including 15 ADEOAD cases, two unknown non synonymous mutations (one missense, one nonsense) were retrieved, thus yielding to a total of 7/29 unknown mutations in the combined sample. Using in silico predictions, we conclude that these 7 private mutations are likely to have a pathogenic effect. SORL1 encodes the Sortilin-related receptor LR11/SORLA, a protein involved in the control of Aβ peptide production. Our results suggest that besides the involvement of the APP and PSEN genes, further genetic heterogeneity, involving another gene of the same pathway is present in ADEOAD.

C11.2

Combination of positional cloning and new generation sequencing identifies two novel genes in spastic paraplegia involved in lipid metabolism

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Hereditary spastic paraplegias are heterogeneous neurological disorders. Known causative genes account for the majority of dominantly inherited cases, but for less than 40% of the recessive forms. We previously mapped the SPG28 locus in a Moroccan family to chromosome 14. Capture and next generation sequencing of all exons of the SPG28 interval allowed us to identify a homozygous truncating mutation in the *DDHD1* gene, encoding for a phosphatidic acid (PA)-preferring phospholipase A1 that was shown to segregate with the disease in patients. In 2 Saudi-Arabian families, genome wide linkage studies mapped a new disease locus, *SPG49*, to chromosome 4. Classical Sanger sequencing of the 22 assigned genes allowed us to identify a missense mutation in the *CYP2U1* gene, encoding for extra hepatic cytochrome P450 protein. The mutation segregates in patients in the 2 families, while 2 other mutations were identified in the same gene, including a frameshift mutation in a kindred from Egypt. All mutations were absent in large series of healthy unrelated controls.

The SPG28 and SPG49 mutations were associated with a decreased mitochondrial respiratory rate in patient lymphoblasts. Interestingly, these 2 new genes are involved in the same metabolic pathway related to lipid metabolism, paving the way for a better understanding of the mechanisms involved in these diseases. Our study underlies the power of next generation sequencing combined with linkage data in rare and genetically heterogeneous disorders.

C11.3

Exome sequencing reveals causal gene for spinocerebellar ataxia 19

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Spinocerebellar ataxia type 19 (SCA19) is characterized by a late-onset, slowly progressive, mild cerebellar ataxia, postural head tremor, myoclonic movements, and cognitive impairment. In 2002, we mapped the SCA19 disease gene to chromosome region 1p21-q21 in a Dutch family. This candidate region contained ~500 genes, but prioritized candidate gene sequencing yielded no success.

In a further attempt to identify the disease gene, we performed exome sequencing in two patients of the SCA19 family originally studied. After quality control and other exclusion criteria, we validated 5 missense mutations in potentially interesting candidates. After screening 400 Dutch controls, only one missense mutation remained unique for all SCA19 patients in the family (n=12). In addition, we screened the coding region of this candidate gene in 200 Dutch ataxia cases and identified two more families with missense mutations that had not been reported before. All of these mutations change highly conserved amino acids.

Immunohistochemistry in SCA19 autopsy cerebellum showed a significant loss of Purkinje cells and altered localization of the mutant protein. Analysis of mRNA and protein levels showed increased expression in patient cerebellum compared to controls. The different mutations alter the subcellular localization of the SCA19 protein and lead to reduced protein stability. We were able to rescue the mislocalization of the mutant proteins and increase their protein stability by co-expressing auxiliary subunits. Whether these mutations induce a gain- or loss-of-function is now being investigated.

The identification of new SCA genes remains important as they advance our understanding of the disease etiology.

C11.4

Defective presynaptic choline transport underlies hereditary motor-neuropathy

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The neuromuscular junction (NMJ) is a specialized synapse with a complex molecular architecture that serves to achieve reliable transmission between the nerve terminal and muscle fibre. Using linkage analysis and whole-exome sequencing of DNA samples from subjects with distal hereditary motor neuropathy (dHMN) type VII, we identified a mutation in the presynaptic choline transporter (CHT, SLC5A7), a critical determinant of synaptic acetylcholine (ACh) synthesis and release at the NMJ. This dominantly-segregating, CHT mutation truncates the transporter just beyond the final transmembrane domain, eliminating sequences in the cytosolic C-terminus known to mediate surface transporter trafficking. Our choline transport assays in both transfected cells and patient monocytes revealed significant reductions in hemicholinium-3 (HC-3)-sensitive choline uptake, findings most consistent with a dominant-negative mode of action. The paradigm hereditary disorder of the NMJ is the congenital myasthenic syndrome, a genetically heterogeneous neuromuscular disorder resulting from mutations in genes encoding pre-, post- and synaptic NMJ proteins. The discovery of CHT dysfunction associated with motor neuropathy is an unexpected finding which identifies a new biological basis for this group of conditions and widens the spectrum of disorders that derive from impaired NMJ transmission. Our efforts compel consideration of mutations in CHT or its functional partners in relation to idiopathic forms of the disorder.

C11.5

Mutations in *C8orf37*, encoding a ciliary protein, are associated with autosomal recessive cone-rod dystrophy and retinitis pigmentosa with early macular involvement

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Cone-rod dystrophy (CRD) and retinitis pigmentosa (RP) are clinically and genetically overlapping heterogeneous retinal dystrophies. By using homozygosity mapping in an individual with autosomal-recessive (ar) RP from a consanguineous family, we identified three sizeable homozygous regions, together encompassing 46 Mb. Next-generation sequencing in these three regions revealed a homozygous nonsense mutation (c.497T>A; p.Leu166*) in *C8orf37*, located on chromosome 8q22.1. This mutation was not present in 150 ethnically matched control individuals, single nucleotide polymorphism databases or the 1000 Genomes database. Immunohistochemical studies revealed C8orf37 localization at the base of the primary cilium of human retinal pigment epithelium cells and at the base of connecting cilia of mouse photoreceptors. *C8orf37* sequence analysis of individuals with retinal dystrophy which carried conspicuously large homozygous regions encompassing *C8orf37* revealed a homozygous splice site mutation (c.156-2A>G) in two siblings of a consanguineous family, and homozygous missense mutations (p.Arg177Trp and p.Gln182Arg) in siblings of two other consanguineous families. The missense mutations affect highly conserved amino acids, and *in silico* analyses predicted that both variants are likely pathogenic. Clinical assessment revealed CRD in four individuals, and RP with early macular involvement in two individuals. The two CRD siblings with the c.156-2A>G mutation also showed unilateral postaxial polydactyly. These results underline the importance of disrupted ciliary processes in the pathogenesis of retinal dystrophies and demonstrate the power of next-generation sequencing combined with homozygosity mapping to identify new disease genes.

C11.6

SUN protein interactions at the nuclear envelope and their role in Emery-Dreifuss muscular dystrophy

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Emery-Dreifuss muscular dystrophy (EDMD) is a rare neuromuscular disorder characterized by early contractures, slowly progressive muscular weakness and life-threatening cardiac arrhythmia that can turn to cardiomyopathy. EDMD is genetically heterogeneous, patients can have *LMNA*, *STA*(*EMD*), *FHL1*, *SYNE1* or *SYNE2* mutations. Onset, course and severity of EDMD can vary remarkably, even among patients with mutations in the same gene or carrying the same mutation. Lamin A/C, emerin and nesprins have been shown to interact with SUN1 and SUN2, resulting in a LINC termed complex that connects the nucleoskeleton with the actin cytoskeleton via the nuclear envelope. Thus, the *SUN1* and *SUN2* genes have been considered as functional candidates for EDMD association. We analyzed the *SUN1* and *SUN2* genes in a cohort of 175 unrelated EDMD patients that had previously been excluded from carrying pathogenic mutations in *LMNA*, *STA*, *FHL1*, *SYNE1* and *SYNE2*, and in 70 EDMD patients with defined mutations in these genes. The complete coding region including intron/exon boundaries of both genes was amplified and used for direct Sanger sequencing. As a result, mutations have been found in both patient cohorts. One patient has compound heterozygous mutations in *SUN1*. Additional mutations in *SUN1* or *SUN2* have been found in EDMD patients in combination with *LMNA* or *STA* mutations and are associated with increased disease severity in these individuals. Functional analysis of mutants showed a weakening of binding to other LINC proteins. Our results indicate that *SUN1* and *SUN2* mutations can cause EDMD and worsen the clinical features of the disease.

C12.1

Mutated *GPD1*, encoding Glycerol-3-Phosphate Dehydrogenase 1, causes transient infantile hypertriglyceridemia with fatty liver and hepatic fibrosis

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The molecular basis for primary hereditary hypertriglyceridemia has been identified in fewer than 5% of cases. We describe a hitherto unreported autosomal recessive condition manifesting as severe but transient infantile hypertriglyceridemia and fatty liver followed by hepatic fibrosis. We identified the mutated gene responsible for this condition in 10 individuals originating from the same isolated highly inbred population. A single large continuous segment of homozygosity on chromosome 12q13.12 containing 35 OMIM genes was identified in the affected individuals using SNP array-based homozygosity mapping. Candidate gene sequencing revealed a homozygous splicing mutation, c.361-1G>C, in *GPD1*, which encodes glycerol-3-phosphate dehydrogenase 1. This mutation is predicted to result in a truncated protein lacking essential conserved residues including a functional site responsible for initial substrate recognition. Functional consequences of the mutation were evaluated by measuring intracellular concentrations of cholesterol and triglycerides as well as triglyceride secretion in HepG2 (hepatocellular carcinoma) human cells lines overexpressing normal and mutant *GPD1* cDNA. Overexpression of mutant *GPD1* resulted in increased secretion of triglycerides (P = 0.01), supporting the pathogenicity of the identified mutation. *GPD1* mutation may lead to hypertriglyceridemia by limiting the conversion of glycerol-3 phosphate to dihydroxyacetone phosphate, and thus causing an increase in the amount of hepatic glycerol-3 phosphate available for triglyceride synthesis. The transient nature of the hypertriglyceridemia in the individuals described in this study is compatible with the fact that the rates of triglyceride secretion by hepatocytes are higher in neonates than in adults.

C12.2

Familial diarrhea syndrome caused by an activating *GUCY2C* mutation

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Chronic diarrhea is a frequent health problem, but knowledge about underlying etiological mechanisms is insufficient, and treatment is often ineffective. Rare inherited diarrheas are usually severe recessive diseases. Here we describe the clinical picture and genetic cause of a novel autosomal dominant disease in 32 members of a Norwegian family. Their chronic diarrhea is of early onset, relatively mild, and may in some patients be mistaken for irritable bowel disease. However, the diarrhea is combined with increased susceptibility to inflammatory bowel disease, ileus and oesophagitis. We performed SNP-linkage analysis to identify a candidate region on chromosome 12. This region contained GUCY2C, encoding guanylyl cyclase C, an intestinal receptor for bacterial heat-stable enterotoxins. We identified a heterozygous missense mutation (c.2519G>T) in GUCY2C in all affected family members. Exome sequencing was performed to rule out the possibility of other rare variants in the candidate region. Functional studies of the mutant receptor in HEK293T cells showed markedly increased formation of cellular cGMP in response to endogenous ligands and toxin (ST). This may cause hyperactivation of the cystic fibrosis transmembrane regulator (CFTR) and consequently increased secretion of chloride and water into the intestinal lumen, resulting in chronic diarrhea. In conclusion, increased guanylyl cyclase C signalling disturbs normal bowel function and seems to have a pro-inflammatory effect, either through increased chloride secretion or additional effects of elevated cellular cGMP. The importance of genetic variants in the guanylyl cyclase C/CFTR pathway for conditions like Crohn's disease and irritable bowel syndrome should be further explored.

C12.3

Exome sequencing identifies nonsense mutations in AGK as a cause of Sengers syndrome

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Sengers syndrome is a rare autosomal-recessive disorder hallmarked by cardiac and skeletal myopathy combined with congenital cataracts. Lactic acidosis and exercise intolerance are frequently observed while mental development is normal. The clinical course varies from fatal neonatal to more benign forms with survival into the fourth decade of life. Cause of death is invariably heart failure due to a hypertrophic cardiomyopathy.

By exome sequencing of one individual with Sengers syndrome we discovered two nonsense mutations in the gene encoding mitochondrial acylglycerol kinase (AGK). Genetic testing of AGK in additional individuals with congenital cataracts and cardiomyopathy identified numerous loss-of-function mutations in ten families, substantiating the causal nature of AGK deficiency in Sengers syndrome. Western blot experiments confirmed the absence of full-length AGK in muscle from several individuals. Interestingly, absence of AGK causes decreased levels of the adenine nucleotide translocator in the inner mitochondrial membrane in patient's skeletal muscle and a number of patients displayed reduced activities of several respiratory chain complexes. The biochemical phenotype was not expressed in patient's myoblast or fibroblast cell cultures. Our findings suggest a distinct function of AGK in mitochondrial phospholipid metabolism essential for the assembly of inner membrane proteins.

C12.4

Molecular diagnosis in mitochondrial complex I deficiency using next-generation sequencing

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Next-generation sequencing has become the core technology for gene discovery in rare inherited disorders. However, the ability to discriminate between pathogenic and benign variants remains a challenge. We used complex I deficiency, one of the most common variants of mitochondrial diseases, as an example to assess the power of exome sequencing in combination with stepwise filtering of gene variants.

Ten unrelated individuals were selected for this study. The first filter criterion was "The presence of known pathogenic variants". This revealed homozygous mutations in NDUFS3 and ACAD9 in two individuals. A second criterion was "The presence of two potentially pathogenic variants in the same structural gene of complex I", which discovered rare variants in NDUFS8 in two unrelated individuals and in NDUFB3, a hitherto unknown disease gene, in a third. Expression of wild-type cDNA in mutant skin fibroblast cell lines rescued complex I activity and assembly, thus providing a function validation of their pathogenicity. Using the third criterion "The presence of two potentially pathogenic variants in the same gene encoding a mitochondrial protein" we discovered in two patients loss-of-function mutations in MTFMT. In three patients the molecular genetic correlate remained unclear and follow-up analysis is ongoing. Appropriate in silico filtering of exome sequencing data, coupled with functional validation of new disease alleles, is effective to rapidly identify disease-causative variants in known and new complex I-associated disease genes.

C12.5

Mitochondrial ribosome assembly defect underlies infantile-onset mitochondrial cardiomyopathy

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Mitochondrial diseases display a progressive course, often manifesting and deteriorating after infection or trauma. Here we investigated the molecular background of cardiomyopathy in siblings with combined deficiency of mitochondrial respiratory chain complexes I and IV. The first patient died at six months of age of cardiomyopathy and cardiac failure manifesting after a respiratory infection. The second patient is a teenager with a clinically stable, currently asymptomatic cardiomyopathy. Using whole-exome sequencing, we found both patients to have a homozygous missense mutation in a novel disease gene, *MRPL44*, which encodes for a protein component of the mitochondrial large ribosome subunit. The mutation affected a conserved amino acid, leading to MRPL44 protein instability and its reduced levels in patient's heart, skeletal muscle and fibroblasts. Only a low level of assembled large ribosome subunit was found in patient fibroblasts, whereas the small subunit was present at normal level. Retroviral expression of wild-type MRPL44 in patient cells rescued all identified defects. The result suggests that MRPL44, which has no homologue in bacteria and has only emerged in the mitochondrial ribosome of eukaryotes, is essential for translation in human mitochondria. Our findings also demonstrate that only a fraction of mitochondrial ribosomes are required to assemble to maintain sufficient respiratory chain function. In conclusion, we have identified a new genetic cause of infantile-onset mitochondrial hypertrophic cardiomyopathy.

C12.6

Different sequencing strategies in the analysis of new ENU-derived mouse models for metabolic bone disease

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Animal models are required to understand the molecular mechanisms of metabolic bone disorders in which imbalances of bone metabolism and mineralization lead to a variety of different phenotypes. Within a large-scale genome-wide Munich ENU (N-ethyl-N-nitrosourea) Mutagenesis Project mutant mouse models for metabolic bone disease were identified using three blood parameters (total alkaline phosphatase activity (ALP), total calcium (Ca), and inorganic phosphate (Pi) levels) which were commonly used as biochemical markers in patients with metabolic bone disease. Here, we describe three different sequencing strategies, (1) a candidate gene approach, (2) whole chromosome sequencing, and (3) exome sequencing, to identify novel genes involved in phosphate homeostasis and bone metabolism. In two mutant mouse lines (BAP012, BAP024) we identified novel *Phex* alleles (*Phex* c.148A>T, p. Lys50* and *Phex* c.2197T>C, p.Csy733Arg) by capillary sequencing of the candidate gene and extend the available mouse models for X-linked hypophosphatemia (XLH, OMIM 307800) to the number of 9. In two mutant mouse lines (BAP004, BAP005) the disease causing mutations were identified by linkage analysis, chromosome sorting and whole chromosome sequencing (BAP004: *Jak1* c.1933T>C, p.Ser645Pro and BAP005: *Asgr1* c.815A>G, p.Tyr272Cys). In 20 mutant mouse lines and one C3H wt line we performed exome sequencing using an exome capture kit from Agilent on a HiSeq2000 genome analyzer from Illumina. We identified in each mouse line between 3 and 42 SNVs (single nucleotide variants). We started with the evaluation of the different variants in mouse mutant and control cohorts and will present the data of the SNV evaluation.

C13.1

Mutations in PIGO, a member of the GPI anchor synthesis pathway, cause hyperphosphatasia with mental retardation syndrome

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More than a hundred cell surface proteins are attached to the plasma membrane by covalent attachment to a glycophosphatidylinositol (GPI) anchor that is assembled in the endoplasmic reticulum (ER) and added to the C-terminus of the proteins. Biosynthesis of GPI anchors involves more than 30 different genes. Genetic defects in various components of the GPI pathway have been identified in a number of phenotypically diverse diseases.

We have recently identified mutations in PIGV in individuals with hyperphosphatasia mental retardation (HPMR) syndrome, an autosomal recessive form of mental retardation with facial dysmorphism, seizures, brachytelephalangy, and persistent elevated serum alkaline phosphatase (hyperphosphatasia). However, not all patients with HPMR syndrome harbor mutations in PIGV. The purpose of the current study was therefore to investigate the molecular etiology of HPMR syndrome in PIGV-negative patients and to establish a next- generation sequencing based screening approach for GPI pathway diseases.

We employed whole-exome sequencing of two siblings with HPMR and identified compound heterozygous PIGO mutations. Screening of further PIGV-negative patients detected compound heterozygous PIGO mutations in another affected individual. The characteristic facial appearance, developmental delay, hypoplastic or even absent terminal phalanges including nails and hyperphosphatasia were present in all affected. In a cell based assay, two of three PIGO mutations had a deleterious effect on PIGO function. Furthermore, by Fluorescense-activated cell sorting analysis we demonstrated that PIGO is essential for GPI anchoring of attached proteins such us CD 59 and uPAR.

Our findings extend the range of reported phenotypes associated with GPI anchor synthesis defects

C13.2

Identification of de novo variants in 51 sporadic patients with unspecific severe intellectual disability and 20 controls by exome sequencing

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Due to the absence of morphological or positional clues, the etiology of severe intellectual disability (ID) remains elusive in the majority of patients. We analyzed 51 children (32 girls, 19 boys) with severe non-syndromal ID and their healthy parents for de novo SNVs and small indels by exome sequencing. 20 trios with children and/or parents affected by diabetes mellitus type 2 were investigated as controls. Exomes were enriched with SureSelect Human All Exon 50 Mb kits and sequenced to an average read depth of 100. We detected de novo non-synonymous variants in 84% of patients. The number per individual varied between 0 and 4. The average number was higher in the disease (1.4/individual) than in the control group (0.8/individual). Specifically, the disease group showed a considerably higher number of nonsense/splice/indel variants (0.37/individual) than the control group (0.1/individual). 16 patients showed de novo mutations in the known ID genes IQSE2, SATB2, SNC2A, SCN8A, SETBP1, SLC2A1, STXBP1, SYNGAP1, TCF4, and MECP2. We regarded at least 8 variants in 8 novel genes to be disease causing because they fulfilled 4 of the following criteria: mutation type (nonsense/splice/frameshift), location in regions known for de novo microdeletions, haploinsufficiency predictions, brain expression, and functional evidence. In summary, this study clearly demonstrates the power of exome sequencing in identifying a sizable fraction of disease causing mutations in both known and novel genes.

De novo variants

	Cases n=51	Controls n=20
missense	54	14
nonsense	5	0
splice	1	1
frameshift	13	1
synonymous	12	5

C13.3

Dosage imbalance of nonsense-mediated mRNA decay factors is associated with intellectual disability

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Nonsense-mediated mRNA decay (NMD) functions to degrade transcripts bearing premature stop codon and is a crucial regulator of gene expression. We implicated NMD and the UPF3B gene as the cause of various forms of intellectual disability (ID) and various other psychiatric traits. In expanding our inquiry, we identified three patients with global developmental delay who carry deletions of the genomic regions encompassing the UPF2 gene, another important member of the NMD pathway. We hypothesized that loss of one allele of UPF2 and likely loss of other NMD factors impair NMD and result in neurological phenotype. Using RNA-SEQ on lymphoblastoid cells from UPF2 del patients we identified 1009 differently expressed genes (DEGs). 38% of these DEGs overlapped with DEGs identified in UPF3B patients. More importantly, 95% of all DEGs in either UPF2 or UPF3B patients share the same trend of de-regulation. This suggested that the transcriptome de-regulation in UPF2 and UPF3B patients is similar. To gauge into the role of other NMD factors we performed a comprehensive search for copy number variations (CNVs) encompassing all known NMD factors in ID patients and controls. Our data indicate that CNVs, especially for UPF2, UPF3A, Y14, SMG6 and EIF4A3 are frequent in patients with neurological disorders and ID in particular. This tells that the changes in copy number of these factors, and as such dosage imbalance of NMD genes, are likely the causes or predisposing factors to ID. Our data strengthen the importance of NMD in normal learning and memory.

C13.4

A truncating mutation of *CEP135* causes primary microcephaly and disturbed centrosomal function

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Autosomal recessive primary microcephaly (MCPH) is a rare congenital disorder of mental retardation, reduced brain and head size but usually without defects in cerebral cortical architecture and other syndromic abnormalities. MCPH is heterogeneous with 7 known loci: MCPH1-MCPH7. The underlying genes code for centrosomal proteins. We collected 49 MCPH families from Pakistan. Sequencing of *ASPM* and *WDR62*, the two most frequently mutated genes, and exclusion of homozygosity for all known MCPH loci by typing of appropriate microsatellite markers resulted in 14 families with a putatively new disease locus. Out of these, we studied one family with two microcephalic children using homozygosity mapping and found suggestive linkage for regions on chromosomes 2, 4 and 9. We sequenced two positional candidate genes and identified a homozygous frameshift mutation in centrosomal protein135 kDa (*CEP135*), located in the linkage interval on chromosome 4. Post-hoc whole-exome sequencing corroborated this mutation to be the causal variant. Immunostaining of CEP135 showed strong signals in the developing neuroepithelium of the cerebral cortex during embryonic stages E11.5 through E15.5. Fibroblasts obtained from one of the patients showed multiple and fragmented centrosomes, disorganized microtubules, and reduced growth rate. Similar effects were reported after knockdown of CEP135 through RNA interference; we could provoke them also by ectopic overexpression of the mutant protein. Our findings suggest a new locus, MCPH8, at HSA 4q12, further strengthen the role of centrosomes in the cause of MCPH, and place CEP135 among the essential components of this important organelle in particular for a normal neurogenesis.

C13.5

Dysregulation of Rho GTPases in the alphaPix/Arhgef6 mouse model of X-linked intellectual disability is paralleled by impaired structural and synaptic plasticity and cognitive deficits

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Mutations in the ARHGEF6 gene, encoding the guanine nucleotide exchange factor alphaPIX/Cool-2 for the Rho GTPases Rac1 and Cdc42, cause X-linked intellectual disability (ID) in humans. We show here that alphaPix/Arhgef6 is primarily expressed in neuropil regions of the hippocampus. To study the role of alphaPix/Arhgef6 in neuronal development and plasticity and gain insight into the pathogenic mechanisms underlying ID, we generated alphaPix/Arhgef6-deficient mice. Gross brain structure in these mice appeared to be normal, however, analysis of Golgi-Cox stained pyramidal neurons revealed an increase in both dendritic length and spine density in the hippocampus, accompanied by an overall loss in spine synapses. Early-phase long-term potentiation was reduced and long-term depression was increased in the CA1 hippocampal area of alphaPix/Arhgef6-deficient animals. Knockout animals exhibited impaired spatial and complex learning and less behavioral control in mildly stressful situations, suggesting that this model mimics the human ID phenotype. The structural and electrophysiological alterations in the hippocampus were accompanied by a significant reduction of active Rac1 and Cdc42, but not RhoA. In conclusion, we suggest that imbalance in activity of different Rho GTPases may underlie altered neuronal connectivity and impaired synaptic function and cognition in alphaPix/Arhgef6 knockout mice.

C13.6

The functional spectrum of *SRGAP3* in cognitive development

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Disruption in the *SRGAP3* gene has been associated with abnormal cognitive function. srGAP3 is a member of the Slit-Robo GAP family and is implicated in repulsive axon guidance and neuronal migration through Slit-Robo mediated signal transduction, making it an ideal candidate for orchestrating the formation of functional neural networks during brain development. To uncover the importance of srGAP3 in the underlying neurodevelopmental processes required for normal cognition, a *srGAP3* knockout mouse model was generated (Waltereit et al. submitted). *srGAP3*$^{-/-}$ mice exhibit a complex behavioural phenotype including impaired social interaction along with various neuroanatomical defects. To gain more insight into the molecular processes underlying these defects, we identified novel binding partners of srGAP3. This avenue led us to the discovery that srGAP3 and its novel interacting partner lamellipodin co-localise to the leading edge of cell protrusions and that srGAP3 inhibits protrusion dynamics through lamellipodin. Additionally, *srGAP3*$^{-/-}$ hippocampal neurons exhibit excessive lamellipodia formation. To take the story further, we examined the role of srGAP3 in axon guidance using commissural axon crossing in the spinal cord as a model system. We were able to show that axons were not normally positioned after crossing, implicating srGAP3 in axon repulsion from the floor plate. Taken together, our results have begun to uncover the underlying processes in which *srGAP3* is playing a role during brain development, thus shedding light on the importance of this gene in cognitive processes.

C14.1

ASB10 variants are associated with open-angle glaucoma and silencing impairs ocular outflow

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The molecular events responsible for obstruction of aqueous humor outflow and the loss of retinal ganglion cells in glaucoma, one of the main causes of blindness worldwide, remain poorly understood. We identified a synonymous variant, c.765C>T (Thr255Thr), in ankyrin repeats and suppressor of cytokine signaling box-containing protein 10 (ASB10) in a large family with primary open angle glaucoma (POAG) mapping to the GLC1F locus. This variant affects an exon splice enhancer site and alters mRNA splicing in lymphoblasts of affected family members. Systematic sequence analysis in two POAG patient groups (195 US and 977 German) and their respective controls (85 and 376) lead to the identification of 26 amino acid changes in 70 patients (70 of 1172; 6.0%) compared with 9 in 13 controls (13 of 461; 2.8%; $P = 0.008$). Molecular modeling suggests that these missense variants change ASB10 net charge or destabilize ankyrin repeats. ASB10 mRNA and protein were found to be strongly expressed in trabecular meshwork, retinal ganglion cells and ciliary body. Silencing of ASB10 transcripts in perfused anterior segment organ culture reduced outflow facility by 50% compared with control-infected anterior segments ($P = 0.02$). In conclusion, genetic and molecular analyses provide evidence for ASB10 as a glaucoma-causing gene.

C14.2

Seven New Loci Associated with Age-Related Macular Degeneration

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Age-related macular degeneration (AMD) is a prevalent disease of complex aetiology and one of the leading causes of vision impairment in industrialized countries. To further our understanding of AMD genetics, 18 groups from 12 countries formed the AMDGene Consortium in 2010.

This endeavor brought together 15 genome-wide association studies comprising 7,650 advanced AMD cases and 51,812 controls of European and Asian ancestry. The meta-analysis revealed SNPs at 32 genomic loci with P < 10-5 that were followed up in 18 additional studies consisting of 9,531 advanced AMD cases and 8,230 controls. In the joint analysis, a total of 19 loci reached genome-wide significance (P < 5x10-8) which included all 12 previously established and the seven novel AMD loci COL8A1/FILIP1L (4x10-13), IER3/DDR1 (2x10-11), SLC16A8 (3x10-11), TGFBR1 (3x10-11), RAD51B (9x10-11), MIR548A2 (5x10-9), and B3GALTL (2x10-8). Pathway analyses of the 19 loci indicated an over-representation of genes involved in complement activity, lipid metabolism and inhibition of angiogenesis thereby falling well into place with known AMD pathomechanisms. While the sensitivity analyses indicated that several loci had differences in disease risk between males and females, or in European and Asian ancestry or disease subgroups, the overall predictive value of these variants displayed similar effectiveness in all samples examined (0.69 < AUC < 0.79).

Our findings can guide future biological and genetic AMD studies, allow better classification of individuals at risk, and might ultimately lead to improved disease treatment and prevention.

C14.3

Meningococcal disease and age-related macular degeneration are genetically related

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Background and aims: Host genetic variation in complement factor H (CFH) show very strong evidence of association with individual susceptibility to meningococcal disease (MD).

Methods: We performed a meta-analysis of two GWAS in Spain (ESIGEM-network) and the UK totaling 894 MD cases and 5,645 controls,with replication in a further 565 MD cases and 2,600 controls of West European descent. The MD cases were genotyped using the Illumina Human-610K Quad Bead Chips for UK and the Illumina Human-660W Quad Bead Chips for Spain. Replication genotyping was done using the Sequenom-MassArray platform.

Results: We note strong evidence of association at the previously reported *CFH* locus on Chromosome 1(rs1065489, *P*=1.18 x 10-8 and rs11582939, *P*=1.95 x 10-8).The second most significant SNP was observed within *ABCA4* (rs544830, *P*=2.93 x 10-6, per-allele OR=1.30).Strong statistical association of this locus with MD was corroborated by two other neighboring SNPs (rs550060, *P*=4.48 x 10-6, per-allele OR=1.29 and rs497511, *P*=4.55 x 10-6, per-allele OR=1.29). These findings within *ABCA4* were further replicated in the Western European collection (*P*=8.72 x 10-5, *P*=1.81 x 10-4, and *P*=6.59 x 10-5 respectively), leading genome-wide significant findings for all three *ABCA4* SNPs (*P*=8.46 x 10-10, *P*=5.28 x 10-9, and P=2.36 x 10-9, respectively) when data from all MD sample collections were jointly analyzed.

Conclusion: As mutations in both *CFH* and *ABCA4* also confer susceptibility to macular degeneration, our observation points to shared mechanisms of pathogenesis between macular degeneration and MD.

C14.4

First genome-wide meta-analyses of nonsyndromic cleft lip with or without cleft palate identify six new risk loci including one subtype-specific locus

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Nonsyndromic cleft lip with or without cleft palate (NSCL/P) is one of the most common birth defects in humans. In the last years, the etiology of this phenotypically variable malformation, which involves both environmental and genetic factors, has been elucidated by the discovery of six genetic susceptibility loci in large genome-wide association studies (GWAS). To identify additional loci we conducted the first meta-analyses using the two largest GWAS on NSCL/P that are available to date. Our analyses confirmed all previously identified loci, and identified six new susceptibility regions for the European population (1p36, 2p21, 3p11.1, 8q21.3, 13q31.1, and 15q22). Five of these loci were shown to also play a role in the Asian population. Analyses of the phenotypic subgroups NSCLO (nonsyndromic cleft lip only) and NSCLP (nonsyndromic cleft lip with cleft palate) revealed that a locus on chr. 13q31.1 was a strong susceptibility factor for NSCLP (rs8001641, P_NSCLO = 0.163, P_NSCLP = 6.51x10-11; RRhet_NSCLP = 1.63 (95% CI: 1.28 - 2.07), RRhom_NSCLP = 2.41 (95% CI: 1.84 - 3.16)). The present study is the first to identify a genome-wide significant locus that is specific for NSCL/P subtypes and emphasizes the power of genetic studies when detailed clinical and / or phenotype information is available.

C14.5

Variants in *RUNX3* contribute to susceptibility to psoriatic arthritis exhibiting further common ground with ankylosing spondylitis

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Psoriatic arthritis (PsA) is a common inflammatory joint disease distinct from other chronic arthritides and frequently accompanied by psoriasis vulgaris (PsV). In a first genome-wide association study (GWAS), we were able to identify several genetic risk factors. But even combined with previously identified factors, only a fraction of genetic contribution to disease is explained. Therefore we pursued further 16 loci from our GWAS with several SNPs showing association in the range of $5E^{-8}$ (= genome-wide significance level) < p-value < $1E^{-5}$ as well as one functional candidate.

20 of 21 SNPs at 18 loci were successfully genotyped in independent European cohorts of 1,748 PsA cases and 3,926 control probands, furthermore in

a group of 961 German PsV patients. Association to a *RUNX3* variant was replicated and resulted in a combined (GWAS + replication) p-value of $1.52E^{-06}$ in a Cochran-Mantel-Haenszel test and an OR of 1.20 (1.11-1.29). Further analyses based on linkage disequilibrium at *RUNX3* could pinpoint the most significant association to the initial SNP located in the first intron of one isoform. In the smaller patient group of PsV patients and corresponding German control individuals, p-value was $2.5E^{-02}$ and OR 1.22 (0.96-1.56), indicating also a role in skin manifestation of psoriasis.

Our analyses suggest that variants in *RUNX3* contribute to susceptibility to PsA, a genetic factor already described to be associated with another spondylarthritis, ankylosing spondylitis. RUNX3 is a transcription factor involved in CD8 lymphozyte differentiation and therefore a good candidate for PsA and PsV as T-cell mediated diseases.

C14.6

Genome-Wide Association Analysis Identifies the MTHFR-CLCN6-NPPA-NPPB Gene Cluster as an Importance Influence on BNP Levels - Implications for the Use of BNP levels in the Diagnosis and Therapeutic Monitoring of Heart Failure.

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Brain Natriuretic Peptide (BNP) levels provide insight into Left Ventricular (LV) filling pressures, and are therefore increasingly used in the diagnosis and management of heart failure.

We conducted a genome wide association analysis to identify genetic variants associated with BNP levels in 737 hypertensive caucasian individuals. 318,000 SNPs were genotyped and an additional 1,972,462 SNPs were imputed from HapMap. Serum BNP levels were measured by immunoassay. LV filling pressures were measured using the echocardiographic derived ratio of early diastolic transmitral flow velocity to mitral annular velocity (E/E'). Linear regression analysis were adjusted for major cardiovascular risk factors.

17 SNPs spanning 150kb of MTHFR-CLCN6-NPPA-NPPB (MCNN) gene cluster were significantly associated with BNP levels. Median BNP levels for AA homozygotes, AG heterozygotes and GG homozygotes for the top-hit SNP (a MTFHR gene SNP), were 25, 32 and 34, respectively ($p=4.9X10^{-9}$). Despite no associations observed between these 17 SNPs and E/E', carriers of gene cluster variants were much more likely to have BNP levels above the cut-off regarded as diagnostic of heart failure (>100 pg/ml). Results showed that individuals who are minor allele homozygotes (GG) had three times elevated BNP levels when compared to major allele homozygotes (AA) (7% *versus* 2%, p=0.01). This is the first study to demonstrate that genetic variants in the MCNN gene cluster influence BNP levels independently of LV filling pressures. Combining MCNN genotyping with BNP measurement is likely to improve the sensitivity and specificity of algorithms that use BNP levels in the diagnosis and management of heart failure.

C15.1

Identification and characterization of genetic disorders with ID in diagnostics and diagnostic related research

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The etiology of intellectual disability (ID) is unknown in about 50% of the patients. Knowing the cause enables anticipation on associated health and behaviour problems, and is essential for adequate counseling regarding recurrence risk and prognosis. In recent years, advances in genetic technologies have provided great new opportunities for the identification of genetic defects. We aimed to identify genetic causes of ID in a unique cohort of 254, mainly adult, patients with unexplained ID, selected from a large cohort of individuals from Dutch residential settings. Our studies included a multidisciplinary clinical evaluation, followed by specific genetic diagnostic tests if indicated, and standard genome-wide array analysis and a metabolic screen. In the diagnostic part of the study a genetic diagnosis was established in 17.5%, comprising of 12% chromosomal abnormalities and 5% monogenetic defects, mostly fitting syndromes for which the causative genes were identified recently, such as Pitt-Hopkins and Kleefstra syndrome. In a small minority (<1%) a primary metabolic cause was established. About 60 patients were selected for further research studies, mainly comprising different next generation sequencing approaches. So far, these led to the identification of 14 additional likely molecular diagnoses, including both known and novel genetic defects. Pertinent diagnoses included *de novo* mutations in *PDHA, GRIN2A, DYNC1H1* and *GATAD2B*. This has increased the current diagnostic yield to over 23%. Since several studies are pending, more diagnoses are expected. The majority of selected individuals have an age above 40 years, providing useful information on natural progression and comorbidity of these disorders.

C15.2

High yield of massive parallel exome sequencing in 25 families with autosomal recessive intellectual disability

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To elucidate the genetics of autosomal recessive intellectual disability, we undertook systematic autozygosity mapping in 69 large, consanguineous families. We focused on 25 families with small candidate regions of 10 to 45 Mb. After enriching the exomes with the Agilent SureSelect Kit (13 exomes using the 38Mb and 19 using the 50Mb versions, whereas 7 were enriched using both for comparison), we undertook massive parallel sequencing on Solexa or SOLiD. We were able to clarify the causes of intellectual disability in 12 families. We described one new intellectual disability gene, AP4S1, and characterized the AP4 deficiency syndrome. Furthermore, in seven families we identified mutations (two in-frame deletions, two frameshifts, and three missense mutations) in EDC3, ENO2, c9orf4, FAR1, HMG20A, PPFIA1, and SPATA5. In silico analysis predicted pathogenic effects of the mutations, and frequencies in 280 ethnic matched controls were null. The functions of c9orf4 and SPATA5 are unknown, while all other genes are highly expressed in brain and functionally relevant. In addition, we clarified the etiology in four further families by identifying pathogenic mutations in the known genes AIH1, ALDH5A1, GPR56, and HGSNAT. As a consequence of the enriching method, only 67% of the exons were properly covered (i.e. depth >5x at >80% of the nucleotides). To identify the mutations in the rest of the families, ensuring complete and high coverage of all exons in the linkage regions, we designed targeted sequencing enriching sets (Agilent, SureSelect). Sequencing on SOLiD is ongoing.

C15.3

Brain malformation and clinical finding in autosomal recessive primary microcephaly: genotype - phenotype correlation

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Background: Autosomal recessive primary microcephaly (MCPH) has underlying genetic defects, of which some are defined to date. However, fewer studies till now have tried to answer the questions regarding the genetic defect in MCPH gene family and the specified brain architecture changes.

Methods: From Iranian cohort of mentally retarded -Intellectual disability (ID) - patients, 51 families with concomitant primary microcephaly in at least two members and modest neurological signs were selected and MRI was performed for the proband of each family. In the previous study, homozygosity mapping, coupled by sanger sequencing or next generation sequencing (NGS) were used to define underlying genetic defects.

Results: Genetic defect was successfully defined in 13 out of 51 families. 4 novel genes were identified using NGS, two of which were responsible for syndromic (TMEM135, TAF2) and two for non-syndromic (CAPN10, ZBTBYO) primary microcephaly. 2 families had defects in recently identified AP-4 complex (AP4M1, AP4E1). Remainings were previously known genes: three WDR62, two CENPJ, one Microcephalin and one PDHX

Moreover, 19 out of 51 families showed different types of brain abnormalities on MRI: Cerebellar hypoplasia in eight, small or hypoplastic corpus callosum in six. Heterotopia, abnormal white matter, enlargement of ventricles and thickening/thinning/agenesis of corpus callosum, each in one.

Conclusion: MCPH can be associated with neurological signs relevant to MRI changes despite spared architecture of the brain, but due to the underlying basis of the disorder. Here we demonstrate new findings of imaging in both recently identified genetic defects and MCPH genes.

C15.4

The utility of exome sequencing in Primary Immunodeficiency Diseases and Immunodysregulative Disorders

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Mutations in more than 200 different genes have been reported causing various primary immunodeficiency diseases (PIDs) including immunodysregulative/autoinflammatory disorders. Knowing the exact molecular genetic diagnosis is valuable to direct protocols for immunoreconstitution, immunotherapies, and prophylaxis, and may predict clinical outcomes. For combined T- and B-cell deficiencies and for isolated B-cell deficiencies, phagocyte disorders and defects in innate immunity there are multiple genes known to be causal, and patients with different immunodeficiencies may have overlapping immunological and clinical phenotypes.

We examined the utility of high throughput next generation DNA sequencing with exome capture in the diagnostic workup and research of PIDs, including SCID, severe autoinflammatory disorder, severe congenital neutropenia, hyper IgM syndrome, autosomal recessive agammaglobulinemia and other immunodeficiency diseases with unknown etiology. Based on the clinical and immunophenotypical data, family history and knowledge from similar PID cases, we varied between using candidate gene testing with relevant known PID genes, triotesting (patient + parents) in the assumed *de novo* cases, or focused on genomic regions with unknown candidate genes in the assumed autosomal recessive cases when loss of heterozygozity or copy number variations had been found.

Pathological variants were detected in both well known and less characterized genes. We address the advantages and limitations of this approach i.e. regarding detection of low grade mosaicism. Our project illustrates the capability of targeted exome sequencing to efficiently identify novel variants in a large set of candidate genes, and reinforces the method's clinical utility to identify causal variants of PIDs and other rare immunological disorders.

C15.5

NGS-panel targeting 150 cilia-related disease genes improves diagnostic testing for the broad spectrum of ciliopathies

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Cilia-related disorders (ciliopathies) are characterized by great clinical and genetic heterogeneity. Literally all organs can be affected, frequent cilia-related manifestations are cystic and polycystic kidney disease, retinal degeneration, situs inversus, polydactyly, skeletal features, and defects of the central and peripheral nervous system. These can occur isolated or as part of syndromes, such as Bardet-Biedl, Joubert, Meckel, Jeune, Ellis-van-Crefeld, and Sensenbrenner syndrome. Variable expressivity and overlaps between different entities often make it difficult to give a clear diagnosis. Genotype-phenotype correlations are usually not convincing and mutations in the same gene can cause very different phenotypes. Overall, it is more the rule than the exception that multiple, often dozens of genes are to be considered to be disease-relevant in a patient with suspected ciliopathy. Second-site modifiers are expected to exert an aggravating effect in an epistatic way. In this scenario, altered dosage of disease proteins may disturb cell homeostasis and network integrity contributing to early and more severe disease expression. We designed a "ciliopathy panel" for next-generation sequencing (NGS) that allows the parallel investigation of about 150 cilia-related disease genes in a time- and cost-efficient manner. We present families from a broad spectrum of cilia-related disease phenotypes in which we have used our NGS-panel and identified convincing disease-related mutations in different genes corroborating our hypothesis. This novel genetic testing approach considerably improves genetic diagnostics of ciliopathies and deserves increased attention in genetic counselling and the management of affected families.

C15.6

Next generation sequencing of 105 genes associated with retinal dystrophy: A new era for diagnostic testing

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Retinitis Pigmentosa (RP) is a group of highly genetically heterogeneous retinal dystrophies which lead to reduced vision and eventual blindness. RP affects approximately 1:3500 people in the UK. Current diagnostic testing is largely restricted to conventional Sanger sequencing of a small number of genes. As a result of its heterogeneity, RP was an ideal candidate to use a targeted enrichment next generation sequencing approach, technologies not previously utilised in a diagnostic setting, as the basis for a diagnostic service.

We describe our approach to the validation of the entire workflow (assay design, sample preparation, bioinformatic analysis and scientific analysis) of a next generation sequencing targetted enrichment of 105 genes known to cause RP or associated conditions. The process was validated using 50 patients. The validation process included: developing criteria for transcript choice during assay design, documenting laboratory sample processing, defining minimum coverage criteria, assigning quality and coverage thresholds for SNP and indel calling, defining criteria for filtering of benign polymorphisms and assessing SNP concordance between next generation sequencing and Sanger sequencing.

We identified likely pathogenic mutations in 42% (21/50) of patients tested, 64% (9/14) in adRP, 32% (6/20) in sporadic and 37% (6/16) in sporadic patients. We have developed a diagnostic NGS testing strategy that will extend clinical testing to a wider range of RP referral types and improve detection rates across all patients. Furthermore we propose a model on which to base future validation of large scale next generation sequencing target enrichments in a diagnostic laboratory.

C16.1

Non-invasive prenatal detection of fetal autosomal aneuploidies using massively parallel sequencing: a collaborative study in Europe

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Objectives: Recent advances in non-invasive prenatal diagnosis show that massively parallel sequencing (MPS) of maternal plasma DNA allows an accurate detection of common fetal aneuploidies. Here, we describe a large-scale clinical study, which will be finished in March 2012. The aim of the study is to validate the diagnostic accuracy of our non-invasive prenatal test based on MPS for detecting fetal autosomal aneuploidies.

Method: Maternal blood samples were collected from more than 500 pregnant women prior to invasive prenatal procedures at 7 clinics located in Germany and Switzerland. The extracted maternal plasma DNA was analyzed using Illumina sequencing platform HiSeq2000 in a multiplexed fashion. For data analysis a z-score equation was used to distinguish samples with fetal aneuploidies from samples with a set of normal fetal chromosomes. The results of MPS analysis have been compared with the fetal karyotype obtained from chorionic villi sampling or amniocentesis.

Results: We will present the results of the collaborative, blinded study including sensitivity and specificity of the well-established non-invasive prenatal test for detection of fetal autosomal aneuploidies. Furthermore, advanced algorithms for bioinformatics for detection of these aneuploidies will be discussed.

Conclusions: MPS of maternal plasma DNA is a very promising approach for non-invasive detection of fetal aneuploidies. Their implementation in prenatal care of high risk pregnant women will decrease the use of invasive procedures. However, future clinical studies are required to validate the use of MPS for the detection of broader spectrum of fetal chromosomal abnormalities and fetal genomic imbalances.

C16.2

Further development and larger validation of non-invasive prenatal diagnosis for trisomy 21 using MeDIP real time qPCR

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Nowadays, prenatal diagnosis of Down syndrome is performed using invasive procedures such as amniocentesis or chorionic villi sampling, which are associated with a significant risk of fetal loss (1%). Many attempts have been made in the last two decades towards the development of a non-invasive prenatal diagnostic (NIPD) test. One of the most promising technologies is the application of the Methylated DNA Immunoprecipitation (MeDIP) real-time qPCR-based approach developed by Prof. Philippos Patsalis and his team. The MeDIP methodology was initially combined with high-resolution tiling oligonucleotide array, to identify DNA methylation differences between maternal and fetal DNA across chromosomes 13, 18, 21, X and Y. Thousands of Differentially Methylated Regions (DMRs) were generated and the best were used for the development and validation of the first universal non-invasive prenatal diagnosis for trisomy 21 (NIPD^{21}), using MeDIP real-time qPCR for chromosome 21. The team has recently described an improved version of the diagnostic formula and a much larger validation study (unpublished data). The improved version was created using old and new DMRs and taking into account the genomic position and copy number variation of DMRs. An additional advantage of the new diagnostic formula is that it consists of only seven DMRs, simplifying the diagnostic assay and further reducing the cost. In conclusion, the MeDIP real time qPCR-based approach is an accurate, reliable NIPD test for Down syndrome. It is simple, fast and easy to perform in every genetic diagnostic lab worldwide as it does not require expensive equipment, software or special infrastructure.

C16.3

Analysis of PCR-based monogenic preimplantation genetic diagnosis (PGD) by follow up of untransferred embryos - A multi-center study

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PGD, an established reproductive alternative for couples with high-risk of transmitting a monogenic disorder, currently involves PCR-based methods. PCR protocols must be robust, sensitive and highly accurate, completely precluding misdiagnosis. Misdiagnosis can be adverse (affected pregnancy/baby) or benign. The twelve adverse misdiagnoses reported to the ESHRE PGD-Consortium are likely an underestimate. This study assessed validity, diagnostic value and accuracy of PCR-based PGD, through re-analysis of untransferred embryos from monogenic-PGD cycles. Data from 7 centres included: disease, embryology, assay type (singleplex/multiplex, one- or two-cell biopsy), genotype concordance at PGD and follow-up. Reasons for discordance included ADO, contamination, mosaic embryo, other. Data from 1352 untransferred embryos was analysed for: sensitivity (Se), specificity (Sp), false negative (FN) and positive (FP) rates, Negative (NPV) and Positive predictive value (PPV), diagnostic odds-ratio (OR). Impact of 1- versus 2-cell biopsy, and type of assay (singleplex versus multiplex) on PGD outcome was also assessed. In addition, frequencies of factors leading to discordant results (FP or FN) were estimated.Diagnostic outcomes were better for multiplex assays versus singleplex (OR 2116 versus 154), and for two-cell versus one-cell biopsy (OR 1036 versus 407). However, Sensitivity and NPV of singleplex/multiplex assays compared to one- or two-cell biopsy were not significantly different, indicating that 2-cell biopsy is not essential for more accurate clinical results. Inherent risks of PCR based PGD methods (ADO, contamination) accounted for 40.68% of discordant results, whereas mocaisism (biological risk) accounted for 57.63%. This study demonstrates the validity, robustness and high diagnostic value of PCR-based PGD.

C16.4

Genetic basis of intrauterine fetal demise: the role of cardiac channelopathies

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Background

Intrauterine fetal demise (IUFD) or stillbirth, accounts for 60% of perinatal mortality, and its incidence is 1:200 pregnancies. Despite extensive postmortem investigations, nearly 50% of IUFDs remain unexplained. Since 10-15% of sudden infant death syndrome (SIDS) cases carry functionally relevant long QT syndrome (LQTS)- or Brugada syndrome (BrS)-associated genetic variants, we hypothesized that sudden arrhythmic death may underlie some IUFD as well.

Methods

Ninety-eight IUFD cases with negative diagnostic work-up (gestational age at death >=15 weeks; average 26.3±8.6 w.) were collected. Molecular screening of *KCNQ1* (LQT1), *KCNH2* (LQT2), *SCN5A* (LQT3, BrS1) genes was performed using DHPLC and sequence analysis on genomic DNA extracted from frozen tissue. Each genetic variant identified was evaluated in ethnically-matched controls and reference databases. Novel variants were expressed in mammalian cells and studied using patch clamp technique.

Results

Four missense mutations, absent in >1,000 controls, were identified in 4 IUFD cases (4%). The genetic variants detected in KCNQ1 (-A283T,-R397W) and in the 1b splicing-isoform of KCNH2 (-R25W) were associated with markedly reduced I_{Ks} and I_{Kr} current respectively, consistent with in utero LQTS type 1 and 2. The mutation identified on SCN5A (-T220I), induced a significant reduction in the late I_{Na} current, consistent with a BrS phenotype.

Conclusions

This study represents the largest cohort of stillbirths in which a molecular autopsy was performed. The results of this study highlight that 4% of unexplained fetal demise may be due to life-threatening arrhythmias secondary to underlying cardiac channelopathies, as LQTS or BrS.

C16.5

Metabolic reprogramming of the epigenome by intrauterine exposure to gestational diabetes

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The offspring of women with gestational diabetes mellitus (GDM) are at greater risk of developing metabolic disorders later in life. Epigenetic processes such as DNA methylation are primary candidates when searching for mechanisms that can stably modulate gene expression and metabolic pathways due to fetal overnutrition. Umbilical cord blood and placenta tissue were obtained from 88 newborns of mothers with dietetically treated GDM, 98 with insulin-dependent GDM and 65 without GDM. Bisulfite pyrosequencing was used to study the methylation levels of seven imprinted genes involved in pre- and postnatal growth, four genes involved in energy metabolism, one anti-inflammatory gene, one tumor suppressor gene and one pluripotency gene. In addition, we examined global DNA methylation of the ALU, LINE1 and alpha-satellite repeat families. The maternally imprinted MEST gene and the non-imprinted glucocorticoid receptor NR3C1 gene as well as ALU repeats showed significantly decreased methylation levels in both GDM groups, compared with controls, in both analyzed tissues. Decreased blood MEST methylation was also observed in adults with severe obesity, compared with normal-weight controls. The fact that two of 14 analyzed genes and ALU repeats showed significant hypomethylation in newborns of GDM mothers suggests that the effects are minor (in the order of several percentage points) but widespread. Epigenetic changes in children exposed to a hypergylcemic intrauterine environment provide a molecular link between GDM and life-long predisposition to metabolic disorders. Overall our results support the idea that adverse early life conditions can have long-term effects on the epigenome and phenotypic consequences.

C16.6

SPOC1 is involved in meiotic sex chromosome inactivation (MSCI)

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Meiotic sex chromosome inactivation (MSCI) and meiotic silencing of unsynapsed chromatin (MSUC) are two barely understood mechanisms responsible for transcriptional silencing of unsynapsed chromatin during male meiosis. It has been shown that both mechanisms are essential for normal spermatogenesis and fertility. Recently, we identified SPOC1 (PHF13) as a novel gene whose expression is negatively correlated with the survival time in patients with ovarian cancer. SPOC1 associates dynamically with chromatin in cell lines (probably via H3K4me3 binding) and plays a role in chromosome condensation and cell division. We demonstrated that male Spoc1-/- mice show pronounced hypoplasia of the testis with progressive loss of germ cells due to apoptosis in the pachytene stage. Here, we report data of microarray-based gene expression analyses performed with testis tissue from Spoc-/- strains and wild type controls. We identified a chromosome-specific dysregulation of transcripts with a highly significant disproportionate number of X- and Y-linked genes overexpressed in Spoc1-/- tissue. The results were verified using qPCR. Together with the increased apoptosis rate observed during pachytene stage the microarray data strongly suggest an essential function of SPOC1 in meiotic sex chromosome inactivation (MSCI). The formation of the XY-body, the double strand break (DSB) formation/repair, as well as chromosome synapsis seems normal in the knockout testis, which suggests a direct epigenetic effect SPOC1 on X- and Y-linked genes. In conclusion, our data indicate that SPOC1 is a novel factor involved in MSCI, and is essential for the epigenetic control of male germ cell development.

C17.1

The MHC association to celiac disease can be mostly explained by six amino acids in the HLA-DQ heterodimer

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The major histocompatibility complex (MHC) containing the HLA genes has been associated to immune-related diseases. However, pinpointing the causal variants is challenging due to its characteristic genetic structure. To refine the association signal in HLA in celiac disease we genotyped >10,000 MHC SNPs in 12016 cases and 11920 controls using a custom genotyping array (Immunochip).

We imputed classical HLA-A, B, C, DQA1, DQB1, DRB1, DPA1 and DPB1 alleles and polymorphic amino acids in these genes using a European reference panel. Logistic regression and conditional analysis was used to identify independent associations.

The strongest association was to amino acid 55 in the DQβ1 protomer (x2=9528, df=3, -log(p)=2067), located near the binding groove. Adjusting for the effects of position 55, we found additional associations to positions 57, 203 and 71 in the DQβ1 subunit and to positions 72 and 22 in DQα1. The haplotypes formed by these six positions recapitulate the known risk-conferring effects of the classical DQ2.5 (OR= 10.49), DQ2.2 (OR= 2.02), and DQ8 (OR= 4.4) alleles. After controlling for these HLA-DQ effects, we found 6 other associated variants within HLA-A and HLA-B class I, and in DRB1, DPB1 and DPA1 in class II.

Our results provide a structural basis for the well-known HLA-DQ associations and confirm that six amino acids can explain the bulk of the observed MHC association.

Population	**Sample**	**Cases**	**Controls**	**Male(%)**	**Female(%)**	Lambda gc
Netherlands	2323	1150	1173	1035(44.6)	1288(55.4)	1
Italy	2756	1486	1270	722(26.2)	2034(72.8)	1
Poland	1062	521	541	505(47.5)	557(52.5)	1
Spain1	1793	1131	662	722(40.2)	1071(59.8)	1
Spain2	1793	1131	662	722(40.2)	1071(59.8)	1
UK	16002	7728	8274	6153(38.4)	9849(61.6)	1.08
Total	23936	12016	11920	9137(38.2)	14799(61.8)	1.04

C17.2

Haplotype phasing using next-generation sequencing reads

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Next-generation sequencing is now widely used in many studies of human disease and population genetics. Sequencing is still relatively expensive so many studies are collecting low-coverage data that necessitates the use of phasing and imputation methods to call genotypes and haplotypes at polymorphic sites. Sequencing reads carry valuable phase information when spanning two or more heterozygous sites. For example, in the Phase I 1000 Genomes Project data we have found ~33% of the heterozygous sites are covered by phase informative reads. This information is not used by current methods. We have extended SHAPEIT to incorporate the sequencing read information and boost performance. To compare methods and investigate parameters such as coverage, read length and insert size we have carried out an extensive simulation study of sequence read level data using the SFS_code. For example, with 5x paired-end 100bp reads (500bp insert size) using phase-informative reads increases the mean distance between phasing errors from 85.2kb to 94.0kb. The mean switch distance for BEAGLE was much lower at 63.5kb. By adjusting the distributions of read length and insert size performance can be substantially improved. These results indicate the likely benefit of future advances in sequencing technologies. On real data we have achieved similar performance but have found necessary to carefully account for poorly mapped reads and poor calibration of base qualities. Overall our results highlight the gains that can be achieved by using phase-informative reads when estimating haplotypes from next-generation sequence data.

C17.3

Bayesian multivariate phenotype modeling for genome-wide association studies.

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The majority of genome-wide association studies have been carried out using a single binary or single quantitative trait as the phenotype of interest. For many traits several potential phenotypes may be available so it is natural to ask the question of how best to test for association in the presence of multiple phenotypes. We have developed a Bayesian model averaging approach with sparsity inducing priors that allow the set of phenotypes to be partitioned into an associated and un-associated set. In this way we combine both model comparison and model selection. Additional properties are that we allow for correlations between the residuals of both the associated and un-associated phenotypes and allow for multiple cohorts to be analysed together. We have shown using simulated data that these methods lead to an increase in power to detect effects, over and above using single phenotype analysis, and are able to accurately uncover the true set of associated phenotypes. We will present results from a genome-wide association of eight hematological parameters collected on the TwinsUK, KORA and UKBS Common Control collection.

C17.4

Case-control maximum weighted bipartite matching in genome wide association studies

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Population stratification in samples of genome wide association studies give rise to large obliterations in the results of statistical tests. In order to correct for stratification effects we have implemented a pairwise case--control matching that is based on the identity-by-state matrix. We obtain a ‚maximum weighted bipartite matching' by making use of an improved Kuhn-Munkres „hungarian" algorithm which solves the assignment problem of weighted bypartite graphs in polynomial time.

A quality control on the matched pairs as well as a rematching of residual sample elements makes sure that we do not loose power due to reducing the sample. In this way, the pairwise matching is extended to tiny clusters with at least one case and one control. Association P-values are obtained by within cluster case-control permutation.

The matching can be performed both genome-wide and window-wise (‚localized matching'). The latter will be applied to the analyses of rare variants, where one would expect that the amount of stratification vary according to

genomic location.
As it turns out from simulation studies the statistical niveau is maintained: local window sizes of a few thousand SNPs are enough to guarantee identification of strata. Thus, our method leads to an increase of power and simultaneously to a reduced false-positive rate in simulations compared to unstratified analyses. As a byproduct, our implementation strongly outperforms common covariate approaches based on multidimensional scaling in runtime, and makes genome-wide application possible. Our method for stratified analyses is implemented in the genome-wide interaction analysis software INTERSNP.

C17.5

Variants in exons and in transcription factors affect gene expression in trans

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In recent years many genetic variants have been found to be associated with gene expression (eSNPs). However, unraveling the causal variants and the regulatory mechanisms by which they act is still limited. Here we present a computational framework to study *trans*-eSNPs, integrating SNPs that are fully ascertained from sequencing data along with publicly available RNA-seq for 50 Yoruban samples. We focus on pairs of eSNP located within a source gene: anywhere along the span of a Transcription Factor (TF) or within any known exon. We map the source and its *in trans* target onto a Protein-Protein Interaction (PPI) network and study their topological properties.
When considering pairs of exon-source with their corresponding eQTL targets, the more likely their association, the closer they are within the PPI network and the higher the rank of target. These effects are significantly different than chance expectation, as demonstrated by randomly permuted data. These results suggest a mechanism for *trans* regulation by coding variation, thereby pinpointing the causal SNPs.
In addition, the more likely is the association of a TF source eSNP to its target, the higher is its rank. These results pinpoint a causal SNP within a TF and suggest a global property of TF sources as hubs in the PPI, even more so than other TFs. The targets of eSNP TFs are enriched for proteins sorted to intracellular membrane bounded organelles.

C17.6

eQTL mapping in 5,300 blood samples reveals downstream pathways in non-hematological traits

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Genome-wide association studies (GWAS) have revealed many SNPs that are associated with complex traits. The next challenge is to identify the functional consequences of these variants. To systematically map these downstream effects, we used expression quantitative trait locus mapping and meta-analysis to identify cis-eQTLs (distance between SNP and mid-probe position < 5Mb) and trans-eQTLs in a cohort of 5,311 peripheral blood samples across seven independent cohorts. When confining our analysis to 4,500 SNPs that are known to be associated with complex traits and diseases, we detected over 1,000 SNPs (24%) with trans-eQTL effects (FDR 0.05) and over 600 SNPs (14%) with both cis and trans-eQTL effects on at least one gene.
Although we studied peripheral blood (?leukocytes) and identified many plausible downstream effects for hematological and immune-related traits, we also detected significant trans-eQTLs for other traits as well. For example, a SNP (rs174550) in FADS1_a gene known to affect lipid and glucose levels_showed significant association in trans with LDLR gene expression levels (p-value: 4.27*10-14), and we furthermore observed a significant correlation between LDLR gene expression and phospholipid levels. When relaxing the FDR to 0.5, we also observed trans effects on two additional genes involved in cholesterol metabolism (INSIG1 and TLE3).
GWAS meta-analyses have revealed a substantial amount of previously unknown biology. Our results indicate that a meta-analysis of eQTL datasets is equally promising, and can reveal downstream effects for genetic variants that are associated with a wide range of complex traits.

C18.1

Dominant missense mutations in potassium channel cause Cantú syndrome

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Cantú syndrome, also known as hypertrichotic osteochondrodysplasia, is characterized by congenital hypertrichosis, characteristic facial features and cardiac defects. Cantú syndrome is an autosomal dominant disorder with a thus far unknown genetic cause. We used trio-based exome sequencing to detect candidate de novo variants in a selected patient with Cantú syndrome and his healthy parents. We identified a de novo missense mutation in a subunit of a potassium channel. Subsequent sequencing of the candidate gene in additional cases was performed to confirm the initial findings. Sanger sequencing revealed heterozygous missense mutations in the same gene in 13 out of 15 cases. The mutations had occurred de novo in all 7 cases where genetic material from both parents was available. In one male Cantú patient, the mutation was inherited from an affected mother, providing further support for causality of this gene in Cantú syndrome. We built a molecular model to gain insight on the effect of the mutations on protein structure. All mutations affect residues in or close to the transmembrane part of the protein. Using patch clamp experiments we show that the mutations in the potassium channel reduce ATP-mediated inhibition of this channel. Our findings may have direct implications for the treatment of Cantú patients because the channel is a known pharmaceutical target.

C18.2

PNPLA1 mutations cause autosomal recessive congenital ichthyosis in golden retriever dogs and humans

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Ichthyoses comprise a heterogeneous group of genodermatoses characterized by abnormal desquamation over the whole body, for which the genetic causes of several human forms remain unknown. We used the spontaneous dog model to perform a genome-wide association study in the golden retriever breed, which is affected by a lamellar ichthyosis resembling human autosomal recessive congenital ichthyoses (ARCI). We identified a new gene, PNPLA1, in which a homozygous indel mutation leads to a premature stop codon in all affected golden retriever dogs. We then found one missense and one nonsense mutation in the catalytic domain of the orthologous human gene in six ARCI patients from two families. Further experiments highlighted the importance of PNPLA1 in the formation of the epidermal lipid barrier. In addition to the identification of a novel gene in human ichthyoses, these results provide insights into the localisation and the function of this yet uncharacterized member of the PNPLA protein family.

C18.3

Mutations in FKBP14 cause a variant of Ehlers-Danlos syndrome with progressive kyphoscoliosis, myopathy and hearing loss

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We report on an autosomal recessive variant of Ehlers-Danlos syndrome (EDS) characterized by severe muscle hypotonia at birth, progressive scoliosis, joint hypermobility, hyperelastic skin, myopathy, sensorineural hearing impairment and normal pyridinoline excretion in urine. Clinically the disorder shares many features with the kyphoscoliotic type of EDS (EDS VIA) and Ullrich congenital muscular dystrophy. Linkage analysis in a large Tyrolean kindred identified a homozygous frameshift mutation in FKBP14 in two affected individuals. Based on the cardinal clinical characteristics of the disorder four additional individuals originating from different European countries were identified who carried either homozygous or compound heterozygous mutations in FKBP14. FKBP14 belongs to the family of FK506-binding peptidyl-prolyl cis-trans isomerases (PPIases). ER-resident FKBPs have been suggested to act as folding catalysts by accelerating cis-trans isomerization of peptidyl-prolyl bonds and to act occasionally also as chaperones. We demonstrate that FKBP14 is localized in the endoplasmic reticulum (ER) and that deficiency of FKBP14 leads to enlarged ER cisterns in dermal fibroblasts in vivo. Furthermore, indirect immunofluorescence of FKBP14 deficient fibroblasts indicated an altered assembly of the extracellular matrix in vitro. These findings suggest that a disturbance of protein folding in the ER affecting one or more components of the extracellular matrix might cause the generalized connective tissue involvement in this disorder. FKBP14 mutation analysis should be considered in all individuals with apparent kyphoscoliotic type of EDS and normal urinary pyridinoline excretion, in particular in conjunction with sensorineural hearing impairment.

C18.4

Homozygosity mapping and candidate prioritization identify mutations, missed by whole-exome sequencing, in SMOC2, causing major dental developmental defects

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Inherited dental malformations constitute a clinically and genetically heterogeneous group of disorders. Here, we report on a severe unique dental phenotype that results in dentin dysplasia associated with major microdontia in the primary dentition, oligodontia in the permanent dentition, teeth shape/size abnormalities and thin enamel in a highly consanguineous family. Classical homozygosity mapping (GeneChip Human 250K SNP Affymetrix) revealed a unique zone on 6q27-ter containing 70 genes. The two affected children were found to carry a homozygous mutation at the exon 1 /intron border (c.84+1G>T) in the canonical-splice donor site of intron 1 of SMOC2 gene coding for the SPARC related modular calcium binding 2 matrix protein. The parents of both affected children were heterozygous for this mutation. The SMOC family is well conserved through evolution. Smoc2 gene is indeed expressed throughout mouse odontogenesis. Knockdown of smoc2 in zebrafish showed pharyngeal teeth that had abnormalities reminiscent of the human phenotype. Moreover, smoc2 depletion in zebrafish affected also the expression of three major odontogenesis genes: dlx2, bmp2, and pitx2.
The ultimate proof through whole exome sequencing failed to reveal the mutation because of insufficient coverage of the GC rich region containing the disease causative mutation !

C18.5

Mutations in ROGDI cause epileptic encephalopathy and amelogenesis imperfecta (Kohlschütter-Tönz syndrome)

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Kohlschütter-Tönz syndrome (KTS) is an autosomal recessive disease characterized by the combination of epilepsy, psychomotor regression and enamel defects. The molecular basis has not yet been elucidated. Here we report that KTS is caused by mutations in ROGDI. Using a combination of autozygosity mapping and exome sequencing we identified a homozygous frameshift deletion c.229_230del (p.Leu77Alafs*64) in ROGDI in two affected individuals from a consanguineous family. Molecular studies in two additional individuals with KTS from two unrelated Austrian and Swiss families revealed homozygosity for a nonsense-mutation c.286C>T (p.Gln96*), and compound heterozygosity for the splice site mutations c.531+5G>C and c.532-2A>T in ROGDI, respectively. The latter mutation was also found heterozygous in the mother of the Swiss affected individual in whom KTS was reported for the first time in 1974. ROGDI is highly expressed throughout the brain and other organs but its function is largely unknown. Possible interactions with DISC1, a protein involved in diverse cytoskeletal functions, have been suggested. Our finding that ROGDI mutations cause KTS indicates that the protein product of this gene plays an important role in neuronal development as well as amelogenesis.

C18.6

Mutations in GRIP1 cause Fraser syndrome

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Fraser syndrome (FS) is an autosomal recessive malformation syndrome characterized by cryptophthalmos, syndactyly and urogenital defects. Thus far, mutations in *FRAS1* and *FREM2* have been identified as a cause of FS. Both *FRAS1* and *FREM2* encode extracellular matrix proteins that are essential for the adhesion between epidermal basement membrane and the underlying dermal connective tissues during embryonic development. Mutations in murine *Grip1*, which encodes a scaffolding protein that interacts with Fras1/Frem proteins, result in FS-like defects in mice. We therefore tested *GRIP1* for genetic variants in FS families that did not have mutations in *FRAS1* and *FREM2*. In three unrelated families *GRIP1* mutations were found to segregate with the disease in an autosomal recessive manner (donor splice site mutation NM_021150.3:c.2113+1G>C in two families and a 4-bp deletion, NM_021150.3:c.1181_1184del in the third). RT-PCR analysis of the *GRIP1* mRNA showed that the c.2113+1G>C splice mutation causes skipping of exon 17 leading to a frame shift and a premature stop of translation.

The FS phenotype of the three probands presented here appears to be indistinguishable from the phenotype that results from mutations in *FRAS1* or *FREM2*. This is in line with the assumption that Fras1, Frem2 and Grip1 are indispensable for the integrity of the Fras1/Frem protein complex, and that lack of one of the components leads to a defective complex.

We conclude that mutations in *GRIP1* cause classic FS in humans. Our findings expand the possibilities for diagnostic testing, carrier testing and early prenatal diagnosis for FS patients and their families.

ESHG Posters

P01. 01. Genetic counseling, including Psychosocial aspects, Genetics education, Genetic services, and Public policy

P01.01

Access to assessment of familial cancer risk by people from minority ethnic communities

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Background: Patients from minority ethnic groups diagnosed with, or are at risk of, cancer with a genetic component appear less likely to access screening and other services to assess their higher level of risk than the mainstream population in the UK.

Aim: To explore why people from minority ethnic groups with a significant family history of cancer are under-represented in NHS clinical genetics services to inform interventions and service development to improve quality of care, and review and dissemination of findings at stakeholder and community levels.

Methods: Qualitative study using semi-structured interviews and focus groups with: patients with direct experience of familial cancer risk assessment; and community members from Black Caribbean, South Asian, and White Irish communities with, or at familial risk of breast and ovarian, bowel, and prostate cancer; and clinical genetics and other key NHS staff. Transcripts were analysed using constant comparison of data and processes for validation and feedback with respondents.

Results: Data were generated with a purposeful sample of 58 respondents (15 patients, 20 community members, 23 health and other professionals). Some findings appeared common to all patients, but were amplified for people from minority ethnic communities, for example in relation to the challenges of sharing information and decision-making within families about cancer and genetic risk. Factors further preventing people being empowered to negotiate health services effectively, obtain appropriate referral or further assessment included: language barriers and cultural sensitivities relating to stigma; accessibility of family medical histories; and a non-directive emphasis in genetic counselling.

P01.02

Applying the Estonian Biobank to estimate the potential impact of genomic testing

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The Estonian Biobank is a population-based biobank of the Estonian Genome Center of the University of Tartu with genotypic, phenotypic, and health information for over 51,000 participants aged 18 years and over. The age, sex, and geographical distribution of the cohort reflects the structure of the Estonian adult population. Besides promoting the development of genetic research, collecting health and genetic information from the Estonian population, the genome center aims to use the collected biobank data and results of associated genetic research to improve public health. The health information has been collected by medical personnel with access to the electronic health records. The health data are expanded periodically through linking to national registries as well as through follow-up.

By having the genotypic and longitudinally collected health data for 5% of the Estonian adult population it is possible to estimate the impact of the genomic information on public health. We attempt to present what portion of the population would benefit from a specific genomic test for an actionable disease by studying the population allele frequencies of the markers associated with the disease and the impact of these markers in the context of the predictive value. This analysis can be done for a large number of diseases. We present it for the type 2 diabetes, hypertension, lactose intolerance, glaucoma, age related macular degeneration to demonstrate how a biobank with a comprehensive database can be applied to estimate the impact of genomic information. This approach is expected to be superior to the simulation studies.

P01.03

Reasons to participate to a biobank study: a systematic review

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The implementation of cohort biobank studies is intrinsically dependent on the successful recruitment of participants. Studies have shown that the decision to enroll is only partly influenced by the information provided during recruitment and mainly relies on individual attitudes and motivations. Cost-benefit analyses (which include the potential benefit of receiving information about one's health) also play a significant role in the decision-making process. Whether the (potential) participant' perception of benefit corresponds to what the researchers communicate in the informed consent procedure is a question that has received increased attention in the last years. Empirical studies addressing individuals' motivations to enroll have been implemented in different biobank settings. For the scope of this review, we focus on the motivations expressed by apparently healthy and legally autonomous participants who actually took part to a biobank study.

To this end, three literature databases (PudMed, Embase and Web of Science) as well as Google Scholar have been searched with standardized keywords. To guarantee its systematic approach, the search had been done twice using the same strings by two different teams of authors. 157 articles considered relevant have then been read independently by four of the authors to decide on their inclusion in the review. The selected articles have been analyzed and their content has been coded and organized by themes independently by two of the authors. The outcome of this work is to provide a comprehensive overview of the reasons to participate to biobank studies as they appear in the literature up to now.

P01.04

Respect of guidelines for breast and ovary surveillance and management in women with a BRCA mutation: a french study

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Pilot follow-up studies in people with a genetic predisposition for cancers, were created in France in 2009. In this context, a specific database was set up for the follow-up of these patients in two French regions. Observance of surveillance recommendations for breast and ovary cancer in patients with a BRCA mutation was studied using the collected data.

The patients were at least thirty years old and had known their genetic status for at least 2 years. Concerning the breast, poor observance was defined as an interval of more than 18 months between breast follow-up examinations. Concerning ovary cancer, poor observance was defined as not having ovariectomy after the age of 50 years. The 2 centers followed 248 women with a BRCA mutation: 232 from 30 to 50 years, and 116 over 50 years.

Prophylactic mastectomy was performed in 11/232 women (5%); nine had already had cancer, and two were asymptomatic. Breast follow-up data were obtained for 101/232 women; 62% of patients followed the guidelines closely and 84% had a follow-up MRI. Ovary follow-up data were available for 73/116 women: only 6/73 of the women over 50 (8%) did not undergo adnexectomy.

In conclusion, the vast majority of women with a BRCA mutation followed the recommendations concerning management of risk of ovarian cancer. 1/3 of patients had insufficiently regular breast surveillance. These results show the importance of setting up pilot studies to improve the management of people with a genetic predisposition for cancer. Data collection is continuing.

P01.05

What do counselees know about (hereditary) breast cancer? - And which role can an Interactive Personal Health Record (IPHR) have in this?

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In order to enhance the realistic expectations and participation during genetic counseling we developed an IPHR.

To study the effects of the IPHR we developed a study which will be conducted in four phases. In the first phase of this study we studied how informed counselees, referred for breast cancer (BC), are prior to their first consult at

the Cancer Genetic Clinic.
A total of 49 counselees completed a questionnaire on topics about knowledge, risk perception (RP), Perceived Personal Control (PPC), and anxiety for BC (Cancer Worry Scale).
The general risk for BC is overestimated by 63% of all counselees (mean 27.8%; SD 21.2), and the chance on having a BRCA1/2 mutation by 84% of the counselees with no mutation in the family (mean 44.2%; SD26.1). The counselees also show a low baseline knowledge level (BKL)(46%). Analysis between different groups of counselees showed that the low educated counselees (LEC)have a poorer RP, and BKL than high educated counselees (HEC). 81% of the LEC overestimated the general risk for BC, and the LEC have a significant lower score on knowledge (38%) than HEC (62%)(p<0.001).
The counselees have no low scores on the PPC-questionnaire (mean score 10.5) and were not anxious (mean score 30).
Counselees, especially the low educated, have a poor RP and BKL prior to their first genetic consult. We expect that the implementation of the IPHR will have a positive effect on RP and BKL, which results in a more active role of the counselee.

P01.06

Group Genetic Counseling for Cardiomyopathy patients is well accepted

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Background: Group Genetic Counseling (GGC) may have benefits for counselees and counselors (more information, psychosocial support, increased efficiency), although its use has not yet been reported in cardiogenetics. We therefore set up GGC sessions for cardiomyopathy index patients.
Aim: To see whether the quality of care provided by GGC is acceptable to counselees.
Methods: GGC was offered in regional hospitals with few referrals of cardiomyopathy patients to our academic center in the past years. Sessions were led by two counselors: a clinical genetics expert and a group leader. Patients completed questionnaires before and after counseling, measuring sociodemographics, Personal Perceived Control (PPC), State-Trait-Anxiety-Inventory (STAI), Clinical Genetics Satisfaction Indicator, etc.
Results: 53 patients and 36 relatives attended a course of eight GGC sessions. PPC scores (range 0-2) increased in 81% of patients, mean item score (SD): before 0.92 (0.54), after 1.29 (0.38). STAI scores (range 1-4) decreased in 51% of patients, mean item score (SD): before 1.89 (0.58), after 1.68 (0.49). Median score of the Clinical Genetics Satisfaction Indicator (range 1-5) was 4.93.
Conclusion: This is the first report of GGC being used for cardiomyopathy patients. On average, personal perceived control increased and anxiety was lowered. Mean changes in PPC and STAI were comparable to reports for group and individual counseling in oncogenetics. Satisfaction scores were high, patients reported their questions were answered during the sessions and they received no undesired information. Our study indicates GGC is well accepted. We will next investigate whether GGC is more efficient than individual counseling.

P01.07

Progress of the Clinical Utility Gene Card initiative

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As of January 2011, the Clinical Utility Gene Card (CUGC) project receives support by EuroGentest 2 and the European Society of Human Genetics. Based on this support the CUGC initiative can be continued, including the publication, in spring 2012, of the first set of guidelines having undergone updating.
CUGCs are disease-specific guidelines authored by international expert groups. Based on the ACCE framework they are dealing with the risks and benefits of the application of genetic tests in the clinical setting. Each document represents a balanced summary of the analytical and clinical validity, the clinical utility and cost-benefit issues. CUGCs offer quick guidance to all stakeholders, including clinicians, clinical geneticists, referrers, service providers and payers. Each CUGC is peer-reviewed and published by the European Journal of Human Genetics. CUGCs are also freely available on the websites of EuroGentest, the European Society of Human Genetics and Orphanet.
The feedback from the scientific community and the CUGC download rates are promising: the European Journal of Human Genetics counted between 600 and 1,500 downloads, with an average above 1,000, per gene card and year. From 2011 to 2013, 300 new CUGCs are planned to be established. To ensure that all published documents reflect the state-of-the-art, all published CUGCs are annually revised.

P01.08

Developing a genetics-genomics education framework for midwives: a consensus approach using individual/family stories

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Competence frameworks in genetics exist for UK health professionals and include a combined framework for nurses, midwives and health-visitors. As part of a review of this framework, the development of a set of competences specifically for the midwifery profession was seen as essential. A national consensus meeting was held involving midwives in practice and management, educators, policy makers and lay representation (n=18). Electronic voting was used to capture opinions anonymously and stimulate discussion.
All but one attendee agreed that “good midwifery care is currently compromised by midwives’ level of genetic competence”. Individual/family and professional stories illustrating a range of experiences and conditions were reviewed and the content mapped to the original framework. Attendees looked for topics missing from the framework and considered whether any of the existing statements should be re-focused. Eight themes [ongoing-care, advocacy, multi-professional team, listening, timeliness, client knowledge, broad knowledge and key indicators (of disease)] were identified and, following discussion, voted on. All themes were to be included within existing statements.
Seven statements now set out the minimum level of competence that should be required of all midwives in the UK at the point of registration. With learning outcomes aligned to the stages of pre-registration training, and practice indicators, this framework will provide guidance to educators, practitioners and managers. The importance of genomics within healthcare is explicit and anticipating that the implementation of new knowledge and technology will impact the midwifery role, the team have endeavoured to ‘future-proof’ the framework.

P01.09

Attitudes of health care professionals towards carrier screening for Cystic Fibrosis. A review of the literature

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Recently, commercial companies have started to offer preconceptional carrier tests directly to consumers. This increasing commercial offer creates the background which makes reflection necessary about the desirability to offer population carrier screening in the healthcare system. A positive attitude of potential providers is vital to the success of a screening program.
Therefore, a literature review of the attitudes of healthcare professionals, focused on the attitudes towards carrier screening for Cystic Fibrosis (CF), was performed.
The databases Pubmed, Web of Science, as well as the interface Google Scholar, were searched for the period 1990-2011. Studies were selected if they were published in a peer reviewed journal in English and described the attitudes of potential providers toward carrier screening. Eleven studies were retrieved describing the attitudes toward carrier screening for CF. Studies reported attitudes toward the best time for screening, the best setting to offer screening, the willingness to be involved in a screening program and the concerns about offering screening. Ten papers described a general attitude toward carrier screening.
We can conclude that health care providers are willing to be involved in a carrier screening program, but there is need for appropriate education as well as adequate support. The prospect of an increasing number of genetic disorders for which screening becomes possible and the potential increasing demand for such screening in the future calls the need for further debate on the desirability of carrier screening, and relevant questions such as the conditions screened, the providers involved, the information provision and counseling.

P01.10

The views of CF patient’s parents on genetic testing

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Mandatory newborn screening for CF is spent in Russia since 2006. To estimate patient's parents opinion concerning genetic testing we have spent questioning 93 CF patient's parents.Majority of parents (90 %) have learnt about hereditary character of their child disease from the pediatrician, only 64 % of them have been referred to geneticist, and 49 % have been held DNA testing. However 90% respondents have consider, that they have understood the information about repeated genetic risk for CF. However only 51% of them could correctly specify the value of recurrence risk of CF, and only 37 % of them could correctly attribute a risk category. Majority of respondents (82%) have consider that prenatal testing is very useful procedure and 65% of them wanted to use it. Only 1.1 % of respondents have answered that they didn't want to terminate the CF foetus pregnancy. Acceptance of prenatal testing was correlated with the age and educational level of woman, whether or not she was referred for genetic counselling, and whether or not she has received an explanation about repeated risk.

P01.11

Diagnostic and counselling dilemmas in newborn screening for cystic fibrosis

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Introduction: Newborn screening (NBS) for cystic fibrosis (CF) has been implemented as a nationwide immunoreactive trypsinogen (IRT)/DNA/IRT scheme in the Czech Republic since X/2009. DNA testing is associated with inherent drawbacks such as detection of infants with atypical mutations resulting in variable phenotypes.

Objectives: We evaluated a clinical status in individuals (from NBS and non-NBS group) carrying either R117H or D1152H allele in trans with another CF-causing mutation and utilized these data for CF-NBS and genetic counselling.

Methods: The Czech CF registry and an "in house" clinical-genetic database were used.

Results: Of 13 individuals with CF-causing mutations/R117H on a 7T background, 6 symptomatic adults (1 suffering from respiratory symptoms (RS), 1 from pancreatitis, 4 with azoospermia) and 1 child with RS were reported before implementation of CF-NBS. 1 adult with unknown clinical status was identified due to cascade screening. 5 infants were identified in CF-NBS without having CF symptoms. There has been no case with CF-causing mutation/R117H on a 5T background yet.

Of 5 individuals with F508del/D1152H genotype, 2 children and 2 adults suffer from RS and 1 adult from pancreatitis and azoospermia. These cases were reported before implementation of CF-NBS and none from CF-NBS, so far.

Conclusions: Although phenotype in individuals with CF-causing mutation/R117H-7T genotype is usually mild, we follow consensus guidelines and monitor infants on a long-term basis. Albeit limited knowledge exists on phenotypes associated with D1152H, this mutation is considered to be CF-causing mutation and long-term follow up in a CF specialist is essential.

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P01.12

Genetics and its Implications for Clinical Dental Practice and Education, Report of Panel 3 of the Macy Study: Are Dental Students Prepared?

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Objective: In the Report of Panel 3 of the Macy Study, funded by the NIH and the American Dental Education Association "Knowledge, Skills, and Attitudes Required for Oral Health Professionals to Care for Patients with Genetic Conditions" were outlined. The aim of this study is to investigate the genetic knowledge, skills and attitudes of West Virginia University School of Dentistry's (WVU SOD) students utilizing this report as the source for the specific questions.

Methods: All dental students (195) were invited to participate by answering 16, primarily Likert style questions (1= Strongly Agree to 5 =Strongly Disagree). Questions included Knowledge: of genetic transmission; molecular biology of the human genome; principals of population genetics; Skills: to perform a head/neck exam with special attention to signs of major genetic disorders; to recognize when to refer a patient for genetic screening, testing, and counseling; Attitude: to understand the potential for genetics to contribute to the development of new approaches to prevention, diagnosis and treatment.

Results: 89 (45.6%) filled out questionnaires. When it came to Knowledge of transmission, biology of the human genome, principals of population genetics and Skills to perform a head/neck exam and when to refer, 54.2%, 39.7%, 82.4% and 69.1% disagreed respectively. Attitude, however, revealed that 71.0% agreed they understood the potential for genetics to contribute to new approaches of disease.

Conclusion: Although baseline knowledge and skills of the WVU SOD students were lacking, the students recognized this new technology could potentially contribute to new approaches in prevention, diagnosis and treatment.

P01.13

Direct to consumer testing - a review of the available evidence

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As part of the EuroGentest2 project, we are developing European guidelines on direct to consumer testing for potential consumers and health professionals. We conducted a series of systematic reviews focussed on consumers (17 papers), health professionals (four papers) and current recommendations and position statements (11 documents) to inform this process. Findings indicate a low level of awareness of direct-to-consumer genomic testing in both users and some health professionals. Consumers appeared motivated to purchase tests to obtain information to guide their own health management while some wanted to avoid disclosure of genetic risk to health professionals. Potential users expressed concerns about privacy, reliability of genomic tests and the nature of results. Many consumers preferred to discuss the test with a health professional before and after testing, while health professionals expressed concerns for the consumer such as misinterpretation of results and increased anxiety due to perceived risks. In the review of policies, more potential harms than benefits were cited. An area of concern was the overstatement of the actual predictive power or utility of the results. Strong recommendations were made about the need to involve health professionals and to regulate test quality. We conclude that there is public interest in direct-to-consumer genomic tests. However, while consumer autonomy may dictate freedom of choice in undergoing such tests, health services need to ensure that potential benefits are maximised and harms prevented. Further research into the impact of testing and the views of stakeholders is required to implement appropriate regulation, guidelines and education.

P01.14

"It's our DNA, we deserve the right to test!" A qualitative analysis of a petition for the right to access direct-to-consumer genetic testing without the intermediate of a health care professional

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As a relatively new model of genetic test provision, the offer of direct-to-consumer (DTC) genetic testing (GT) has fueled a number of scientific, ethical and policy debates. Proponents of DTC GT claim that the benefits of such services include, among others, increased access for consumers, increased genomics education, and added support for consumer autonomy. A few studies have been published regarding the public's view of these services but none have specifically addressed the public's desire for "unrestricted" access to their genomic information. The goal of this study is to increase our understanding of the public's views of accessing GT without the intermediate of a health care professional. We have conducted an exploratory analysis of comments written by individuals who have signed a public online petition initiated by DIYgenomics.org to support "personal access to genetic information" (http://www.thepetitionsite.com/1/mydna/). Of the 522 individuals who have signed the petition to date, 240 individuals have also written comments; these were the focus of our content analysis. Preliminary results reveal that the main themes raised by the petitioners include: i) the notion that each individual "owns" their DNA and ii) that individuals have the right to access information about their DNA; iii) the notion that regulation of DTC GT falls outside of the realm of the government's control; and iv) the belief that involving health care professionals as intermediates provides no added value for consumers. These results contribute to understanding the public's view of DTC GT services and lay the foundation for further research.

P01.15

Genomics and the prevention of antisocial behavior: A comparative ethical analysis

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Current research in the genomics (and neurobiology) of antisocial behavior (ASB) trigger great hopes and expectation concerning the development of new forms of early detection of children at risk and of targeted early prevention. Children as young as preschoolers, toddlers and babies are considered the most important target-group of such efforts. While scientific research progresses continuously, it is of great importance to pro-actively consider the social and ethical implications of potential applications. This presentation reflects on this development from an ethical point of view.

It investigates whether and when it may be justified to expect that early detection and prevention is mainly for the good of those identified and intervened upon, what are relevant caveats and dangers and how both are to be balanced. Issues that will be discussed encompass increased support, empowerment and emancipation as well as dangers of stigmatization and labeling, surveillance and repression, privacy concerns, and possible negative impacts on children's development and (self)perception.

However, unlike much other ELSI research, this presentation wants to avoid any kind of gene-exceptionalism. That is, it will argue that from an ethical point of view it is of secondary interest whether future prevention practices are informed by either new genomical or traditional social/psychological findings. Instead, the ethical evaluation should focus on the characteristics, specificities and conditions of use of any measure applied. To this end, the proposed ethical analysis of early prevention practices will be conducted in a comparative way.

P01.16

Poster for ESHG: Comprehensive embryo screening. Results from two focus group studies

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Genetic testing of embryos is done in two contexts. preimplantation genetic diagnosis (PGD) is done when one or both of the prospective parents are known carriers of a genetic disease, be it a Mendelian condition or a chromosomal translocation. Preimplantation genetic screening (PGS) is the screening of embryos from infertile or subfertile couples undergoing IVF to select the embryo which is most likely to lead to a successful pregnancy. The technique of PGS is not standard offered to couples, because it is still uncertain whether it really leads to improving implantation rates. However, with the introduction of microarray technology, and single cell whole genome sequencing, embryos could also be screened for Mendelian disorders, susceptibility genes and potentially non-medical traits. Hence, the aim of PGS may shift from choosing the best embryo for transfer in order to ensure a successful pregnancy, to choosing the embryo most likely to develop into a healthy child, to even selecting the 'best child'. In order to understand the ethical questions arising from the introduction of comprehensive screening techniques in clinical practice, we have conducted two focus group studies. One study, which was performed in October 2011, explored the opinions of top scientists in the field of embryo testing regarding the technical possibilities and associated ethical questions. The other study, performed in March 2012, was conducted with gynecologists and genetic counselors and explored opinions about possible dilemmas and their solutions in IVF practice. This poster presents the major findings and conclusions of the two studies.

P01.17

Predictive genetic testing for Familial Adenomatous Polyposis (FAP) in young children

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Objective. Predictive genetic testing for familial adenomatous polyposis (FAP) is routinely offered to children at-risk from the age of 10 years onwards. Because of absence of medical benefits, potential psychosocial harm and respect to the child's autonomy, predictive testing for FAP at younger age is reluctant. As a result, there is a lack of experience in predictive testing of children at the young age.

Patients and methods. We evaluated 13 children from 8 families, tested for an *APC* mutation at the age younger then 10 years (the male to female ratio was 1.6:1; mean age was 6,4 years (2-9 years); 7 APC-carriers and 6 non-carriers). All parents were re-contacted and structural interviewed.

Results. None of the contacted parents regretted the timing of genetic testing. Ten children were tested at the same moment with an older sibling. The main reasons for testing were 1) testing all children in a family at the same moment (4:13); 2) a possibility to prepare a child for future surveillance (3:13); 3) certainty about the future (3:13). According to the parents none of the children showed changes of mental and physical health after testing.

Conclusion. Genetic testing for FAP at a young age is desired by some parents and is experienced as causing no harm. However, the effects of early genetic testing on children and their own experience should be evaluated in future studies.

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P01.18

Factors affecting the utilization of genetic counseling services among Arab Israeli women

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High rate of consanguineous marriages, underutilization of prenatal diagnosis services and low rate of pregnancy termination of an affected fetus are the main risk factors leading to high prevalence of infant morbidity and mortality in the Israeli Arab community. The purpose of genetic counseling services is to allow the pregnant couple having informed decisions about their pregnancy, by discussion of various diagnostic tests and preventive measures, expected to decrease congenital morbidity rate. The aim of the study was to identify the factors affecting the utilization of genetic counseling services in the „Triangle region" of North Israel, among Arab pregnant women who were referred by their doctors for genetic counseling. In multivariate analysis, identified factors affecting women's utilization of genetic counseling service were level of income, access to service, abortions in the past, attitudes towards genetic counseling and the level of religiosity. Easier access of genetic counseling services, abortions in the past and woman's positive attitudes toward genetic counseling were proved to be significant predictors to utilizing genetic counseling services. On other hand, low level income and the more religious families were the main factors associated with none utilizing genetic counseling services. We recommend developing and strengthening wide-scale community-based genetic counseling service for the Arab population, preferably operated by professionals who are Arab speakers, with the background support and encouragement of religious leaders.

P01.19

Genetic counseling role in historicist middle east cultures

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Genetic counseling is a process of communication and education that considering expression and transference of genetic disorders. Achieving to this communication is depended to knowledge about culture and traditions of society and psychological situation of consultant. This article is an inspection about social reactions against genetic counseling as a new branch of medical science in historic and historicist Middle East societies and their own cultures.

Public belief to "paternal big family" and "God willing destiny" are two major challenges of genetic counseling in Middle East. In case of paternal big family Middle East people especially Arab tribes of Persian Gulf region believe that familial marriage especially children of two brothers makes stable and honorable family. For example familial marriage rate in Saudi Arabia and Kuwait are 12 times more than Europe. In the other hand belief to "God willing destiny" that is based on some Quran sentences made kind of religious historicism. In this ideology called "Taghdir", no person can predict about illness or healthy of newborn child because it is part of God desigens and theology.

Now Middle East geneticists are facing with public distrust about genetic counseling; also increasing rate of recessive genetic disorders patients and decreasing the gene pool diversity. In the absence of state programs for public education, "perceived personal control" has a completely different definition from European societies. It seems so that spiritual influence of pioneer Muslim clerics and their fatwa (religious decree) could help to improve people's trust to genetic counseling.

P01.20

A comparison study of the practices of genetic counsellors between France and Canada

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Genetic counsellors are Health Professionals with specialized training and experience in the areas of clinical genetics and counselling. They did work as members of a multi-disciplinary healthcare team that provides genetic services. In France, the profession of genetic counsellor is relatively recent (2005). This profession has been created due to the increasing number of genetic consultations but also face to the decrease of medical professional in this field. About Canada, the profession has been created, for the same reason, since 1985 by a genetic counsellor graduated in the United-states and by the auspices of geneticists who exercise their profession in Canada. Members of different groups (French and Canadian) have received an electronic survey based on their background, the role and the practice of the profession of genetic counsellors in their own country. The questionnaire was sent to the Association of Genetic Counsellors which transmitted it to all the members. Data were collected during the year 2011. We are looking to see if there are major differences in the practice of exercising the profession, but also in the education and in the collaboration established between genetic counsellors and medical geneticist.

P01.21

Genetic education in Brno, Mendel 190

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The Department of Medical Genetics at the University Hospital Brno (www.fnbrno.cz) has besides its core activities (comprising genetic counseling, molecular diagnostics, pre-graduate teaching of medical genetics at the Masaryk and Mendel Universities in Brno) devoted itself to the popularization of medical genetics to the lay public(http://www.mendelmuseum.muni.cz/cz/lekarska-genetika/).

Since 2009 successful series of public lectures, conducted in association with our partner institutions, on various subjects of medical genetics are held at the premises of the St Augustine‘s Abbey - Mendel Museum, i.e. at the place where in between 1843 -1884 Gregor Johann Mendel spent most of his professional live.

Lecture series „Medical Genetics for the Public“ has been linked to the GenEthics project lectures within the Mendel‘s Refectory that are carried out since 2006 (http://www.mendel-museum.com/eng/7lectures/lectures-ref6.htm). Within these series we have covered various domains of genetic counseling, diagnostic methods in medical genetics, significance of genetic diseases and our collaboration with patient organizations. This year‘s series is dedicated to rare diseases and development of the CZ National Plan ec.europa.eu/health/rare_diseases/national_plans/detailed/index_en.htm. Since 2010, this lecture cycle also takes place under the auspices of the Czech Parliament (MP- J. Husák) and the Olga Havel Foundation (www.vdv.cz; Ms. M. Cerná).

In 2012 the Mendel Museum and the Masaryk University will celebrate the 190th Johann Gregor Mendel´s birthday anniversary (July 20) through an event known as “Mendel 190”, within which planned lecture series will be dedicated to his tribute. Associated events will be organized in Prague and at Mendel´s birthplace (www.vrazne.cz). Supported by CZ.2.16/3.1.00/24022 to MM.

P01.22

Creating an agenda for effective genetic educational strategies: Needs assessment and prioritization in primary care

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Purpose

General practitioners (GPs) are increasingly expected to deliver genetics services in daily patient care. Education in primary care genetics is considered suboptimal and in urgent need of revision and innovation.

Aims of our study: exploring the role of genetics in primary care and the need for genetics education and prioritization of GPs' genetics education.

Methods

Three types of focus groups were held (n=44): mono-disciplinary groups of GPs and midwives, respectively and multidisciplinary groups composed of a diverse set of experts. Recurrent themes were identified after verbatim transcription and content analysis.

Consecutively, a Delphi consensus procedure was conducted. A purposively selected heterogeneous panel (n=18) of experts participated. Educational needs regarding genetics in general practice in terms of knowledge, skills and attitudes, were rated and ranked in a Top 10.

Results

Four themes emerged from the focus group study: (1) genetics knowledge, (2) family history, (3) ethical dilemmas and psychosocial effects in relation to genetics and (4) insight into the organisation and role of clinical genetics services. These themes reflected a shift in the role of genetics in primary care with implications for education.

The entire Delphi study panel completed all three rounds. Kendall's coefficient of concordance indicated significant agreement regarding the top ten genetic educational needs ($P<0.001$). “Recognising signals potentially indicative of a hereditary component of a disease” was rated highest.

Conclusion

Results help to develop effective genetics educational strategies (including input for case-based education). Enhancement of primary care providers' competences in genetic patient care could actually become possible.

P01.23

Developing best practice guidelines for provision of clinical genetic service - Examples of testing for monogenic subtypes

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Introduction: In 2010 the Council of Europe described that “The development of genetics in health care services has a major impact on the organisation of health care, leading to shifting from curative to preventive services, from in-patients to out-patients treatment, from specialised genetic services to genetics as an integral part of general health services.” The responsibilities of expert geneticists, both in laboratory and clinic, will change. Because of this shifting, new guidance on genetic service provision is urgently needed in many countries.

Methods: In Workpackage 8 within Eurogentest2 an expert meeting, questionnaire and online discussion forum were used to develop recommendations which describe the optimal practice and interaction between different parties involved, including:

- (genetic) patients and their families, the users of the services;
- medical professionals such as primary care workers and other non-genetic specialists;
- genetic professionals from clinical and laboratory background.

Results of this ongoing project include a “Temple of genetic services” depicting interactions between different stakeholders in genetic services. Also experiences with testing for monogenic subtypes have been discussed as best practices in an expert meeting. By describing practices of service provision from oncogenetics, cardiogenetics and MODY we hope to show that different fields ask for different approaches and have their own opportunities and threats when it comes to good genetic service provision.

Implications: The tools that will be developed within this project could help health care stakeholders in different countries to improve genetic services for their citizens.

P01.24

An overview of the genetic testing offer in Europe: trends and forecast

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Genetic testing services are now offered internationally, through both the public and private sectors. Physicians prescribing these tests and biologists receiving samples need to know which tests are available, where they are performed and whether identified laboratories meet quality standards. To fulfill this need, www.orpha.net was launched fifteen years ago to set up a database of medical laboratories in the field of rare diseases. Data was collected in 1 country in 1997, 15 in 2003, 26 in 2006 and 36 in 2011, with resources from the EC DG Public Health. In collaboration with the EuroGentest Network of Excellence, information on quality management has been added over the past six years. Information on genetic testing in Orphanet can be searched by disease name or by gene as well as by laboratory or by professional. The website can be freely queried and the complete dataset downloaded from www.orphadata.org. In September 2011, 1,056 laboratories offering tests for 1,811 genes were registered. The test offer differed greatly from one large country to another: from 1,449 genes (Germany) to 541 genes (UK). In medium and small-sized countries, it ranged from 1 to 355 genes. A comparison with the information available at Genetests for the USA highlights the need for a worldwide coordination of cross-border healthcare, as 584 genes are only available for testing in the EU member states and not in the USA. The capacity to access genetic testing on an international scale, however, both increases the availability of testing and raises significant policy issues.

P01.25

Alignment and Assessment Problems in the Undergraduate Genetics Curriculum: A View from the United States

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'Backward design' is a model of curriculum development that relies on identifying learning outcomes and defining what constitutes evidence of learning before planning the teaching. Although backward design is widely considered best practice, it is often overlooked by university faculty and may help explain the inconsistency between faculty teaching behavior and the genetics concepts they claim are most important. This paper will review the state of genetics instruction in the United States from the perspective of backward design, with particular attention to the goals and assessments that inform curricular practice. An analysis of syllabi and leading textbooks indicates that genetics instruction focuses most strongly on the structure and function of DNA and Mendelian genetics. At the same time, a survey of faculty indicates that other concepts, such as the application of genetics to society or the environment, are viewed as equally or even more important than certain foundational concepts. This disconnect suggests a need for more explicit goal setting prior to curriculum development. Preliminary analysis of existing assessments, specifically concept inventories developed for higher education, indicates that assessments are poorly aligned with faculty goals for instruction and need to be modified into more valid and reliable measures of student conceptions.

P01.26

Considerations in the review of ethical guidelines pertaining to human genome research in Japan

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Ethical guidelines for human genome research in Japan were formulated in 2001, by three government ministries: the Ministry of Education, Culture, Sports, Science and Technology; the Ministry of Health, Labour and Welfare; and the Ministry of Economy, Trade and Industry.

The present (2012) review has taken into consideration advances in genome research such as the implementation of studies involving large amounts of genomic information, diversification of the study design such as genome cohort studies, and the advent of next-generation sequencing technologies.

The main aspects reviewed are the use of existing samples, method of collection and distribution of resources, the drafting of informed consent so that it will also be applicable to future genome research, and the disclosure of genetic information.

With respect to the use of existing samples, institutions that do not possess a correspondence table can handle samples anonymized in a linkable fashion in the same way that they handle those anonymized in a non-linkable fashion. In terms of collection and sale of samples, requirements and administrative procedures have been revised to enable the more effective use of existing samples and other resources. Regarding the disclosure of genetic information, the revisions adhere to the basic principle of disclosure with respect to the Personal Information Protection Law, but some disclosure-related issues remain unresolved.

New regulations have also been established for safety management measures related to the handling of genetic information, compliance rules for the outsourcing of genetic research, and education and training for researchers and members of ethical review boards.

P01.27

One disorder is not like another - the importance of taking a disorder-centred approach to introducing high-throughput sequencing into the clinic

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Now that next generation sequencing has become a reality, it is time to examine how this technology will change the way medicine is practiced in the clinic. A particular concern is that the rapid penetration of systematic technologies into genetic medical departments blurs established frontiers between research and clinics. The benefits and risks of using the technology as part of the diagnostic toolkit for each disorder need to be considered in relation to the characteristics of the patients themselves as well as the societal context in which the tests will be offered. It is vital to consider each patient group in their own right - the different motivations for testing and expectations for the process, and the meaning of results in terms of screening, management, prevention and knowledge. During the course of the European Techgene project, which aimed to develop clinically applicable high throughput sequencing tests, a series of 7 semi-structured interviews was performed with clinicians and researchers dealing with one of four clinical areas: mental retardation, breast cancer, sensory disorders and neurodegenerative disorders. Through these interviews areas of particular and specific concern for each disorder, were highlighted. Proposals were then developed to allow an approach to the introduction of NGS technologies into the clinic in a manner which is tailored to the categories of disorder, to ensure that we are properly prepared to meet the challenges of the new technology and harness its potential for the greatest benefit for patients.

P01.28

A proposal: a family-driven social network model for clinical data sharing and research in intellectual deficiencies and other neurodevelopmental disorders with specific genetic causes

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CGH-array has led to a rapidly growing number of genetic diagnoses of intellectual deficiency (ID) associated or not to autism. High-throughput sequencing will further accelerate the detection of mutations in ID-related genes. This will be useful for genetic counseling (when penetrance is known to be high). But the extraordinary genetic heterogeneity of ID will render extremely difficult the determination, for each specific cause (recurrent CNV or mutated gene), of genotype-phenotype correlations and natural history, the estimation of penetrance and expressivity variation, and the organization of clinical trials, except for the most frequent causes (see recent work on 16p11.2 del or dup). Symptomatic treatments will be proposed, with little chance to evaluate whether their efficacy depends on the specific genetic cause. It will be difficult to motivate busy MDs to establish and maintain the wide-ranging databases required for such studies. We propose genetic ID databases organized in a social network model, whereby clinical information would be entered mostly by the patient's family. 23andMe or PatientsLikeMe have recently shown that such data can lead to useful research. Contacts between families affected by the same genetic cause could be established in an initially anonymous way as for Relative finder in 23andMe, creating gene- or CNV-specific micro-networks to which interested professionals could be associated, akin to disease-specific patients associations. Anonymized data could be accessible to professionals for specific projects approved by a comity composed of health or research professionals and of family representatives. Concerned families could then decide whether to partipate in such projects.

P01.29

The legal landscape of stratified medicine

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This paper examines the extent to which there is a 'lack of fit' between the existing framework of European legislation (and the devolved national legislation which rests thereon), and emerging applications of genomic knowledge in stratified health care. For example, the advent of stratified medicines has enabled the co-development of therapeutic products and 'companion diagnostics' which are used together to prospectively target individuals who through genetic factors, are at risk of disease. This has the potential to increase drug efficacy and safety whilst ensuring greater cost effectiveness. Current legal and regulatory frameworks do not provide a clear route for developing these linked applications and the safeguards, protections and incentives for developing packages of diagnosis, treatment and care sometimes lack coherence.

Other emerging technologies, such as the diagnostic algorithms that support the process of stratification, may not be within the remit of existing legislative frameworks at all, or may be protected by non-patent intellectual property rights, such as copyright. In other respects, the regulatory framework is predicated upon outdated assumptions, such that products and devices can always be easily differentiated, or that the processes in bringing a product, device or test to market will take place entirely within Europe, when increasingly this is a global exercise.

In combination, these factors have the potential to negatively impact upon clinical translation and national healthcare economics. This paper offers an analysis of the existing regulatory gaps and inconsistencies within Europe in the context of stratified medicine, and suggests some proposals for reform.

P01.30

Development of the Hellenic Neuromuscular Disorders (HNDR) Registry

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Over the recent years, a growing number of patient databases are being created to accommodate patient data for various diseases. Registries acting as a hub for information and social awareness, can prove very beneficial to the patients registered and society in general, especially since they help disease organizations with government lobbying. They also provide valuable information to scientists, helping them to perform research on a bigger scale, having access to accumulated medical and genetic data. For neuromuscular disorders in particular, patient registries have played a significant role facilitating clinical trials designed to test for new therapeutic strategies and thus have promoted research regarding those types of diseases. This project aims to create and coordinate the first national neuromuscular disorders registry in Greece. The main concept behind the HNDR is to organize a reliable electronic database, containing clinical and personal data of all patients in order to provide important data for the study and research of neuromuscular disorders in Greece. Ultimately, the goal is to connect the Hellenic registry to the global network of patient registries that is universally being developed ensuring that patients who register in the HNDR can be retrieved if their profile fits to a clinical trial.

P01.31

Incidental findings in genetic testing: current laboratory reporting procedures

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Incidental findings from genetic testing are defined as those that have potential health or reproductive importance and are discovered in the course of testing but beyond the aims of the initial test. Since genetic testing was initiated there has been a potential for incidental findings, but numbers are likely to increase with the emergence of array and sequencing techniques. A genetic incidental finding is unlikely to require immediate treatment; most often it will affect the relative risk of a patient developing a disease. This raises questions as to which genetic incidental findings should be returned to the patient, at what time and by whom. These issues are passionately debated in the ethical literature, however there is a paucity of empirical data on which to base recommendations. The aim of this study was to determine current practice and ascertain the views of many different stakeholders as to future management of incidental findings. In the first phase a systematic review indicated a dearth of empirical evidence on dealing with incidental findings. In Phase 2 we surveyed staff of national health service genetics laboratories in the United Kingdom to determine how incidental findings were reported. Our initial findings indicate a lack of consensus; no universally accepted set of guidelines were used and decisions were made on a case-by-case basis. This individualised approach may interfere with equity of patient care. Findings will be used to shape the qualitative collection phase of the study and generate guidelines concerning the reporting of genetic incidental findings.

P01.32

Ethical implications on disseminating complex genetic information to relatives at risk

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Background: Genetic counselling focuses on providing patients with qualified professional assistance in understanding and responding to their inherited risk. Genetic information is both individual but at the same time highly familial. Decisions regarding how and by whom relatives at risk for the disorder should be informed are included in the counselling.

Aim: To examine how aspects of respect for autonomy are addressed by genetic counsellors when patients have relatives deemed to be at risk.

Method: Data from empirical observations of genetic counselling, insight in medical charts, and discussion with health care personnel regarding information strategies for relatives was gathered and analysed through a structured, philosophical-theoretical approach.

Results: When relatives are informed by a tested patient, the outcome of their experience and perception of the information is influenced by *how* they are informed.

Counselees are affected by their own diagnosis and may be challenged in disseminating complex genetic information correctly to relatives.

Problems particularly arise when lack of communication in families is present and when relatives are in conflict.

Health care professionals are reluctant to contact relatives at risk directly, to respect their right to not know.

Discussion: Existing procedures for informing relatives at risk may be inadequate. Complexities of genetic information, combined with tested patients' emotional reactions, reduce relatives' access to relevant information, necessary for making autonomous and rational choices about their future.

A more proactive role for health care professionals seem warranted, towards increasing accurate information and knowledge of options, enabling patient and relatives to make informed, autonomous decisions.

P01.33

The challenge of education in birth defects: managementof orofacial clefts

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Cleft lip and/or palate (CL+/-P) has an incidence of 1/650-1000 live births. It is often accompanied by comorbidities, needing multi professional assessment. It was investigated the knowledge on this matter in a random sample of 292 students attending the last degree of Medicine, Nursery, Speech Therapy and Odontology courses in a Brazilian University. It was used a pre validated auto applicable questionnaire on information of both anatomy and physiology of the motor oral apparatus, either of carriers and non-carriers of CL+/-P, existing feeding resources, indication of particular feeding methods, and skills to give genetics orientations. The questionnaire form applied to the respondents was retrieved immediately it was filled in with his/her answers. The results were treated by descriptive and analytical statistical methods, adopting the 5% significant level. As a whole, there were no significant differences among the students from the different courses. Student's auto evaluation achieved 58.6% of them referring sufficient notion on anatomic alterations of CL+/-P, as well as 51.0% on functional ones. It was observed the lack of systematization on the knowledge of the various topics herein investigated, leading 96.2% of the respondents to not consider them capable, in their particular health field, to deal with affected individuals. Also, 48.3% referred knowledge on CL+/-P etiology, though only 8.6% and 9.9% were able to give genetics orientation for families of carriers of CL+/-P and CP, respectively. A specific multi professional discipline, which fits each particular course, would be an alternative to increase academic capacitation of health professionals.

P01.34

The psychological impact of pancreatic cancer surveillance in high-risk individuals

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Background: The success of pancreatic cancer (PC) surveillance depends to a large extent on the commitment of participants to adhere to the repeated follow-up investigations. We aimed to investigate possible changes in distress in high-risk individuals participating in a PC surveillance program.
Methods: Questionnaires were sent before and after PC-screening tests to high-risk individuals participating in a multicenter nationwide endoscopic ultrasonography (EUS)-MRI-based PC-surveillance study. Distress was assessed with the Cancer Worry Scale, and the Hospital Anxiety and Depression scale.
Results: Forty-seven individuals (87%) completed both pre- and post-surveillance questionnaires (38% male, mean age= 52 yr., range 20-74 yrs.); 44 participated in the PC screening and 3 declined. The expected burden of EUS was significantly higher than the actual experienced burden (p<.001). The number of participants with clinical levels of anxiety and/ or depression was low (n=5) and remained stable over time. The possibility of developing cancer themselves (29% at both time points) and the chance that relatives would develop cancer (19% and 21%, respectively) were the most frequently reported worries. The 3 decliners indicated that they were not fearful of the MRI or EUS, and had low levels of distress.
Conclusion: The results of this prospective study indicate that: (1) the expected burden of EUS is higher than the actual experienced burden; and that (2) mean levels of distress are not significantly influenced by participating in the PC screening program. This finding is of great importance for this high-risk group that might benefit from participation in a life-long repeated PC-surveillance-program.

P01.35

Focusing on patient needs and preferences may improve genetic counseling for colorectal cancer

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During genetic counseling, different items which counselors consider important are discussed. However, little is known about the needs and preferences of counselees. Therefore, counselees with a personal and/or family history of colorectal cancer (CRC), who were referred for genetic counseling regarding CRC, received a slightly adjusted version of the QUOTE-GENE^{ca} questionnaire prior to their first visit at the Hereditary Cancer Clinic. Response rate was 60% (48/80 counselees). Participants rated the importance of 45 items discussed during counseling. Participants considered the items regarding information about their familial CRC risk (100%) and preventive options (98%) important or very important. Fewer participants considered the items of general information on genetics as important. No major differences were seen between participants in relation to individual characteristics. Our data suggest that focusing on familial CRC risk and surveillance options may lead to better satisfaction with genetic counseling.

P01.37

E-learning in Romania - a chance for improving information about rare diseases

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Introduction - Based on new scientific data up to 6% of total EU population are affected by rare diseases. In Romania, **more than 95% of patients do not have a complete diagnosis or do not receive adequate care and treatment.** All statistics showed that patients are open to use electronic resources in a same way that specialists do. **Aim -** This paper focuse on web solution that helps rare disease patients to learn about their disease and doctors to know how can they get assistance. Material - Our e-university www.edubolirare.ro want to provide information on rare diseases and care of patients suffering from these diseases and for all the interested doctors. The training method used is **blended learning** - a modern concept implemented in the University of Tromso - Norway by the combination of classical face to face with modern Internet-based learning. Results - The project platform offers a wide range of short courses and informations: Legal regulations on patients rights, E-Learning courses, and scientific information about diseases. This virtual platform give quality training programs, presented in an attractive, encouraging continued teaching progress, a lifelong learning, skills needed for diagnosis and management of rare diseases. Conclusions - **Most of rare diseases not currently benefit from specific treatment,** and for other several hundred **there only exist measures** allowing patients to improve quality of life. Access to information about diagnosis and disease management are crucial in rare diseases field and web-based solution can be effective.

P02. Clinical genetics and Dysmorphology

P02.001

Seven items flowchart (7-iF) for the clinical indication to GCK genetic test

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We propose a simple diagnostic flowchart aiming to easily identify, among a diabetic pediatric population, patients with the highest probability to carry a pathological mutation in the GCK gene. Heterozygous individuals for these mutations are affected by a monogenic diabetes, characterized by a moderate increase in fasting glucose and HbA1c levels with, usually, no micro- or macro-vascular complications and no need of pharmacological intervention. The molecular diagnosis provides a perceptible impact on both patient's quality of life (no need of treatment) and health care costs (less frequent follow up visits, no stick for glyceamic controls or drugs to provide).
The proposed 7-item flowchart (7-iF) takes into account the most recent criteria for the etiological diagnosis of diabetes, including the autoimmune pancreatic antibodies, the HbA1c and the familiarity. We validated this approach in one of the largest Italian pediatric diabetes outiclinic cohort.
Of 921 patients, 21 (2.3 %) received positive indication to GCK testing according to the 7-iF. Seventeen underwent to genetic testing and 13 (76 %) carried a pathological mutations 5 were novel mutations. The flowchart had a specificity of 99% and a sensibility of 92%, basing on the estimated frequency of GCK-MODY2 in Italian diabetic patients.
The proposed flowchart successfully identified diabetic patients with high risk to carry a pathological GCK mutation. The flowchart is extremely handily and can be implemented in any clinical setting. The flowchart is implemented in a webtool http://www.geneticamedica.unina.it/diabsun/

P02.002

Mutiple congenital anomalies in a male fetus with a 5.6 MB deletion of 10q25.1-q25.3

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We report on a 34-week-old male fetus with multiple congenital anomalies including hydrocephaly, hypoplastic olfactory bulbs, agenesis of diaphragm, coarctation of aorta, lung hypoplasia, hydronephrosis, hypospadias, short lower limbs, webbing of knees and deep gap between first and second toes. The facial features were dysmorphic with: prominent forehead, hypertelorism, low set and posterior rotated ear, dysplastic ears small mandible and broad based nose. Radiological findings were: markedly thin ribs, flat acetabular roof of pelvic, no talus or calcaneus ossification, hypoplastic pubic ossification, asymmetric femoral size and narrow thorax. Parents are first cousins with history of two spontaneous abortions. Cytogenetic analysis, performed by array-CGH revealed a 5.6 Mb region deletion of chromosome 10q25.1-q25.3. Chromosomal studies of both parents were normal confirming the abnormality to be of de novo origin.
To the best of our knowledge this phenotype with major anomalies which had lead to intrauterine fetal demise (IUFD) has not been reported before with this deletion. The unique presentation of our case notably, agenesis of diaphragm is a very rare finding and not reported previously in 10q deletion cases. It should be taken into consideration that most cases with agenesis of diaphragm had normal chromosomes based on karytoype, and it is possible that minor chromosomal abnormalities were missed with routine cytogenetic techniques.

P02.003

Delineation of a novel, recognizable microdeletion syndrome on 13q12.3 in three unrelated patients with intellectual disability

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Proximal deletions of the long arm of chromosome 13 have been reported only rarely. Here we present three unrelated patients with intellectual disability carrying novel heterozygous interstitial deletions encompassing 13q12.3. The proximal and distal breakpoints are similar and the deletions span about 1.4 Mb, comprising at least 11 RefSeq genes.

The patients present with moderate intellectual disability, secondary microcephaly and short stature as the leading symptoms. In addition, they experienced pronounced feeding difficulties in early infancy and later on eczema/atopic dermatitis. They display strikingly similar facial features such as fullness of the periorbital region, a flat malar region, a characteristic nose with a bulbous nasal tip and hypoplastic alae nasi, a smooth philtrum and thin upper lip.

Heterozygous deletions of 13q12.3 overlapping about 1 Mb of the distal part of the deletions in our patients have been described in healthy carrier parents of patients with Peters-Plus syndrome (an autosomal recessive disorder caused by inactivation of the *B3GALTL* gene). We therefore propose that the critical region of the 13q12.3 microdeletion syndrome contains only three genes, namely, *KATNAL1*, *LINC00426* and *HMGB1*. So far, little is known about the function of the *KATNAL1* and *LINC00426* genes in humans. HMGB1, however, is an evolutionarily conserved chromatin-associated protein which has been implicated in various disease processes.

In summary, we suggest that microdeletion 13q12.3 represents a clinically recognizable condition characterized by intellectual disability, microcephaly, short stature, a disposition for atopy and characteristic facial features. The critical region encompasses about 300kb with 3 genes assigned to it.

P02.004

Proximal and distal 15q25.2 microdeletions - genotype-phenotype delineation and confirmation of two neurodevelopmental susceptibility loci

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Cooper and coworkers have recently reported distal 15q25.2 microdeletions as a potential novel CNV locus for neurodevelopmental and neuropsychiatric disorders with variable outcome. Previously, more proximal microdeletions of 15q25.2 have been described by Wat et al. as a susceptibility locus for cognitive deficits, congenital diaphragmatic hernia, and Diamond-Blackfan anaemia (DBA).

We present two new 15q25.2 deletion patients and compare them to the 18 patients reported in the literature. Our patient 1 with a deletion overlapping both, the distal and proximal 15q25.2 deletions, presented with mild learning deficits, portal vein thrombosis, iron deficiency anaemia, short stature, and Noonan syndrome aspect. DBA is thought to be caused by reduced copy numbers of RPS17, which is normally present in four copies (two on each allele) in the proximal 15q25.2 region. We demonstrate a 50% reduction in copy number of RPS17 in our patient 1 by quantitative real-time PCR. Loss of two copies of RPS17 might be responsible for at least some of our patients features. As the clinical spectrum of DBA includes individuals without DBA who have other DBA-associated congenital anomalies, patients with proximal 15q25.2 deletions should be monitored for development of anaemia and DBA-associated malignancies.

Patient 2 with the more distal 15q25.2 deletion presented with severe psychomotor retardation, microcephaly and epileptiform signs. He carries two additional microdeletions, the 1q21.1 recurrent microdeletion and a hemizygous deletion on the X-chromosome encompassing OPHN1.

We contribute to the genotype-phenotype delineation for 15q25.2 microdeletions and further expand the characterization of these two novel microdeletion syndromes.

P02.005

Microdeletion in chromosome band 20p13 associated with moderate developmental delay and minimal facial dysmorphism

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The subtelomere screening studies in the patients presenting with mental disability and developmental delay has let to only few reports of cases harboring pure subtelomeric terminal deletions in the 20p13 region. Clinical findings reported for these cases suggest that the phenotypic consequences of this chromosomal imbalance are very variable, in many cases however, the clinical data are rather limited. Hence, to establish the genotype-phenotype correlation of 20pter monosomy is still a challenge.

We report a 9-year-old girl with subtelomeric 20p microdeletion. She was referred to the genetic counseling because of learning difficulties. During the further evaluation: short stature, hypoplastic fingernails, submucous cleft palate with cleft uvula, flat foot, tendency to infections and large fontanelle after birth were noted. No specific facial dysmorphic features were observed. The diagnosis of deletion of 20p13 was established by MLPA, and delineated by arrayCGH, as 46,XX.arr20p13(1-1150000)x1 dn with the size of 1.15 Mb.

Our report is the second, giving the detailed molecular and clinical characteristics of pure, smallest deletion 20p13. In this presentation we have delineated the phenotype of this aberration, providing possible candidate genes and giving data supporting genotype-phenotype correlation.

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P02.006

The clinical overlap between 22q11.2 deletion syndrome and CHARGE syndrome: they are more alike than often anticipated

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CHARGE syndrome is a highly variable congenital malformation syndrome that shows considerable clinical overlap with other syndromes. The most striking co-occurrence of clinical features is seen with the 22q11.2 deletion syndrome, including congenital heart defects, cleft palate, ear abnormalities, hearing loss, growth deficiency, developmental delay, renal abnormalities, hypocalcaemia and immune deficiency.

We further explored the clinical similarities between the two syndromes by studying patients clinically diagnosed with CHARGE syndrome, but who were shown to carry a 22q11.2 deletion (n=6). Subsequently we analysed the 22q11.2 deletion features in our large patient cohort of *CHD7* mutation carriers (n=834). In 29 patients (3.5%) typical 22q11.2 deletion features, like hypocalcaemia, thymus anomalies and severe immunological problems, were mentioned. This prompted us to study *CHD7* in 20 patients clinically diagnosed as 22q11.2 deletion syndrome but without deletion or mutation of *TBX1*. We found truncating *CHD7* mutations in five of these patients.

Our results demonstrate that the clinical diagnosis of these two highly variable syndromes is challenging. They should therefore be included in a common differential diagnosis and we strongly recommend *CHD7* analysis in patients with a 22q11.2 deletion phenotype without a deletion or mutation of *TBX1*. Conversely, a genome-wide array for 22q11.2 deletions in clinical CHARGE patients without a *CHD7* mutation is recommended. The results also show that there is strong clinical evidence that the molecular pathways of *CHD7* and *TBX1* are linked.

P02.007

The co-occurrence of abnormal movement and 22q11.2 Deletion Syndrome: more than a coincidence?

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We report five patients who present co-occurrence of abnormal movement and 22q11 Deletion Syndrome (22q11 DS).

Three of them (mean aged 35 years) were addressed to neurologic department for extra pyramidal features leading to diagnosis of early onset Parkinson Disease (PD) with good responsiveness to L-Dopa therapy and dopaminergic denervation on DAT Scan.

The fourth patient presented myoclonic tremor.

Facial appearance and learning difficulties led to diagnosis of 22q11 DS. For the fifth patient, 22q11 Deletion Syndrome was established for several years and myoclonic tremor appeared during evolution.

To date, three cases of extra pyramidal features without relation with neuroleptic therapy, one Tourette syndrome and one myoclonic tremor have been reported in 22q11 DS.

Is it more than a coincidence?

Pathophysiology and signaling pathways involved are unknown. Among the commonly deleted region, which spans over 40 genes, COMT (catechol-O-methyltransferase) polymorphism could represent a good candidate based upon its pharmacological involvement in L-Dopa pathway (sequencing is in progress).

Parkinson disease or other abnormal movement may be occasional features of 22q11 DS.

Geneticists as well as neurologists and physicians caring patients with 22q11 DS should be aware of this possible association.

Genetic testing may be relevant in cases of early onset PD and preexisting learning and behavioral problems and/or facial dysmorphism.

Pathogenesis and etiology remain to be explained.

P02.008

De novo microdeletion 2p14-p15 in a boy with developmental delay, facial dysmorphisms and sensorineural hearing loss with dysplasia of the inner ear

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During the past years interstitial microdeletions of various segments of the short arm of chromosome 2 were recognized as a cause of mental retardation. However, most of these deletions have no recurrent breakpoints which hampers precise genotype phenotype correlation. In this situation thorough clinical characterization of patients with overlapping deletions and comprehensive database search for gene centred information may give insight into the contribution of single genes to the clinical phenotype.

Recently, Wohlleber et al. (2011) described two patients with small interstitial microdeletions 2p14-15 who presented with mild mental retardation and dysmorphisms. We report on a third patient with a de novo 2p14-15 microdeletion. The boy presented at the age of 22 months with muscular hypotonia, developmental delay, absent speech development and facial dysmorphisms (high forehead, sparse eyebrows, short palpebral fissures, hypertelorism, thin vermillion of the upper lip, deep set ears). Measurements (height, length, OFC) were in the lower normal range. He had bilateral deafness with dysplasia of the semicircular canals and was supplied with cochlear implants. Molecular karyotyping (HumanCytoSNP-12 array, Illumina, Ca) revealed a de novo 2.9 Mb microdeletion 2p14-p15 encompassing 12 genes. This deletion overlaps with those previously described, but, in addition, affects the homeobox gene *MEIS1*. The latter has recently been shown to be strongly expressed in the semicircular canals of the developing inner ear in chicken and therefore is a good candidate for dysplasia of the inner ear in our patient. This case contributes to further delineate the 2p14-p15 microdeletion phenotype.

P02.009

Molecular cytogenetic characterization of a family with 3p deletion and 3p duplication cases

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3p deletion syndrome is a rare disorder involving developmental delay and dysmorphic physical features. Most cases are de novo. Here we report a family with 3p-deletion syndrome and a rare 3p-duplication case. Two brothers with intellectual disability (ID) presented fully similar features of 3p-deletion syndrome, including developmental delay, muscular hypotonia, epichantal folds, flat and long philtrum, micrognathia, dolychocephaly, microcephaly, and hypertelorism. From family histories of non-consanguineous parents, a paternal aunt showed ID, delayed speech, and obesity. Karyotype analyses showed the deletion of 3p not only in affected sibs but also in their healthy father. Characterization of the rearrangement extent was performed by InfiniumHD whole-genome genotyping assay (HumanOmniExpress BeadChip arrays). The analysis of brothers showed identical 3p25.3 terminal hemizygous deletion of 10.9 MB. The deletion encompasses 49 RefSeq genes, including proposed 1,5 Mb minimal terminal deletion with causative CRBN and CNTN4 genes, and two others proposed as major candidates for ID: CHL1 mapped at 3p26.3 distally and SRGAP3 mapped at 3p25.3 proximally to minimal terminal region. FISH mapping using whole chromosome paint, MCB and subtelomere probes detected balanced translocation 46,XY,t(3;8)(p25.3;p23.3) in father and a partial 3p25.3 terminal trisomy in the paternal aunt. The duplicated region contains GHRL and PPARG genes which contribute to obesity and behavioral problems presented in the aunt. Despite of controversial results related to candidate regions for 3p-deletion syndrome, this is the biggest deleted region among reported few familial cases, encompassing all candidate genes responsible for ID with apparent clinical consequence of 3p-deletion. Supported by FP7-CHERISH-223692, DFG-LI 820/38-1.

P02.010

A familial case of developmental delay/intellectual disability, variable psychiatric disorders and optic atrophy due to a novel 1.5 Mb deletion on 3q29

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We report on a family segregating a 3q29 deletion centromeric to the classic "Chromosome 3q29 deletion syndrome" (OMIM #609424). The proband, a 4 yr. old girl, presented with severe global developmental delay and autism. Family history was positive for psychiatric/ophthalmologic disorders.

Array-CGH analysis (Agilent, 44K) revealed a 1.5 Mb deletion on chromosome 3q29 (194,529,547-195,888,674 bp, hg18). The deletion, confirmed by a real-time PCR assay, was inherited from the mother, affected by mild depression, and was also present in the maternal uncle (anxiety/depression), two maternal aunts (schizophrenia), the maternal grandmother (microcephaly, depression and visual deficit), and her brother (schizoaffective disorder).

The deletion encompasses 14 genes, including the OPA1 gene, whose haploinsufficiency causes autosomal dominant optic atrophy type 1 (OMIM#165500). Complete ophthalmologic evaluation performed in three deleted subjects of the family (mother, uncle, grandmother) confirmed the presence of a variable degree of optic atrophy. Among the deleted genes, HES1, which encodes for a basic helix-loop-helix transcription factor, is essential for neurogenesis. This gene has been suggested to have a role in the determination of autistic spectrum disorders and could be related with the psychiatric diseases observed in the family.

In conclusion, we detected a novel 3q29 deletion associated with optic atrophy and variable neuropsychiatric manifestations, ranging from mild depression to schizophrenia.

P02.011

EMX2 haploinsufficiency and ambiguous genitalia in a retarded boy

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We report a 28 month-old boy, born to unrelated parents of French ancestry, presenting at birth with 46, XY disorder of sex differentiation (DSD): posterior hypospadias, micropenis (1.2 cm) bifid scrotum, atrophic testes(one undescended), a left hypoplastic ectopic kidney on ultrasound, and no useful mullerian duct residue on genitoendoscopy. Hormonal data supported a diagnosis of testicular dysgenesis: low antimullerian hormone at day 2, undetectable basal testosterone at minipuberty, slightly stimulated by HCG (1,35 nmol/L), and normal gonadotrophins. No significant alteration of *SF1, WT1, Sox9* and *MAMLD1* sequences was found, nor rearrangement of *SRY* or *Dax1*. As the decision was taken to rear him as a male, surgery was limited to Onlay uretroplasty. Testis biopsy showed atrophic testicular tissue with widely spaced seminiferous tubules and rare spermatogoniae. When referred at 28m for developmental delay, no language, bruxism and clumsiness were observed, alongside with a small head (OFC -3SD). Array-CGH (Agilent 180 K) then indicated a 3.85 Mb 10q25.3-q26.12 *de novo* microdeletion encompassing 28 genes, including *EMX2* and *BAG3*. We postulate that *EMX2* haploinsufficiency is responsible for the masculinization defect observed in our patient, similar to what has been described in the mouse by Chung in 1998 and Miller in 2009. There are only a few descriptions of 10q25.3 microdeletion and DSD in medical literature, all but one before the array-CGH era. Our patient represents thus the second case. We recommend considering *EMX2* haploinsufficiency in case of 46, XY DSD with testicular dysgenesis left without a definite molecular diagnosis.

P02.012

5q31 Microdeletions: Definition of a Critical Region and Analysis of *LRRTM2*, a Candidate Gene for Intellectual Disability

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Microdeletions in 5q31 have been reported in only few patients to date. Apart from intellectual disability / developmental delay (ID/DD) of varying degrees, which is common to all reported patients, the clinical spectrum is very wide and includes short stature, failure to thrive, congenital heart defects, encephalopathies and dysmorphic features.

Here, we report a male patient with a 0.9-Mb *de novo* deletion of 5q31.2, the smallest microdeletion of 5q31 reported thus far. His clinical presentation includes mild DD, borderline short stature, postnatal microcephaly and mild dysmorphic signs including microretrognathia. In conjunction with data of seven reported overlapping microdeletions, analysis of our patient enables the tentative delineation of a phenotype map for 5q31 deletions. In contrast to the mild phenotype of small microdeletions affecting 5q31.2 only, carriers of larger microdeletions affecting subbands 5q31.1 and / or 5q31.3 are more severely affected with congenital malformations, growth anomalies and severe encephalopathies.

A 0.24-Mb smallest region of overlap (SRO) in 5q31.2 is delineated which contains only two genes. We propose *LRRTM2* as the most promising candidate gene for ID/DD in this SRO due to its expression pattern, its function as a key regulator of excitatory development and its interaction with Neurexin 1. However, mutational analysis of *LRRTM2* in 330 patients with ID/DD revealed no sequence alterations, excluding intragenic mutations in *LRRTM2* as a frequent cause of ID/DD in patients without microdeletions.

P02.013

A second case of 7p22.1 microduplication: clinical and molecular characterization

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The use of high-resolution microarray technology for investigation of patients with intellectual disability and/or congenital anomalies provided the possibility to identify new microdeletion/ microduplication syndromes and discover the dosage sensitive genes, which are implicated in the manifestation of various genetic conditions. Microduplication of the 7p22.1 region, 1.7 Mb in size, has very recently been reported, representing the smallest interstitional 7p duplication, associated with specific facial features and speech delay. We report on a new case of even smaller 7p22.1 *de novo* microduplication, 1Mb in size in position 5337072:6316915 (NCBI build 36), detected in a 14.5 years of age patient with mild intellectual disability and similar facial dysmorphism, including macrocephaly, ocular hypertelorism, low set ears and other features. To our knowledge, this is the second report of 7p22.1 microduplication, characterized by array CGH, however more than 60 cases of 7p duplications detected by routine karyotyping and differing in their size and position have been reported to date. Many of them share developmental delay, typical craniofacial and skeletal abnormalities as common clinical features. We suggest that 7p22.1 might be a critical region, representing a novel clinically recognizable microduplication syndrome caused by overexpression of dosage sensitive genes within this chromosomal alteration. There are 15 RefSeq genes included in this duplication. *ACTB* gene is a strong candidate gene for the disturbance of craniofacial development. Further cases with similar duplications will contribute to the delineation of a potential new microduplication syndrome of 7p22.1.

P02.014

Phenotypic Evaluation of 8q11.1-q11.23 Deletion In a Mental Retardation Patient

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We report on a 17 years old male patient with mental retardation, speaking disability and dysmorphic features. He has hyperextansibility, deep set eyes, upslunting palpebral fissures, prominent dysplastic ears, short philtrum, prognatism, narrow and highly arched palate, broad thumb, short and thick hand fingers and macrotestis. Family members of the patient was evaluated genetically, and we found that his mother has 3 spontaneous abortus occured in third trimester. Molecular karyotyping made using array CGH method with CytoSure Syndrome Plus(v2)4x44K microchips and scanned with Agilent Microarray Scanner. Obtained data were analyzed using Cytosure Analysis Software,v.2.0.8. and revealed a heterozygous 7.183 Mb deletion of 8(q11.1-q11.23) region containing PRKDC, RB1CC1, SNAI2 genes. For verification of the array results, FISH analysis was performed with Bluegnome region spesific probes and confirmed the deletion in the region. We concluded that 8q11.1-q11.23 region may contain candidate genes for the related clinical findings.

P02.017

Recognizing adult Aarskog-Scott syndrome carrier females based on craniofacial measurements using interaction variables and a surrogate covariance matrix

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Aarskog-Scott syndrome is an X-linked recessive syndrome caused by FDG1 mutations and characterized by dysmorphism, shawl scrotum, short stature and brachydactyly. We demonstrate that carrier females are distinguishable based on craniofacial measurements. We evaluated 20 adult females, out of which 16 are obligatory carriers, 1 is a molecularly verified carrier, and 3 are verified non-carriers. First, we compute the likelihood to be sampled from the matched control population for each of 21 craniofacial measurements. The combined likelihood scores demonstrate a moderate recognition rate with Area Under ROC Curve (auc) of 0.74. In order to improve the recognition rate, we consider the interaction variables that correspond to all pairs of measurements. Since the individual measurements of the control population became unavailable once the statistics of each measurement were computed, we cannot directly estimate the distribution of the interaction variables. Instead, we employ a second data set of 21 adult female Navajos, for which five cranio-facial measurements that are common with the ones of the Aarskog-Scott syndrome carrier dataset are available per-person. Employing only these five measurements and correcting for the correlations among the derived interaction variables, we are able to obtain an improved recognition rate (auc of 0.78). This is significantly higher than the recognition rate obtained from these five measurements without considering the interaction variables (auc of 0.65). Therefore, our results highlight the utility of interaction variables in evaluation of facial features and support the usage of surrogate correlation matrices when such data are unavailable.

P02.018

Factors of mortality and morbidity in congenital abdominal defects

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Congenital abdominal wall defects are one of the most common malformations. The purpose of this study is to assess the main factors that may influence the outcomes of the treatment of omphalocelle and laparoschizisul. Between 1999 and 2010, 96 patients (53 boys and 43 girls) were admitted in the Pediatric Surgery Departments of two of the major pediatric hospitals in Romania. There were 62 with laparoschizis and 34 with omphalocelle. Primary closure of the abdominal defect was possible in 49 cases of laparoschizis (79%) and 21 cases of omphalocelle (60%). Complications occurred in 57 patients with infectious one being the most prevalent. Overall mortality rate was 62,5 %, 69% for laparoschizis and 50% for omphalocelle. In table 1 and 2 are summarized the main prognostic factors assessed by us. Conclusions: The main predictors for complications are: low APGAR score, masculine gender, incongruence between abdominal cavity and the herniated bowel, postoperative anemia, thrombocytopenia and renal insufficiency during postoperative course. The main predictors for an unfavorable outcome are: low birth weight, low APGAR score, masculine gender, incongruence between abdominal cavity and the herniated bowel, associated malformations, postoperative anemia, thrombocytopenia, renal insufficiency, pulmonary infections and sepsis during the postoperative course

	Mann-Whitney U	Wilcoxon W	Z	p
Sex	894,000	1674,000	-1,884	0,050
Gestational age	813,500	2088,500	-,554	0,579
Birth weight	878,000	2418,000	-1,306	0,192
APGAR	444,500	1572,500	-2,578	0,010
Prenatal diagnostic	1032,000	1812,000	-1,175	0,240
Natural birth/ C section	1089,000	2742,000	-,234	0,815
Presentation	1110,000	1890,000	-,029	0,977
Maternal age	683,500	1673,500	-,745	0,456
Heredo-colateral	73,000	128,000	-,590	0,555
Alcohol/ cigarettes	99,500	144,500	-,223	0,823
Type of the defect	814,500	2467,500	-2,675	0,007
Size of the defect	778,500	1306,500	-,358	0,720
Herniated organs	1056,000	1836,000	-,478	0,632
Chromosomal aberrations	1075,500	2728,500	-,561	0,575
Cardiac malformations	981,000	1761,000	-1,411	0,158
Intestinal atresia	1105,500	2758,500	-,078	0,938
Scheletale malformations	1069,500	1849,500	-,547	0,584
Renal malformations	1093,500	2746,500	-,388	0,698
Malformations overall	883,500	2536,500	-1,786	0,717
Surgical intervention	1090,500	1870,500	-,374	0,709
Age at operation	264,000	792,000	-1,058	0,290
Surgical procedure	794,000	1497,000	-1,941	0,052
Reinterventions	818,500	1521,500	-1,500	0,134
Concomitant operations	908,000	2183,000	-,216	0,829
Anemia	846,000	1626,000	-2,299	0,021
Thrombocytopenia	850,500	1630,500	-2,299	0,022
Renal insufficiency	855,000	1635,000	-2,588	0,010
Hidro-electrolitic disturbances	922,500	1702,500	-1,641	0,101
Positive cultures	880,500	1660,500	-2,080	0,038

	Mann-Whitney U	Wilcoxon W	Z	p
Sex	846,000	1512,000	-2,056	0,040
Gestational age	660,000	2145,000	-1,631	0,103
Birth weight	586,000	2297,000	-3,404	0,001
APGAR	307,000	1633,000	-3,718	0,000
Prenatal diagnostic	1062,000	1728,000	-,270	0,787
Natural birth/ C section	1038,000	1704,000	-,444	0,657
Presentation	1074,000	2904,000	-,118	0,906
Maternal age	555,500	1731,500	-1,936	0,053
Heredo-colateral	56,000	92,000	-1,421	0,155
Alcohol/ cigarettes	56,000	462,000	,000	1,000
Type of the defect	876,000	2706,000	-1,864	0,062
Size of the defect	760,000	2245,000	-,224	0,822
Herniated organs	873,000	1539,000	-1,810	0,070
Chromosomal aberrations	1080,000	2910,000	,000	1,000
Chromosomal aberrations	882,000	1548,000	-2,171	0,030
Cardiac malformations	1056,000	2886,000	-,317	0,751
Intestinal atresia	912,000	1578,000	-2,219	0,026
Scheletale malformations	1056,000	2886,000	-,525	0,600
Renal malformations	966,000	1632,000	-1,709	0,087
Malformations overall	860,000	2690,000	-1,727	0,050
Surgical intervention	972,000	1638,000	-1,949	0,051
Age at operation	178,000	958,000	-1,603	0,109
Surgical procedure	705,500	1371,500	-2,786	0,005
Reinterventions	883,500	2368,500	-,649	0,517
Concomitant operations	853,500	2284,500	-,612	0,540
Postoperative bowel occlusion	986,000	2526,000	-,045	0,964
Anemia	978,000	2808,000	-,896	0,370
Thrombocytopenia	828,000	1494,000	-2,251	0,024
Renal insufficiency	810,000	1476,000	-2,764	0,006
Hidro-electrolitic disturbances	1020,000	1686,000	-,528	0,597
Positive cultures	900,000	1566,000	-1,644	0,100
Blood transfusion	1008,000	1674,000	-,631	0,528
Fever	972,000	1638,000	-1,018	0,309
Enterocolitis	966,000	1632,000	-1,709	0,087
Pneumonia	540,000	1206,000	-4,825	0,000
Sepsis	642,000	1308,000	-3,848	0,000
Wound infections	1074,000	2904,000	-,118	0,906
Overall complications	630,000	1296,000	-4,003	0,000

P02.019

Aicardi-Goutières syndrome type 1 in a Russian family

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Aicardi-Goutières syndrome (AGS) is a rare early-onset hereditary encephalopathy with some features of congenital virus infection which often leads to misdiagnosis. AGS is genetically heterogeneous, five genes are known and genotype-phenotype correlations exist. *TREX1* mutations produce AGS type 1, a severe neonatal-onset form making up 25% of AGS cases. Inheritance is autosomal recessive but few cases due to heterozygous *TREX1* mutation *de novo* are known. We diagnosed AGS type 1 in a non-consanguineous family with two affected children, a 1.5-year-old girl (index case) and a 9-year-old boy (whom we did not examine personally). Both children had typical presentation: uncomplicated pregnancy and delivery, normal condition in first weeks of life, unexplainable fever >38ºC, leukocytosis and severe motor and mental delay with variable neurological signs since 1-1.5 months, stabilization and partial improvement with time, multiple petrificates and white matter lesions on CT/MRI. Along with intrafamilial likeness few differences in MRI and clinical signs were seen. There were no characteristic skin chilbains. The boy's previous diagnosis was 'cerebral palsy due to congenital CMV infection', and risk for the second child was mistakenly considered low. AGS was first recognized in the girl. Homozygosity for *TREX1* mutation c.342G>A (p.Arg114His) was detected. The mutation is most common in European populations and is supposed to have common origin due to founder effect [Crow et al, 2006]. Evidently, ours is the first AGS Russian case which shows its underestimation in practice. AGS should be considered in 'CNS congenital infection' with negative virusological tests.

P02.020

A novel heterozygous *ALX4* gene mutation in a familial case presenting parietal foramina and a mild frontonasal dysplasia phenotype

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ALX4 encodes a transcriptional regulator involved in cell-type differentiation and development during embryogenesis, playing an important role in the processes of cranial development. We will illustrate the clinical heterogeneity of the *ALX4*-related craniofacial malformations by describing the first familial case segregating a heterozygous mutation and with an intermediate phenotype between isolated parietal foramina and the severe *ALX4*-related frontonasal dysplasia with alopecia and genital abnormality phenotype (*ALX4*-related FNDAG).

The proband, the second child of non-consanguineous parents, has had normal cognitive development and his craniofacial features included hypertelorism, telecanthus, epicanthus, broad nasal bridge, notch at the tip of his long nose and fronto-parietal alopecia. These features strongly resembled his mother. He also presented with bilateral cryptorchidism and broad thumbs. Cranial and limbs X-rays disclosed only parietal foramina in both the patient and his mother.

The coding region sequencing of the *ALX4* gene disclosed a novel heterozygous mutation on the proband and his mother: p.Asp326fs (c.1080-1089_delGACCCGGTGCinsCTAAGATCTCAACAGAGATGGCAACT; cDNA reference: NM_021926.3; protein reference: NP_068745.2). This mutation was considered causative of the phenotype based on the facts that it is a frameshift mutation with generation of a stop-codon and it segregates only in the affected individuals within this family.We have, then, expanded the phenotype of the *ALX4*-related craniofacial anomalies by describing a patient harboring a heterozygous loss-of-function mutation in the *ALX4* gene that presents a mild frontonasal dysplasia associated with parietal foramina. This illustrates the broad phenotypic spectrum associated to mutations in *ALX4* gene, ranging from isolated parietal foramina to a severe frontonasal involvement.

P02.021

A homozygous novel WDR72 mutation in two siblings with amelogenesis imperfecta and short stature - coincidence or expansion of the clinical spectrum?

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Amelogenesis imperfecta (AI) is a clinically and genetically heterogeneous group of inherited defects of enamel formation. In isolated AI (no additional segregating phenotype), mutations in at least 6 genes are known so far, causing dominant, recessive or X-linked AI and allowing the identification of the molecular etiology in ~40% of affected families. We report on two siblings (11 year-old female and 7 year-old male), born to consanguineous Turkish parents, with mild proportionate short stature, normal OFC, mild developmental delay and amelogenesis imperfecta. Both parents have normal teeth, but mother, maternal grandmother and great-grandfather are/were also of short stature. Affymetrix GenomeWide SNP6.0 Array analysis excluded pathogenic copy number changes but showed that both siblings share large homozygous regions. One of those regions is located on chromosome 15q21.3 and contains the WDR72 gene. Mutations in WDR72 are a very rare cause of autosomal-recessive hypomaturation type of isolated AI. The WDR72 protein is critical for dental enamel formation but its exact function is still unknown. By now, only 6 different truncating mutations have been published. WDR72 sequence analysis in both siblings revealed homozygosity for a novel stop mutation in exon 10 (c.997A>T, p.Lys333X) explaining the AI phenotype. Patient reports with AI and mild short stature due to a brachyolmia were found in the literature. But a spine X-ray performed in the girl excluded brachyolmia. Therefore, it remains unclear whether the short stature in the two siblings is due to the mutant WDR72 or segregates as an independent trait in this family.

P02.022

Identification of a new mutation in exon 1 of androgen receptor gene in a Turkish patient with complete androgen insensitivity syndrome

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We report a 22 months old girl with the diagnosis of complete androgen insensitivity syndrome (CAIS). From her medical history, we learned that she had been operated because of left inguinal hernia. Its pathological examination revealed immature testis without malign transformation. Ultrasonography and MRI had shown testis in right inguinal channel, but no wolffian derivatives. In laboratory, basal testosterone was 57,6 ng/dL, stimulated testosterone was 264 ng/dL, stimulated DHT was 30 ng/dL. Accordingly, stimulated T/DHT ratio was calculated as 8,8. Her basal LH was 2,22 mIU/mL, basal FSH was 0,94 mIU/mL. Direct sequence analysis of the androgen receptor (AR) gene showed c.88G>A mutation in exon 1 causing p.val30met in N-terminal domain of the AR. It may disrupt receptor activity in two ways. Firstly, val30 residue is too close to ^{23}FQNLF^{27}-primary androgen dependent motif which stabilizes hormone-receptor complex via N/C interaction. Although both amino acids are hydrophobic in nature, methionine is a bigger molecule than valine. Hence, it may distort the delicate three dimensional structure of the protein and interfere with receptor activity. Secondly, c.88G>A creates a noncanonical start codon. Translation initiation from this noncanonical start codon removes ^{23}FQNLF^{27} motif and disrupts N/C interaction. There are very few missense mutations in exon 1 shown to cause CAIS, and p.val30met mutation has never been reported before.

P02.023

First report of aortic aneurysm in infancy in a new family with Aneurysms-Osteoarthritis Syndrome due to a *SMAD3* mutation: further delineation of the clinical phenotype

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Recently, mutations in the *SMAD3* gene were found to cause a new autosomal dominant aneurysm condition similar to Loeys-Dietz Syndrome (LDS), mostly with osteoarthritis, called Aneurysms-Osteoarthritis Syndrome (AOS).

Our proband is a 3-year old boy who underwent correction of an inguinal hernia at 3 months and substitution of the ascending aorta for pathologic dilation at 12 months of age. He also presents LDS-like facial features and elongation and kinking of the thoracic aorta on MRI. Family history reveals aortic dilation in his mother, death due to aortic dissection of an 18-year old maternal aunt, surgical replacement of the ascending aorta because of aneurysm in a maternal uncle at 19 years of age, postpartum death of the maternal grandmother at 24 years and surgical intervention because of thoracic aortic aneurysm in a brother of the proband's grandmother at 54 years. Other clinical findings in affected individuals include pes planus, striae, easy bruising, dural ectasia, degenerative disc disease, hiatus hernia, premature loss of teeth. No radiologic evidence of osteoarthritis was present in the family. Molecular testing of the *TGFBR1* and *TGFBR2* genes, involved in LDS, resulted negative, but analysis of *SMAD3* disclosed the novel heterozygous loss-of-function mutation c.1170_1179del (p.Ser391AlafsX7) in exon 9 in all affected family members, confirming the diagnosis of AOS. *SMAD3* mutations should be considered in patients of all ages with LDS-like phenotypes and negative *TGFBR1/2* molecular tests, especially in the presence of early-onset osteoarthritis. Molecular diagnosis allows early identification of patients and relatives at risk of major cardiovascular complications.

P02.024

Neuroleptic susceptibility in Angelman syndrome

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We report four patients with Angelman syndrome (Ube3a microdeletion confirmed in 15q11 locus) who present severe extra pyramidal syndrome after neuroleptic medication.

Symptoms resolved with discontinuation of treatment.

Those observations suggest high susceptibility to antipsychotic agents in this population and could be move closer to description of two Angelman patients who develop in adulthood extra pyramidal features consistent with Parkinson Disease with spectacular improvement with L-Dopa therapy (Harbord 2001).

It could be useful to perform DAT-Scan in Angelman patients.

In the same way, in the mouse model (maternal loss of Ube3a), number of neurons in the substantia nigra is significantly reduced and motor deficits could be attributed to the dysfunction of the nigrostriatal pathway (Mulherkar et al, 2010).

Furthermore the dysregulation of CaMKII could be responsible of neurological phenotype and could be improved by Levodopa.

In this population, if neuroleptic medication turns out necessary, we suggest to use atypical neuroleptic with different pharmacokinetic pathway with less affinity to Dopaminergic receptors D2.

Those results could improve our understanding of pathophysiological mechanisms involved in Angelman syndrome and lead to finalize new therapeutic strategies.

P02.025

A case of mosaic paternal uniparental disomy 15 identified by SNP-array analisys.

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Angelman syndrome (MIM 105830) is a complex neurodevelopmental disorder caused by loss of function of the imprinted UBE3A gene in 15q11-q13 region. Approximately 7% of cases are due to paternal uniparental disomy (UPD). Our case is the first child of healthy non consanguineous Italian mating. He was born at term after uneventful pregnancy. At birth no anomalies were referred, birth weight = 3540g (50° centile). Growth curve was normal and at 6 yrs his parameters were: weight = 37Kg (>>97°), height = 125cm (50°-75°) and OFC = 52cm (50°-75°). He had normal motor development (sitting = 6m, walking = 10m). He was evaluated for absence of speech. All audiological investigations were normal. He had friendly personality. No facial dysmorphysms were evidenced and no seizures were referred, although sleeping EEG showed anomalies. Molecular karyotype analysis using "HumanCyto-12 BeadChip" ILLUMINA, revealed a paternal UPD 15 mosaicism. Data analyzed with GenomeStudio 2011.1 (cnv Partition 3.1.6) and PennCNV, showed a 80% percentage of mosaicism, which was calculated from B allele frequencies. Results confirmed by UPD study. Is the first time that we can describe a somatic paternal UPD 15 mosaicism. UPD 15 patients normally have milder phenotype with a better prognosis, and our case is a very mild condition with mainly severe speech delay and truncal obesity. If we compare with other cases due to somatic mosaicisms of the imprinting center, our case could clinically overlap although maybe is still milder. Our work suggests that maybe many cases of AS could have been undiagnosed.

P02.026

Array-based genome-wide genotyping in patients with anorectal malformations and intellectual disability and/or malformations of the brain

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Anorectal malformations (ARM) occur in about 1 in 2,500 live births. Similar to most congenital defects, ARM can occur as an isolated malformation or associated with other defects or syndromes. To date, no causative genetic or non-genetic factor has been unequivocally identified in humans. Since intellectual disabilities (ID) are frequently associated with chromosomal aberrations, we suggested that a cooccurrence of ARM and ID will make it more likely to detect causative CNVs (copy number variations).

Molecular karyotyping, utilizing 1,134,514 SNPs (single nucleotide polymorphisms), was performed to screen 30 non-isolated ARM patients with ID and/or malformations of the central nervous system. To identify potential CNVs, the SNP fluorescence intensity was analyzed with QuantiSNP using an Objective-Bayes Hidden-Markov model for calling putative CNVs.

Preliminary results revealed probably causative deletions in five patients encompassing chromosomal regions: 6q14.3-q16.3 (16.5 Mb; 51 RefSeq genes), 13q31.2qter (28.5 Mb; 85 RefSeq genes), 17q12-q21.2 (2.1 Mb; 81 RefSeq genes), and two regions on chromosome 22q11.2 (2.54 Mb; 43 RefSeq genes and 2.52 Mb; 43 RefSeq genes, respectively). In one patient we identified a 7.9 Mb duplication on chromosome 3q26.32-q27.2 harboring 56 RefSeq genes. All findings were confirmed by quantitative PCR. These observations support the previously described association of microdeletion on 22q11.2 and the occurrence of ARM and/or ID suggesting this region to harbor genes, which contribute to both phenotypic features. Studying larger cohorts is likely to detect additional CNVs that might give further information on chromosomal regions and genes involved in the etiology of ARM and/or ID.

P02.027

Specific transglutaminase 1 mutation profiles in bathing suit ichthyosis and self-improving collodion ichthyosis

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Bathing suit ichthyosis (BSI) and self-improving collodion ichthyosis (SICI) are two minor variants of generalized autosomal recessive congenital ichthyosis (ARCI). BSI is characterized by scaling of the skin in a bathing suit pattern, mainly limited to the trunk, whereas SICI is characterized by complete disappearance of the skin lesions. We report genotypic and phenotypic data from a series of 9 patients born as collodion babies, who developed BSI or SICI due to mutations in the transglutaminase-1 gene (TGM1), including 3 previously unreported missense mutations. All our BSI or SICI patients carried at least one specific missense mutation in TGM1 concerning an arginine at position 307 or 315. In two patients the disease evolved in two phases (BSI to SICI or BSI to ARCI). The other 7 patients exhibited a stable BSI phenotype after shedding of the collodion membrane. This study highlights the possibility of variable evolution of the phenotype of patients with identical mutations in the same gene. Combined with data from the literature, this confirms the hypothesis that only a restricted spectrum of TGM1 mutations is susceptible to lead to a BSI and/or SICI phenotype. This phenotypic variability also depends on other genetic and external factors.

P02.028

Application of high resolution array CGH in newborns with multiple congenital anomalies and isolated cleft lip/palate

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Molecular karyotyping by array CGH has now been widely used for genetic analysis in the setting of both multiple (MCA) as well as isolated congenital birth defects. The technique has the potential of providing diagnosis in up to 27% of newborns with MCA and accompanying dysmorphism and a significant number of neonates with isolated anomaly [i.e. cleft lip/palate(CL/P), heart defect], where it can also identify new candidate genes.

To assess the frequency of array-detected copy number variations (CNVs) in neonates with congenital birth defects we screened two cohorts of 33 newborns with MCA and 40 newborns with isolated CL/P using whole genome Agilent 180k (hg18) microarray with mean resolution of 16kb.

We have found 19 CNVs, including 17 deletions and 2 duplications ranging in size from 27 kb up to ~16Mb. The prevalence of aberrations in the group with MCA was 25% whereas in the group with isolated CL/P it reached 21%. All CNVs found (except 4 deletions exceeding 5Mb) were unique and did not encompass any known microdeletion/microduplication syndrome regions. Novel candidate genes (T brachyury, SLFN12) were suggested in the group with isolated CL/P, whereas in the MCA group one of the interesting findings was del2q35 (STK36 gene). Our results demonstrate that high resolution array CGH is a powerful diagnostic tool in newborns with MCA and isolated CL/P. It can also help in recognition of genes involved in pathogenesis of congenital anomalies.

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P02.029

1p22 microdeletion represents a novel contiguous gene syndrome associated Diamond-Blackfan anemia

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Diamond-Blackfan anemia (DBA) is characterized by a profound normochromic and macrocytic anemia with normal leukocytes and platelets, congenital malformations, and growth retardation. The phenotype varies from a mild form to a severe form of fetal anemia resulting in hydrops fetalis. DBA is associated with an increased risk of hematological malignancy and solid tumors including osteogenic sarcoma. Other genetic forms of anemia, such as Fanconi anemia, need to be considered and ruled out as appropriate. The mutations of nine genes encoding ribosomal proteins have been recognized to be responsible for DBA. Most of the mutations are detected by sequence analysis except for the RPS19. We present a patient with mild intellectual disability, chronic normochromic anemia, mild neutropenia, normal platelets, and multiple congenital malformations including characteristic facial appearance, finger-like thumbs, atrial septal defect, growth retardation, and vaginal atresia. The detection of chromosomal aberrations in cells after culture with a DNA interstrand cross-linking agent (MMC) failed to determine the diagnosis as Fanconi anemia. Bone marrow examination revealed hypoplastic, but showed normal balanced hematopoiesis. Microarray CGH analysis revealed 7.9 Mb deletion at 1p22.1-p22.3, involving ribosomal protein L5 (RPL5), and transcriptional repressor protein GFI1. RPL5 is responsible for Diamond-Blackfan anemia, and GFI1 is responsible for autosomal dominant severe congenital neutropenia, where both genes are adjacent to each other. These results indicated that the 1p22 microdeletion represents a novel contiguous gene syndrome associated Diamond-Blackfan anemia, but overlapping with clinical features of Fanconi anemia.

P02.030

Array CGH with normal karyotype. Utility in pediatrics diagnosis.

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Microarray-based comparative genomic hybridization (array CGH) has provides a relatively quick method to scan the genome for gains and loses of chromosomal material. This new methodologies have led to identification of novel genomic disorder in patients with developmental delay/mental retardation and/or multiple congenital anomalies (DD/MR/MCA), with a significant increase in diagnostic yield .

In this study we present the result of array CGH obtained in 150 children with normal karyotype but DD/MR/MCA. The array CGH 60K from agilent platform was performed.

In 34 patients (22,66%) was detected a chromosomal deletion or duplication on previously described like pathogenic copy number variants (CNVs),. In 15 cases (10%) was necessary the analysis of parental samples, showing that 6 anomalies (40%) had occurred de novo and was classified as pathogenic and in 9 cases (60%) appeared to be inherited from an unaffected parent. In a total of 40 patients (26,66%) was possible to detect a pathogenic CNVs .

Recent studies suggest that when aCGH is performed with an apparently normal karyotype, the diagnostic yield increases by an additional 8-17%. In our study we have obtained a 26,66% of children with pathogenic CNVs that is higher than the results obtained by other authors. This increase at the detection rate probably is due to the array type utilized.

As a conclusion, array CGH can be implemented routinely after a normal chromosome result when a phenotype of DD/MR/MCA is present, but is also appropriate for patients with autism and apparently balanced translocations.

P02.031

Human body asymmetry spectrum - dysmorphic and genetic perspectives for five rare disorders

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Human body asymmetry is a diagnosis with a large spectrum of features, with a multifactorial aetiology: physiological, osseal, neurological, genetic. There are many aspects to discuss involved in asymmetry: situs anomalies, asymetric cell division, laterality, asymetric embriopathy, hemiasymetries, asymetric vascular syndromes, somatic mosaicism.

Asymmetric entities are classified in non-syndromic and syndromic, congenital or acquired, total or limited. Left-right asymmetry has important implications for human health and development.

We report our clinical experience of some rare entities with body asymmetry:

1) an infant with *Beckwith-Wiedemann syndrome* (MIM: 130650)
2) a newborn with probably *Proteus syndrome* (MIM: 176920)
3) three cases with *Klippel-Trenaunay-Weber syndrome* (MIM: 149000)
4) a girl with short stature, left-right asymmetry and radiologic signs of skeletal dysplasia, with clinical diagnosis of *Conradi-Hünermann syndrome* (MIM: 302960)
5) a six years old female patient with *diploid-tetraploid mixoploidy* (92,XXXX/46,XX), demostrated by cytogenetic studies in blood cultures, with asymmetric overgrowth, abnormal skin pigmentation, mental retardation, the first child of a family with primary subfertility.

Different mechanisms involved in the development of asymmetry in these disorders are discussed: mutations of imprinted genes, mosaicism for a somatic activating mutation, pathogenic gene for vascular and tissue overgrowth, random X-inactivation in affected tissues in heterozygous female, mosaicism versus chimerism.

The diagnostic were given according to the associated clinical features. Clinical delineation of the cases with congenital growth asymmetry remains essential until pathophysiological mechanisms are elucidated. Other laboratory tests like chromosomal analysis, biochemical studies, molecular assay, applied on different tissues, help in the differential diagnosis.

P02.032

ATP6V0A2-related cutis laxa: Case report and review of the literature

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We report on a 2-3/4 year-old Afghanian male patient diagnosed with ATP6V0A2-related cutis laxa whom we first saw postnatally and who has come for routine follow-up consultations. The parents are double first cousins. The boy initially presented with excessive skin wrinkling, microcephaly with a sloping forehead, large anterior fontanelle, dysmorphic facial features (hypertelorism, downslanting palpebral fissures, broad nasal bridge, long philtrum, micrognathia as well as low-set posteriorly rotated and protruding ears), myopia, atrial septum defect and small penis. At the age of 2 months, an inguinal hernia occured. Meanwhile the furrowing of the skin has markedly improved. Our patient has fortunately not shown any signs of developmental delay which is often present in patients with ATP6V0A2-related cutis laxa. All these findings are consistent with the clinical diagnosis of Wrinkly skin syndrome, the clinically mild phenotype of this disorder. Molecular genetic analysis revealed a homozygous c.1A>T mutation of the ATP6V0A2-gene. This is a yet undescribed pathogenic mutation presumably leading to an impairment of initiation of translation. The 20 exons of the gene encode the a2-subunit of the V-type H+-ATPase complex. Immunolabeling with an antibody against the N-terminal domain of ATP6V0A2 demonstrated loss of the a2-subunit in the patient's fibroblasts.

The ATP6V0A2-related cutis laxa is a rare autosomal recessive disorder first described in 1973 by Dr. Gazit. Up until now approximately 60 cases of ATP6V0A2-related cutis laxa have been reported. (OMIM # 278250).

We compare the phenotype and findings of our case to previously published cases.

P02.033

An autosomal recessive form of atrophoderma vermiculatum: Clinical and genetic characterisation

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Atrophoderma vermiculatum (AV) is a rare, benign skin disorder characterized by the occurrence of pitted atrophic and depressed scars in a reticular pattern. AV usually begins in childhood by symmetric reticular or honeycombed atrophy of the cheek. The genetics behind AV is unclear as most cases appear to be sporadic and the few cases with mendelian inheritance seem to follow an autosomal dominant inheritance pattern.

We have identified a consanguineous Pakistani family segregating autosomal recessive AV. In total, the extended family contains four affected individuals, three affected siblings and their first cousin. Parents to affected individual were healthy. Affected individuals are born with normal skin. Symptoms starts at approximately one year of age by tear shedding provoqued by sunlight, breeze and cool air, followed by the development of characteristic skin changes. The facial skin shows pit like areas of atrophy distributed most prominently over the cheeks, extending to the chin, upper lip, forehead and nasal ridge. The atrophic pits are separated by ridges of normal looking skin.

We have recently initiated homozygosity mapping and exome sequencing to identify the mutation behind autosomal recessive AV. The results from these efforts will be presented.

P02.034

Two cases with different microaberrations of the long arm of chromosome 15 and autism

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Autism is a complex neurodevelopmental disorder of the immature brain with unknown origin that manifests in early childhood. The exact aetiology of autism remains speculative, although it is likely to result from a complex combination of multiple non genetic and/or genetic factors. Advances in high-resolution comparative genomic hybridization (CGH) microarray technology have revealed sub-microscopic aberrations that lead to identification of many disease-causing genomic copy number variants (CNVs) in autism. Numerous reports have implicated duplications or deletions of proximal chromosome 15q as significant risk factors for autism and autism-related disorders.

We report two autistic children with 15q11-13 rearrangements. We have used genomic array CytoChip Oligo (BlueGnome, Cambridge, UK), format 2x105K, version 1.1. and BlueFuse Multi software, version 2.2. The 2x105K array detects 35 kbp imbalances on the backbone and has tiling of 20 probes over 137 OMIM disease loci. In the first case a de novo cryptic deletion of 2q36.3 region spanning 1,456 Mb and amplification of (15)(q12q13.1) region spanning 3,473 Mb were found in 12 years old girl with autism, severe mental retardation and dysmorphic features. The second case showed deletion of 15q11.2 region spanning 494, 905 bp in a boy with idiopathic autism. FISH experiments with BAC clone confirm the CytoChip results.

These data strongly support the implication of 15q11-13 rearrangements as a predisposing factor for autism.

P02.035

The clinical and genealogical diagnosis of autosomal dominant spinocerebellar ataxias in Yakutia

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According to genetic-epidemiological investigations of Yakut population in Republic Sakha (Yakutia), the frequency of hereditary diseases in Yakuts are very high. Most common AD disease in Yakuts is a spinocerebellar ataxia. 80% of SCA are SCA 1 type. The high incidence of the SCA1 in Yakutia (38.6 per 100000), compared to 1-2:100000 in the world population.

The aim of study was to establish of the spectrum of genetic form of AD SCA in Yakutia.

A computer database of patients with cerebellar syndrome was established. 83 families with SCA1 and 9 familial and 66 sporadic cases of undifferentiated SCA identified by clinical and genealogical research. Differential diagnosis was performed on the five forms of AD SCA: SCA2, 3, 6, 17 and DRP-

LA. The expansion of CAG-repeats in the genes SCA 2, 3, 6, 17 in the studied sample while was not found.
In four patients from one of the Yakut families the expansion of CAG-repeat in the gene of DRPLA (DRPLA) were discovered. In the proband`s DNA molecular analyses of the DRPLA gene showed two alleles of 23/64 CAG-repeats, in the mother`s DNA - 19/61, in the sibs`s DNA - 19/61 and 19/63 repeats (normal range ≤ 36). This is a first description of family with genetically established DRPLA reported in Yakutia. Most members presented with ataxia and chorea and some of these developed dementia late in the course of the disease. These clinical symptoms of Yakut patients with DRPLA are identical with the cases on another patients with DRPLA.

P02.036

Jervell and Lange-Nielson Syndrome: Homozygous missense mutation in KCNQ1 in a Turkish family

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Long QT syndrome is one of the most common cardiac ion channel disease which its morbidity and mortality rate can be lessened with an early diagnosis and proper treatment. It is a cardiac repolarization abnormality that is characterized by prolonged OT interval and propensity for ventricular tachycardia (VT) of the torsades de pointes type are the charecteristics of the disease. This syndrome represent high risk of presyncope, syncope, cardiac arrest and sudden death. Jervell and Lange-Nielson syndrome (JLNS) is one of the inherited form of long QT syndromes. It is inherited recessively and characterized by profound sensorineural deafness and prolongation of the OT interval, thus representing abnormal ventricular repolarizasiton. JLNS has been shown to occur due to homozygous and copmpound heterozygous mutations in KCNQ1 or KCNE1. There was one clinical report on JLNS in Turkey; however, it was not confirmed by a molecular study. We identified a homozygous mutation in KCNQ1 in a 3.5-yr-old female child with JLNS, who visited the hospital due to recurrent syncope and seizures and had congenital sensorineural deafness. Her electrocardiogram revealed a markedly prolonged QT interval. The sequence analysis of the proband revealed the presence of homozygous missence mutation (c.728G>A, p.R243H). Heterozygous mutation in KCNQ1 was identified on the maternal, paternal and sister side. Even if with a high dose β-blocker therapy the patient has twice VT attacks, because of this reason the implanted cardiac defibrillator (ICD) was planned and implanted. We suggest early genetic diagnosis for proper management of the disease and genetic counseling.

P02.037

Axenfeld-Rieger Syndrome Type 1 - family case report

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INTRODUCTION: The **Axenfeld-Rieger syndrome** is a rare, autosomal dominant disorder, characterized by corneea defects, iris defects and glaucoma. More than 50% of pacients become blind because of glaucoma complications. Other associated developmental defects involve the teeth and facial bones.
MATERIAL AND METHODS: We present a family with **Axenfeld-Rieger syndrome** on four generations. Complete ophthalmic examination (biomicroscopy, gonioscopy, oculo-orbitar ultrasound, corneal topography) and general examination of the patients showed ocular and non-ocular manifestations. The craniofacial and oral examination involved cephalometric radiographs and orthopantomograms. All the patients were screened for mutations in PITX2 and FOXC1 by DNA Analysis.
RESULTS: DNA Analysis of all the patients revealed a mutation of the PITX2 gene.
CONCLUSIONS: We present a family with **Axenfeld-Rieger syndrome** type 1 on four generations, a very rare disorder with clinical and genetic variability.

P02.038

Muscle hemangiomatosis as a severe presenting feature in a patient with *PTEN* mutation: Expanding the phenotype of vascular malformations in Bannayan-Riley-Ruvalcaba syndrome

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Bannayan-Riley-Ruvalcaba Syndrome (BRRS) is a rare autosomal dominantly-inherited hamartoma syndrome with distinct phenotypic features. Mutations in the *PTEN* gene have been identified in PTEN hamartoma tumor syndromes. Our aim was to determine the correlation of phenotype-genotype relationships in a BRRS case. We have evaluated a *PTEN* mutation in a patient with vascular anomalies and the phenotypic findings of BRRS. We described an eight-year-old girl with the clinical features of BRRS, specifically with vascular anomalies. The mutation in the *PTEN* gene was identified by DNA sequencing. In our patient, we defined a de novo non-sense R335X (c.1003C->T) mutation in exon 8, which results in a premature termination codon. Due to vascular anomalies and hemangioma, the patient’s left leg was amputated one year after hemangioma diagnosis. In conclusion, BRRS patients with macrocephaly and vascular anomalies should be considered for *PTEN* mutation analysis and special medical care.

P02.039

Bardet-Biedl Syndrome with disorder of sex development: A new case report from Tunisia

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Bardet-Biedl syndrome (BBS) is a ciliopathy causing multivisceral abnormalities. It is a rare and heterogeneous genetic condition characterized by obesity, mental retardation, dysphormic extremities, retinal dystrophy, hypogonadism and renal anomalies. Prevalence rates are estimated of 1:140000 to 1:160000 in North America and Europe, respectively but in Asian, Australian and African continents, there are only case reports and small serials. To date, mutations in 18 different loci are responsible for BBS phenotype. Here, we report a new case of BBS from Tunisia characterized by sexual ambiguity without renal abnormalities. The patient who is 24 years old was referred to our genetic counselling because of aspermia. Patient history reveals bilateral orchiopexy one year ago. Physical examination showed poor visual acuity, strabismus and gaze nystagmus (with retinal degeneration), obesity, tetramelic postaxial polydactyly, superficial dilatation of veinules, dental problems, short neck, low hairline at the nape of the neck, impaired coordination and ataxia and moderate mental deficiency. At sexual level, the patient had a female voice, small empty scrotums that look like labia majora with a severe microphallus. Cytogenetic evaluation reveals a 46,XY male formula. Tetramelic polydactyly which is described in our patient and in some other members of his family seems to be associated to BBS chromosome 3 locus. Molecular diagnosis will lead to familial genetic counseling as well as to surveillance of the patient who need a multidisciplinary medical care especially to detect renal abnormalities which is the main life-threatening features and the other complications including hypertension and Type 2 diabetes.

P02.040

Phenotype /(Epi-) Genotype correlation in 265 cases of Beckwith-Wiedemann syndrome

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Beckwith-Wiedemann syndrome (BWS) is characterized by cancer predisposition and a variable association of overgrowth, macroglossia, abdominal defects, renal anomalies, *nevus flammeus*, ear malformations, hypoglycemia, hemihyperplasia, and organomegaly. BWS molecular bases are heterogeneous as several mechanisms lead to unbalanced transcription of genes regulated by 11p15 chromosomal region Imprinting Center IC1 and IC2. We searched for epigenotype-phenotype correlations in 265 BWS patients with diagnostic criteria and proven molecular defect. Patients’ characteristics were compared among molecular subclasses: KvDMR1 hypomethylation (IC2, n=153), H19/IGF2 hypermethylation (IC1, n=27), chromosome 11p15 paternal uniparental disomy (UPD, n=65), CDKN1c mutation (n=10), and Multiple Differently Methylated Region Methylation Defect (MDMD, n=9). The table summarizes the differences among groups. Each showed different growth patterns: neonatal macrosomia in IC1 patients, postnatal overgrowth in IC2/CDKN1c/MDMD patients, and hemihyperplasia in UPD patients. Exomphalos was more common in IC2/CDKN1c patients, whereas *diastasis recti* and umbilical hernia were associated with IC1 defects, consistent with organomegaly and polyhydramnios. Renal defects were typical of UPD/IC1 patients, and uretheral malformations of IC1 cases. Ear anomalies and *nevus flammeus* were associated with IC2/CDKN1C/MDMD genotype. Macroglossia, almost always present, was less common in UPD. Wilms' tumor was associated with IC1 defects and never observed in IC2 patients. Hepatoblastoma was typical of UPD and other tumors were randomly scattered among molecular subclasses. In BWS is definable a clear phenotype-epigenotype correlation allowing tailored follow-up and cancer screening procedures.

	IC2		**UPD**		**IC1**		**CDKN1C**		**MDMD**		***p***
	n	***%***	***n***	***%***	***n***	***%***	***n***	***%***	***n***	***%***	
	153	***57,7***	**65**	***24,5***	**27**	***10,2***	**10**	***3,8***	**10**	***3,8***	
Neonatal macrosomia	65	*42,5%*	28	*43,1%*	**21**	***77,8%***	4	*40,0%*	4	*40,0%*	***<0,001***
Postnatal overgrowth	**64**	***41,8%***	14	*21,5%*	8	*29,6%*	**4**	***40,0%***	**7**	***70,0%***	***0,002***
Polydramnios	24	*15,7%*	9	*13,8%*	**10**	***37,0%***	0	*0,0%*	0	*0,0%*	***0,005***
Hemihypertrophy	70	*45,8%*	**57**	***87,7%***	12	*44,4%*	1	*10,0%*	3	*30,0%*	***<0,001***
Exomphalos	**50**	***32,7%***	4	*6,2%*	1	*3,7%*	**8**	***80,0%***	1	*10,0%*	***<0,001***
Rectum diastasis	47	*30,7%*	13	*20,0%*	**15**	***55,6%***	2	*20,0%*	3	*30,0%*	***0,004***
Umbilical hernia	40	*26,1%*	16	*24,6%*	**13**	***48,1%***	0	*0,0%*	4	*40,0%*	***0,021***
Organomegaly	43	*28,1%*	22	*33,8%*	**18**	***66,7%***	1	*10,0%*	4	*40,0%*	***<0,0001***
Hypoglycemia	47	*30,7%*	23	*35,4%*	9	*33,3%*	1	*10,0%*	4	*40,0%*	*NS*
Macroglossia	141	*92,2%*	**44**	***67,7%***	25	*92,6%*	6	*60,0%*	9	*90,0%*	***<0,001***
Ear pits/ creases	**76**	***49,7%***	17	*26,2%*	6	*22,2%*	**5**	***50,0%***	**5**	***50,0%***	***<0,001***
Naevus flammeous	**71**	***46,4%***	19	*29,2%*	6	*22,2%*	**6**	***60,0%***	**4**	***40,0%***	***0,002***
Heart defects	23	*15,0%*	4	*6,2%*	4	*14,8%*	2	*20,0%*	1	*10,0%*	*NS*
Renal abnormalities	17	*11,1%*	**22**	***33,8%***	**10**	***37,0%***	2	*20,0%*	0	*0,0%*	***<0,001***
Ureteral abnormalities	7	*4,6%*	5	*7,7%*	**6**	***22,2%***	1	*10,0%*	0	*0,0%*	***0,005***
Wilms'' tumor	0	*0,0%*	2	*3,1%*	**6**	***22,2%***	0	*0,0%*	0	*0,0%*	***<0,001***
Hepatoblastoma	0	*0.0%*	**4**	***6.2%***	0	*0.0%*	0	*0.0%*	0	*0.0%*	***0.004***
Other malignancies	3	*2.0%*	1	*1.5%*	1	*3.7%*	0	*0.0%*	0	*0.0%*	*NS*
Benign tumors	3	*2.0%*	2	*3.0%*	2	*7.2%*	0	*0.0%*	0	*0.0%*	*NS*

P02.041
Beckwith-Wiedemann syndrome - clinical findings in Polish patients with IC2 (KvDMR) hypomethylation in 11p15 region

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Beckwith-Wiedemann syndrome (BWS) is characterized by overgrowth, macroglossia, abdominal wall defects and a high risk of childhood tumors. BWS is caused by various 11p15 genetic or epigenetic defects leading to defective expression of imprinted genes. The genes in 11p15 region are organized into two imprinted domains controlled by two Imprinting Centers: IC1 (H19DMR) and IC2 (KvDMR). The most common defect in BWS (~50%) is loss of methylation at IC2. Paternal UPD of 11p15, gain of methylation at IC1, mutations in *CDKN1C* gene encoded on 11p15 and chromosomal rearrangements also result in the BWS phenotype. Some specific phenotype-(epi)genotype correlations are observed in BWS. However, there is a marked phenotypic variability within molecular subgroups.
We present fourteen Polish patients with BWS and IC2 hypomethylation. A molecular analysis was performed by methylation sensitive multiplex ligation-dependent probe amplification (MS-MLPA) in a group of thirty five unrelated BWS patients. The analysis demonstrated loss of methylation at IC2 in 40% of investigated patients. All the patients presented clinical features typical for BWS, although in various degree. The study underlines correlation between IC2 hypomethylation and the presence of BWS features such as: macroglossia (14/14 cases), characteristic face (14/14 cases), abdominal wall defects (12/14 cases) and anterior ear lobe creases and/or posterior helical pits (12/14 cases). An interesting finding is a relatively high prevalence of cryptorchidism (5/9 male patients). The further investigations of the genetic background of BWS in Polish population are under way.
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P02.042
Ophthalmic status of the patients with Bloch-Sulzberger syndrome

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Bloch-Sulzberger syndrome (BSS) or Incontinentia pigmenti (MIM 308300) is an X-linked dominant syndrome with cutaneous, neurologic, ophthalmologic, and dental manifestations. It is caused by mutations in the IKK-gamma gene (IKBKG; MIM 300248, Xq28). A.Garrod reported the first probable case of incontinentia pigmenti in 1906. Subsequently, Bloch and Sulzberger further defined the condition in 1926 and 1928, respectively, as a clinical syndrome with unique features of typical cutaneous manifestations. IKK-gamma is the regulatory subunit of the inhibitor kappa kinase (IKK) complex and is required for the activation of the transcription factor NF-kappaB (NF-kB). NF-kB is central to many immune, inflammatory, and apoptotic pathways. The incidence of BSS is believed to be 1 case per 40,000. Here we reported on 5 female patients with BSS aged from 4 to 20 yr. Proband mothers (3/5) had multiple male miscarriages. All patients had hypopigmented, atrophic patches, conical forms of their teeth and some ophthalmologic findings. Two patients had strabismus and nystagmus due to congenital ectopy of the macula and optic nerve atrophy. Their visual acuity was insufficient and rehabilitation process was difficult without positive prognosis. Three patients had vitreoretinal abnormality as congenital hyperplastic persistent vitreous body complicated with traction retinal detachment. These probands have been observed formerly as the patients suffered from retrolental fibroplasias. Vitrectomy with scleral buckle was performed with successful anatomical results and preservation visual function

P02.043
Molecular analysis of patients with Bohring-Opitz syndrome

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Bohring-Opitz symdrome (BOS) is a rare condition comprising distinct facial features including bulging forehead over the metopic suture, frontal nevus flammeus, exophthalmos, retinal abnormalities, hypertelorism, upslanting palpebral fissures, and cleft lip and/or palate, intrauterine growth retardation, severe failure to thrive, flexion deformities of the upper limbs, lower limb deformities, severe developmental delay, and death often early in childhood. Recently, ASXL1 nonsense mutations were identified in 7/13 suspected BOS cases as the cause of the syndrome [Hoischen et al., 2011]. Here we report on 10, clinically undoubtful cases with BOS. All of them fulfilled the diagnostic criteria proposed by Bohring et al. [2006] and all carry a private, previously undescribed heterozygous nonsense/frameshift ASXL1 mutation. As far as parental DNA was available for analysis, the mutations were shown to be de novo. Thus, our data further support ASXL1 as the main cause of BOS and confirm exon 13 (NM_015338) as mutational hotspot in this syndrome. In addition, our data show that in clinically well characterized cases the detected mutation rate may be 100%. Further carefully performed genotype-phenotype studies are necessary to specify the most appropriate key symptoms and to differentiate between BOS and other phenotypically overlapping syndromes or to prove genetic heterogeneity.

P02.044
Clinical Spectrum and Natural History of Bohring-Opitz (BOPS) (BOS) syndrome, in the three first Italian Patients

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Bohring-Opitz Syndrome; BOPS (#605039), also known as C-like syndrome, is a very rare malformation disorder, reported until now in about twenty, unrelated, subjects. The delineation of BOS was made by Bohring, in 1999, on the basis of a complex phenotype characterized by IUGR, feeding problems, severe intellectual ad motor disability, trigonocephaly, frontal nevus flammeus, exophthalmus, cleft palate, flexion of elbows and wrists, hirsutism. More recently (2011) Hoischen , sequencing the exomes of 13 unrelated subjects with BOS, identified mutations of ASXL1 gene, in 7 of them, suggesting the genetic heterogeneity of this disorder. Here we report the follow-up study of three, unrelated, Italian subjects with BOS, and we briefly underline the natural history steps, and the major clinical aspects useful for the diagnosis at different ages. The first two children (a female and a male) are still alive, respectively aged 3 and 7 years; the third patient (a female) passed at age 22. Only the male is able to walk. Absolute lack of language in all patients. Extremely similar, clinical phenotype at birth, with characteristic face, frontal nevus flammeus, severe myopia, BOS "attitude". The face was decidedly less typical in the third patient since the age 15. Severe mental retardation in cases 1 and 3: the male interacts by using cards with images and alphabetical letters.Sequencing analysis of ASXL1 gene in the first and second child, revealed new mutations, respectively c.2407_2411del5 [p.Q803TfsX17] and c.2893C>T[p.R965X]. We are looking for biological samples of the third patient to screen the ASXL1 gene.

P02.045

Unusual presentation of combined saggital-metopic synostosis represents the second case of the Boston-type craniosynostosis syndrome

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Background. Craniosynostosis, caused by early fusion of cranial sutures, can include premature fusion of the saggital (scaphocephaly) or metopic suture (trigonocephaly). Though often occurring as isolated findings, their co-existence in a craniosynostosis syndrome is infrequent and mostly sporadic.

Case description. The male proband presented with premature fusion of the saggital and metopic suture. Imaging revealed also coronal synostosis and multiple endocranial hypoplastic areas. Radiographs demonstrated bilateral agenesis of the middle phalanges in the feet. Family history revealed the father, his sister and half-sister, to have scaphocephaly with 3-4 syndactyly. The paternal grandfather did not have a phenotype, though the great grandfather had bilateral 3-4 syndactyly. Molecular analysis revealed a mutation (p.P148L) in the MSX2 gene, which has been associated with the Boston-type of craniosynostosis in a single family (Warmer et al., 1993). Segregation analysis confirmed non-penetrance in the grandfather.

Conclusion. In this four-generation family with various expression of scaphocephaly and severe trigonocephaly, molecular analysis revealed a missense mutation in MSX2, previously described in the Boston craniosynostosis family. Besides unique features such as incomplete penetrance, limb abnormalities and the cranial sutures involved, our patients share with the original family autosomal dominant inheritance with anticipation and the endocranial hypoplastic areas. Though these findings are diagnostic clues for MSX2-related craniosynostosis, the initial patients in this family presented with isolated scaphocephaly and syndactyly. MSX2 analysis should therefore be considered in patients with scaphocephaly, especially if a positive family history for craniosynostosis or syndactyly is present.

P02.046

Unusual Phenotype of Brachydactyly in a Patient with a GDF5 Splice Mutation

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Brachydactyly (BD) is characterised by shortening of digits due to abnormal development of phalanges and/or metacarpals. BD has been classified on an anatomic basis into five types (A to E) with several subgroups. This group of inherited hand and foot malformations usually follows an autosomal dominant inheritance.

In recent years, mutations affecting the GDF5, a signal protein of the bone morphogenic protein family, have been shown to result in skeletal malformation syndromes like brachydactyly type A2 (BDA2), brachydactyly type C (BDC), proximal symphalangism, multiple synostoses syndrome 2 and acromesomelic dysplasias of the Hunter-Thompson, Grebe and DuPan types.

Here we report on 21-year-old male with hypoplasia of all middle phalanges, shortening of all metacarpals except of the 2nd metacarpal, normal height, scoliosis, shortening of his arms' length and Madelung deformity. Complete analysis of the growth/differentiation factor 5 (GDF5) gene identified heterozygosity for a novel splice mutation in intron 3 (c.631+2T>G).

Until now, no splice mutations have been described for GDF5. The phenotype of our patient could not be classified as one of the well-known types of BD, since there are overlapping features of several skeletal malformation syndromes. Hence, our findings extend the spectrum of phenotypes caused by mutations in the GDF5 gene.

P02.047

6p24.2 microdeletion involving TFAP2A without classic features of branchio-oculo facial syndrome.

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Branchio-oculo-facial syndrome (BOF, MIM 113620) results from haploinsufficiency of TFAP2A on 6p24.2. The cardinal features of BOF are pseudo-cleft of the upper lip, brachial sinus/post auricular linear skin lesion, auricular/lip pits, lacrimal duct obstruction, short stature and intellectual disability. Other features described include coloboma of the iris/retina and preaxial polydactyly. Here we describe a mother and daughter with a deletion at 6p24.2 involving TFAP2A but with a clinical presentation that would not have led to a diagnosis of BOF.

The proband was seen at 3-months of age. She was growth restricted at birth (1985gm at 37 weeks gestation). Examination revealed a non-dysmorphic infant with no evidence of a branchial sinus, linear skin lesion or pseudo-cleft of the lip. A blocked left lacrimal duct was diagnosed in the first few weeks of life. Numerous investigations were performed because of growth restriction and poor feeding. Hearing was normal and renal ultrasound revealed mild pelvicalyceal dilatation. Array CGH revealed a 593 kb deletion at chromosome 6p24.2-p24.3 involving TFAP2A.

The infant's 26-year-old mother was also found to carry the deletion. She has a mild intellectual disability and good general health. She had lacrimal duct obstruction until 11 years of age, requiring surgical intervention. She had normal hearing and normal kidneys and was of normal stature. Close examination of her branchial region and lips/philtrum was normal.

This case adds to the growing list of atypical presentations of "classical" single gene disorders which have only come to light in the array CGH era.

P02.048

BRESEK/BRESHECK syndrome and IFAP syndrome are allelic disorder caused by mutation in MBTPS2

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BRESEK/BRESHECK syndrome is a multiple congenital malformation characterized by brain anomalies, retardation, ectodermal dysplasia, skeletal deformities, ear or eye anomalies, and kidney dysplasia/hypoplasia, with or without Hirschsprung disease and cleft palate/cryptorchidism. The syndrome is quite rare, but the combination of ectodermal dysplasia, vertebrae anomaly and Hirschsprung disease is unique to this syndrome.

Here, we report the fourth male patient presenting with brain anomaly, mental and growth retardation, ectodermal dysplasia, vertebral (skeletal) anomaly, Hirschsprung disease, ear anomalies (low-set and large ears), cryptorchidism, and kidney hypoplasia; these manifestations fulfill the clinical diagnostic criteria of BRESHECK syndrome. Since all the patients with BRESEK/BRESHECK syndrome are male, and X-linked syndrome of ichthyosis follicularis with atrichia and photophobia (IFAP) syndrome sometimes associates with some of the features of BRESEK/BRESHECK syndrome, such as mental retardation, vertebral and renal anomalies, and Hirschsprung disease, we analyzed the causal gene of IFAP syndrome, MBTPS2, in our patient and identified an R429H mutation.

This mutation has been reported to cause the most severe type of the IFAP syndrome, including neonatal and infantile death. These results demonstrate that the R429H mutation in MBTPS2 causes BRESEK/BRESHECK syndrome.

Since the original description of IFAP syndrome did not include structural abnormalities, and photophobia, which is one of the triad of IFAP Syndrome, is hardly diagnosed in severely intellectually disabled patients as present case, we propose BRESEK syndrome remains as a clinical entity for diagnosis of congenital anomaly syndrome.

P02.049

Detailed analysis of IGF2 isoforms in patients with imprinting defects

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As part of the network „Imprinting Defects“ we aim to understand the regulatory mechanisms of the human organism to achieve monoallelic gene expression and how this is altered in patients with the imprinting defects Beckwith-Wiedemann (BWS) and Silver-Russell syndrome (SRS). They mostly show epigenetic alterations within two clusters of imprinted genes within the chromosomal region 11p15.5. Altered methylation at additional imprinted gene loci can be found in a significant proportion of BWS and SRS patients with unknown molecular cause or pathogenic consequence. One designated effector gene involved in BWS and SRS growth defects is IGF2. It is hyperactivated by loss of imprinting in most BWS patients and silenced in most SRS patients with 11p15.5 epimutations. The IGF2 gene is transcribed from five promoters, each of which drives the transcription of the coding region with an individual first exon. They differ in allele- and tissuespecificity. In addition an alternative splice site lies within the second coding exon present in all promoter isoforms. We analysed the distribution of IGF2 splice-isoforms and promoter usage in selected human fetal tissues and in primary fibroblasts of BWS and SRS patients as well as in patients with multi locus hypomethylation, a patient with an unrelated overgrowth phenotype and normal controls to determine disease associated isoform usage. In addition we established genetically engineered HEK293 cells with isoformspecific IGF2 overexpression and analysed the potential of enhanced IGF2 thresholds in triggering tumorigenesis by critically altered Akt/mTOR and Erk1/2 pathways.

P02.050

Investigation of notch3 in cadasil in 10 patients in Iran

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Cerebral autosomal dominant arteriopathy with sub cortical infarcts and leukoencephalopathy (CADASIL is an inherited cerebrovascular disease due to mutations of the Notch3 gene at the chromosome locus 19p13. The symptoms of CADASIL are attributed to mutations in this gene. . Allelic variants of Notch3 . In addition to the clinical workup for genetic testing for this mutation can be used in familial or sporadic ischemic disorders of undetermined cause to assist in confirming a diagnosis. our lab‘s CADASIL DNA Sequencing Test will detect approximately 85% of the mutations resulting in the phenotype of the disease. CADASIL experts recommend that people under 65 with the following characteristics should be tested: Depression, memory loss, behavior change, migraine, and/or stroke-like symptoms, such as recurrent stroke at a young age (<29 years old) when associated with prominent white matter disease/diffuse white matter hyperintensities on MRI. • Lack of significant vascular risk factors. It is important to note that because there is no treatment for CADASIL, a similar counseling protocol to Huntington‘s disease should be followed for presymptomatic patients. It is understood that presymptomatic genetic testing for CADASIL can have potential benefit and clinical utility. Often under appreciated, however, are the types of possible adverse outcomes and, the severity and duration of the problems. The American Academy of Neurology has published recommendations and practice guidelines

We have checked eighteen patients since then (referred to our lab or diagnosed by Dr. Aryani). Six of the eighteen cases were affected by CADASIL and the others were normal

P02.051

Eleven patients with Camptodactyly-Arthropathy syndrome in a kindred Turkish family caused by homozygous deletion in PRG4 gene

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The camptodactyly-arthropathy-coxa vara-pericarditis syndrome (CACP) is an autosomal recessive condition characterized by the association of congenital or early onset camptodactyly and noninflammatory arthropathy with synovial hyperplasia. Progressive coxa vara deformity and/or noninflammatory pericardial or pleural effusions have been observed in some patients. CACP is caused by mutations in Proteoglycan 4 (PRG4) gene, encodes a protein that presents in synovial fluid and at surface of articular cartilage and acts as lubricating glycoprotein in protecting joints. We describe two siblings at age of 2 and 9, and two patients who are their second cousins at ages between 14 and 35. It was noticed that 7 distant relatives of these patients share same findings. Swelling of wrist and elbows were earliest symptoms of the disease, even in first years of life. The age of arthropathy onset was 1 years, and camptodactyly began around 4 years old. Severe hip and vertebral involvement were developed during at the age of 20’s. Patients had mild coxa vara. Although none of the patient had pericarditis, sister of one of the patients with same findings died due to cardiac problems at 34 years old. A novel frameshift mutation including 1 bp homozygous deletion (c.1068delA) in PRG4, predicting early truncation of the protein (p.Thr358fs), was found. This is the first report of PRG4 mutation in Turkish family with CACP and supports the hypothesis that only termination mutations cause phenotype since all previously reported PRG4 mutations are predicted to produce a premature termination.

P02.052

A novel missense mutation (c.1442C>A) in the BRAF gene caused Cardio-facio-cutaneous syndrome: Case report

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Cardio-facio-cutaneous syndrome (CFCS) is a multiple congenital anomaly disorder characterized by craniofacial features, cardiac defects, ectodermal anomalies and neurocognitive delay. CFCS is caused by mutations in BRAF, MEK1, MEK2, KRAS genes encoding proteins of the RAS/ MAPK signaling pathway. In more than 70% of CFCS patients, BRAF gene mutations are detected. BRAF is an oncoprotein and somatic mutations occur in BRAF in approximately 8% of all human cancers. The most common germline BRAF mutations have been identified in 7 out of 18 exons (6, 11-16). In this case report, we present a ten-year-old boy who had characteristic craniofacial features of CFCS, short stature, hypertrophic cardiomyopathy, café au lait spots, developmental delay and severe mental retardation. A novel, de novo missense mutation (c.1442C>A) leading to A481E aminoacid substitution in exon 12 of the BRAF gene was detected by sequence analysis. This mutation was not detected in both parents.

It is considered that the novel mutation defined in the case presented causes CFCS with severe mental retardation and the sequence analysis of exons 6, 11-16 of BRAF gene is recommended in the first step for the molecular diagnosis.

P02.053

Copy number variations shape human brain: The contribution of the array-CGH in the understanding of the genetic bases of congenital brain malformations and cognitive disorders

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Copy number variants (CNVs) are genomic segments which are duplicated or deleted among different individuals.

Following the recent technological advances leading to the development of molecular cytogenetic techniques and the emergence of the array comparative genomic hybridization (aCGH), CNVs has gained considerable interest as a source of genetic variation likely to play a role in phenotypic diversity and disease.

Several new genomic disorders caused by CNVs of genes whose dosage is critical for the physiological function of the central nervous system (CNS) have been recently identified.

We applied aCGH in a group of patients presenting CNS anomalies to map novel loci involved in brain malformations. Here, we describe some evidence that CNVs are responsible for congenital CNS anomalies ranging from size anomalies to structural brain malformations and neural migration disorders illustrated, respectively, in patients with microcephaly, agenesis of corpus callosum, and pachygyria.

Here, we discuss the mechanisms mediating these rearrangements and suggest candidate genes for the respective disorders within the mapped loci.

P02.054

Report of one case of cerebro-oculo-nasal syndrome

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Cerebro-Oculo-Nasal syndrome (CONS) is characterized by structural anomalies of the central nervous system, by ocular alterations ranging from anophthalmia/microphthalmia to normal eyes, and by proboscis-like nares. It was first reported by Richieri-Costa and Guion-Almeida in 1993 in two patients with clinical anophthalmia, abnormal nares, central nervous system anomalies, and mental retardation.

In this report, we present an additional sporadic case of this syndrome. A 1-month old boy from non-consanguineous parents with a pregnancy complicated by a gravid diabetes had unilateral anophthalmia, hypertelorism, single nostril orifice and asymmetric mouth. He also had a faun-like ear and three preauricular appendages. Additional findings were umbilical hernia and abnormal dermatoglyphics. CNS malformations will be explored by MRI. Other investigations including cardiac and abdominal ultrasonographies were normal. Karyotype showed a de novo mosaic reciprocal translocation between chromosomes 2 and 3: 46,XY,t(2;3)(p22;p12)[10]/46,XY[46].

Until now about twenty cases of Cerebro-Oculo-Nasal syndrome were reported. Despite marked variability, CONS is so unique that differential diagnosis is extremely limited because of its characteristic nasal configuration. Indeed, our patient presents also proboscis-like nares. Orofacial clefting was variable among reported cases, it was absent in our patient. All cases reported so far have been sporadic suggesting that the syndrome may be due to a new dominant mutation. Characterization of chromosomal breakpoints is planned.

P02.055

Congenital myopathy caused by a novel missense mutation in the *CFL2* gene

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Nemaline myopathy and myofibrillar myopathy are heterogeneous myopathies that both comprise early-onset forms. We present two sisters from a consanguineous Iraqi Kurdish family with predominant axial and limb girdle weakness. Muscle biopsies showed features of both nemaline myopathy and myofibrillar myopathy. We performed homozygosity mapping in both siblings using an Affymetrix 250K Nspl SNP array. One of the overlapping homozygous regions harboured the gene *CFL2.* Because a mutation in *CFL2* was identified in a family with nemaline myopathy, we performed sequence analysis of the gene and a novel homozygous missense mutation in exon 2 (c.19G>A, p.Val7Met) of *CFL2* was identified in both siblings. *CFL2* encodes the protein cofilin-2, which plays an important role in regulation of sarcomeric actin filaments. To our knowledge, this is the second family in which a mutation in *CFL2* causes an autosomal recessive form of congenital myopathy with features of both nemaline and myofibrillar myopathy. Given the clinical variability and the multitude of histological features of congenital myopathies, *CFL2* sequence analysis should be considered in patients presenting with an autosomal recessive form of congenital myopathy.

P02.056

Charcot-Marie-Tooth families analysed with High-Throughput Sequencing

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Background

Charcot-Marie-Tooth (CMT) is the most common inherited neuropathy, affecting 1 per 1,214 persons in the general population. 47 CMT related genes are known to cause CMT by altered gene dosage (20-50 %) or point mutations.

Genetic analysis to discover point mutations have traditionally been performed with Sanger sequencing, a time consuming and expensive analysis. Most genetic laboratories choose to sequence only the most frequently involved genes, meaning that more than 50% of the patients remain genetic undiagnosed.

High-Throughput Sequencing (HTS) opens for the possibility to sequence many genes at the same time, fast and to a low cost compared with traditional methods.

Method

HTS has been used to analyse point mutations in 68 CMT families from a defined epidemiological population. This has been performed by designing a panel containing the known CMT genes, enriching for CMT gene areas. Compared to exome sequencing, sequencing a panel of genes means more samples at shorter time at a lower cost, and fewer problems with unrelated findings.

Results

To our knowledge this is the first report to sequence all the presently known genes related to CMT with HTS for a large population based material. These results will establish the CMT gene frequencies in the Norwegian population which probably might be transferred to other populations.

The results will be presented at the meeting.

P02.057

The clinical presentation of a newborn girl with isodicentric chromosome 22

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We assessed a ten days-old girl with bilateral praeauricular skin tags, downslating palpebral fissures, micrognathia, deep-seated ears and an anal atresia with vaginal fistula. We suspected cat eye syndrome (CES) which was confirmed by subsequent ophthalmologic evaluation describing coloboma of iris, choroid and retina. Accordingly, cytogenetic analysis identified an extra bisatellited marker chromosome in all metaphases of a lymphocyte culture (47,XX,+idic(22) (pter-q11::q11→pter)). The finding was confirmed by FISH and MLPA analyses. Further clinical studies revealed an open foramen ovale and a ductus arteriosus as well as biliary atresia. Jung Min Ko et al. (2010) reported a patient with the same partial tetrasomy of chromosome 22q11.1, but with milder clinical features.

CES is a complex malformation syndrome with a significant clinical variability ranging from severely affected children to phenotypically unaffected parents carrying the same cytogenetic abnormality. Accordingly, only 40% of the CES-patients present the classical triad of symptoms iris coloboma, anal anomalies, and praeauricular pits or tags. In addition, CES may be associated with cardiac defects, hepatic, renal and skeletal abnormalities and mental retardation.

The cause of this clinical variability remains to be determined. The classical CES is present in most cases and characterized by supernumerary bisatellited and isodicentric marker chromosome containing duplicated material of chromosome 22. The phenotype does not correlate with the size of the marker chromosome. It may be speculated whether mosaic conditions, the genetic background and/or epigenetic factors affect the phenotypic presentation. However, life threatening problems to consider are severe cardiac, renal and/ or biliary defects.

P02.058

Chromosome 9p deletion syndrome and sex reversal: Novel findings and redefinition of the critically deleted regions

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Deletions of the short arm of chromosome 9 are associated with two distinct clinical entities. Small telomeric 9p24.3 deletions cause genital anomalies in male subjects, ranging from disorder of gonadal sex to genital differentiation anomalies, while large terminal or interstitial deletions result in 9p-malformation syndrome phenotype. The critical region for non syndromic 46,XY sex reversal was assigned to a 1Mb interval of chromosome 9p, extending from the telomere to the *DMRT* genes cluster. The 9p- syndrome was assigned to band 9p22.3p24.1, but a phenotypic map has not been established for this condition, probably because of the lack of detailed molecular and/or phenotypic characterization, as well as frequent involvement of additional chromosome rearrangements. Here we describe a unique patient with a small isolated 9p terminal deletion, characterized by array-CGH and FISH, who shows a complex phenotype with multiple physical anomalies, resembling the 9p- syndrome, disorder of sex development with gonadoblastoma, congenital heart defect and epilepsy. The observed deletion includes the 46, XY sex-reversal critical region, excluding the region so far associated with the 9p- syndrome. Genotype-phenotype correlations are tentatively established comparing our patient to seven other previously reported males with isolated terminal 9p deletions, finely defined at a molecular level. Our observations expand the 9p deletion clinical spectrum, and add significantly to the definition of a 9p- syndrome critical region.

P02.059
Spondyloepiphyseal dysplasia with luxations, CHST3 type (Recessive Larsen syndrome): Further clinical characterization and report of a novel mutation

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Spondyloepiphyseal dysplasia with luxations, CHST3 type (OMIM143095) is an autosomal recessive chondrodysplasia predominantly affecting joints, spine and epiphyses. Mutations in carbohydrate sulfotransferase 3 (*CHST3*) (OMIM603799) have been identified to cause this condition. We describe the clinical features of three patients in a large highly inbred Indian family with a novel homozygous mutation g.G144S (p.431 G>A) in *CHST3*. All of them had thickened mitral valves and short metacarpals. We emphasize thickened mitral valves, short metacarpals, hallux valgus and bifid epiphysis of distal phalanx of thumbs are additional features of this condition that should be looked into while evaluating patients with multiple joint dislocations.

P02.060
Advances in Cohen syndrome diagnosis using Next Generation Sequencing

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Cohen syndrome is an autosomal recessive disorder characterized by developmental delay, visual impairment, typical facial gestalt (wave-shaped/down-slanting palpebral fissures, short-upturned philtrum, prominent incisors, beak-shaped nose, low-hairline), intellectual deficit (ID) and neutropenia. The conventional mutation screening, performed by DHPLC and/or Sanger sequencing, is time-consuming and has relatively high costs because of the absence of hot-spots and to the high number of exons of the causative COH1 gene. Thus, we designed a Next-Generation Sequencing protocol enabling detection of variants in the whole coding region. We used a method coupling selective amplification to the 454 Roche DNA sequencing platform (Genome-Sequencer-junior). This technology allowed us to identify the second mutation in patients previously analysed by DHPLC and MLPA and to diagnose new patients. Overall the clinical picture of our cohort of 23 patients indicates that the key features of the syndrome are ID, typical facial gestalt, narrow hands/feet with tapering fingers, myopia and/or retinopathy that are present in 100% of cases. Neutropenia, joint hyperlaxity and microcephaly are present in more than 80%. Truncal obesity and social behaviour are present in about 70%. Interestingly, we identified mitral insufficiency in about 20%. In one case aortic and tricuspidal insufficiency was also present. Cardiac anomalies have not been previously reported in patients with documented COH1 mutations. Overall, after the use of a combination of the most sensitive available molecular techniques, the phenotype of Cohen syndrome due to COH1 mutations is quite homogeneous. We thus recommend to request COH1 analysis only for patients with the core phenotype.

P02.061
Monitoring of Congenital Malformations and Hereditary Pathology

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In order to study the frequency of congenital and hereditary diseases developed a computerized data base „Genetic Registry". The register contains information about 1,500 patients and fetuses with birth defects, genetic disorders, their complex inspection and diagnosis. The structure of the register: birth defects - 759 (51%), chromosomal abnormality - 338 (22.5%), monogenic disorders - 302 (20%), pregnant women at genetic risk - 101 (6.5%).

The 19 registered nosologies that according to the list of the International Registry EUROCAT: anencephaly - 2 (0,2%), spina bifida - 16 (2.1%), encephalocele - 2 (0.2%), hydrocephalus - 43 (5.6%), microtia - 4 (0.5%), cleft palate, cleft lip - 71 (9.3%), congenital heart diseases - 78 (10.2%), atresia of the esophagus - 14 (1.8%), reduction deformities of limbs - 2 (0.2%), polydactyly - 8 (1%), diaphragmatic hernia - 31 (4%), renal agenesis and dysgenesis - 94 (12.3%), herniated umbilical cord - 6 (0.7 %), gastroschisis - 8 (1%), Down's syndrome - 178 (23.4%), multiplex birth defects - 110 (14.5%).

Thus, the register will allow determining the frequency and structure of the congenital malformations and hereditary diseases, to monitor the dynamics of the load of hereditary and congenital disorders in the population of the Republic of Kazakhstan.

P02.062
New TUBB3 gene mutation in a woman with CFEOM3 and brain abnormalities

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Congenital fibrosis of extraocular muscles type 3 (CFEOM3) is a cranial dysinnervation disorder characterized by ophthalmoplegia and ptosis, thought to result from defect in axonal guidance. Isolated CFEOM3 results from mutations in microtubules-associated KIF21A and TUBB3 genes. CFEOM3 caused by TUBB3 gene mutation may also be associated with facial weakness, congenital contractures, intellectual deficiency, progressive polyneuropathy and brain anomalies as dysgenesis of the corpus callosum, anterior commissure, internal capsule, corticospinal tracts and basal ganglia. Other mutations of the same gene are reported in patients without CFEOM who present a wide range of cortical dysplasia including neuronal migration disorders associated with pontocerebellar hypoplasia.

We report on a 26 years old woman who suffered from severe congenital strabismus, ophthalmoplegia, unilateral ptosis and psychomotor retardation. CFEOM3 was diagnosed during strabismus surgery. She also has minimal pyramidal syndrome with conserved muscular strength but fatigability and slowness. Electroencephalogram, somatosensory evoked potentials and intelligence were normal. Magnetic resonance imaging showed dysplastic and hypoplastic vermis, dysmorphic and small corpus callosum, hypoplastic right cerebral hemisphere and peduncle, absent anterior commissure, asymmetric caudate nuclei and dilated ventricles.

Therefore, we sequenced TUBB3 gene in this patient and found a de novo heterozygous missense mutation. This mutation affects a highly conserved amino acid, is predicted to be damaging by Polyphen2 software and was not reported in patients with CFEOM3 (Tischfield, 2010) nor cortical dysplasia (Poirier, 2010) until now. Our finding will help to determine the genotype-phenotype correlation of the TUBB3-related spectrum.

P02.063
Unknown CNVs in cohort of 52 Bulgarian patients with learning disability and congenital malformations

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Introduction. The increasing resolution of DNA-microarrays and the techniques optimization allow detection of larger (> Mb) aberrations and a large number of small CNVs, whose clinical significance in some cases is unknown. The interpretation of CNVs with unknown clinical significance remains still a challenge.

Materials and methods. Oligo array-CGH was applied in 52 patients with developmental delay and multiple congenital anomalies in order to unravel the underlying genetic abnormalities. We have used BlueGnome CytoChip oligo 2X105K microarray, v1.1, with 35kbp backbone resolution.

Results. A total of 247 CNVs were detected, of which 15 pathogenic (7 deletions, duplications 8), 124 normal (62 deletions, 62 duplications) and 108 with unknown clinical significance (68 deletions, 40 duplications).

Discussion. Due to insufficient data for the Bulgarian population we made an individual assessment of each variant. In our study 19 variations in chromosomal loci 2q37.3, 10q11.22, Xp22 were found in over 5% patients, which gave us a reason to suppose that they were probably not pathogenic. Twenty-five of the variations occurring in patients with established large pathological aberrations associated with specific phenotype. Therefore, we have assumed that probably they have no pathogenic nature. Thirty-five of the other variants do not contain OMIM genes.

Conclusion. So from total 108 unknown CNVs as potentially pathogenic remain to be seen only 29 variations. Of these, only one aberration in Xq22.1 can be directly related to patient's clinical phenotype. This gave us a reason to accept this deletion as potentially pathogenic.

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P02.064

Congenital myasthenic syndromes: impact of genotype-phenotype correlation on strategy and efficiency of genetic testing

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Congenital myasthenic syndromes (CMS) are clinically and genetically heterogeneous disorders characterized by a neuromuscular transmission defect. Even though CMS are genetic disorders they are highly treatable, and the appropriate drug treatment depends on the underlying genetic defect. This highlights the importance of genetic testing in CMS. In recent years, the molecular basis of CMS has constantly broadened and disease-associated mutations have been identified in 14 genes encoding proteins of the neuromuscular junction. In the dawn of novel sequencing strategies we report on our 14-year experience in traditional Sanger-based mutation screening of a large cohort of 680 independent patients with suspected CMS. In addition to most known CMS-causing genes, we analyzed the functional candidate genes LRP4, VACHT, and CNTN1. In total, we identified disease-causing mutations in 299 patients (44%) of patients in various known CMS genes, confirming the high degree of genetic heterogeneity associated with the disease. Apart from four known founder mutations, and a few additional recurrent mutations, the majority of variants are private, found in single families. Genotype-phenotype correlations reported previously in the literature were extended and refined. Based on these results, we propose an algorithm for genetic testing in CMS.

P02.065

Management of Pain and Fatigue in The Joint Hypermobility Syndrome (Ehlers-Danlos Syndrome, Hypermobility Type): Principles and Proposal for a Multidisciplinary Approach

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Joint hypermobility syndrome (JHS), or Ehlers-Danlos syndrome hypermobility type (EDS-HT), is a underdiagnosed heritable connective tissue disorder characterized by generalized joint hypermobility and a wide range of visceral, pelvic, neurologic and cognitive dysfunctions. Deterioration of quality of life is mainly associated with chronic pain and fatigue. Except for the recognized effectiveness of physiotherapy for some musculoskeletal features, there are no standardized guidelines for the assessment and treatment of pain and fatigue. In this work, a practical classification of pain presentations and factors contributing in generating painful sensations in JHS/EDS-HT is proposed. Pain can be topographically classified in articular limb (acute/subacute and chronic), muscular limb (myofascial and fibromyalgia), neuropathic limb, back/neck, abdominal and pelvic pain, and headache. For selected forms of pain, specific predisposing characteristics are outlined. Fatigue appears as the result of multiple factors, such as muscle weakness, respiratory insufficiency, unrefreshing sleep, dysautonomia, intestinal malabsorption, reactive depression/anxiety and excessive use of analgesics. A set of lifestyle recommendations to instruct patients as well as specific investigations aimed at characterizing pain and fatigue are identified. Available treatment options are discussed in the set of a structured multidisciplinary approach based on reliable outcome tools.

P02.066

Cornelia de Lange syndrome with mutation in NIPBL and mosaic Turner syndrome in the same individual

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Cornelia de Lange syndrome (CdLS) is a dominant inherited disorder characterized by facial dysmorphism, growth and cognitive impairment, limb malformations and multiple organ involvement. Mutations in *NIPBL* gene etiologically account for 60% of CdLS patients. This gene encodes a key regulator of the Cohesin complex, which controls sister chromatid segregation. TS affects about one in 2000 live born females and results from complete or partial absence of one of the X chromosomes, frequently accompanied by cell-line mosaicism.

Here, we report a patient with CdLS due to a mutation in the *NIPBL* gene (c.1445_1448delGAGA) and mosaic TS (mos 45,X/46,XX karyotype). This patient showed the classical and predominant phenotype of CdLS, although without limb reduction. She was also clinically diagnosed with TS because of two typical recognizable features: the peripheral lymphedema and the webbed neck. Molecular characterization showed that the *NIPBL* mutation was present in all the tissues analyzed from different embryonic origins (mesoderm and ectoderm); while FISH analyses revealed that the percentage of cells with monosomy X was low and tissue-specific.

These findings indicate that, ontogenically, the *NIPBL* mutation appeared before the mosaic monosomy X. Moreover, the recent identification of frameshift *NIPBL* mutations in colon cancer cells associated with chromosome aneuploidy suggests that it could affect faithful chromosome segregation.

The coexistence in several patients of both these rare disorders raises the issue of whether there is indeed a cause-effect association. We hypothesize that the NIPBL mutation might be responsible for the loss of one of the X chromosomes in this patient.

P02.067

Costello syndrome - a long way to diagnosis

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Costello syndrome is an autosomal dominant disorder associated with postnatal growth retardation, facial dysmorphic features, intellectual deficit as well as skin and cardiac abnormalities. Only a small number of cases with Costello syndrome has been identified so far. The majority of cases is caused by de novo mutations in the ras family oncogene (HRAS - localized in 11p15.5). Here we report a case that took a long time to be diagnosed correctly.

The 26 months old girl presented with respiratory and feeding problems, postnatal growth retardation with relative macrocephaly, typical facial dysmorphism, cardiac arrhythmia, deep creases of hands and feet, loose abundant skin and sparse curly hair. Conventional chromosome analysis revealed a normal female karyotyp 46,XX. Previously performed testing including array-CGH, muscle biopsy, enzyme histochemistry, quantitative Western blot, FAMILION screening for various heart related ion channel mutations and amino acid screening in liquor were without pathological findings.

Due to the patient`s phenotypical characteristics we initiated the molecular analysis of the HRAS gene. After more then two years of diagnostic procedures Costello syndrome could finally be confirmed by a heterozygous mutation c.34G>A, p.G12S, in exon 2 as a de novo mutation. Equal contribution of the first and the second author.

P02.068

Two cases of craniofrontonasal dysplasia

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Craniofrontonasal dysplasia (CFND) is an X-linked craniofacial disorder with an atenuated manifestation in males, in which affected females show distinct picture of skeletal malformations incuding striking hypertelorism, synostosis of coronal suture, syndactyly and preaxial polydactyly but without intelectual deficit.

We report 2 new cases - the first girl without positive family history shows typical craniofacial features with aditional nonfrequent signs - hypoplasia of corpus callosum, asymetrical mammils, axilar pterygium and slight hypoplasia of pectoral muscle on the left side combined with synpolydactyly of left thumb.

Mutation analysis revealed a new non published mutation c.161>A (change p.Pro>Gln in codon 54) in exon 2 of EFBN1 gen. The father didnot show hy-

pertelorism and later was excluded as a carrier of causal mutation.
Second case - newborn girl expresed craniostenosis of coronal suture with typical hypertelorism, hypoplasia of corpus callosum and suspection from hearing loss (patological TEOAE). First suspection from craniofacial dysmorphism was on prenatal ultrasound screening where picture of cloverleaf skull was seen.
Mutation analysis revealed known causal mutation c.451G>A in exon 3 of EFBN1 gen. The father has significant hypertelorism, MG findings in father and other family members are not available yet.

P02.069
Identification of a novel EFNB1 mutation in a patient with Craniofrontonasal Syndrome and right hallux duplication

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Craniofrontonasal syndrome (CFNS) is a rare X-linked dominant disorder characterized by a more severe manifestation in heterozygous females than in hemizygous males. Typical manifestations involve hypertelorism with telecanthus, widow's peak, frontal bossing, craniosynostosis, a bifid or broad nasal tip, wiry hair, and grooved fingernails. Besides, anterior cranium bifidum, axillary pterygia, joint abnormalities, cleft lip and palate, unilateral breast hypoplasia, diaphragmatic hernia, asymmetric lower limb shortness, and agenesis of the corpus callosum are within the rare manifestations of CFNS. Most CNFS patients have mutations in the *EFNB1* located at chromosome Xq13.1. This gene encodes a member of the ephrin family protein, Ephrin B1, which interacts with Eph tyrosine kinase receptors. It functions in the formation of tissue boundaries. Here, we report a 7-month-old female patient who has brachycephaly, frontal bossing, hypertelorism, telecanthus, downslanting palpebral fissures, broad nasal root and bifid nasal tip. She also had large anterior fontanelle and broad right hallux. To confirm CFNS diagnosis, *EFNB1* was sequenced and a novel *de novo* c.402 T>C heterozygous mutation in exon 2 was detected. This change is not reported in the SNP databases. Isoleucine is a highly conserved amino acid and its replacement to threonine in codon 134 may result in a conformational change in the protein. Parents were normal both clinically and genotypically, confirming *de novo* mutation in the patient. This mutation was not previously reported for CFNS.

P02.070
Can *FGFR2* mutations explain craniosynostosis with hydroureteronephrosis?

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Fibroblast growth factor receptor 2 (FGFR2) mutations are well known in syndromic craniosynostosis. We report the first case of *FGFR2* mutation associated with craniosynostosis and bilateral hydroureteronephrosis.

During the second pregnancy of unrelated parents, a megacystis and a megaureter were detected by ultrasound examination at 10 weeks-of-gestation (WG). Subsequently craniosynostosis of the metopic, sagittal and coronal sutures was seen at 26 WG. Chromosomes on amniotic fluid were normal. Termination of pregnancy was performed at 28 WG. Pathology confirmed the renal malformation and a trigonocephaly. Molecular analysis found a *de novo* heterozygous *FGFR2* mutation that had been previously reported in Crouzon, Pfeiffer and Jackson-Weiss syndromes.

Rare renal malformations have been previously clinically reported with syndromic craniosynostosis. *In vitro* studies have shown that FGFRs are expressed in the ureteric bud and in the metanephric mesenchyme of the developing kidney. Exogeneous FGFs affect growth and maturation of both organs in cultured tissues. *In vivo* studies on mice demonstrated that the loss of *fgfr2* often lead to multiple ureteric buds, renal dysplasia and obstructed hydroureter. Therefore the *FGFR2* signaling pathway is critical at early and later stages of kidney development.
Other studies showed *FGFR1* and *FGFR2* overexpression in renal cell carcinoma. A case of bladder papillary carcinoma with Apert syndrome and a germline *FGFR2* mutation has also been previously reported.

Thus, we think that *FGFR2* loss-of-function mutation in this case could explain the whole phenotype. This report highlights the probable relationship between *FGFR2* and renal and vesicoureteral abnormalities in human embryonic development.

P02.071
Ventricular septal defect in crouzon syndrome: case report

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Crouzon Syndrome (CS) is an autosomal dominant hereditary disease, which is characterized by clinical triad of cranium deformity, facial anomalies and exophthalmia. Crouzon Syndrome is caused by the mutations in the FGFR2 gene. Same gene mutations are observed in Apert, Pfeiffer and Jackson-Weiss syndromes as well. Cardiac anomalies are detected in 10 % of the patients with Apert Syndrome. According to the literature, cardiac anomalies have been reported in only three patients with CS, but there is no report on cardiac anomalies, neither in patients with Pfeiffer nor the ones with Jackson-Weiss syndrome. We describe here a 10 year old CS patient with ventricular septal defect. A heterozygous 868G>C (Trp290Arg) mutation was detected by sequence analysis in the FGFR2 gene. The patient's mother with similar craniofacial features had the same mutation.
Key words: Crouzon Syndrome, FGFR2 gene, ventricular septal defect

P02.072
A CSF1R mutation in a family with hereditary diffuse leukencephalopathy with spheroids

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Mutations in the colony stimulating factor 1 receptor gene (*CSF1R*) have recently been shown to cause hereditary diffuse leukencephalopathy with spheroids (HDLS), an autosomal-dominant disease leading to progressive cognitive and motor dysfunction. We describe a family from Northern Germany with an apparently autosomal-dominant early onset frontotemporal dementia syndrome. Multiple MRI scans of a 38 year old clinically presymptomatic woman showed slowly progressive bifrontotemporal cortical atrophy and asymmetric hyperintense white matter lesions. Her father and paternal grandfather both suffered of early onset and rapidly progressing dementia and personality change starting in their late 40s, leading to death within a decade. An underlying CADASIL in the family was excluded as molecular analysis of *NOTCH3* showed no pathogenic mutation. Next-generation-sequencing of nine known early-onset dementia genes identified the very likely heterozygous disease causing mutation c.2381T>C (p.I794T) in the *CSF1R*-gene in the female index patient.

P02.073
Danon disease - different phenotypic expression a family with hypertrophic cardiomyopathy

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The rare X-linked dominant lysosomal disorder called Danon disease, is the consequence of the mutation of the lysosome - associated membrane proteine 2 gene (LAMP2), affecting myocytes and skeletal muscles by accumulation of intracytoplasmatic autophagic vacuoles. The characteristic clinical triad of the Danon disease include mental retardation, severe hypertrophic cardiomyopathy, skeletal myopathy.
We describe a case of Danon disease, belonging to a family with known hypertrophic cardiomyopathy with 3 affected generation. The grandmother and mother are known with mild hypertrophic cardiomyopathy. A sister and brother of the affected mother, were both diagnosed with hypertrophic cardiomyopathy and died at 24 years of age, respectively at 36, despite a pacemaker implantation.
The son was diagnosed at the age of 14 years with mild mental retardation, limb-girdle dystrophy, severe hypertrophic obstructive cardiomypathy, WPW syndrome. He presents a massive concentric ventricular hypertrophy of 45 mm, and 2 years later atrial flutter occurred. The parents refused heart transplantation or myomectomy and a defibrilator was implanted. The patient has 2 healthy sisters, were the genetic counseling is of greatest importance.
Conclusion: The LAMP2 mutation was proved as an important cause of massive cardiac hypertrophy with high mortality in the absence of heart

transplantation. The variable phenotypic expression with a usually milder clinical presentation in females is the case in our patients too.

P02.074

Darier Disease - a family case report

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INTRODUCTION: Keratosis follicularis, also known as Darier disease or Darier-White disease, is an autosomal dominantly inherited genodermatosis characterized by greasy hyperkeratotic papules in seborrheic regions, nail abnormalities, and mucous membrane changes.
The disease was first reported independently by Darier and White in 1889.
MATERIAL AND METHODS: We present a family with Darier Disease on three generations.
Dermatological examination and complete general examination of the patients showed **skin rash,** lesions on the hands and nails and lesions affecting the mucous membranes.
Skin Biopsy, histopatologically examination of the skin and DNA Analysis was necessary to confirm the diagnosis.
RESULTS: A skin biopsy show characteristic degeneration of cells in the epidermis (acantholysis) and abnormally increased keratinisation (hyperkeratinisation).
DNA Analysis of all the patients revealed mutations in the ATП2A2 gene.
CONCLUSIONS: We present a family with Darier Disease on three generations, a rare disorder with variable penetrance, clinical and genetic variability in the same family.

P02.075

Clinical manifestation of congenital bilateral, profound sensorineural hearing loss and adult-onset retinitis pigmentosa is not always Usher syndrome

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Usher syndrome is an autosomal recessive condition characterized by a combination of congenital hearing impairment and retinitis pigmentosa (RP). Three types of Usher syndrome are known and differ by the time of onset of the symptoms, severity and progressiveness of deafness and additional vestibular dysfunction. Several genes are known to be associated with the disease. Patients with type II Usher syndrome have congenital bilateral sensorineural hearing loss that is mild to moderate in the low frequencies and severe to profound in the higher frequencies, intact vestibular responses, and bilateral retinitis pigmentosa (RP).
We describe a 30 years old female whose parents are first cousins of Moroccan origin. She has congenital deafness and has been referred by her ophthalmologist because of recently diagnosed RP. A clinical diagnosis of Usher syndrome type II has been suggested. However a molecular workup including testing of the three genes known to cause the disease (*USH2A, GPR98* and *DFNB31)* were all negative.
A founder mutation (c.1355_6delCA, p.Thr452SerfsX3) in the RP gene *FAM161A* has been later described in Moroccan-Jews. Our patient was found to be homozygous to the founder mutation and her parents were heterozygous to this mutation. Information was given to the family during genetic counseling explaining that Usher syndrome was ruled out and a plausible explanation would be that she is expressing two independent diseases. Recently she was tested for mutation in the TMC1 gene associated with autosomal recessive deafness and was found homozygous for the c.1939T > C deleterious mutation.

P02.076

Interstitial 9q34.11-q34.13 deletion in a patient with severe intellectual disability, hydrocephalus and cleft lip/palate

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Interstitial deletions of chromosome bands 9q34.11-q34.13 are rare. We report on a 16-year-old female patient with severe intellectual disability, congenital hydrocephalus, cleft lip and palate, talipes equinovarus, epilepsy, kyphoscoliosis, convergent strabismus, severe short stature, dystrophy and facial dysmorphic signs. Array analysis revealed a 3.7 Mb interstitial deletion in 9q34.11-q34.13. The deletion harbors more than 60 genes, including SPTAN1, DYT1/TOR1A, ABL1, ASS1, LAMC3, POMT1, DOLK and GLE1, mutations in which have previously been associated with monogenic disorders. This is the first patient with a deletion of this size and position in 9q34.11-q34.13. Reports of additional patients with aberrations in this region will be needed to establish karyotype-phenotype correlations and to gain information on the contribution of individual genes for the clinical manifestations.

P02.077

Interstitial deletion of 3p22.1p24.1 including haploinsufficiency of the *MLH1* gene in a boy evaluated for developmental delay, short stature and physical anomalies

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Introduction: Molecular karyotyping is a well established method in the evaluation of children with developmental disorders and/or physical anomalies leading to the diagnosis of a relevant chromosomal imbalance in 10-15% of cases. However, unanticipated findings may be a major challenge. We present the case of a boy with developmental delay and multiple physical anomalies in whom array analysis revealed a concomitant diagnosis of a tumor disposition syndrome.
Case Report: A boy of 4 years of age was evaluated for short stature, psychomotor retardation, hypotonia and physical anomalies (e.g. hypospadias, pectus excavatum). Physical examination also showed facial dysmorphism (flat face, down-slanting palpebral fissures), inverted nipples and hyperemia of fingertips and toes. Array analysis (Affymetrix® CytoScan HD) showed a *de novo* 9.5 Mb interstitial deletion on chromosome 3p22.1-p24.1 not detectable by G-banding analysis. The deletion was confirmed by FISH analysis with probes specific for 3p22.3. The deleted region comprised about 60 refseq genes among which *MLH1* (confirmed by MLPA).
Discussion: Overlapping interstitial deletions of 3p22-p24 so far have been rarely reported and most often predate molecular karyotyping. They are associated with global developmental delay, CHD, short stature and mild dysmorphic features. In the present case, based on haploinsufficiency of the mismatch repair gene *MLH1,* a diagnosis of Lynch syndrome (HNPCC) was also established and surveillance guidelines were discussed with the parents.
Conclusion: This case illustrates that any chromosomal imbalance has to be evaluated carefully for alterations potentially affecting cellular key pathways as deletions of tumor suppressor genes with implications for health management.

P02.078

After a long diagnostic journey: Duplication 17q25.1 in a child with multiple disabilities

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About 20% of the children with syndromic appearence show submicroscopic duplications and deletions, which are detectable by high resolution Array CGH-diagnostic platforms. We give an account of a meanwhile nearly eight years old disabled girl, who had already been presented to a genetic counsellor as a new born child because of prematurity (34th weeks of gestation), microcephalus, atrial septal defect, stenosis of the left bronchus, sickle feet, muscle weakness, dysmorphic features and feeding problems. A chromosomal analysis was performed and showed a normal female karyotype. In the further course of her development, the girl showed profound psychomotor retardation, speech delay, mental retardation, recurring infections and early behavioural problems. On the occasion of the second presentation at the age of 2 ½ years to the genetic counsellor the first array-CGH was performed using a low-resolution BAC array (resolution 0,44 Mb). The result was interpreted as normal. Because of the persisting impression of a syndromal clinical picture the parents continued presenting her daughter to the genetic counsellor. An Angelman-Syndrome as well as a Rett-Syndrome were excluded. Finally the Array-CGH-examination was repeated using a higher resolution (resolution 25 to 100 kb) and revealed a 830 kb duplication on 17q25.1 that is thought to be causal. The results of the parental analysis will be presented. This case emphazises the benefit of applicating a high resolution array after a normal result with a low resolution array.

P02.079

22q13.3 deletion syndrome - report of three cases

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Phelan-McDermid syndrome (22q13.3 deletion syndrome) is characterized by neonatal hypotonia, global developmental delay, absent to severely delayed speech, and normal to accelerated growth. Most individuals have moderate to profound intellectual disability. Other features include large fleshy hands, dysplastic toenails, and decreased perspiration that results in a tendency to overheat. Behavior characteristics include mouthing or chewing non-food items, decreased perception of pain, and autistic-like affect.

Phelan-McDermid syndrome results from terminal or interstitial deletion of chromosome 22q13.3. On rare occasion an apparently balanced chromosome rearrangement or a mutation disrupts SHANK3. We performed MLPA and whole genome array CGH analysis in 68 patients with mental retardation/congenital anomalies.

In three patients the analysis revealed the presence of interstitial deletion of 22q13.3 chromosome region encompassing SHANK3 gene. In two of them this finding was combined with duplication of 20p chromosome. The severity of the clinical presentation varies among the three patients. All of them have abnormal behavior phenotype (autistic like). The impact of the additional finding of 20p duplications to the clinical features will be discussed.

P02.080

A unique case of familial Diamond-Blackfan anemia

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Diamond-Blackfan anemia (OMIM #105650) is a rare, autosomal dominant condition characterized by congenital erythroid aplasia with normal leukocytes and platelets. Half of the patients have associated malformations of upper limb and craniofacial region and are growth retarded. DBA cases are mostly sporadic, only 10-25% are familial. DBA is genetically heterogeneous. Causal variants have been identified in nine ribosomal genes, and diagnostic tests are clinical available for only a limited number of them.

We present a 6 year old boy with classical DBA. He was diagnosed in early childhood with severe anemia, is currently transfusion dependent and on steroid treatment. He has short stature and dysmorphic facial features. His paternal grandfather had DBA and died of liver sarcoma, 26 years old, after androgen treatment. The boy's father is asymptomatic.

Exome sequencing (Agilent 38Mb exome capture and 2x100bp Illumina sequencing) detected a novel heterozygous deletion in *RPS26* (c.6_9del). The deletion leads to a framshift, and will most likely cause a premature stop-codon and induce nonsense mediated decay. Sanger sequencing showed that the variant was paternally inherited. Variants in *RPS26* are estimated to cause 2.6% of the DBA cases (DBA10; OMIM #613309). A molecular diagnosis should enable prenatal diagnostic testing, however predicting the diagnostic outcome of an affected fetus is challenging in this family.

This case illustrates the force of targeted exome sequencing to efficiently identify novel variants in a large set of candidate genes, and demonstrates its clinical utility to identify causal variants in rare dominant disorders.

P02.081

Two patients with Diamond-Blackfan anemia: a novel point mutation in RPL5 and a microdeletion encompassing RPL5

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Diamond-Blackfan anemia (DBA; MIM #105650) is a rare congenital red blood cell aplasia with an increased risk of malignancy. DBA exhibits an autosomal dominant pattern of inheritance with incomplete penetrance. Approximately 50% of affected individuals show congenital malformations. DBA has been associated with mutations in nine genes that encode ribosomal proteins, amongst others RPL5. Mutations in RPL5 and RPL11 are more frequently associated with additional congenital malformations compared to the other genes.

We present two patients with DBA: patient 1 carries a missense mutation in RPL5 inherited from the unaffected mother. The mutation c.625C>T (ENST00000370321;p.Arg209Cys) has not been reported in the literature to date but was predicted as disease causing by the prediction tool MutationTaster (www.mutationtaster.org). The girl was hypotrophic at birth, and presented with triphalangeal thumbs as well as unilateral renal agenesis. She did not show any facial dysmorphism or cardiac anomalies.

In patient 2 array CGH analysis detected a de novo 594kb microdeletion on chromosome 1p22.1 encompassing RPL5. The girl was also hypotrophic at birth, and was additionally diagnosed with complex cardiac malformations. All developmental milestones were delayed. At age three years she presented with short stature, brachycephaly, arched eyebrows, left-sided ptosis, short neck, and finger-like thumbs.

We compare the phenotypes of our two patients with distinct mutations affecting the RPL5 gene.

P02.082

Diencephalic-mesencephalic junction dysplasia: A novel recessive brain malformation

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We describe six patients from three unrelated consanguineous Egyptian families with a novel characteristic brain malformation at the level of the diencephalic-mesencephalic junction (DMJ). Diagnostic testing including high resolution karyotyping and extended metabolic screening were normal. Brain MRI demonstrated a dysplasia of the DMJ with a characteristic "butterfly"-like contour of the midbrain on axial sections. Additional imaging features included variable degrees of supratentorial ventricular dilatation and hypoplasia to complete agenesis of the corpus callosum. Diffusion tensor imaging showed diffuse hypomyelination and lack of an identifiable corticospinal tract. All patients displayed severe cognitive impairment, postnatal progressive microcephaly, axial hypotonia, spastic quadriparesis and seizures. Autistic features were noted in older cases. Talipes equinovarus, non-obstructive cardiomyopathy and persistent hyperplastic primary vitreous were additional findings in two families. One of the patients required shunting for hydrocephalus, however, this yielded no change in ventricular size suggestive of dysplasia rather than obstruction. We propose the term diencephalic-mesencephalic junction dysplasia (DMJD) to characterize this autosomal recessive malformation.

P02.083

De novo duplication 15q22.21-24.1 in patient with mental retardation, congenital heart defect and dysmorphic features

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We report on a 25 years old female referred for evaluation because of intellectual disability, atrioventricular communication, muscle weakness, neurogenic urinary incontinence and dysmorphic features (long trunk, low forehead with hypertrichosis, strabismus, depressed nasal bridge, full cheeks, retrognathia, high narrow palate, malposition of one central incisor, malocclusion of teeth, brachydactyly of the fingers, small and flat feet). Proband's mother had a congenital heart defect. The propositus was born in a normal delivery but with lower birth-weight (2800 g). In infancy she had poor suck reflex, considerable hypotonia and severely retarded psychomotor development.

Initial karyotype analysis revealed normal female karyotype. Array-CGH (400K) showed and FISH confirmed a *de novo* tandem interstitial 9.0 Mb duplication of 15q22.21-24.1. The duplicated region is gene-rich, encompassing more than 140 genes. At least 11 of duplicated genes are associated with known diseases, including *CLN6, NCH4, KBTBD13, MAP2K1.* The other relevant genes (*MEGF11, ZNF609, TIPIN, PAQR5*) are involved in brain development and functioning. Proband's phenotype is similar to previously reported overlapping duplication (22,23 Mb) in *DECIPHER.*

The abnormal phenotype could be determined by overdose of duplicated genes, the disturbance of genes regulatory sequences, or the excess genetic material which may disorganize chromatin conformation affecting the expression of distance genes. Given its large size, high gene content and de novo origin, the observed duplication is considered as pathogenic and responsible for the clinical phenotype manifesting in our patient.

P02.084

The influence of donor DNA level in blood of simultaneous pancreas kidney allograft recipients as a diagnostic factor for rejection

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Grafted organ releases into circulation system some cells, DNA and proteins which are partially destroyed by the recipient's phagocytes but they can be detected in spleen dendritic cells. The aim of our research was to study the possible effects of microchimerism in pancreas and kidney rejection. In our previous studies, we looked for the presence of donor-specific STR loci in the recipient blood. In this study we have examined plasma and mononuclear whole blood cells of recipients to assess the amount of SRY gene in sex-mismatched combinations and phospholipase A2-HUMPLA2A1(AAT)n in the same sex recipients after simultaneous pancreas and kidney transplant, in relation to the patients probes before grafting by the use of the Real-time PCR method. Recipient's blood and donors spleen samples were collected before transplantation and at different times after grafting. Genomic DNA was isolated from blood and tissues. The amount of DNA in different samples after tx was calculated by using the comparative Ct method with GAPDH as internal control. We observed increase donor's DNA level in recipient's blood already on day 1 after grafting (STR loci). The quantitative indication of donor DNA in recipient's blood (SRY gene) was possible in 7 day after graft. In case of HUMPLA gene, our results showed that the relative amount of that gene was higher in mononuclear blood cells than in plasma 14 days after transplantation. Donor DNA is present either in passenger cells or recipient's phagocytes. An open question remains whether it may be incorporated into recipient cell genome.

P02.085

Molecular diagnosis of common mutations in COL7a1 Gene among Iranian patients suffering from Epidermolysis Bullosa

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Background: The dystrophic forms of Epidermolysis Bullosa (DEB), a group of heritable blistering disorders, show considerable phenotypic variability, and both autosomal dominant and autosomal recessive inheritance can be recognized. DEB is derived from mutations in the type VII collagen gene (COL7A1),

It has been reported that most mutations detected in the recessive disease form are nonsense mutations or small insertions or deletions leading to frame shift and premature translational termination, which tend to produce severe phenotypes. In contrast, missense mutations causing amino acid substitutions, which result in variable phenotypes, predominate in the dominant form of dystrophic Epidermolysis Bullosa.

Methods: DNA specimens (Genomic DNA from the patient) from 50 affected patients, clinically diagnosed, were subjected to mutation analysis by PCR using designed primers for hotspot exons of COL7a1, followed by sequencing of the PCR products.

Result: Studying COL7a1 hotspot exons, 73, 74, 75, showed that 11 out of 50(27%) of probands have mutation. Among these mutations 2 cases were compound heterozygotes, the other 2 cases were deletions and the rest were missense substitutions. A novel mutation has been identified within all mentioned mutations above.

P02.086

Molecular findings in patients with isolated ectopia lentis - results of *FBN1* and *ADAMTSL4* mutation analyses

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Ectopia lentis (EL) can occur as an isolated condition or as a feature of syndromal diseases, such as Marfan syndrome (MFS). Isolated ectopia lentis has been associated with mutations in *ADAMTSL4* (autosomal-recessive) and *FBN1* (autosomal-dominant).

We have analyzed 8 patients from 6 families presenting with isolated EL. 4 patients had homozygous mutations in *ADAMTSL4*: 2 unrelated patients (14 and 34 years) carried the common mutation c.767_786del20, p.(Gln256Profs*38); in two siblings (4 and 5 years), the homozygous mutation c.1162dupG; p.(Ala388Glyfs*8), which has not been yet reported, was identified. None of the patients had a history of systemic involvement reported with syndromal EL. We additionally identified 2 heterozygous mutations in *FBN1*. A de novo *FBN1*-mutation c.4043G>A ;p.(Cys1348Tyr), that has not been reported previously, was detected in a 4 year-old child with EL; other features of MFS were not (yet) obvious. The mutation c.2415T>G; p.(Cys805Trp) was identified in a 13-year-old girl with EL not fulfilling MFS diagnostic criteria. She inherited the mutation from a parent who had a history of bilateral lens extraction in childhood and no further clinical signs of MFS. Furthermore, there was a family history of aortic (abdominal) aneurysms. One child with EL neither had a *FBN1* nor *ADAMTSL4* mutation.

These results emphasize the importance of mutation analysis of *FBN1* and *ADAMTSL4* in patients with ectopia lentis, especially in young children since patients with mutations in *ADAMTSL4* do not need the extensive screening exams (especially regarding cardiovascular complications) as patients with a *FBN1* mutation.

P02.087

Translation initiation factor *EIF3A* haploinsufficiency appears to be a neutral variant despite *de novo* occurrence in a patient with developmental delay

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In a 5 year old boy with idiopathic congenital hypothyroidism detected at birth, mild developmental delay, normal stature (25 centile) and microcephaly (1 cm < 2.5 centile), a 0.1 Mb *de novo* duplication was found seemingly disrupting the *EIF3A* gene of which was later confirmed by molecular investigations and supposed pathogenic. In another patient a routine SNP-array test revealed a 1.4 Mb 10q26.11 deletion affecting 12 protein coding genes, including *EIF3A*. This patient was a 15 year old girl with mild ID, short stature (2.5 centile) and normal head circumference (25-50 centile). The deletion turned out to be maternal, and the mother was completely healthy and could report of average or above-average school performance. Our initial assumption that the *de novo* disruption of the *EIF3A* gene was likely to be pathogenic, given the importance of eIF3A for e.g. normal translation and ERK signal transduction, had to be revised. The dissimilar phenotypes additionally support the hypothesis that *EIF3A* deletions are neutral. This is especially evident in the lack of microcephaly in the girl with the 10q26.11 deletion and the lack of short stature in the boy with the *EIF3A* disruption.

P02.088

An atypical Williams-Beuren deletion encopassing *ELN* and *LIMK1* genes

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Williams-Beuren syndrome is a multisystemic developmental disorder caused by hemizygous deletion of a segment on chromosome 7q11.23, spanning 1.55 Mb or 1.8 Mb, and encoding 28 genes. A few individuals have smaller deletions of the region. with phenotype ranging from supravalvular aortic stenosis syndrome to classic Williams-Beuren Syndrome.

We report a family with dominant supravalvular stenosis. The mother presents supravalvular aortic stenosis, distinctive facies and shows strong anxiety; she also have history of school difficulties and impulsivity and short attention span. Her youngest son have supravalvular aortic stenosis, involvement of the pulmonary branch, and mesenteric, coeliac and renal stenosis; her eldest son have supravalvular pulmonary and aortic stenosis. They both had surgery for bilateral inguinal hernia, normal calcemia, no classical WBS facial dysmorphism when seen at the genetic department, and attention deficit and hyperactivity. The youngest daughter was diagnosed at birth with severe supravalvular aortic and pulmonary stenosis, and facial dysmorphism. Calcemia was normal. Her karyotype and FISH of the 7q11.23 region were negative. QMPSF analysis of the 7q11.23 region found an atypical deletion, confirmed by array-CGH (Agilent 244k), of the entire *ELN* gene and part of the *LIMK1* gene cosegregating with the familial phenotype. Although the loss of an *ELN* allele produces the cardiovascular pathology of WBS, the phenotypic consequences of losing other alleles within the WBS chromosome region are much less clear. Based on study of patients with atypical deletion and mouse models, loss of *LIMK1* gene has a possible putative effect on impaired visuospatial abilities.

P02.089
Emanuel syndrome: breakpoint determination.

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The constitutional translocation between chromosomes 11 and 22 is the most common non- Robertsonian translocation in humans. Clustered breakpoints involving chromosome regions 11q23 and 22q11 have been reported in unrelated families. Balanced translocation carriers are clinically normal. Their offspring have a risk to herit a supernumerary der (22)t(11;22) chromosome, which results in a rather very constant phenotype. This very rare genomic syndrome was named Emanuel Syndrome.

We report a case of a boy with classical features of Emanuel Syndrome: mental retardation, microcephaly, failure to thrive, some organ malformations, ear anomalies, and dysmorphic features with a typical round face, prominent forehead and deep round eyes. After array CGH and caryotyping of patient and his parents and sibs, we also analysed the chromosomal restructuration with a breakpoint - specific PCR to determine its precise localization and to confirm that the breakpoint lies in the region of the recurrent translocations, with a variation of only several nucleotides, if compared to other Emanuel Syndrome patients. The mechanism of the recurrent translocation may be explained by the fact that it lies in a region with a palindromic nucleotid sequence.

P02.090
ECO Syndrome Without ICK Mutation: Genetic Heterogeneity?

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Endrocrine-cerebro-osteodysplasia (ECO) is a recently described neonatal lethal recessive disorder characterized by multiple congenital anomalies involving the endocrine, central nervous and skeletal system. Six affected individuals from a single consanguineous Old Order Amish family have been reported so far. All affected individuals carried a homozygous missense mutation in the ICK gene. We report a single case, offspring of non-consanguineous parents, with multiple congenital anomalies equalling those of ECO syndrome. Malformations included micromelia, ulnar deviation of hands, brachydactyly, midface hypoplasia, midline cleft-lip and -palate, holoprosencephaly and absence of the adrenal glands. The external phenotype was indistinguishable from that of the published cases of ECO syndrome. Molecular analysis failed, however, to identify mutations in the ICK gene. We therefore think that ECO syndrome might be genetically heterogeneous and not a "private" condition. Consequently the diagnosis of ECO syndrome should be considered in prenatal/neonatal cases of multi-system disorders involving the endocrine glands, the cerebrum and skeletal system.

P02.091
Recurrence risk and (epi)genetic complexity in Silver-Russell and Beckwith-Wiedemann syndrome

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Silver-Russell (SRS) and Beckwith-Wiedemann syndrome (BWS) are growth disorders mainly caused by defects in the epigenetically regulated region 11p15.5, containing the imprinting control regions 1 and 2 (ICR1 and ICR2). SRS is characterised by severe intrauterine and postnatal growth retardation, contrary to BWS which presents as an overgrowth disorder associated with embryonal tumors. While the most frequent known aberration in SRS is an ICR1-hypomethylation (>30%), most BWS cases (50-60%) are caused by an ICR2-hypomethylation. In addition, a variety of other genetic and epigenetic alterations in 11p15.5 are known to result in deficient epigenetic regulation.

NLRP2-mutations have recently been identified as a rare heritable cause for methylation defects resulting in BWS in children of unaffected female homozygous mutation carriers. Even though familial cases of BWS and SRS are rare, the identification of heritable factors affecting genomic imprinting in these disorders is crucial to estimate the recurrence risk in affected families.

For illustration of the (epi)genetic complexity, we present several familial and sporadic SRS and BWS cases with rare disturbances in 11p15.5. Furthermore, we refer to Next-Generation Sequencing (NGS) as a suitable testing approach for future detection of genes involved in the regulation of genomic imprinting and further epigenetically regulated regions involved in BWS and SRS.

P02.092
A new case of genome-wide paternal uniparental disomy emphasizing the need of multilocus-testing in imprinting disorders

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While non-mosaic genome-wide paternal uniparental disomy (UPD) is not consistent with life and leads to hydatidiform mole, UPD of single chromosomes is a well-known cause for a group of congenital diseases usually called imprinting disorders (IDs). Chromosomes associated with IDs are 6, 7, 11, 14, 15 (Transient Neonatal Diabetes Mellitus, Silver-Russell syndrome, Beckwith-Wiedemann syndrome, upd(14)-syndromes, Prader-Willi syndrome, Angelman Syndrome). The clinical outcome of a UPD depends on the transmitting parent. Whereas maternal and/or paternal UPD of the aforementioned chromosomes have clinical consequences, others are not associated with clinical phenotypes (if not carrying a recessive mutation). Therefore it can be argued that UPDs of other chromosomes are underdiagnosed and are detected only by chance. We here present a 19-year old woman carrying a mosaic genome-wide paternal UPD that was coincidentally identified in a multi-locus screening for aberrant methylation. The patient was initially diagnosed as BWS due to a mosaic upd(11p15)pat but presented additional clinical findings including nesidioblastosis, fibroadenomas, hamatomar of the liver, hypoglycemia and ovarian steroid cell tumour. So far, only single cases with similar clinical and molecular findings mainly diagnosed in early childhood have been reported. Based on these data it can be concluded that the mosaic genome-wide paternal UPD (also known as androgenic/biparental mosaicism) in our patient explains the unusual BWS phenotype. These findings emphasize the need for multilocus testing in imprinting disorders to efficiently detect cases with disturbances affecting more than one chromosome.

P02.093
Eight alterations in the genes FOXG1, TCF4 and CDKL5 in a cohort of 70 patients with seizures and intellectual disability

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To date many genes have been identified to be associated with epilepsy, intellectual disability (ID) and other features such as dysmorphism, movement disorders, microcephaly or brain malformations. CDKL5, TCF4, SLC9A6, ARX and FOXG1 are important genes belonging to this group. The conditions caused by mutations in these genes are especially relevant differential diagnoses to Rett and Angelman syndromes. The incidence of these conditions is unknown. In addition, there is only insufficient data available concerning the phenotypic variability, caused by haploinsufficiency of these genes.

In a cohort of 70 patients with seizures or EEG abnormalities and ID, we sequenced the genes CDKL5, TCF4, SLC9A6, ARX and FOXG1. Further we performed MLPA analysis for CDKL5, TCF4, ARX and FOXG1. We found heterozygous pathogenic alterations in eight patients (11,4 %). Four patients showed alterations in FOXG1 (one frameshift, two missense mutations and one deletion) and two in CDKL5 (one splicesite mutation and one deletion). Two patients had the same recurrent missense mutation in the TCF4 gene. We present detailed clinical and molecular data of our patients. We compare the results to the literature and discuss on novel insights into the phenotypes of patients with FOXG1, TCF4 and CDKL5 mutations.

Our study contributes to the delineation of the phenotypes of these rare conditions. Further investigations with new molecular techniques such as next generation sequencing will identify much more patients. The evaluation of the clinical findings in these patients will help to define more precisely the incidence and phenotypic variability in these disorders.

P02.094

Screening of SCN1A and MDR1 polymorphisms in epilepsy

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Genetic variability in drug metabolism affects the treatment with Anti Epileptic Drugs (AEDs). Allelic variations in genes include SCN1A and MDR1, encoding AEDs target and drug transport proteins, may affect efficacy and tolerability of antiepileptic drugs. This study designed to evaluate frequency of ABCB1 and SCN1A selected SNPs in genotype and haplotype combination in Iranian population who were affected by Idiopathic Refractory Epilepsy (IRE).About 81 healthy normal samples and 34 probands, clinically diagnosed as one type of IRE, were selected. The genotype of two SNPs in SCN1A (rs2298771, rs7601520) and one in MDR1 (rs1045642) were determined in two groups by ARMS-PCR, PCR- RFLP and confirmed by direct sequencing.

The data analysis shows no statistically significant difference and so the predicted haplotype frequencies including three SNPs did not show significant difference between patients and control group.

P02.095

Whole exome sequencing to identify genes involved in epileptic encephalopathy

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Epileptic encephalopathy is a severe condition occurring often at a very young age. Patients suffer from epileptic seizures, and, presumably as a consequence, show developmental retardation.

When no external cause is prevalent, the condition is assumed to be genetic. In some families, multiple sibs are affected. A few genetic causes for epileptic encephalopathy are known, but many cases cannot be explained. Probably, there is high genetic heterogeneity.

In order to identify more genetic causes for this disorder, we have collected patients with healthy parents. Five families had multiple affected sibs, whereas ten patients had no affected relatives. We performed exon sequencing to look for genetic causes, assuming a recessive model, either homozygous or compound heterozygous in the multiplex families, while in the sporadic cases a de novo mutation is an additional possibility.

So far, we have identified X-linked mutations in CDK16 and GABRE as possible candidates in male patients. Further analysis of the remaining families may identify more candidate mutations. To confirm their pathogenicity, we will combine our results with those of other research groups.

P02.096

Establishment of Diagnostic Decision Support System (DDSS) in Clinical Diagnosis of Genetic Diseases: The FaceGP DDSS Methodology and Its Applications

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A face develops under the influence of many genes. Thus, facial appearance can be a significant clue in the initial identification of genetic anomalies associated with especially cognitive impairments. It may be possible to diagnose a good number of syndromes correctly by using computer-assisted face analysis. For dysmorphic syndromes -with - known genetic causes, cyto- and/or molecular genetic analysis is the appropriate route of investigation in order to confirm a diagnosis. In this study, in terms of helping non experienced practitioners in diagnosing process as well as supporting experts in their decisions, we established a methodology to ease the process and we refer to our method as FaceGP DDSS. In the methodology, digital facial image processing methods are used to reveal facial features with disorders indicating genotype-phenotype interrelation. A great number of genetic disorders indicating a characteristic pattern of facial anomalies can be typically identified by analyzing specific features with the aid of facial image processing methods such as PCA in order to determine reference values (eigenfaces) and train the system. Distance algorithms such as Euclidean, Mahalanobis are used to construct the correlation of the input image with the trained images in matching. Some image enhancement methods such as histogram equalization and median filter are implemented on detected degraded images to capture better features. This study proposes a novel computer-assisted and cost-effective method by merging several methods in the characterization of the facial dysmorphology, in particular a method relying primary on face image capture and manipulation to diagnose genetic diseases.

P02.097

A Patient with 3q26.33q27.3 monosomy presenting with intellectual disability, facial dysmorphism and diaphragm evantration

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A Patient with 3q26.33q27.3 monosomy presenting with intellectual disability, facial dysmorphism and diaphragm evantration

We herein describe a 7-year-old female patient who was referred us for facial dysmorphism, intellectual disability and diaphragmatic eventration. The patient was born 2050 grams, to nonconsanguineous parents, as their first liveborn by normal delivery, at term. Prenatal history revealed oligohydramnios and intrauterine growth retardation. Postnatally the patient had irregular respiration and tachypnea. She had a delay in both motor and mental developmental milestones. Fluoroscopy detected eventration of the right diaphragm. Perfusion scintigraphy of the lungs demonstrated segmental perfusion defects bilaterally. Magnetic resonance imaging revealed hypoplasia of corpus callosum and cerebral atrophy. On physical examination body weight was 17.7 kgr (3-10th centile), head circumference was 46 cm (<-2SD), and height was 106 cm (<3rd centile). She had facial dysmorphic features including thin lips, broad base to nose, low set ears, bilateral epicanthi. The patient was clinically suspected to have a chromosomal disorder. The patient had a normal karyotype; however, array-CGH analysis (Agilent 8X60K Array) revealed a 4.3 Mb deletion in 3q26.33q27.3. Previously six cases of microphthalmia or anophthalmia in association with deletions/rearrangements of chromosome 3q involving 3q26.33q27.3 have been reported. The described region was estimated to be 6.7 Mb and was assumed to have an anophthalmia gene at 3q26.33-q28 locus. Our patient did not have any eye malformation. Therefore, the previously descibed region suspected to harbor the anophthalmia gene may be further narrowed down to the 3q27.3-q28 region with the findings of the present patient.

P02.098

Clinical and genetic characteristics of the chest and pleuropericardial manifestations in Armenian children with Familial mediterranean fever

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Familial Mediterranean fever (FMF) is an autosomal-recessive disease characterized by febrile aseptic polyserositis. FMF, as ethnic disorder for Mediterranean sea ancestors region, is widespread in Armenians. Chest pain attacks are 2-nd manifestation after febrile abdominal attacks.Objective; to investigate clinical and genetic characteristics of the chest manifestations (pleuritis and pericarditis) in children with FMF. Results: We performed clinical and genetic (MEFV gene mutations) investigations in 715 children (438 boys, 277 girls, mean age 8,64±0,17). Chest pain attacks (pleuritic or/and pericardial in origin), mainly unilateral, short lasting, with dyspnea, superficial, painful inspiration, often developed recurrent pleurisy (81.7%), pericarditis (13.8%). Pleurisy as 1st manifestation was observed in 23.1% of patients. In 1% of cases lung atelectasis and asthma attacks were observed. We detected the most frequent mutations of MEFV gene. Frequency of V726A mutation in Armenians is higher (22.3%) than in other populations with high level of FMF: Jews (3.0%), Turks (2.9%). Risk of leurisy in contrast to other serosities was associated mainly with V726A/M694V compound-heterozygous genotype (81,6%) and was 2.3 time higher than in M694V-homozygotes (71.9%) and M694V-heterozygotes (68,9%) with benign clinical course. Recurrent pericarditis was revealed in 13.8% and associated with M694V-homozygotes in compare to patients without M694V mutation. Conclusions: Taking into consideration our data about more frequent association of V726A mutation with pleurisy, we suppose that the prevalence of V726A compound-heterozygous genotype might be considered as a risk factor for development of the pleurisies. Pericarditis as rare FMF manifestation, was associated with M694V mutation and considered as unfavorable FMF prognosis.

P02.099

Feingold syndrome type II in a family with deletion 13q31q32 comprising the microRNA 17~92 cluster

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Feingold syndrome is characterized by developmental delay/intellectual disability, microcephaly, short stature, characteristic shortening of middle phalanges II/V and various congenital defects of the heart, kidney and gastrointestinal tract. In the majority of patients this syndrome is caused by mutations of N-MYC. Recently, germline deletions of microRNA 17~92 cluster have been identified to be responsible for the Feingold syndrome phenotype in three patients. This variant is called Feingold syndrome type II. Here we report on a family with a 4.5 Mb deletion of the chromosomal region 13q31.3q32.1 comprising the microRNA 17~92 cluster. This deletion was identified by array CGH and confirmed by FISH. The index patient and his mother show the typical phenotypic pattern of Feingold syndrome including developmental delay/intellectual disability in combination with microcephaly, short stature and the digital abnormalities. N-MYC mutations were excluded in the index patient. The 13q31.3q32.1 deletion emerged de novo in the affected mother of the index patient. To clarify whether phenotypic features could be caused by a single copy loss of other involved genes in the deleted chromosomal region we compared the clinical findings of our patients with those of reported patients carrying 13q31q32 deletions. Interestingly, we could not find any further remarkable clinical feature which is not belonging to the phenotypic spectrum of Feingold syndrome in the here reported family. Our findings confirm deletions of the microRNA 17~92 cluster as a further cause of Feingold syndrome.

P02.100

Heterotopic bone formation not related to POH/FOP disease: a new entity.

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We present a peculiar case of multiple and massive congenital periarticular calcification in a normally developed female, aged 2 years, the first child of Italian non consanguineous parents. Since her first month, she presented progressive diffuse joints limitation. Skeletal survey and a CT scan at 8 months assessed shoulder, elbow, wrist, hip, knee and ankle joints fixed by periarticular calcifictions. Ectopic bone tissue was present between the occipital skull base and C3 vertebral body. At 10 months, total body MRI showed respiratory and deglutitory muscles calcification, with progressive cranio-caudal involvement. On physical examination at 2 yr., diaphragmatic breathing underlaid gradual respiratory deterioration. Her posture was forced in flexion of elbows, knees and ankles, and movements of the head were completely abolished.

She also had brachydactyly of hands and feet, camptodactyly of the hands, very short fingers of feet. Her eyes are deep-set, the philtrum is short and smooth, but she has no other striking dysmorphisms.

Neurocognitive milestones were properly achieved.

Extensive metabolic workup gave normal results: serum and urine calcium levels were normal, as well as serum and urine phosphorum, sodium, potassium, magnesium, cratinine, PTH, 1-25-OH-Vit D, 25-OH-VitD levels.

Molecular analysis of ACVR1 and GNAS genes ruled out both Fibrodysplasia Ossificans Progressiva (FOP) and Progressive Osseous Heteroplasia (POH), two distinct severely disabling heritable disorders of connective tissue characterized by progressive heterotopic ossification.

Exome sequencing: pending.

P02.101

Filamin A associated periventricular nodular heterotopia in males

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Introduction: Filamin A (FLNA) associated periventricular nodular heterotopia (PVNH; MIM 300049) is an X linked dominant inherited neuronal migration disorder with high perinatal lethality of hemizygous males. Occasional reports of older male patients were associated with hemizygous hypomorphic FLNA alleles or somatic mutations.

Methods: neurological examination, cerebral MR imaging, sequence analysis of the entire FLNA coding region and MLPA.

Results: we here present the neurological findings, selected MR images and results of FLNA mutation analysis for three new male patients. Patient A at the age of 4 years presented with a severe global developmental delay, dolichomicrocephaly, muscular hypotonia, complex cerebral malformations including polymicrogyria and PVNH in MR imaging and was hemizygous for a FLNA missense mutation inherited from his mother. A severe and complex phenotype was also identified in 37 year old patient B with intractable seizures, skeletal features within the OPD spectrum and severe obstructive lung disease, resulting from a mosaic frameshift FLNA mutation. In contrast, a mosaic FLNA splice site mutation was observed in the 63 year old father (patient C) of two daughters with PVNH. MR imaging confirmed for him very subtle PVNH; he has a University degree, under antiepileptic medication is without seizures and otherwise healthy.

Discussion: FLNA mutations in males are rare and should also be considered in patients with more complex phenotypes including PVNH as shown in patients A and B. Furthermore, mosaic Filamin A mutations may be clinically silent, but still be associated with a high recurrence risk as demonstrated in patient C.

P02.102

De novo FMR2 (AFF2) deletion encompassing exons 2 and 3 in a boy with a mild intellectual disability

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Alterations of the Fragile Mental Retardation 2 gene (FMR2, synonym AFF2) can result in a mild to moderate intellectual disability (ID), speech delay, hyperactivity, and autistic behaviour. The well-known underlying molecular mechanism of this condition, also referred to as FRAXE, is a (CCG)n trinucleotide repeat expansion which leads to silencing of the FMR2 gene. Additionally, deletions within the FMR2 gene were described in handful number individuals with ID.

Here we report on a de novo 131 kb deletion of FMR2 gene in a 2-years-old boy with a mild developmental delay, behaviour changes, hypotonia, and discrete facial dysmorphism. The deletion, detected by SNP-array analysis (Affymetrix 250 K Nsp I), spans between the base pairs 147454718-147586323 (NCBI36). The aberration and its de novo origin were confirmed by MLPA. RNA analysis on the patient showed a 994 bp deletion of AFF2 transcript, resulting in the complete loss of exons 2 and 3.

In conclusion, this case report further confirms the role of FMR2 gene deletions as a FRAXE phenotype underlying mechanism. RNA analyses demonstrate that the deletion within the FMR2 gene ceases the transcript production.

P02.103

Detection of serum anti-neuronal antibodies in patients with Fragile X syndrome

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Fragile X syndrome (FXS) is the most common form of familial mental retardation and known cause of autism. The mutation responsible for FRAXA is a large expansion of the trinucleotide CGG repeats at 5' end of the *FMR1* gene resulting in the transcriptional silencing of the gene. There are close similarities between autism profiles in idiopathic and comorbid autism (FXS).

Aim: The aim of the study was to investigate the frequency of serum antineural antibodies in a group of 23 Fragile X males.

Material and methods: Serum anti-neuronal antibodies were measured by Western blot technique (Anti-neuronal Antigens EUROLINE-WB EUROIMMUN) in 23 patients with FXS (full mutation in the *FMR1* gene), aged between 10 and 32 years, in comparison to 19 healthy-matched males

Results: We detected the presence of antibodies in the serum of 10 FXS males and in 1 from the reference group. FXS males had significantly higher percent of elevated levels of serum anti-neuronal antibodies (43,48%) than healthy controls (5,26%).

Conclusion: Serum anti-neuronal antibodies were found in a subgroup of FXS patients. Autistic symptoms in FXS may be, in part, caused by autoimmune factors. Further wide-scale studies are necessary to shed light on the role of anti-neuronal antibodies in autistic syndromes

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P02.104

Offspring of a fully mutated fragile-X male patient: a premutated female baby

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A 28 y-old male originating from eastern Algeria with a diagnosis of Fragile X mental retardation established at the age of 12 y during evaluation of severe learning disabilities married his second-cousin. He required special education in France and is currently working in a shelter. After an arrangement has been decided by his family, his second-cousin who was educated in Algeria was married to him. Although he suffers moderate mental retardation, his wife is of normal intelligence. A pregnancy developed soon thereafter. A male with the full mutation is expected to transmit alleles of premutation size to his daughters, based on an experience of less than ten reported cases. This is why prenatal testing is still recommended for a female fetus of a fully mutated genitor. Fetal DNA was extracted from cultured amniocytes and submitted to Southern blotting and (CGG)n PCR analysis. While the father is mosaic for a methylated full mutation in lymphocytes (around 20% of the cells are not methylated), his wife has two normal alleles. The female fetus harbored a normal allele and a 105 CGG repeats premutation. She was therefore predicted to be unaffected with Fragile X syndrome and the pregnancy was pursued. Repeat ultrasound examination of the fetus was normal. After an uneventful pregnancy, the woman gave birth to a healthy female baby. Reproduction in Fragile X males is exceptional. This case report gives further support to this unusual reverse instability mechanism as a common one.

P02.105

A rare case of Fraser syndrome followed 9 years alive

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BACKGROUND: Fraser syndrome is a rare autosomal recessive disorder (just over 250 cases reported) characterized by cryptophthalmos, syndactyly and uro-genital anomalies (as major criteria). Survival of affected subjects seems to be up to 25% by 1 year of age and up to 15% at 1-10 years. CASE PRESENTATION: A girl with Fraser syndrome was followed from birth to the time of death (at 9 years of age). Parents were young, healthy, unrelated but originating in the same relatively isolated village. The patient had a healthy brother (4 years older), and a brother with Dandy-Walker malformation (2 years younger). On examination she presented major criteria for Fraser syndrome: bilateral criptophtalmos, syndactyly - both hands and both feet, clitoromegaly, associated with multiple other anomalies. Brain imaging (ultrasound, CT, MRI) revealed no abnormalities. She had clinical progression of multi-sensory impairment, along with learning difficulties and physical disabilities. No cytogenetic anomalies were found and there were no molecular studies conducted. DISCUSSION: Fraser syndrome has a very low frequency. The syndrome can be caused by mutations in two genes, FRAS1 (4q21) and FREM2 (13q13.3). This case posed multiple medical problems determined by multi-sensory involvement and physical and mental retardation. A speculative element of the case presented consists of the familial association of a brother with Dandy-Walker malformation; Fraser syndrome is associated in 20% of cases with Dandy-Walked malformation. CONCLUSION: The case presented could contribute to a better understanding of this rare syndrome, given phenotypical characteristics and survival up to 9 years of age.

P02.106

Frontonasal dysplasia; characterization of a family with an evident pattern of an autosomal dominant inheritance.

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Frontonasal dysplasia is due to disturbance in the development of frontonasal, medial nasal and maxillary prominences, structures derived from the neural cresta. It is a clinically and genetically heterogeneous disease that presents large hypertelorism, broad nasal bridge, bifid hidden skull, nose bifid and cleft lip/palate. By far, most cases show an autosomal recessive pattern. In the present study, we describe a family in three generations with 15 affected members with frontonasal dysplasia and alopecia. The proband was a woman of 22 year old with high forehead, mild alopecia, hypertelorism, broad nasal bridge, nose bifid, hypoplastic midface, sparse eyebrows. She underwent reconstructive surgery of the nose at 19 years. The rest of the family members harbour similar clinical characteristics. There are few reports of frontonasal dysplasia with dominant autosomal inheritance, practically all of them have an autosomal recessive pattern. In the present family we confirm the automosal dominant inheritance in frontonasal dysplasia, linkage analysis is necessary to identify the gene responsible of the disease.

P02.107

Severe FX deficiency caused by a 4-bp deletion compound heterozygous with a large deletion in 13q34

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We report the case of a newborn who presented with major bleedings due to severe FX deficiency. The infant was the first child of unrelated caucasian parents. FX levels were markedly reduced (<1%). Both parents had borderline low FX levels. The *FX* gene of the patient and her parents were examined by direct sequencing. The analysis revealed a homozygous 4-bp deletion in exon 2 of the girl. This deletion leads to a frameshift and subsequently to a premature stop codon. The father is a heterozygous carrier of this pathogenic mutation, whereas the mother's sequence for exon 2 was wild type, raising the possibility of a large deletion involving the *FX* gene on one maternal allele, which she could have passed on to the child. Subsequent SNP analysis showed that the patient was homozygous for a variant in intron 2 of the *FX* gene, whereas the father was heterozygous and the mother carried the homozygous wild type allele at this position. As MLPA analysis of the *FX* gene is currently unavailable and deletions involving both *FVII* and *FX* genes have been reported, MLPA analysis was performed for the *FVII* gene, which is located in close proximity to the *FX* gene. This analysis revealed a complete heterozygous deletion of the *FVII* gene which is likely to also involve at least part of the *FX* gene including exon 2, in both the mother and the child. Together, these findings account for severe FX deficiency in the patient.

P02.108

Efficacy and safety of biphosphonates in a Romanian pediatric clinical trial with genetic disorders affecting bone mineralization

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Background: Most genetic disorders with increased bone fragility (congenital rickets, osteogenesis imperfecta), include close to normal growth and autosomal dominant inheritance. Previous randomized controlled trials revealed that the majority of children and adolescents with mild and moderate forms of disease have low areal bone mineral density (BMD) at the lumbar spine and that cyclical treatment with biphosphonates has beneficial effects.

Methods: In a two years longitudinal study, we prospectively followed up 35 pediatric patients recruited from a Romanian primary care setting between November 2009 and November 2011. Inclusion criteria: positive family history - presence of at least one fracture or dentinogenesis imperfecta, children > 10 yr of age. Patients were randomized to either risedronate (N=18) or alendronate (N= 17) and study visits occurred every 3 month.

Results: The main efficacy variable was the change in lumbar spine (L_1- L_4) areal BMD Z-score; risedronate increased it by 0.76, whereas patients receiving alendronate therapy experienced an increase of 0.45 ($p > 0.05$). Regarding safety, the incidence of gastrointestinal side effects due to alendronate was higher (47%) when compared with the risedronate group (28%). These results suggest that the skeletal effects of oral alendronate are weaker but still lead to an increase in lumbar spine areal BMD.

Conclusions: In a single dose, pharmacokinetic study, data showed that biphosphonates were well tolerated and reduced fracture rates in a population-based cohort study of children with genetic disorders affecting bone mineralization.

Key words: genetic disorders, bone mineralization, pediatric patients, biphosphonates.

Abbreviations: BMD - bone mineral density

P02.109

Audiological analysis in deaf patients homozygous for the splice site mutation IVS1+1G>A in *GJB2* gene

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Congenital deafness is one of the most frequent sensory disorders which accounts for about 1 in 1000 newborns, and approximately half of all cases have a genetic etiology. 15-20 cases of congenital/early onset hearing impairment (HI) in 14000 - 15000 live births (~1:900-1:750 newborns) are detected per year in the Sakha Republic (Yakutia) located in Eastern Siberia (Russian Federation). In recent study we registered a large cohort of Yakut patients homozygous for the IVS1+1G>A mutation (70 unrelated deaf subjects in total) [Barashkov et al., 2011].
We collected audiometric data on 40 patients with genotype IVS1+1G>A / IVS1+1G>A. Audiograms from available medical documents were analyzed retrospectively for each ear separately. All 40 patients (80 ears) underwent otoscopic examination, tympanometry, and audiometric testing. Analysis of the audiological characteristics obtained from 40 subjects with genotype IVS1+1G>A / IVS1+1G>A revealed significant association of this genotype with severe to profound HI (85% of severe to profound versus 15% of mild to moderate, $p<0.05$) with mostly symmetrical bilateral hearing loss (29 out of 40 examined patients, $p<0.05$). Moreover, detailed audiological analysis showed a variability in hearing thresholds on different frequency ranges among subjects homozygous for IVS1+1G>A mutation.The study was supported by Russian Foundation for Basic Research ([1]09-04-01123-à, [1]11-04-01221-à), Federal Programs «Scientific and educational staff for innovative Russia» [1]02.740.11.0701 for years 2010-2012, [1]16.740.11.0190 and [1]16.740.11.0346 for years 2009-2013, and the State Scholarship of the Academy of Sciences of the Sakha Republic (Yakutia).

P02.110

Variability in the level of erythrocyte glucose uptake in two patients with the same SLC2A1 mutation

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Purpose: Glut1 deficiency syndrome (Glut1-DS) is a treatable epileptic encephalopathy diagnosed by hypoglycorrhachia, impaired erythrocytes glucose uptake, and heterozygous mutations in SLC2A1 gene. Here we report two patients with the same SLC2A1 mutation and different results in the erythrocyte glucose uptake to reveal clinical heterogeneity in Glut1-DS.
Patient 1: A 15-year-old girl, born to healthy non-consanguineous parents. Atypical absence seizures and dystonic posturing were seen since 2 years of age, and astatic episodes after a long walk has seen since 10 years of age. Hypoglycorrhachia and decreased erythrocyte glucose uptake were observed. She was diagnosed with Glut1-DS and started on the ketogenic diet.
Patient 2: A 17-year-old girl, born to healthy non-consanguineous parents. Since 4 years of age, she showed myoclonus, ataxia, and loss of consciousness with drooling. Such events were mostly seen in the late afternoon, and recovered with eating. Hypoglycorrhachia was observed while erythrocyte glucose uptake was normal. She has not started ketogenic diet yet, and her developmental status is severely retarded. Both patients had the same mutation in SLC2A1 (R330X). Discussion and conclusion: This is the first report of a patient with normal erythrocyte glucose uptake caused by the R330X mutation. There is variability in the level of erythrocyte glucose uptake even in patients with the same SLC2A1 mutation. Factors causing the variability remain to be clarified.

P02.111

Gomez-Lopez-Hernandez Syndrome - a further case report

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Gomez-Lopez-Hernandez syndrome (GLHS), also known as cerebellotrigeminal-dermal dysplasia, is a rare, possibly underdiagnosed neurocutaneous syndrome of unknown origin. GLHS is characterized by the triad of rhombencephalosynapsis, trigeminal anesthesia and partial alopecia of the scalp; however, only rhombencephalosynapsis and partial alopecia have been recognized as consistent features and are obligate diagnostic criteria. Rhombencephalosynapsis is defined as agenesis of the cerebellar vermis and fusion of the cerebellar hemispheres, the superior cerebellar peduncles and the dentate nuclei. Inconsistent features of GLHS include characteristic craniofacial features (brachy-turricephaly, midface hypoplasia, down-slant of palpebral fissures, hypertelorism, lowset posteriorly rotated ears), strabism, short stature, cognitive impairment, ataxia and muscular hypotonia. 27 patients have been published to date, all of them sporadic cases.
We report one further male patient, 6 years of age, to expand awareness of this rare disorder. He presented with short stature, hypospadias, atrial septal defect, mild muscular hypotonia and motor coordination problems, strabism, bilateral parietal alopecia, craniofacial dysmorphic signs, and congenital hypothyroidism also present in his brother. He showed a normal cognitive development. Chromosome analysis, FISH for 22q11 microdeletion and SNP-array analysis yielded no relevant results. A MRI of the brain performed for the evaluation of short stature showed rhombencephalosynapsis and thus led to the diagnosis of GLHS.

P02.112

Gómez-López-Hernández syndrome: description of an additional case with typical phenotypic features and normal cognitive function

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Gómez-López-Hernández syndrome (GLHS) also named cerebello-trigeminal-dermal dysplasia is a rare neurocutaneos syndrome with unknown etiology. Based on current data, only 27 patients have been described so far, all sporadic cases. GLHS is characterized by the triad of rhombencephalosynapsis, trigeminal anesthesia and bilateral parietal or parieto-occipital alopecia. Bilateral alopecia is already present in the neonatal period and is highly suggestive of GLHS. Trigeminal anesthesia seems to be very variably in its expression and can be easily missed. Rhombencephalosynapsis is a consistent neuroimaging sign, comprising fusion of the cerebellar hemispheres with agenesis of the cerebellar vermis. Further features described so far include short stature, hypertelorism, down-slanting palpebral fissures, brachy-turricephaly, craniosynostosis, midfacial hypoplasia, mild mental retardation, dyspraxia, ataxia and corneal opacities.
Here we report on an additional patient with typical GLHS. The seven year old girl presented with trigeminal anesthesia with distinctive corneal lesions and visual impairment and mild parietal alopecia. Rhombencephalosynapsis was diagnosed in retrospect. Other phenotypic features were brachy-turricephaly, low set and dorsally rotated ears, strabismus convergens, down-slanting palpebral fissures, hypoplastic end phalanges and interrupted transverse palmar creases. Cognitive function was normal (IQ 104). Based on literature and our additional case we propose that the presence of partial alopecia, trigeminal anesthesia and rhombencephalosynapsis is required for the diagnosis of GLHS. Intellectual impairment is reported in patients but seems not to be a consistent feature of GLHS.

P02.113

Calcaneonavicular coalition in patients with Gorlin syndrome

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Basal cell nevus syndrome (BCNS), also known as Gorlin syndrome, is an infrequent autosomal dominant disorder characterized by a predisposition to neoplasm's and other developmental abnormalities.
Key features are jaw keratocysts, calcification of the falx or ectopic calcification, palmar/plantar pits and multiple basal cell carcinomas (BCC). A large proportion of affected patients have a recognizable appearance with macrocephaly, bossing of the forehead, coarse features and facial milia. Gorlin syndrome is caused by mutations in PTCH gene. Complete penetrance and variable expressivity are seen.
Calcaneonavicular coalition is one of the most common types of tarsal coalition and may be a fibrous, cartilaginous or bony union of the two bones.
Calcaneonavicular coalition was seen in three patients from two different families, with known pathogenic PTCH mutations (c.2287dupG and c.1142-1145delATGT).
Family A: An affected father and his two affected daughters all had classical

clinical manifestations of Gorlin syndrome. In addition, they had pain and reduced range of motion in their feet. Unilateral calcaneonavicular coalition was seen in the father and bilateral coalition in one of his daughters.
Family B: A father and son with classical manifestations of Gorlin syndrome. The son was diagnosed with bilateral calcaneonavicular coalition at the age of 38.
One major criteria of Gorlin syndrome is the ectopic calcifications and congenital skeletal malformations. To our knowledge there have not been any reports of tarsal coalition in individuals with Gorlin syndrome in the current literature. Although tarsal coalition is a common condition, this may be manifestation of Gorlin syndrome.

P02.114
Gorlin-Chaudhry-Moss syndrome: a case report

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The first description of the Gorlin-Chaudhry-Moss syndrome (GCMS) was published in 1960. Two sisters with craniosynostosis, hypertrichosis, hypoplastic labia majora, dental defects, eye anomalies, and normal intelligence were presented. Two other female unrelated cases have been documented. The inheritance is still not clear, both, autosomal recessive and X-linked dominant inheritance (lethal in males) were proposed. In 2011, Aravea et al. reported two sisters with some similarities to GCMS. However, they had neither craniosynostosis nor hearing loss, but had additional manifestations not previously depicted in GCMS: aplasia cutis, ossification defect of the skull and early mortality.
We report a two-year-old girl born to apparently unrelated parents. She fulfils the clinical criteria of GCMS with the following main clinical features: microsomia, hypertrichosis, midface hypoplasia, brachycephaly, coronal craniosynostosis, low frontal hairline, coarse hair, small ears, short and downslanting palpebral fissures and hypoplastic labia majora. Other features included loose skin, umbilical hernia, high arched palate, microdontia, somewhat hypoplastic and hyperkeratotic toe nails. Radiological evaluation showed hypoplasia of the distal phalanges of the left hand. Karyotype and metabolic screening were normal. Our patient, similarly to the patients described by Aravea et al. has not got hearing loss, the feature observed in the all first three patients with GCMS.
We would like to bring this condition to the attention of clinical geneticists. Future case reports can stimulate detailed clinical and molecular investigations into aetiology of GCMS with all implications for genetic counseling .

P02.115
Mutation-based growth charts for SEDC and other *COL2A1* related dysplasias

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In a large international collaborative study we have constructed a growth chart for patients with molecular confirmed congenital spondylo-epihyseal dysplasia (SEDC) and other *COL2A1* related dysplasias. The growth chart is based on longitudinal height measurements of 79 patients with glycine substitutions in the triple-helical domain of COL2A1. In addition, measurements of 27 patients with other molecular defects, such as arginine to cysteine substitutions, splice mutations and mutations in the C-terminal propeptide have been plotted on the chart. Height of the patients progressively deviate from that of normal children: compared to normal WHO charts, the mean length/height is -2.6 SD at birth, -4.2 SD at 5 years and -5.8 SD at adult age. The mean adult height (male and female combined) of patients with glycine substitutions in the triple-helical region is 138.2 cm, but there is a large variation. Patients with glycine to cysteine substitutions tend to cluster within the upper part of the chart, while patients with glycine to serine and valine substitutions are situated between +1 SD and -1 SD. Patients with carboxy-terminal glycine substitutions tend to be shorter than patients with amino-terminal substitutions, while patients with splice mutations are relatively tall. However, there are exceptions, and specific mutations can have a strong, or the reverse, a relatively mild negative effect on growth, The observation of significant difference in adult height between affected members of the same family indicates that height remains a multifactorial trait even in the presence of a mutation with a strong dominant effect.

P02.116
Severe growth retardation in an 8.5 year old boy with dup 2p16.2→p22.1

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So far, interstitial duplications of the short arm of chromosome 2 have been reported rarely and there is no specific phenotype. Here we describe an 8.5-year-old boy with severe growth retardation (110 cm (<< P3)) associated with delayed bone age (age 3 years for wrist and 5 years for forehand at a chronological age of 8 years 3 months), moderate intellectual disability (IQ 79 tested by nonverbal intelligence test), and facial dysmorphisms including relative macrocephaly (54 cm (P75)) with a high, prominent, and broad forehead, and a large anterior fontanel. Hormone values for fT3, fT4, TSH, HGH (basic and stimulated), ACTH, Cortisol, LH and FSH were within normal ranges, whereas IgF1 and IgFBP3 were normal in the lower ranges. Cardiac ultrasound and cerebral imaging were normal. SNP-microarray analysis with the Illumina CytoChip-12v2.1 revealed a duplication of approximately 14.6 Mb carrying 63 genes, with breakpoints between rs2540240 (39,791,569 bp) and rs2540229 (39,797,559 bp) in 2p22.1 and between rs843622 (54,401,986 bp) and rs1682139 (54,410,054 bp) in 2p16.2, respectively, and a formation in maternal meiosis.
Comparison of our patient with cases from the literature and the DECIPHER database allowed the identification a region of around 4 Mb carrying 48 genes, where one or more genes relevant for growth might be located. So far, none of these genes has been associated with growth retardation.

P02.117
MTHFR 677T is a determinant of the degree of hearing loss among Polish males

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Hearing impairment (HI) is the most common sensory handicap. Whereas congenital HI has often a genetic basis the etiology of nonsyndromic postlingual HI (npHI) usually remains unidentified. Our purpose was to test whether the *MTHFR* C677T (rs1801133) polymorphism affecting folate metabolism is associated with the occurrence or severity of npHI. We studied rs1801133 genotypes in 647 npHI patients (age < 40, sudden sensorinural loss excluded, HI characterized as mean of better ear hearing thresholds for 0.5-8kHz) and 3273 adult controls from background population. Genotype distribution among patients and controls was similar but among male cases (N=302) we found a dose dependent correlation of *MTHFR* 677T with degree of HI (mean thresholds in dB: 38.8, 44.9 and 53.3, for CC, CT and TT genotypes, respectively; P=0.0013, $P_{cor.}$=0.017). Among male patients rs1801133 TT significantly increased risk for severe/profound HI (OR=4.88, P=0.001). Among controls the known effect of *MTHFR* 677T on homocysteine concentration was more pronounced in men than women (P<0.00004 for genotype-sex interaction) suggesting that in Poland folate deficiency is more prevalent in males. In conclusion, we report a novel effect of *MTHFR* 677T among males with npHI. The functional significance of rs1801133 suggests these patients may benefit from that folate supplementation.

P02.118
Screening for miRNA and common mutations in deaf Brazilian patients

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Mutations in the genes coding for connexin 26 (*GJB2*) and connexin 30 (*GJB6*) are the main cause of autosomal recessive nonsyndromic sensorineural hearing loss (AR-NSNHL). Lately, mutations in a noncoding microRNA (miRNA) gene, miR96, a member of the miR-183 miRNA cluster that is expressed in the inner ear sensory epithelium, were linked with progressive hearing loss in humans and mice. In the present study, we screened mutations in the *GJB2* gene and two deletions in the *GJB6* gene in 566 unrelated Brazilian patients, with moderate to profound NSNHL. Besides these common mutations we included the investigation of mutations in miR-183 genes family. We have found 13 different mutations in *GJB2*. Thirty-four patients were homozygous for the most frequent mutation, 35delG. Seventeen patients were compound heterozigotes for *GJB2* gene mutations and 33 patients have been identified carrying mutation in only one *GJB2* allele. Deletions in *GJB6* gene have been found in 6 patients, four of them carried mutation in *GJB2* gene in the other allele. Screening microRNA cluster (miR-96/182/183), we have identified nine single-nucleotide polymorphisms and two novel variant sequences located outside the mature miR-96 sequence. Our findings have showed a mutation spectrum of the *GJB2* gene in Brazilian patients with nonsyndromic sensorineural hearing loss and demonstrate the high genetic heterogeneity for the deafness.

P02.119

Identification of two new alpha globin gene mutations in patients suspected of having alpha thalassemia

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Alpha thalassemia disorder is a hereditary anemias caused by quantitative reduction of the alpha chain of hemoglobin, which the majority is caused by deletion in alpha globin gene cluster and, small group by point mutations. We report two novel point mutations observed in two male from south of Iran with lur ethnic origin, detected during screening for hemoglobinopathies. The patients were initially selected for their hematological indices as belonging to a group suspected for alpha thalssemia. The patients who did not reveal the most common alpha thalassemia deletions (3.7kb, Med, 4.2kb, 20.5kb) by gap-PCR, were subjected for alpha2 and alpha1 globin gene DNA sequencing. Sequence analyses identified Cd 31 A>G and Cd 99 A>T located in alpha 1 globin gene, which the Cd31 was missense and Cd 99 was nonsense mutation. Based on the red cell indices and phenotype, these mutations seem to be associated with a mild alpha-thalassemia (alpha-thal) phenotype.

P02.120

SPG 23: autosomal recessive spastic paraplegia with pigmentation anomalies: further definition in a large Algerian pedigree with pseudodominance.

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Autosomal recessive hereditary spastic paraplegia includes at least fifteen discrete entities differentiated by age at onset of symptoms and presence of distinctive features (pure and complicated forms). Among them, SPG23 has been characterized by presence of skin and/or scalp hypopigmentation. Three autosomal recessive pedigrees have been reported until now, while one is a sporadic case and the other doesn't exclude autosomal dominant inheritance. Hypopigmented macules or scalp hair are present, alongside to spastic paraparesia of variable age at onset. At present, only one pedigree has been subject to a linkage study indicating a locus at 1q24-32 (interval 25cM).

We present a four-generation Algerian family were the index patient, a 34 y-old female with onset of SP at the age of 18y has multiple hypopigmented macules and white forelock. Her maternal grandfather is affected as well in the context of an endogamic population. She married her first cousin and gave birth to a normal baby male with white forelock and hypopigmented macules. Four other remote cousins are affected, all of them born to consanguineous parents.

We suggest that the pedigree is compatible with either pseudo dominance or AD inheritance with incomplete penetrance. In conclusion, we confirm SPG23 as a discrete entity and give further support to an autosomal recessive mode of inheritance.

P02.121

A Microdeletion at the 7q11.23 Locus including HIP1 in a Girl with Developmental Delay, Behavioural Problems, Gait Abnormalities and Facial Dysmorphism

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Recurrent deletions in the proximal region 7q11.23 are common in patients with Williams-Beuren syndrome (WBS). However, only a few patients with a microdeletion including the HIP1 gene, located in the distal region of 7q11.23, have been reported. These patients show a neurodevelopmental and epilepsy syndrome. HIP1 encodes huntingtin interacting protein-1 which is normally expressed in the brain. HIP1-knockout mice develop a progressive neurologic phenotype with tremor and gait ataxia. Therefore, HIP1 haploinsufficiency has been proposed to lead to cognitive and behavioural dysfunction in these patients. We present a 4 year old girl with motor and speech delay, mild ataxia, and behavioral problems including impulsivity, aggression and mild autistic features. She showed facial dysmorphism, including a short nose with anteverted nares, downturned corners of the mouth, and a full lower lip, reminiscent of patients with WBS. Array-CGH analysis revealed an intragenic 14-23kb deletion in the distal region 7q11.23, which leads to loss of exons 5 to 8 of HIP1. The deletion detected in this patient overlaps with a recurrent distal deletion in 7q11.23 in the patients reported by Ramocki et al. (2010). Those patients had similar clinical features, including intellectual disabilities, neurobehavioral problems and in addition epilepsy. However, no information about facial dysmorphism was reported. Our patient appears to be the first with HIP1 haploinsufficiency. Further, our observation supports HIP1 to be a good candidate gene in patients with developmental delay, behavioral and gait abnormalities and the facial dysmorphism described.

P02.122

Zimmermann-Laband syndrome: a case report

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Zimmermann-Laband syndrome (ZLS) is an extremely rare malformation syndrome. It is characterized by dysplastic or absent nails, hypoplasia of the distal phalanges, scoliosis, hirsutism, gingival fibromatosis and mental retardation. Although the genetic basis of ZLS is unknown, previous studies in cases suggested that the gene responsible for ZLS is located at chromosome 3. ZLS is believed to be inherited as an autosomal dominant inheritance. In contrast, there were evidence of autosomal recessive inheritance for ZLS. In the present report, a 15 year-old female was referred to our department due to intellectual disability. There was no relationship between the patient's parents. We performed cytogenetic analysis by using GTG-banding. The patient's karyotype was 46, XX, and no abnormality was found in any chromosome. Clinical examination show that she had thick eyebrows, bulbous soft nose, large upper lip, large floppy ears, high palate, hypoplastic nails, short distal phalanges, hypertrichosis, pektus excavatum and an operation scar on her back due to scoliosis operation. The patient was evaluated for gingival fibromatosis, were identified as minimal gingival fibromatosis. Other system examinations and laboratory investigations were normal. Patient was diagnosed as a Zimmermann-Laband syndrome. This is the first our experience for this syndrome.

P02.123

Holoprosencephaly overview at the Hospital for Rehabilitation of Craniofacial Anomalies in Bauru, Brazil

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Holoprosencephaly (HPE) is one of the most common congenital malformations in humans and it is characterized by the incomplete separation of the cerebral hemispheres into distinct right and left halves. Structural chromosomal anomalies previously compiled from chromosomal HPE predicted at least 12 different HPE loci. Point mutations in the four major genes, SHH, ZIC2, SIX3, and TGIF, were identified in 20% of our HPE patients. The Hospital for Rehabilitation of Craniofacial Anomalies in Bauru, Brazil has 248 patients registered as HPE. From this total, 137 were classified as classical HPE and 111 were classified as microform of HPE phenotype. We performed mutational analysis in the SHH, SIX3, TGIF, ZIC2, GLI2, PTCH, and GAS1 genes in 149 patients. We found 9 mutations on SHH, 7 on SIX3, 1 on TGIF, 5 on ZIC2, 5 on GLI2, 4 on PTCH, and 5 on GAS1 genes. Two patients presenting a double mutation involving SHH and GAS1 genes, and GLI2 and PTCH genes were identified. Further analysis on 20 new patients for SHH, TGIF and SIX3 genes showed a new mutation on SHH (p.Q46P) which was inherited from the mother. No specific genotype-phenotype correlation according to type or location of the mutations was observed. There were no clinical features unique to individuals with a mutation compared with those without a detected mutation. Identification of new genes related to HPE and the interaction between their products will result in a more rationale understanding of the genetic mechanisms involved. Grants: Fapesp Proc n° 2011/07012-9; 2006/60973-9

P02.124

Validation of "triplet repeat" disease detection and quantitation using micro-fluid technology based platform

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Classical molecular genetic diagnosis of Huntington disease and similar diseases caused by unstable repetitive sequences relies mainly on the Southern blotting method. Specifically in the case of Huntington disease that we used as a model, which is transmitted in an autosomal-dominant inheritance, increased number of CAG triplet repeat is a genetic cause for the redundant synthesis of the targeted product. In the normal genotype, the numbers of repetitions ranges from 6-26, and with increasing numbers of repetitions also increase the degree of disease. Development of PCR technology has evolved and the possibility of faster and more reliable diagnosis of diseases of this type. Analysis of the PCR product from conventional gel electrophoresis is generally accepted, but it is not always possible to precisely determine the exact number of CAG repetitions that characterize this disease. In the present study we compared the process of PCR-based analysis using micro-fluid technology based platform (Agilent 2100 bioanalyzer, Agilent Technologies, USA) potentially produce more accurate data on qualitative and quantitative aspect of the mutation.

P02.125

Ischemic stroke in a case with moderate hyperhomocysteinemia

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Increased concentration of homocysteine is a risk factor for stroke, peripheral vascular disease, myocardial infarction, and venous thromboembolism. It seems that hyperhomocysteinemia affects not only the blood coagulation system, but also the vascular wall structure. MTHFR gene encodes a co-substrate for homocysteine remethylation to methionine, but it is also involved in transsulfuration to cystathionine. We present the case of a 42 years old man hospitalized for treatment and functional rehabilitation in Medical Rehabilitation Clinical Hospital Baile Felix, Romania, after ischemic stroke. The patient with unremarkable anamnesis, negative family history, no known diagnosis of homocystinuria developed an acute cerebrovascular ischemic accident. MRI described an ischemic vascular lesion of the left cerebellar hemisphere with edema, herniation phenomena through foramen magnum and supratentorial and amputation of the fourth ventricle. Ultrasonography of the heart and precerebral arteries revealed normal aspects The most significant laboratory finding was moderate hyperhomocysteinemia. Molecular analysis revealed the presence of a heterozygous MTHFR C677T mutation and the absence of A1298C mutation. After drug therapy, he was admitted in our clinic at about one month after the vascular accident, having only coordination problems. Rehabilitation treatment was complex and involved coordination of several team members, with the following main objectives: coordination and balance in physical activities, through stimulation exercises specific for occupational therapy and kinetotherapy. He showed significant improvement after three weeks of intensive treatment. Prompt response to the rehabilitation programme in this case is another argument for early, individualized treatment, which continues at home, over a long period of time.

P02.126

Phenotype-genotype correlation in patients with mutations in the beta-myosin converter domain

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Purpose: Evaluate the genotype-phenotype correlation of mutations located in the beta-myosin converter-domain (aminoacids 709-777). This region is responsible for the elastic distortion of the protein which allows strain to develop within the motor before the cargo is actually moved.
Methods: Identification of mutations in the converter domain of MYH7 was performed in a cohort of more than 800 cases diagnosed either with Hypertrophic (HCM) or Dilated Cardiomyopathy (HCM), followed-up in a single reference unit. We also reviewed the published data about mutations located within this domain.
Results: 6 mutations (G716R, G741R, G768R, I730N [novel mutation], I736T and R719Q) were identified in 11 families (59 relatives-30 carriers). Taking in account our data and data from literature, a total of 21 pathogenic mutations have been identified within this domain. They were distributed in 143 families (470 relatives). 424 relatives were affected or possibly affected (11 with DCM and the rest with HCM) and 382 were mutation carriers. We observed an early onset of disease (27±18 years, 56% males). Thirteen of 21 mutations were associated with a severe adverse event affecting at least one member in 52/143 families: sudden death occurred in 96 patients and at least 56% were younger than 45 years old, heart failure death in 35, cardiac transplantation in 18 and fatal stroke in 6.
Conclusion: Mutations located within the beta-myosin converter domain presented an early onset of disease. A significant proportion of mutations were associated with the occurrence of a serious adverse event and left ventricular dysfunction.

P02.127

Epidemiological and genetic assessment of hypospadias

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Hypospadias is one of the most common birth defects. In several countries, the incidence appears to be increasing, possibly due to increased reporting of minor degrees of hypospadias, but severe cases were also reported. Some reports have linked its high rate to prematurity and low birth-weight. The etiology is still unknown in many cases, pedigree analyses indicating a heterogeneous pattern of inheritance. A genetic predisposition has been suggested. The aim of this study was to perform an epidemiological study focused on this type of pathology, in newborns from Timisoara, Romania, for a period of three years, between 2008 and 2010 and to highlight the etiological aspects of hypospadias. Methods: data selection, family history, clinical, laboratory, cytogenetic and molecular study. Major and minor congenital defects present in examined patients were recorded. The determined incidence was 0.36% of male newborns. Glanular and distal penile locations prevailed. At birth, mean values were 2982 g weight and 46 cm for height. Frequently associated anomalies were genital, skeletal, nervous system and different minor anomalies. A brotherhood with hypospadias was noted. 8.3% of mothers had previous miscarriages or malformed births. Cytogenetic investigation revealed trisomy 21 in one case. A patient had disorder of sexual development. Environmental exposure to diluents was documented in another case. Identifying patients with a genetic susceptibility and further studies regarding gene and environment interactions will play an important role in preventing the occurrence of the defect.

P02.128

Molecular analysis is essential in the hypotonic infant approach detecting high incidence of Genetic diseases

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Neonatal central hypotonia is the lack of spontaneous movement, with or without muscular weakness, and generalized hypotonia during the neonatal period. This condition can be caused by a number of different pathological processes in the brain or defects to any structure in the motor unit. Central hypotonia affects the central nervous system, including the spine, and among its most frequent causes is systemic illness. As part of the central approach to the hypotonic baby, it is important to eliminate syndromic and genetic causes. Some reports have been proposed that 40% of the central hypotonic neonates had Prader Willi the most common genetic cause of obesity however, in children under 2 years of age the diagnosis is especially difficult. It is clear that early diagnosis of PWS o any other genetic entity is crucial to avoid complications and decrease morbidity and life expectancy. Nevertheless, other genetic syndromes have central hypotonia as main clinical manifestation during infancy. So, genetic approach is mandatory during the initial clinical intervention. We present the genetic approach in 30 consecutive pediatric patients with central hypotonia as a major symptom, referred by the neuro-pediatrician. According with the clinical genetic evaluation and presumptive clinical diagnosis, molecular analysis was performed detecting that 70% of the cases had a genetic disorder. We conclude that genetic evaluation is crucial in the central hypotonic clinical approach, proposing that all the central idiopathic hypotonic babies should be evaluated by a clinical genetic professional.

P02.129

Incidence of Beckwith-Wiedemann syndrome

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Background - Beckwith-Wiedemann syndrome (BWS) is the most common genetic overgrowth disorder, with an incidence ranging 1:13,000-15,000 live births. However, as data on its epidemiology are scanty and estimates show wide variability, there is a feeling that BWS could be more common than previously thought. **Objective -** We assessed its incidence in Piedmont, Italy (4,432,571 population) locating BWS cases born in this region. **Methods -** Patient were searched through local genetic counselling services, BWS Italian Association, malformation registries and rare diseases network. Data from the Italian National Institute for Statistics was used for live births assessment. BWS diagnosis was clinical according to Wecksberg's criteria and molecular testing was performed according to currently employed diagnostic flow-chart. **Results -** 45 clear-cut BWS cases (26 females, 19 males) were born across a 13-year period (1996-2009), providing an incidence of 1:10,569 live birth. Fourty patients accepted molecular testing: 72.5% turned positive showing imprinting center 2 (IC2) hypomethylation (30.0%), paternal chromosome 11p15 uniparental disomy (UPD, 25.0%), IC1 hypermethylation (15.0%), CDKN1c mutation (2.5%), whereas 27.5% turned negative. Mean age at diagnosis was 0.49±1.07 years, with 34 patients diagnosed in the first semester of life, providing a birth prevalence of 1:13,989. Four patients of the cohort developed Wilms' tumor (8.9%) and 2 hepatoblastoma (4.4%) during the observation period, resulting in a 14.2%cancer risk. **Conclusion -** We observed a BWS incidence of 1:10,569 live birth in Piedmont. This estimate results higher than previous figures, and represent the first attempt to correlate epidemiologic and molecular data in BWS.

P02.130

Inheritance of the VATER/VACTERL association

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VATER/VACTERL association refers to the non-random co-occurrence of the following component features: vertebral defects, anal atresia, cardiac malformations, tracheo-esophageal fistula, renal abnormalities, and limb defects. Recent observations suggest that in some patients, the disorder may be inherited. The aim of the present study was to replicate these findings by investigating 116 VATER/VACTERL patients and their relatives. The prevalence of anal atresia (OR 41.7, 95% CI 15.5-112.2) and limb anomalies (OR 6.4, 95% CI 1.6-25.7) was significantly higher among first-degree relatives compared to the general population. This confirms other observations of an increased prevalence of component features among relatives. The fact that the two studies report a higher prevalence for differing specific component features might be explained by the differing malformation spectra of the respective index patients. Conclusion: the present study provides independent support for the hypothesis that some cases of VATER/VACTERL have a genetic basis.

P02.131

A novel *INPP5E* mutation in a patient with Joubert syndrome

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Joubert syndrome (JS) is an autosomal recessive condition characterized by ataxia, muscular hypotonia, developmental delay, irregular breathing pattern and eye movement abnormalities. The MRI shows cerebellar vermis hypoplasia with accompanying brainstem malformations resulting in the characteristic "molar tooth sign". JS can also be associated with additional features including retinal dystrophy, ocular colobomas, cystic renal disease, nephronophtisis, hepatic fibrosis and polydactyly summerized as JS-related disorders (JRDS).

JS and JSRD are genetically and clinically heterogeneous disorders, so far 16 different causitive genes have been identified in JSRD. Here, we present a 5 years old Turkish girl with JS. The parents were first degree cousins and the patient was on chronic peritoneal dialysis program since the age of 15 months. We decided first to perform a homozygosity mapping by SNP-Array (6.0 Affymetrix) analysis, which showed "loss-of-heterozygosity" for the JBTS1 locus on chromosome 9q34.3. The analysis of the *INPP5E* gene revealed a homozygeous mutation (c.1303C>G; p.R435G) within exon 6, affecting a highly conserved amino acid within the inositol polyphosphate phosphatase catalytic domain of INPP5E. The genetic and clinical data will be presented and compared to the published cases.

P02.132

Autosomal recessive syndrome characterized by Hypertelorism - Intellectual Disability - Microcephaly - Short stature: clinical delineation and identification of two possible candidate loci

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Autosomal recessive intellectual disability (ID) is particularly prevalent in consanguineous populations. Numerous genes associated with ID have been identified, and for several genes, mutations can result in both syndromic and non-syndromic ID. We describe a consanguineous family with three boys and one girl affected with a hitherto undescribed autosomal recessive syndromic form of ID characterized by, in addition to ID, hypertelorism, broad nasal bridge with a broad and bifid nasal tip, and microcephaly ranging from -4 SD to -2 SD. One patient has multiple milia and another was diagnosed with keratosis pilaris. Three of the patients have short stature. Neurological examination of all the affected individuals was normal; none of them had seizures. Brain MRI studies showed normal results. We were not able to find any reports of this combination of clinical features in the scientific literature.

Consanguinity increases the coefficient of inbreeding, which in turn increases the likelihood of the presence of pathogenic mutations in a homoallelic state. Homozygosity mapping provides a rapid means of mapping autosomal recessive genes in consanguineous families through identification of „homozygous blocks" suspected of harboring the causative mutated genes. We performed homozygosity mapping using SNP array in four affected family members and identified two candidate loci, one on chromosome 6p12.1-q12.2 and one on chromosome 9p23-p24, possibly containing a homozygous mutation responsible for this family's unique phenotype. None of the genes mapping to these two candidate regions are known to cause a similar syndromic form of ID.

P02.133

A partial de novo deletion of GLRB and GRIA2 in an individual with intellectual disability

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Molecular karyotyping revealed a deletion of 102-111 kb in an individual with intellectual disability. This finding was validated as a de novo event by FISH. The aberration partially affects GLRB and GRIA2. Both genes are highly expressed in brain tissues. GLRB encodes the beta subunit of the inhibitory glycine receptor. It has been associated with autosomal re-cessive hyperekplexia. No symptoms of this disorder were found in this case. GRIA2 encodes the GluR2 subunit of a glutamate receptor. Other subunits of glutamate receptors have been associated with intellectual disability. Sequencing did not detect any further mutation in ei-ther of the two genes. Analyses of reversely transcribed RNA from blood revealed the exist-ence of GLRB/GRIA2 fusion transcripts. We were not able to find a fragment that carried an open reading frame across the fusion site. Nonetheless, open reading frames could be restored by different alternative splice events that might occur in neuronal structures. We speculate that either haploinsufficiency of GRIA2 or a GLRB/GRIA2 fusion gene was causing the disorder.

P02.134

Haploinsufficiency of *SOX5*, a member of the SOX (SRY-related HMG-box) family of transcription factors is a cause of intellectual disability

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Intellectual disability (ID) is a clinically and genetically heterogeneous condition; the cause is unknown in most non-specific and sporadic cases. To establish an etiological basis in those patients represents a difficult challenge. Over the last years it has become apparent, that chromosomal rearrangements below the detection level of conventional karyotyping contribute significantly to the cause of ID.

We present three patients with non-specific intellectual disability who all have a microdeletion in the chromosomal region 12p12.1. In two patients these deletions occurred de novo. All three identified deletions have different breakpoints and range in size from 120 kb to 4.9 Mb. The smallest deletion helps to delineate the critical region to a genomic segment (chr12:23924800-24041698, hg19) encompassing only one gene, *SOX5*. *SOX5* is a member of the SOX (SRY-related HMG-box) family of transcription factors shown to play roles in chondroblast function, oligodendrocyte differentiation and migration as well as ensuring proper development of specific neuronal cell types. Because of these biological functions, mutations in *SOX5* were predicted to cause complex disease syndromes, as is the case for several *SOX* genes, but no such mutations have yet been identified. Our findings indicate that haploinsufficiency of *SOX5* is a cause of intellectual disability. To verify this presumption we are performing mutational analysis in a cohort of patients with non-specific and unexplained ID.

P02.135

Analysis of ring finger proteins RNF133 and RNF148 in Intellectual disability

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Intellectual disability (ID), also referred to as cognitive impairment or mental retardation, is characterized by a substantial below-average score on tests of mental ability or intelligence, and limitations in functions related to areas of daily life. Intellectual disability can range from mild to profound and can be associated with other clinical findings or can occur as an isolated trait, with an extensive genetic and phenotypic heterogeneity. Taken together, syndromic and non-syndromic forms of intellectual disability affect 1-3% of the population. More than a hundred genes have been associated so far to ID, and they mainly have roles in brain development, synaptic plasticity and function. The ubiquitin proteasome system plays a fundamental role in maintaining the correct balance of protein levels inside cells and any disruption to this system is likely to have severe consequences, as shown for Angelman Syndrome. Several types of ubiquitin ligase have been identified, the largest group being those proteins containing a ‚RING' motif. We analyzed two of these, RNF133 and RNF148, in a cohort of 36 ID patients selected from the CHERISH consortium and negative at the array-CGH analysis. A novel missense change was identified in one patient in RNF148 and was of maternal origin. The unaffected brother does not carry the variant. We therefore performed an expression analysis of the gene and could prove that the mother expresses only the wild type form in blood. Expression analysis in the patient and in different tissues will be reported.Supported by FP7 grant CHERISH (www.cherishproject.eu).

P02.136

Mutations in the intraflagellar transport component IFT144 cause a broad spectrum of ciliopathies extending Jeune and Sensenbrenner syndrome

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Intraflagellar transport (IFT) along the microtubule core organizes the cargo of proteins into and out of the cilium and is needed for its formation, maintenance and function. An emerging number of diseases is related to the dysfunction of cilia, collectively termed ciliopathies. We describe an 8-year-old girl with a complex phenotype that does not fit properly to any known syndrome. Hypotonia, facial dysmorphism and retardation were noted shortly after birth. Other features include short stature, skeletal anomalies, strabism, deafness, subdural hygroma, hepatosplenomegaly, and end-stage renal failure due to focal-segmental glomerulosclerosis. Ten weeks after kidney transplantation, life-threatening acute respiratory distress due to an E. coli sepsis recently made extracorporeal membrane oxygenation necessary. After array-CGH had revealed no pathogenicity, we used our next-generation sequencing (NGS) "ciliopathy panel" which encompassed at that time 131 cilia-related disease and candidate genes targeting 2335 exons and 644 kb of sequence. By this, we identified the homozygous mutation c.1483G>C (p.Gly495Arg) in *WDR19* encoding the intraflagellar transport protein IFT144. This mutation affects an evolutionarily highly conserved residue, is absent from databases, and predicted by different bioinformatic sources to be pathogenic. Our patient emphasizes the usefulness and efficiency of this NGS panel approach and adds to the recent description of three families with *WDR19* mutations and nephronophthisis, Jeune and Sensenbrenner syndrome suggesting that *WDR19* mutations can cause a broad spectrum of ciliopathies.

P02.137

Hematologic anomalies in a rare case of Jacobsen syndrome

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BACKGROUND: Jacobsen syndrome is a contiguous gene syndrome caused by partial deletion of the long arm of chromosome 11. It is a rare syndrome, first described in 1973 by Jacobsen, reported so far about 200 cases. The incidence is about 1/100000 newborns. The key features of the syndrome are pre- and post-natal growth failure, mental retardation, facial dysmorphism, thrombocytopenia. CASE PRESENTATION: The patient, now a 19 year-old female, has been followed at the Oradea Genetics Department since the age of 3 months. Phenotypically she shows severe mental retardation, characteristic cranio-facial dysmorphism suggesting Jacobsen syndrome (trigonocephaly, hypertelorism, coloboma, down-slanting palpebral fissures, epicanthic folds, small nose, depressed nasal bridge, small, lowset ears, large mouth). Hematological (pancytopenia) and immunological (IgM deficiency) anomalies were absent in infancy, but become apparent at puberty. Karyotype: 46, XX del (11) (q23.3-qter). DISCUSSION: Authors discuss the hematological and immunological abnormalities of the presented case, in terms of delayed onset of these symptoms and problems raised for differential diagnosis. CONCLUSION: This rare case of Jacobsen syndrome shows hematological and immunological features that can be useful both for practice and for better understanding of genotype-phenotype relationships.

P02.138

A 1 Mb deletion within the Jacobsen-Syndrome critical region causes moderate mental retardation in a boy

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Jacobsen syndrome is a contiguous gene disorder caused by partial deletion of the long arm of chromosome 11. It is characterized by developmental delay /mental retardation, physical growth retardation, facial dysmorphism, visceral malformations and thrombocytopenia. Candidate genes for mental retardation in this region include *SNX19, THYN1, OPCML, NCAPD3* and *NTM*. Here we report a boy with moderate mental retardation, behavioral problems and slightly dysmorphic features but without physical growth retardation, with normal platelet count and no further obvious malformation.

Molecular karyotyping (4x180A, Agilent) was performed on the patient sho-

wing an approximately 1Mb loss of genomic material in 11q25 encompassing only the two genes *NTM* and *OPCML*.
Our findings support the hypothesis that *OPCML* and/or *NTM* are candidate genes for mental retardation.

P02.139
Joubert syndrome: clinical variability in a family

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Joubert syndrome is a rare genetic disorder characterized by the underdevelopment or even the absence of vermis cerebelli and a brain malformation (molar sign). Most common manifestations are ataxia, hyperpnea, slep apnea, ocular anomalies, hipotonia.
Presentation: Non-consanguine couple with 6 children of whom 4 with typical manifestations of Joubert syndrome.
Results: Family history reveals one child that died 1 day after birth and one spontaneous abortion at months 2-3 of pregnancy. The affected children have different severity forms of disease in terms of motor coordination, ocular troubles, intellectual impairment, and respiratory problems. Mother presents with retinitis pigmentosa with no other symptoms. Laboratory testing and interdisciplinary consultations are used to staging the disease. MRI done on all brothers reveals the specific form of cerebellum (molar tooth sign). Genetic testing that we had available did not show abnormalities.
Conclusions: The syndrome is managed differently in the four children. Genetic counseling is challenging because of the variable clinical picture as well as the different progression and prognosis of the disease. The children benefited from the adequate and empathic genetic counseling together with psychological counseling of the family who better supported them and differentially addressed their problems.

P02.140
Kabuki syndrome - clinical and genetic study of four new cases

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Kabuki syndrome (KS, Kabuki makeup syndrome, Niikawa-Kuroki syndrome) is an autosomal dominant disorder characterized by distinctive facial features (long palpebral fissures, eversion of the lower lateral eyelid, arched/interrupted eyebrows, depressed nasal tip, abnormal teeth and large/prominent ears), fetal pads, intellectual disability and postnatal growth deficiency. Cardiac, renal and skeletal defects are sometimes associated. Most cases are sporadic, but a few familial cases have been reported, suggesting an autosomal dominant inheritance with variable expressivity.
We present 4 cases with KS in order to show some particularities that could be included in the feature list of the syndrome. Cardiac and renal defects seem more common, whereas skeletal defects have been identified less frequently. Soft skin and moderate/severe intellectual disability seem to be common features. All our cases are males and in 2 of them the mother presented a mild phenotype.
Case 1: postnatal growth retardation, typical face, soft skin, fetal pads, severe vesico-ureteral reflux leading to chronic renal failure, moderate/severe intellectual disability; the mother has typical face and fetal pads;
Case 2: normal growth, typical face, soft skin, fetal pads, heart defect, unilateral renal agenesis, nephrocalcinosis, moderate intellectual disability;
Case 3: normal growth, situs inversus, typical face, fetal pads, cardiac and renal defect, severe intellectual disability with behavioral disturbance;
Case 4: postnatal growth retardation, typical face, soft skin, fetal pads, cardiac and renal defect, gynecomastia, moderate/severe intellectual disability; the mother has typical face and fetal pads.
In conclusion, we present 4 cases with KS to illustrate particular features and to discuss management.

P02.141
Clinical and molecular spectrum of kidney malformations in Kabuki syndrome

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Kabuki syndrome (KS) is a rare syndrome with multiple congenital abnormalities and intellectual disabilities. The most specific feature is a characteristic face. Numerous patients with MLL2 mutations and rare of KDM6A have been reported. Kidney and urologic abnormalities occur in 12 to 43% of KS patients. Kidney function in KS has not been studied. Only 3 cases of terminal renal insufficiency are reported.
Taking advantage of a French cohort including 95 genotyped KS patients, renal ultrasounds and serum creatinine were collected. Renal function was evaluated by estimated glomerular filtration rate. A special attention was given to severe cases and a genotype-phenotype study was conducted for kidney malformation.
Kidney malformations were present in 24% of cases and urinary tract abnormalities in 17%. Renal function was normal except for the two patients with severe renal disease. Patient DIJ002 presented with renal agenesis and controlateral severe hypoplasia. Severe renal insufficiency was diagnosed in the first days of life and progressed to terminal stage at 2 years of age. A MLL2 mutation was found (c.9829C>T, p.Gln3277X). Patient NCK013 presented with tubulointerstitial nephritis during childhood, leading to terminal renal insufficiency at 27 years of age, and no MLL2 or KDM6A mutations or deletions were found.
Kidney malformations were observed in 27% of MLL2 mutation-positive group and 5% of MLL2 mutation-negative group. No correlation was found between renal malformation and the location or type of MLL2 mutation.
Our study emphasizes the need for renal function and ultrasound screening when KS is diagnosed.

P02.142
Investigating KBG Syndrome: Partial deletion of *ANKRD11* results in the KBG phenotype distinct from the 16q24.3 microdeletion syndrome

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KBG syndrome (OMIM 148050) is a very rare genetic disorder characterized by macrodontia, distinctive craniofacial abnormalities, short stature, skeletal and neurologic involvement, and intellectual disability. Approximately 60 cases have been reported since it was first described in 1975. Recently mutations in *ANKRD11* have been documented in patients with KBG syndrome, and it has been proposed that haploinsufficiency of *ANKRD11* is the cause of this syndrome. In addition, copy number variation in the 16q24.3 region that includes *ANKRD11* results in a variable phenotype that overlaps with KBG syndrome that also includes autism spectrum disorders and other dysmorphic facial features. In this report we present a 2 ½ year old African American male with features highly suggestive of KBG syndrome. Genomic microarray identified a 154 kb deletion within *ANKRD11*. The deletion does not involve other nearby genes. This child's mother was mosaic for the same deletion (present in approximately 50% of cells) and exhibited a milder phenotype including macrodontia, short stature and brachydactyly. This family provides additional evidence that *ANKRD11* causes KBG syndrome and the phenotype seems to be dose dependent, differentiating it from the more variable 16q24.3 microdeletion syndrome. This family has additional features that could expand the phenotype of KBG syndrome.

P02.143
Detection of large-scale mtDNA deletions by next-generation-sequencing (NGS) in patients with Kearns-Sayre Syndrome (KSS)

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Kearns-Sayre syndrome (KSS) is a severe early-onset multisystemic mitochondrial syndrome. Prominent features include progressive external ophthalmoplegia, retinophathy, encephalopathy, proximal muscle weakness, cardiac arrhythmia and ataxia.

In most affected patients heteroplamic large-scale mtDNA deletions can be found. The percentage of mutated mtDNA varies between patients and from tissue to tissue within the same individual. A high proportion of mtDNA deletions are found consistenly in the most affected tissues (e.g. central nervous system, muscles), whereas very low or undetectable amounts are found in unaffected tissues (e.g. peripheral blood).

Due to the low amount of deleted mtDNA in peripheral blood confirmation of KSS by deletion specific PCR or Southern Blot from blood samples is not possible in most cases. In cases in which a mtDNA deletion is confined to affected tissues (e.g. skeletal muscle), the molecular diagnosis of KSS requires genetic analysis of DNA form muscle biopsy.

To overcome this limitation, we have established a next-generation-sequencing protocol on the Roche 454J platform, which allows the detection of very low amount of deleted mtDNA from peripheral blood samples. With this approach we are able to test for the three most common mtDNA deletions found in approx. 80% of KSS patients.

We could show that this new approach is able to characterize mtDNA deletions in patients with KSS from peripheral blood samples with a very high sensitivity. It allows clincial testing for KSS without muscle biopies.

P02.144

Neurocognitive phenotype and personality profile in men with Klinefelter syndrome and their vulnerability to psychiatric symptoms

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Klinefelter syndrome (KS) is associated with increased risk of psychiatric disease and behavioral problems. The background for these risks is not known.

The aim was to describe the cognitive function, personality traits and the vulnerability to psychiatric symptoms in patients with KS.

41 KS patients and 41 age- and educational-matched control subjects participated in the study. All participants were tested with standardized neuropsychological tests and 4 questionnaires investigating psychological problems.

KS patients scored significantly lower in processing speed, working memory, verbal abilities and showed a selective deficit in executive function compared to control subjects, whereas visual cognitive abilities and cognitive response inhibition was preserved. The KS patients displayed significantly higher levels of cognitive failures, emotional distress and autism traits as reported in questionnaires. Furthermore symptoms of anxiety were also significantly higher among KS patients, whereas there were no difference in depressive symptoms between KS patients and control subjects. On the NEO PI-R personality test KS patients scored high on the neuroticism scale, low on the extraversion scale and low on the conscientiousness scale.

Men with KS have deficits in several cognitive domains and have an altered personality phenotype. Furthermore our results suggest that KS patient may be associated with an increased genetic vulnerability to psychiatric symptoms. In future analyses, we are going to assess the neuroanatomical, neurofunctional, endocrine and genetic basis for the cognitive deficits, altered personality phenotype and increased psychiatric symptoms seen in KS patients. Whether testosterone therapy or other interventions can alleviate these deficits remain to be proven.

P02.145

L1 Syndrome diagnosed in a family with a manifesting female carrier with hydrocephalus and a fetus with agenesis of the corpus callousm.

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L1 syndrome is consistent with a variable clinical phenotype, including hydrocephalus, MASA syndrome, hereditary spastic paraplegia, and corpus callosum agenesis. Mutations in the L1CAM gene located on the X chromosome cause the full syndrome in males while females may manifest minor features such as adducted thumbs and/or subnormal intelligence. We present here a family with an unusual presentation of the L1 syndrome.

The couple was referred to genetic consultation after a pregnancy which was terminated due to a diagnosis of agenesis of the corpus callosum in a male fetus. Medical history revealed that the mother was diagnosed with hydrocephalus at 22 years that necessitated a surgical insertion of a shunt. No other family members had symptoms consistent with L1 syndrome**.** Sequencing of the L1CAM gene revealed that the mother was a carrier of a missense mutation: c.791G>A which is known to be causative for L1 syndrome. The mutation was also identified in the fetal DNA and in an asymptomatic maternal sister. The couple chose to proceed with preimplantation genetic diagnosis.

Conclusions: L1 syndrome can be associated with atypical presentations. A high level of clinical suspicion and active work-up is warranted in some cases.

P02.146

Occipital band heterotopia in an infant with partial merosin deficiency due to novel LAMA2 mutations

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Laminin α2 (merosin) deficiency (MDC1A) is the most common congenital muscular dystrophy in Western countries. Typically patients are hypotonic at birth, with muscle weakness and joint contractures. Many will sit independently, but less than 10% will walk. Lifespan can be shortened due respiratory compromise. Most children with MDC1A will have characteristic cerebral white matter hypodensities detected by MRI after 6 months of age. Neuronal migration defects (cortical dysplasia, polymicrogyria) are rare, occurring in approximately 4% of merosin deficient muscular dystrophies[1].

We report a case of a 1 year old boy with congenital hypotonia and an elevated creatine kinase level. Electromyography was consistent with a myopathic process. MRI of the brain at 14 weeks of age revealed band heterotopia bilaterally in the occipital lobes. There was also the suggestion of polymicrogyria involving the inferior occipital lobes and posterior temporal lobes bilaterally. Sequencing of the LAMA2 gene revealed three novel nucleotide changes of uncertain clinical significance. Two of the mutations were predicted to be probably damaging, and parental mutation analysis confirmed a trans orientation of these two mutations in our patient, which would be consistent with autosomal recessive inheritance. The patient also had one novel splice site mutation in the fukutin gene. Muscle biopsy at 9 months of age showed partial merosin staining and normal alpha dystroglycan staining, which together with the molecular results supports a diagnosis of MDC1A. The finding of band heterotopia in this patient expands the rare cortical dysplasia phenotype seen with this congenital muscular dystrophy.

P02.147

A case of Mandibuloacral Dysplasia type A due to heterozygous LMNA mutation

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Mandibuloacral Dysplasia type A [MADA; OMIM # 248370] is an autosomal recessive disorder, due to mutations in LMNA gene, characterized by peculiar facial dismorphysms, lipodystrophy and progeroid features.

We report a 28-year old Italian female patient with mandibular hypoplasia, scoliosis, lipodystrophy, breast aplasia and metabolic abnormalities. She was found to be heterozygous for a missense mutation c.1045C>T (p.R349W) in exon 6 of the LMNA gene.

She presented a features described in different LMNA-associated entities and a mutation previously described a patient with muscular dystrophy, cardiac involvement and lipodystrophy.

The case underlines the clinical heterogeneity and the unclear genotype-phenotype correlation in laminopathies.

P02.149

Large homozygous interstitial duplication within the LARGE gene in a patient with Walker-Warburg-syndrome

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Mutations in the LARGE gene are a rare condition causing Walker-Warburg-syndrome (WWS). We report on a girl with clinical features of WWS. The 19 months old girl, born to consanguineous parents, shows a very severe global developmental delay and extreme hypotonia. She is not able to turn her head or move any part of her body. Complex brain malformations with ventricular dilatation, absence of the inferior cerebellar vermis, and hypoplastic cerebellum were detected. She has got unilateral microphthalmia and cataract and ureteropelvic junction stenosis with hydronephrosis. Myoclonic seizures started at the age of 6 weeks. CK was highly elevated (>2000 U/l).

The diagnosis of WWS was confirmed by microarray analysis. It revealed an interstitial duplication of a 132 kb segment within the LARGE gene on both alleles (4 copies, 6.0 Affymetrix SNP-Array) in the index patient. The twofold duplication in the index patient was confirmed by MLPA. Both parents have 3 copies analogous to a duplication of one allele. We hypothesize that the partial duplication of the LARGE gene has a loss-of function effect on the duplicated allele with the parents being heterozygote carriers of this autosomal recessive disease, respectively, and the child being homozygous. This is the first case of WWS caused by a large duplication within the LARGE gene emphasizing the need of gene dosis analysis in WWS patients in whom mutations have been ruled out by conventional sequencing analysis. Furthermore it shows that array analysis should be considered even in cases with a presumed autosomal-recessive disease.

P02.150

Distinct and pathogenic substitution of IVS15+5G→A in the SLC26A4 gene in patients with enlarged vestibular aqueduct syndrome or Pendred syndrome in Okinawa islands.

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Background

The SLC26A4 gene, located on chromosome 7q22.3, is responsible for two clinically overlapped syndromes, Pendred syndrome (PS) and enlarged vestibular aqueduct syndrome (EVA). Distinctive SLC26A4 mutations in such patients were described in some ethnic populations. Previous studies revealed that the spectrum of SLC26A4 mutation was different in different ethnic background. For example, a mutation, H723R, was reported as the most common mutation for EVA and PS in Japanese.

We investigated 18 patients from 18 unrelated families with EVA or PS to define the frequency of SLC26A4 mutations and clinical manifestations in Okinawa islands.

Results

Eight patients diagnosed with PS, and 10 patients with NSEVA were examined in the SLC26A gene. Mutations of the SLC26A4 gene were identified in 15 out of 18 patients. Of the 15 patients, four patients were having a homozygous mutation of H723R, six patients were having compound heterozygous mutations of H723R and IVS15+5G>A, four patients were homozygous mutation of IVS15+5G>A, and one patient had heterozygous mutation of IVS15+5G>A.

Among the mutations detected, IVS15+5G→A was most common, accounting for 61.1% (11/18) of the patients. In order to know whether the mutation was pathogenic, we performed a quantitative RT-PCR for SLC26A4 in patients with homozygous mutation of IVS15+5G→A. The result showed that the SLC26A4 gene was not expressed in the patients.

Conclusions

The substitution of IVS15+5G>A in the SLC26A4 gene was most common in PS or EVA patients in Okinawa area. The substitution of IVS15+5G→A caused loss of expression in the gene, which affects PS or EVA.

P02.151

New mutation in PTPN11 gene causing LEOPARD syndrome with prominent cardiac hypertrophy.

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Background: LEOPARD syndrome is a rare autosomal dominant disorder characterized by multiple lentigines and café-au-lait spots, hypertelorism, cardiomyopathy and heart rhythm defects, pulmonary stenosis, abnormalities of genitalia in males, growth retardation, and deafness. Clinical manifestation is highly polymorphic. Mutations in the three genes (PTPN11, RAF1, and BRAF) can be responsible for LEOPARD syndrome. About 85% of all cases are PTPN11-positive.

Clinical case: We did observe 17 y.o. male patient with prominent hypertrophic cardiomyopathy, left outflow tract obstruction, mitral valve insufficiency III-IV, left atrium dilation, ventricular and supra-ventricular extra systoles. The heart rhythm was imposed by pacemaker, implanted in 2005 (at 13 y.o.), the battery was almost discharged. Extra-cardiac symptoms include multiple lentigines, growth retardation, pectus carinatum, and feet deformation. Parents were apparently healthy. Surgery treatment included mitral valve replacement, left ventricular outflow resection, sub-aortic membrane excision, explanation of electrodes, pacemaker and ICD implantation.

Genetic screening results: We did perform Senger sequencing of PTPN11, RAF1 and BRAF genes by direct Sanger sequencing. New genetic variant p.Thr468Met in PTPN11 gene was found in proband's DNA sample but not in a control group. Additionally, several SNPs in RAF1 gene without clear clinical importance were found.

Conclusion: We suspect that new variant p.Thr468Met in PTPN11 gene is a mutation causing LEOPARD syndrome. Clinical phenotype characterized mainly by cardiac and skeletal involvement. It's important for clinicians to make correct and timely differential diagnostics between hypertrophic cardiomyopathy and inherited syndrome accompanied by myocardial hypertrophy.

P02.152

Leri Weill Dyschondrosteosis -The bone microarchitecture in subjects with mutation in the SHOX-gene

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Background: Leri Weill Dyschondrosteosis (LWD) syndrome is characterized by short stature with mesomelic disproportion of the limbs and Madelung deformity caused by SHOX-haploisufficiency. The morphology of the bones is only partly described.

Aim: To assess volumetric bone mineral density, microarchitecture, and strength in subjects with LWD and controls.

Methods: Controls matched on sex and age. A high-resolution peripheral quantitative CT scanner was used to measure volumetric BMD, bone geometry, and microarchitecture of the non-dominant distal radius and the distal part of tibia. Osteoporosis was defined as a T-score<- 2.5 SD on the basis of a DXA scan.

Subjects: Five families comprising 22 individuals (15 females) aged 38 years [IQR: 21-35] were included and controls. SHOX-mutations: c.440G>T (n=2), c.657delA (n=9), del exon 3-4 (n=2), del SHOX (n=9).

Results: Tibial trabecular thickness was lower in cases (0.067 vs. 0.076, $p<0.05$). In radius the cortical areal was larger in cases (74.4 vs. 56.5, $p<0.001$), cortical thickness was increased (1.16 vs. 0.82, $p<0.001$) and the trabecular number decreased (1.61 vs. 1.90, $p<0.05$). Bone strength was similar in cases and controls. The radial cortex adjacent to ulna was absent in 5 cases. Four subjects were osteoporotic based on a T-score but neither reported a previous fracture.

Conclusion: These results suggest that bone microarchitecture is changed in LWD cases. The increased cortical thickness in radius may be caused by a more proximal measurement of the radius of cases due to the mesomelic forearm. 5 cases had a radial cortical defect.

P02.153

A new inactivating LH receptor mutation causes a disorder of sex development in a 46,XY girl and amenorrhea in her 46,XX sister

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Leydig cell hypoplasia (LCH) is a rare autosomal recessive condition that interferes with normal development of male external genitalia in 46,XY individuals. In 46,XX women primary and secondary sexual characteristics are developed normally but they are suffering from amenorrhea and infertility. Here we report a family with two affected sisters suspected to have an inactivating mutation in the LH receptor gene (*LHR*).

A 14-year-old girl was referred with lack of the progression in breast development and amenorrhea. In physical examination her height was 165.7cm and weight 81.5 kg. Breast development was Tanner stage I with lipomastia and pubic hair development Tanner stage III. She had female external genitalia with mild posterior labial fusion.

Hormonal evaluation revealed FSH 2.62mIU/ml, LH 10.94 mIU/ml, E2 <10pg/ml,

Testosterone <20ng/dl. Karyotype was 46,XY. Pelvic MRI demonstrated testicular tissue bilaterally in inguinal regions. She had therapeutic gonadectomy.

Her 21-year-old sister was also evaluated when she was 15 years old for amenorrhea. Her secondary sexual characteristics were well developed. Pelvic ultrasonography showed an uterus with atrophic endometrium. In hormonal evaluation, serum LH level was 30.9 mIU/ml, FSH 7.68 mIU/ml, E2 35.44 pg/ml and testosterone 36.8ng/dl.

Sequence analysis of the *LHR* gene showed a new homozygous mutation p.Gly71Ala in both sisters.

This mutation is located in the extracellular ligand-binding domain in exon 2, probably interfering with binding of LH to the LH receptor. *In vitro* experiments with the mutant receptor will follow to get more insights into the binding capacity and activity of this receptor.

P02.154

Splice mutations of the luteinizing hormone receptor gene (*LHR*) as a cause of 46,XY disorders of sex development (DSD)

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Leydig cell hypoplasia (LCH) is a rare autosomal recessive condition that interferes with normal development of male external genitalia in 46,XY individuals. Inactivating mutations of the human luteinizing hormone receptor (LHR) lead to decreased response of Leydig cells to LH and hCG.

Here we report 17 patients in 4 families with 46,XY disorder of sex differentiation caused by homozygous or compound heterozygous mutations in non-coding DNA sequences of the luteinizing hormone receptor gene (*LHR*). 14 patients in 2 different families were homozygous carriers of a mutation at the exon splice donor site in intron 1 (c.161+4A>G) whereas two sisters were heterozygous for a missense mutation (p.Y623S) in combination with a mutation 34 nucelotides downstream of the splice donor site of exon 4 (c.383+34G>A). One patient was homozygous for a splice acceptor site mutation in intron 1 (c.162-3T>G). In vitro minigene expression was employed to examine the effect of the mutations on pre-mRNA splicing, while for one patient testis RNA was available to validate the minigene expression approach. The results of these molecular studies represent a large increase in the spectrum of mutations that cause Leydig cell hypoplasia in 46,XY disorders of sex differentiation patients and to the tools available for its molecular diagnosis.

P02.155

Evaluating the clinical significance of observations of loss of heterozygosity in SNP-arrays

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SNP-based detection of loss of heterozygosity (LOH) is now supported by most major array providers. Many labs are therefore for the first time challenged with the task of interpreting the clinical significance of areas of LOH in the genome. However, until now no formal guidelines for the interpretation of LOH are available. In this study we present our internal workflow for the handling of observations of LOH.

In this workflow we are grouping LOH observations into three groups: LOH interstitially on one chromosome, LOH on one chromosome extending to the telomere and LOH on multiple chromosomes. For each group of LOH observations, we review the possible biological mechanisms, the possible clinical significance and we suggest a procedure for further investigations of the observation. Each workflow takes into consideration resources and time constraints typically present in a clinical genetics laboratory setting.

Hopefully these workflows can aid other laboratories in setting up their own internal workflow for interpreting LOH observations or add to a discussion of this new “challenge” in clinical genetics.

P02.156

The level of hearing loss among patients with the mutation m.A1555G

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Many commonly used medications may be in a transient and reversible, or permanent adverse effect on the level of hearing impairment in humans. It is believed that some aminoglycoside antibiotics can damage the sensory epithelium in the inner ear. In case of ototoxic aminoglycosides (eg gentamicin, amikin, streptomycin) causing the damage to the bacterial ribosome, mitochondrial ribosome may be destroyed because of its similarity. Susceptibility to such antibiotics effect is passed in the maternal line, indicating the mitochondrial type of inheritance.

Many mutations in the mitochondrial genes 12S rRNA and tRNASer related to „aminoglycoside“ hearing loss were described. One of them is m.A1555G mutation which occurs in a highly conserved region of 12SrRNA molecule, leading to reduced production of ATP in the cells of the cochlea.

We studied a group of 1933 patients of the Institute of Physiology and Pathology of Hearing for the presence of mutation m.A1555G using RealTime PCR technology. In the studied group 25 patients with this mutation were found.

The aim of this study was to assess the level of hearing loss among patients with the mutation m.A1555G

P02.157

Novel c.1731delC mutation in *RIN2* gene in two Turkish siblings with MACS / RIN2 syndrome

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Inherited disorders of connective tissue, such as Ehlers-Danlos(EDS) and the Cutis Laxa syndromes(CLS), are a heterogenous group of disorders which are characterized by hyperextensible skin and joint laxity. Thin/translucent skin with visible veins and easy bruising are also observed in EDS patients. The EDSs involve a genetic defect in synthesis and structure of collagen and collagen fibril assembly while the cutis laxa syndromes(CLs) are characterized by loose/redundant skin and growth retardation and in some types defective biogenesis of elastic fibers have been shown.The mutations in *RIN2* gene were first reported on three patients by Basel-Vanagaite et al.,(2009) in two related families of Israeli-Arab origin who showed macrocephaly, coarse and swollen facial appearance affecting the eyelids, lips, and cheeks, scoliosis, skin hyperlaxity and joint hypermobility. The acronym **MACS**; **M**acrocephaly, **A**lopecia, **C**utis laxa and **S**coliosis; was suggested for this new syndrome related to the cutis laxa group of inherited disorders. Recently novel mutations in *RIN2* gene were identified in three affected patients from Algeria by Syx et al.(2010) who were formerly reported as EDS-like syndrome in 2005 by Verloes et al. The authors discussed that MACS acronym may not be the most appropriate term to sum up the phenotypic spectrum and suggested **‘’RIN2 syndrome’’.**

We here describe the third family with MACS/RIN2 syndrome in two siblings from Turkey also displaying additional findings such as hypogonadism and mediastinal tumor, carrying a novel homozygous c.1731delC mutation resulting in frame shift in *RIN2* gene and causing protein truncation.

P02.158

De Novo triplication of the *MAPT* gene from the recurrent 17q21.31 microdeletion region in a patient with moderate intellectual disability and various minor anomalies

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We report on a 16-year old male patient with moderate intellectual disability, behavioral problems, and further anomalies such as facial dysmorphism, heart defect and urogenital anomalies. By molecular karyotyping we identified the first *de novo* copy number gain to four copies on chromosome 17q21.31 including the *MAPT* gene but not the entire recurrent microdeletion/microduplication region. Recurrent microdeletions of this region including the *MAPT* and the *CHRH1* genes have been shown to be a relatively frequent cause of intellectual disability, while only a few reciprocal duplications in patients with variable cognitive disorders have been published so far.

A common inversion polymorphism in this region has been linked to a distinct H2 haplotype and seems to be associated with an increased risk for microdeletions and -duplications. Our patient and his father were both heterozygous for the H1/H2 haplotype, whereas the mother was homozygous for the H2 haplotype. Interestingly, in our patient the dosage gain apparently occurred on the paternal H1 allele and did not involve the H2 allele as in the previously published cases.

This patient further delineates the genotypic and phenotypic variability associated with copy number variants from the 17q21.31 microdeletion region.

P02.159

Novel mutations causing Marfan syndrome in Czech population and rare case of compound heterozygozity

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Background: Marfan syndrome (MFS) is an autosomal dominant connective tissue disorder mainly involving the cardiovascular, skeletal and ocular systems. The estimated incidence is of about 1:5 000 - 1:10 000 and approximately 25% of cases are associated with *de novo* mutations. MFS is caused by mutations in fibrillin 1 gene (*FBN1*, 15 q15-q21.1.) resulting in defective glycoprotein fibrillin-1. Recently, there are showed that three other genes FBN2 (5q23-q31), TGFBR2 (3p22) and TGFBR1 (9q22) influence MFS.

Aims: In this study we performed SSCP analysis of all 65 exons of FBN1 gene in order to identify novel mutations in 570 unrelated patients with clinical diagnosis of Marfan syndrome.

Materials and Methods: DNA was isolated from whole blood, the molecular analysis includes mlpa (multiplex ligation-dependent probe amplification) and separation of PCR products by SSCP (single-strand conformation polymorphism). Exons with abnormal migrating patterns were sequenced.

Results: We identified 87 *FBN1* mutations, 53 of them have not been previously described. Of the 46 were missense, 15 nonsense, 7 splicing, 17 small deletions or insertions and 2 gross deletions. Than we identified 4 patients with compound heterozygosity at the *FBN1* locus, that is very rare.

Conclusions: We have confirmed 107 cases of Marfan syndrome caused by *FBN1* mutation, which constitutes detection of about 19%. But mutation screening of *FBN1* should yield a result in about 80-85% of MFS patients who meet the Ghent criteria. Our result is much lower due to sensitivity of SSCP and because the patients involved did not meet the Ghent criteria.

P02.160

Interesting case of an atypical Marfan syndrome patient

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The Marfan syndrome is an autosomal dominantly inherited genetic disorder due to mutations in the fibrillin-1 (FBN1) gene, a gene that encodes a connective tissue protein.

Consequently, mutations in this gene cause skeletal and connective tissue symptoms (pectus carinatum/excavatum, hypermobile joints, arachnodactyly), complications of the cardiovascular system (dilatation/ dissection of ascending aorta, mitral valve prolapse), and the eyes (ectopia lentis, myopia). Patients suffering from Marfan syndrome are at risk of aortic rupture.

Here we report an atypical case of Marfan syndrome.

The 44 year old patient was hospitalized in 2007 with a spontaneous dissection of the arteria carotis interna. Last year, an infrarenal aneurysma of the abdominal aorta was diagnosed. Physical examination showed a patient of below average size (167 cm (mother and father: 160 cm)). The arm span/ body height ratio was 0,98 (<1,05), neither skeletal nor ocular abnormalities were present. Thumb signs and wrist signs were negative. Thus classical characteristics of the syndrome were non-existent. Family history showed that the patient's mother died at the age of 56 due to an arterial rupture.

Molecular analysis of the FBN1 gene revealed a missense mutation c.4727T>C (p.M1576T) in exon 37. This mutation leads to an amino acid exchange in the TGF-beta domain of the FBN1 protein.

In conclusion, the life threatening complications of Marfan syndrome can occur in patients without any of the typical Marfan features.

P02.161

Epidemiological study of *MECP2* duplications in France

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Duplication of Xq28 including the *MECP2* gene has been described primarily in male patients with severe developmental delay, progressive spasticity, epilepsy, stereotyped hand movements and recurrent infections. The aim of our study was to carry out an epidemiological study in order to determine the number of cases identified in France since the implementation of targeted molecular study (MLPA) and array-CGH, and to estimate the proportion of patients detected by either method. The 15 French cytogenetic and molecular labs were contacted and biological and epidemiological data were gathered in all cases. 71 symptomatic patients with a *MECP2* duplication of less than 4 MB were collected, including 66 boys and 5 females ranging from 0 to 24 years. The *MECP2* duplication was identified in 45 patients (63%) using a targeted analysis, and in 26 patients (37%) using an array-CGH analysis. The majority was inherited from unaffected mothers and the size of the duplication varied between cases. Within the 5 females, the *MECP2* duplication resulted from an unbalanced X-autosome translocation (3 cases), random X inactivation (1 case) or a partial MECP2 duplication (1 case, exon 4 only). Two cases were detected prenatally by array-CGH, in a context of generalized lymphoedema or polymalformations (*MECP2* triplication). From this study, besides the epidemiological data, we conclude that the diagnosis of *MECP2* duplication can be difficult since 1/3 of cases were detected using non targeted analysis. The second step of this study will gather the clinical data from this cohort in order to search for genotype-phenotype correlations.

P02.162

Dosage changes of MED13L further delineate its role in heart defect and intellectual disability

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A chromosomal balanced translocation disrupting the MED13L (PROSIT240) gene was previously reported to be associated with transposition of the great arteries (TGA) and intellectual disability (ID) and led to the identification of missense mutations in three patients with isolated TGA. Recently, a homozygous missense mutation in MED13L was found in 2 patients with non-syndromic ID (without cardiac involvement) from a consanguineous family. Next to the clinical findings, studies on *c. elegans* imply a specific role for MED13L in regulating transcription of the Wnt and Shh signaling targets which are crucial for embryonic development. Here, we describe 2 new aberrations of MED13L which were detected by means of molecular karyotyping. The first one is a *de novo* 17 kb out of frame deletion of its exon 2 in a patient with complex congenital heart defect, ID, gross and fine motor coordination problems and dysmorphic features. The second aberration is a 1 Mb *de novo* triplication in 12q24.21 including MED13L, several non protein coding RNA genes and MAP1LC3B2, in a patient with a milder phenotype including learning difficulties and perimembranous VSD (closed spontaneously). These findings suggest that abnormal MED13L dosage affects both, cardiac and neurologic developement. While different missense mutations result in either cardiac or intellectual problems, heterozygous copy number variants can lead to both in a dose dependent manner. Haploinsufficiency of the gene is associated with a more severe phenotype including complex heart defect and ID where as the outcome of increased gene dosage is a milder phenotype in both systems.

P02.163

Simultaneous occurrence of medullary cystic kidney disease type 2 and autosomal dominant polycystic kidney disease in a single family due to novel *UMOD* and *PKD1* mutations

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Background: Medullary cystic kidney disease (MCKD) is a rare autosomal dominant renal disease characterized by medullary cysts, tubulo-intertstitial nephritis, progressive chronic renal failure leading to end-stage renal disease in adult life and, in occasional instances, gout. Two loci have been identified: mutations in the uromodulin gene (*UMOD*, 16p12.3), encoding the Tam-Horsfall glycoprotein expressed in the thick ascending and distal tubules, were found to associate with MCKD type 2 (MCKD2). Clinical symptoms might show inter- and intra-familiar variability, making robust genotype/phenotype correlations hard to establish.

Patients and methods: A 3 generation MCKD2 family was evaluated. Eight members (7 affected, 1 non-affected) were available for molecular and phenotype investigation. Immuno-histochemical analyses of kidney biopsies were performed. Genetic screenings were performed by PCR, direct sequencing and MLPA.

Results: We detected a novel mutation in the *UMOD* gene in 6/7 patients tested with intra-familial variability of the phenotype. In one affected individual we did not find any *UMOD* mutations. However, subsequent screening of the polycystic kidney disease (ADPKD) associated genes *PKD1* and *PKD2* in this patient revealed a novel variant in *PKD1*.

Conclusions: We present the clinical, immuno-histochemical and genetic results of a family demonstrating a unique finding of simultaneous appearance of two different cystic kidney diseases (MCKD2 and ADPKD) and with novel mutations in the *UMOD* and *PKD1* genes. Beside the phenotypic variability of MCKD2, our findings demonstrate the possibility of genetic variety as the cause of cystic kidney disease even within the same family.

P02.164

Controversy in the mode of inheritance in Familial Mediterranean fever diseases; Molecular Analysis of *MEFV* Gene in Patients

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Familial Mediterranean fever is an autosomal recessive disorder characterised by episodic fever, abdominal and pleuritic pain, serositis and arthritis. The FMF gene (MEFV) generates a protein found exclusively in granulocytes. In this study, we report our 4-year experience of over 267 Azeri Turkish patients with this disease

in the central region of Algeria

267 Azeri Turkish unrelated subjects clinically diagnosed with FMF from various clinics from north west of Iran were referred by specialists to the Medical Genetic Centre of Tabriz University of Medical Sciences. A clinical diagnosis of FMF was made according to published Criteria. Mutation screening of the MEFV gene was performed for the 2, 3, 5 and 10 exons by using sequencing method.

163 (% 61) of these patients had one or two mutations. Of those with mutations, 83 were compound heterozygous, 46 were homozygous, and 34 had only one identifiable mutation. The most frequent mutations were M694V and E148Q (41% and 23% respectively) of the alleles, followed by V726A (17%), M680I (16%) mutations.

The results show the diversity and the frequency of the FMF mutations in the Iranian Azeri Turkish FMF patients, and indicate that FMF is one of the common inflammatory diseases in the north west of Iran that must be noted in management of cases with episodic fever, abdominal and pleuritic pain have been referred to clinic. Also, the mode of inheritance in almost 30% of FMF patients was Autosomal dominant.

P02.165

Megalencephaly, thick corpus callosum, dysmorphic facial features, and mental retardation in four unrelated patients: a new syndrome?

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Macrocephaly associated with mental retardation is a part of various syndromes including overgrowth conditions, cutis marmorata telangiectatica, Cowden disease, and neuro-metabolic disorders. We report here a new association characterized by progressive macrocephaly, mental retardation, specific facial features and similar magnetic resonance imaging (MRI) findings in four unrelated boys. All patients except one were born from non consanguineous parents. Case 1 walked at 3 years, developed seizures at 5 years. At 15 years, he had limited speech and a large head circumference (59cm, +3SD). Case 2 developed seizures at 3 years, walked at 8. He had limited speech and head circumference was 64 cm (>+4 SD) at 14 years. Case 3 developed seizures at 2 months, walked at 20 months. At 23 years, he had no speech, and head circumference was 62 cm (+4 SD). Case 4 had severe global developmental delay and head circumference curve was above + 2 SD. The four patients had the same dysmorphic features including downslanting palpebral fissures, long and expressionless face, small mouth. Three of them had enamel defect and distal amyotrophy. In all children, brain MRI detected bilateral megalencephaly, a thick corpus callosum, an enlarged white matter and normal ventricles. Whereas CT scans were initially normal, calcifications of basal ganglia were present in case 2 at 15 years, and in case 3 at 23 years. Extensive metabolic and cytogenetic studies were no contributive. We hope that ongoing cytogenetic and molecular studies will further define this new entity distinct from previously known syndromes with megalencephaly.

P02.166

MFRP-related oculopathy in a child and a distinct retinal disease in his mother

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The combination of nanophthalmos and retinal dystrophy is a rare syndrome recently identified as an inherited autosomal recessive disease caused by mutations in the MFRP gene. A 7-year old boy with high hypermetropia and signs of a progressive retinal dystrophy was referred. His 37-year old myopic mother was known to have a retinal dystrophy since childhood classified as M. Stargardt, consanguinity of the parents was denied. The boy was found to have nanophthalmos with the striking aspect of the fundus with a „macular fold“ and clinical and ERG findings indicating disturbed photopic and scotopic vision. Molecular analysis identified a homozygous truncating mutation in the membrane-type frizzled-related protein (MFRP) gene. Complete sequence analysis of the MFRP gene in the mother showed heterozygosity for the mutation identified in the patient, and no further mutation. The heterozygous mutation of the MFRP gene is not sufficient to cause the phenotype of the mother, and we assume that two phenotypically and genetically distinct retinal dystrophies exist in the family. A comprehensive molecular genetic screening of the mother was initiated.

P02.167

Microcephaly-Capillary Malformation Syndrome (MIC-CAP): a new case

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Microcephaly-Capillary Malformation (MIC-CAP) syndrome was first described in 2011 as a disorder combining severe microcephaly with progressive cortical atrophy, intractable seizures, profound developmental delay and multiple small capillary malformations on the skin. An autosomal recessive mode of inheritance was implied in the families of the six described patients. We describe an affected male patient, the first child of non-consanguineous German parents, who was delivered by cesarean section due to pathological CTG at 34 weeks. Oligohydramnios was noted prenatally, a cranial deformity with severe microcephaly was noted after delivery (OFC 29 cm), as well as cutaneous macules. Seizures with tonic or clonic semiology started on the first day of life and have remained pharmacoresistant ever since. His EEG showed multifocal spikes, focal seizure patterns and a sinusoidal alpha activity, often seen in children with severe malformations of cortical development. Brain MRI revealed severely reduced cortical gyration both anteriorly and posteriorly. At the age of 18 months he has no appreciable psychomotor development, no eye contact, no vocalization and spastic quadriparesis with axial hypotonia. He is severely dysmorphic with a disproportionately small cranium and low-sloping forehead, broad nasal bridge, hypertelorism and shallow philtrum. Toes are short and partly overlapping, the big toes have dysplastic nails. The scrotum is hypoplastic with small testes. An international consortium has been established in order to elucidate the molecular genetic cause of the disorder.

P02.168

A de novo 12q13.13 microdeletion in a patient with mild mental retardation, disproportionate habitus, facial dysmorphy and congenital heart defect.

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We report a de novo 12q13.13 deletion in an 4.5-year-old dysmorphic boy with multiple congenital anomalies/mental retardation (MCA/MR) syndro-

me consisting mainly of mild to moderate MR, disproportional habitus with extremely narrow shoulders and long, narrow thorax, facial dysmorphism (long narrow face, hypotelorism, enophtalmus, wide nose), onychodystrophy, fine hair, atypical hand grip between index and middle fingers, ulnar duction of both hands, ventricular septal defect, pulmonary stenosis, and cryptorchism. His behavioural pattern is very remarkable with a sad expression in the face, timidity and balbuties. The deleted region is 0.95 Mb long and encompasses the whole HOXC gene cluster and 19 additional proximally located genes. Although the HOXC cluster was shown to be dispensable in mouse, we speculate that its haploinsufficiency could potentially be responsible for the remarkable disproportionate stature of the proband. Reduced dose of several other genes mapping to the deletion may also influence this specific phenotype, especially RAGR which plays a role in limb bud development and skeletal growth, or SP7 which influences bone formation. MR of the patient could be influenced by neural-expressed genes AAAS and MAP3K12, but the remaining deleted genes could also influence his phenotype. To our knowledge this is the first report of a de novo 12q13.13 microdeletion removing the whole HOXC cluster and several neighbouring genes, which is likely associated with the MCA/MR syndrome in the patient.
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P02.169

Microdeletions in 9q33.3-q34.1 are associated with developmental delay, micro-/brachycephaly, and seizures of incomplete penetrance

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We report four patients with overlapping microdeletions of chromosome 9q identified by molecular karyotyping using various platforms. Their common clinical features include developmental delay with delayed or absent speech and micro- and/or brachycephaly. *De novo* deletions of 1.76, 1.3, and 2.8 Mb, in 9q33.3-q34.11 were detected in patients 1 through 3, respectively, whereas the 432 kb 9q33.3 deletion in patient 4 was inherited from her mother who is reported to have mild intellectual disability.
The smallest region of overlap (SRO) is defined by the deletion in patient 4 and includes only three RefSeq genes. Interestingly, the three larger deletions have 27 genes in common including *STXBP1*, which plays a role in synaptic transmission. *STXBP1* loss-of-function mutations and deletions have been associated with early infantile epileptic encephalopathy (EIEE, or Ohtahara syndrome), and were identified in patients with intellectual disability and non-syndromic epilepsy. In our cohort, the seizure phenotype appears to be incompletely penetrant. Notably, patient 4 had seizures, although her deletion excludes *STXBP1*. Thus, the involvement of *STXBP1* and/or possibly other genes due to deletion of long-range regulatory elements cannot be ruled out. Sequencing of the three SRO genes in a cohort of unrelated patients with mild to severe idiopathic intellectual disability, as well as gene expression analyses in our patients, is underway to identify the causative gene(s).
We suggest that microdeletions of this region on chromosome 9q may cause a clinical spectrum including developmental delay especially concerning speech, micro- and / or brachycephaly, mild dysmorphisms and seizures of incomplete penetrance.

P02.170

Description of 2 patients with overlapping duplication of chromosome 20q11.2 with abnormal shape head and intellectual deficiency

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The current use of microarray-based comparative genomic hybridization allowed the identification of novel cytogenetic abnormalities. Partial trisomies of the long arm of chromosome 20 are rare. Here we report de novo 20q11.2 confirmed in CGH-Array in two boys with intellectual deficiency, speech delay, and distinctive dysmorphic features with abnormal shape head and short hands. The breakpoints and sizes of the duplications are different but included the *ASLX1* gene (MIM612990). *ASLX1* is a human homolog of the Drosophila *asx* gene which is an homeotic gene. Recently, de novo heterozygous nonsense or truncating mutations in the *ASLX1* gene have been reported in 7 of 13 unrelated patients with Borhing-Opitz syndrome (MIM605039), a severe developmental and malformation disorder. The finding of two other patients in the literature with overlapping 20q11 microduplication and clinical features allowed us to reduce the minimal critical region to 7,4MB encompassing 173 genes including *ASLX1* gene. Interestingly, patients display clinical features in common with Borhing-Opitz syndrome, and in particular abnormal shape head. We hypothesize that the duplication of *ASLX1* is responsible of a milder phenotype resembling Borhing-Opitz syndrome. These observations give data in favour of a novel microduplication syndrome and suggest a contribution of *ASLX1* to the phenotype. Reporting of additional patients with molecular characterization will allow more detailed genotype-phenotype correlations.

P02.171

CGH Array in Miller-Dieker syndrome

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Miller-Dieker syndrome is one of the reason of profound neurodevelopmental delay with lissencephaly.A contiguous gene deletion in 17p13.3 is documented.It is characterized by microcephaly,bitemporal narrowing ,small nose,anteverted nostils,prominent upper lip,micrognathia,low set ears,vertical ridging and forrowing in central forehead,hypotonia,seizure,brain malformation like incomplete development of brain often with smooth surface (lissencephaly), hypoplasia of corpus callosom, and profound mental ratardation. we are reporting , two patients aged 1 and 2 years with microcephaly ,profound mental retardation,bitemporal narrowing,low set ears,and lissencephaly.In CGH Array ,we found the deletion in 17p13.3 which documented the disease.in this article we want to emphasize on CGH Array in lissencephaly spectrum.

P02.172

Agenesis of the corpus callosum and gray matter heterotopia in three patients with constitutional mismatch repair deficiency syndrome

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Constitutional mismatch repair deficiency (CMMR-D) syndrome is a rare inherited childhood cancer predisposition caused by biallelic germline mutations in one of the four MMR-genes, *MLH1*, *MSH2*, *MSH6* or *PMS2.* Due to a wide tumor spectrum, the lack of specific clinical features and the overlap with other cancer predisposing syndromes, diagnosis of CMMR-D is often delayed in pediatric cancer patients. We report of three new CMMR-D patients each of whom developed more than one malignancy. The common finding in these three patients is agenesis of the corpus callosum (ACC) and gray matter heterotopia present in two patients. Fifty-seven CMMR-D patients with brain tumors (therefore all likely had cerebral imaging) have previously been reported, one of whom had cerebral malformations. Taken together with our patients, the prevalence of cerebral malformations is at least 4/60 (6.6%). This number is well above the population birth prevalence of 1 to 4/4,000 live births with these cerebral malformations, suggesting that ACC and heterotopia are features of CMMR-D. The presence of cerebral malformations in pediatric cancer patients should alert to the possible diagnosis of CMMR-D. ACC and gray matter heterotopia are the first congenital malformations described to occur at higher frequency in CMMR-D patients than in the general population. Further systematic evaluations of CMMR-D patients are needed to identify possible other malformations associated with this syndrome.

P02.173
Array detection of apparent mosaic monosomy 7 - a marker for underlying disorders of genome maintenance?

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The increasing availability and precision of array-based comparative genomic hybridisation (aCGH) techniques allow detection of subtle abnormalities that may have previously eluded traditional analyses. We present a male baby (Case 1), born at 36+4/40 gestation, with dysmorphic facies, severe micrognathia, cleft palate, choanal stenosis, small extremities, hypoplastic terminal phalanges and normal genitalia. He had a ventricular septal defect and a patent ductus arteriosus. aCGH analysis on blood DNA revealed mosaic monosomy for chromosome 7 (estimated 6% of cells). Mosaicism was not detected in fibroblasts, and there was no overt evidence of a blood dyscrasia on a blood smear. He died at age 4 months.

Monosomy 7 is a common feature in several myeloproliferative disorders and is very rare as a constitutional abnormality. We previously identified three male patients (Cases 2-4) with features reminiscent of IMAGe syndrome (Intrauterine growth retardation, Metaphyseal dysplasia, Adrenal insufficiency and Genital abnormalities), who were found to have monosomy 7 mosaicism in blood or bone marrow. This was associated with a confirmed myelodysplastic process in at least one child, who underwent bone marrow transplantation in the first year, remaining stable at age 7 years. The other two died in early infancy. Case 1 above, however, had normal adrenal function and normal genitalia, possibly suggesting a different underlying disorder.

We hypothesise that these children display defective genome maintenance contributing to their phenotype, with monosomy 7 as a secondary event. Exome sequencing is in progress in two of our cases.

P02.174
Syndromic Moyamoya disease and hemophilia A caused by Xq28 deletion in Czech patient

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There are 5 types of Moaymoya disease (MYMY) with genetical alignment, of which type 4 is syndromic. MYMY4 described patients suffer from MYMY, growth retardation, hypergonadotropic hypogonadism (HH), azoospermia and stigmatization. Cardiomyopathy, cataract and stroke may also occur.

We present a 19-years old Czech patient with normal intellect, who has dysmophic features, growth retardation since 10 years of age, HH with regress of puberty in 15 years and azoospermia, but shows neither neurologic nor cardiologic symptoms. Unlike any of described patients he suffers from severe hemophilia A with level of factor VIII under 1% and low level of inhibitor (antibodies against exogenous FVIII).

The patient has normal karyotype 46,XY and there has been found no mutation in F8 gene. Array CGH investigation was later performed in this patient and cryptic deletion within Xq28 was detected. The deletion removed approximately 100 kb and the deleted region harboured promotor of F8 gene as well as genes MTCP1/MTCP1NT and BRCC3, which might play an important role in syndromic MYMY development.

After obtaining the array CGH result, we immediately performed MRI, MRA and TCCS (transcranial colour coded sonography) with perfectly normal results.

Phenotype - genotype correlation will be further discussed.

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P02.175
The NSEuroNet Database: an online resource for mutation spectrum and phenotype correlations in RASopathies

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Mutations in proteins involved in the RAS/MAPK pathway cause Noonan syndrome, Costello syndrome, cardio-facio-cutaneous syndrome, Noonan syndrome with multiple lentigines/LEOPARD syndrome, Noonan syndrome with loose anagen hair/Mazzanti syndrome, CBL mutation-associated syndrome, neurofibromatosis type 1 and related phenotypes, collectively called RASopathies. While the number of mutations and sequence variants identified in RASopathy genes is still increasing, the probability for novel mutations to be reported to the public has become quite low and the significance of rare variants may even remain unclear. Moreover, phenotypic data of published cohorts is hardly comparable due to the lack of standardization, and individual study cohorts do not reach the statistical power for reliable genotype-phenotype correlations.

To overcome these limitations, the NSEuroNet Consortium has established a database that contains all published germline mutations in the known RASopathy genes (excluding NF1), unpublished mutations observed by the consortium partners and collaborators, as well as polymorphisms and unclassified variants. In addition, standardized clinical datasets on a steadily increasing number of patients with a molecularly proven RASopathy are collected in order to establish genotype-phenotype correlations. Data can be submitted by registered users via an online questionnaire, are reviewed and then added to the database.

The database will be freely accessible. It can be browsed for genes, mutations or phenotypes through a user-friendly graphical surface. We introduce this novel resource which will be of great value for scientists as well as for clinical geneticists involved in counselling of patients with these disorders and their families.

P02.176
Genotype-phenotype correlation in cohort of patients with myotonic syndromes.

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Myotonic syndromes (MS) - a heterogeneous group chloride and sodium channels diseases with marked clinical polymorphism and often overlapping phenotypes. Attempts are being made to optimize the algorithm of finding causative mutations in the genes for the diagnosis of MS.

We have conducted a molecular genetics study in 94 patients from 65 unrelated families with MS. In 44 patients with myotonia congenita (MC) were revealed 26 different mutations in the *CLCN1* gene, in 39 patients with myotonic dystrophy type 1 (DM1) were detected increased number of CTG-repeats (n>50) in the *DMPK* gene and in 8 patients with clinical hyperkalemic periodic paralysis (HYPP) with myotonia - mutations in the *SCN4A* gene.

In the three formed groups, we performed a study of the decrement of compound muscle action potential (CMAP) (50 Hz 200 repetitions): 34 cases of MC, 25 cases of DM1 and 7 cases of HYPP. Decrement of CMAP was observed in all patients with MC and was 68±21%, no significant statistical differences in the values of the decrement between the patients with Thomsen (7cases) and Becker (27cases) myotonias: 74±8% and 67±22% accordingly. In patients with DM1 decrement of CMAP was detected in 15 of 25 patients and was 33±14%. In patients with HYPP decrement was found in one of seven patients and was 34%.

The largest decrement of CMAP allows statistically to distinguish between groups of patients with MC from the DM1 and HYPP (p>0,001).

Statistically significant difference between decrement of CMAP in patients with DM1 and HYPP wasn't established.

P02.177
LMX1B mutations in Nail-Patella Syndrome (NPS)

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Introduction: Nail-Patella syndrome (NPS; MIM 161200) is a rare (1/50000 births) autosomal dominant disorder characterized by dysplastic nails, absent or hypoplastic patellae, elbow dysplasia, iliac horns, glomerulopathy, and glaucoma. NPS is caused by mutations in the LMX1B gene (LIM homeobox transcription factor 1-beta), mapped at chromosome 9q34. The main pathogenic mechanism underlying NPS, particularly of the skeletal defects, is the haploinsufficiency of LMX1B.

Material and Methods: Genomic DNA was extracted from 4 patients with the clinical diagnosis of NPS and an unequivocal autosomal dominant pattern of inheritance. The 8 exons of LMX1B were amplified by PCR and sequenced in an ABI Prism 3500 genetic analyzer. MLPA technique (SALSA P289-A1 LMX1B) was used for detection of deletions and/or duplications.

Results: Four different mutations were detected: two nonsense mutations - p.Q60X, on exon 2, and p.C142X, on exon 3; one missense mutation - p.R223Q, on exon 4; and a duplication of exon 3 (dup Ex.3). Mutations p.C142X and dup Ex.3 are novel.
Conclusions: The genetic diagnosis of NPS was confirmed by the identification of a pathogenic LMX1B mutation in all four patients. Three of those mutations are located in the LIM domains (exons 2_4) and remainder in the homeodomain of LMX1B (exons 4_6), the former causing a decrease in transcriptional activity and the latter leading to loss of DNA binding activity.

P02.178

A review of breast cancer risk and female patients with neurofibromatosis type 1 in the West of Scotland.

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Background: The West of Scotland (WoS) Clinical Genetics service received a referral from a general practitioner asking us to meet with a 46 year old woman with neurofibromatosis type 1 (NF1) to discuss her risk of breast cancer and screening requirements.
Anecdotal evidence and case reports have indicated an associated increased risk of breast cancer and NF 1. A 2006 study showed an increased risk of breast cancer in women under 50 years but failed to show significance overall. In 2007 a population based study showed with significance that women with NF 1 below the age of 50 years had a five fold increased risk of breast cancer in the population studied.
It was decided to review a cohort of female patients with NF 1 from the WoS and compare the observed cases of breast cancer in this cohort with expected figures in the expected population.
Methods: The pedigrees of families affected by NF 1 were reviewed, inclusion and exclusion criteria were applied producing a cohort of female patients with know NF 1 and who were 20 years or older. 4 women were excluded due to incomplete information and 26 were under 20 years of age the final cohort included 119 patients. Statistical significance of the results was shown using the Poisson distribution.
Results: The results from this study showed a 4.13 fold risk of breast cancer over all ages and, a 20.8 fold risk less than 50 years of age. These results could prove challenging in genetic counselling.

P02.179

A 19-year-old man with intellectual disability, neurofibromatosis, multiple exostoses, and a paracentric inversion (inv(9)(q12q22.3))

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Here, we present a man with neurofibromatosis type 1 (NF1) and multiple hereditary exostoses (HME). The patient is affected by multiple exostoses like benign enchondromata of the tubular bones, multiple cutaneous neurofibromas, axillary and inguinal freckling and café au lait spots. In addition, the patient has a global developmental delay with intellectual disability, a disproportionate short stature associated with macrocephaly, facial dysmorphisms, and shortened extremities with brachydactyly and proximal placement of thumb. A paracentric inv(9)(q12q22.3) was identified by conventional karyotyping and fluorescence in situ hybridization in the index patient. The array-CGH (180K) showed no evidence of a clinically significant deletion or duplication. His 22-year-old sister and the 50-year-old father are also affected by NF1. The heterozygous mutation c.4299insTT in exon 25 of NF1 gene could be detected. Only the index patient and his sister are affected of HME and a disproportionate short stature. The molecular genetic analysis of the EXT1 gene revealed the heterozygous mutation c.1723-1G>T in intron 8. The inversion inv (9)(q12q22.3) was not detected in the lymphocytes of the sister
We report this unusual case of a patient with neurofibromatosis type 1, exostoses, disproportionate stature and profound intellectual disability. NF1 and HME are sufficiently explained by the identification of the heterozygous familial mutations in NF1 and EXT1 genes. However, the profound intellectual disability and facial dysmorphisms are found only in the index patient. Whether the inversion inv(9)(q12q22.3) contributes as another piece of puzzle to the complex phenotype of the index patient is not defined yet.

P02.180

Molecular characterization of two similarly emerging NF1 microdeletions with Oligo-Array-CGH: differentiation of a 66,84-84,42 kb intragenic and a 160,69-178,14 kb contiguous gene deletion in two NF1 patients with different clinical characteristics

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Neurofibromatosis type 1 (NF1) results from microdeletions encompassing the entire NF1 gene and a variable number of flanking genes in 5-10% of patients. Three recurrent microdeletion types have been described: 1.4 Mb type-1, 1.2 Mb type-2, and 1.0 Mb type-3 microdeletions. Usually, a more severe phenotype has been reported in patients carrying genomic microdeletions involving the entire NF1 gene compared to patients with intragenic NF1 mutations.
We demonstrate the results of the molecular genetic analysis in two 2 and 4 year old NF1 patients mainly presenting with multiple café-au-lait-spots and a positive family history in one case. MLPA analysis using Salsa-Kit P081/P082 detected an indistinguishable deletion in both cases of at least 48 kb starting in intron 27b and comprising the 3' terminal half of the NF1 gene including the last but one exon 48. Efforts to specify the deletions applying Salsa P122 NF1-area probemix confined the deletion sizes to a maximum of 579 kb in both cases that remained still indistinguishable. Finally, oligo-array-CGH with the microarray kit 244A resolved a 66,84-84,42 kb intragenic NF1 deletion and a 160,69-178,14 kb gene deletion involving two distally adjacent genes, respectively. Atypical microdeletions less than 1 Mb encompassing the NF1 gene and distally adjacent genes are very rare. We want to highlight the clinical manifestations in our patients with regard to both deletion types identified. This study confirms that array-CGH is a sensitive approach for accurate characterization of NF1 microdeletions to differentiate between the types of microdeletions with respect to patients' follow-up care.

P02.181

Nonfracture Osteogenesis imperfecta (or another collagenopathy ?) in a 2 year old Russian boy with a mutation in the COL1A2 gene

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Case Report:
Our patient is the second child of healthy parents.
He had congenital clubfeet, a unilateral hernia inguinalis, a large frontal fontanel, frontotemporal alopecia, blue sclerae, normal teeth.
Clubfeet were present at birth, but so far no osseous fractures have been reported.
At the age of 2 years, height and weight were at P3, OFC at P97 and hypermobility of the hip joints was evident. Serum levels of calcium, phosphate and alkaline phosphatase were normal.
Radiographs showed wormian bones, normal skeletal mineralisation.
As some of the clinical features were suspicious of OI we initiated a molecular analysis. In exon 23 of the *COL1A2* gene a *de novo* not yet described heterozygous missense mutation c.1316G>A (p.Gly439Asp) was identified.
Comment:
The mutation described here is characteristic for osteogenesis imperfecta as Glycin substitutions are typical for OI. Comparable mutations have only been twice recorded in Ehlers-Danlos syndrome - but the published mutations were all located in exon 8 and not in exon 23 as in our case (LOVD; submitted by S. Symoens, Ghent University, Belgium). Collagen structural defects, which are usually glycine substitutions or exon skipping defects, act in a dominant negative fashion. They usually result in a phenotype that ranges from lethal to moderately severe depending on the collagene chain affected, its location within the chain, and the specific amino acid substituted.
In summary, the patient presented here exhibits particular phenotype with only mild osseus involvement, some features reminiscent of a connective tissue disease, alopecia and a novel *COL1A2* mutation.

P02.182

Visual Function and Ocular Manifestations in Noonan Syndrome

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Purpose of study: To determine visual function and characterize ophthalmic manifestations in Noonan syndrome.

Introduction: Noonan syndrome is a developmental syndrome caused by heterozygous mutations in the genes PTPN11, SOS1, RAF1, NRAS, CBL, or KRAS. It is inherited in an autosomal dominant manner. Clinical features in this syndrome include congenital heart defects, short stature, developmental delay of variable degree and ocular involvement.

Methods: Thirty individuals were studied in the Berkeley and Chicago RAS/MAPK symposium by visual function assessment, slit lamp exam and dilated fundus exam.

Results: The Ocular findings in 30 Noonan syndrome patients have been tabulated below. We noted the following parameters.

Conclusion: Ophthalmic manifestations are commonly noted features in Noonan syndrome. Most individuals have good visual function with majority having stereopsis. Annual eye exams are recommended and necessary to correct refractive errors, prevent amblyopia and in monitoring for intracranial complications such as hydrocephalus or rarely intracranial tumors.

Results of Ocular Findings in Noonan Syndrome

Number	Parameters	Results	n=30
1	Eyelids	Ptosis	14
2	Patient with glasses	Astigmatism Mild Myopia High Myopia Hypermetropia	14 1 1 2
3	Nystagmus		4
4	Anterior segment	Normal	30
5	Fundus exam	Tortuosity Wide optic disc Swollen optic disc Tilted optic disc Small optic disc	4 2 1 4 1
6	Color vision	unable to determine	2
7	Happy Contrast	1/4 1/5 1/3 1/2	1 1 2 26
8	Stereo smile test	1 notch-60 2 notch-120 3 notch -240 4 notch-480	14 5 2 1

P02.184
Atrioventricular canal defect in patients with RASopathies

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Congenital heart defect (CHD) affects 60-85% of patients with RASopathies. Pulmonary valve stenosis (PVS) and hypertrophic cardiomyopathy (HCM) are the most frequent anomalies, although the spectrum of cardiac defects is wider. We analysed the clinical and molecular characteristics of atrioventricular canal defect (AVCD) in patients with mutations affecting genes in the RAS/MAPK pathway. Between 2002 and 2011, 101 patients with cardiac defect and a molecularly confirmed RASopathy were collected. Complete or partial (including cleft mitral valve) AVCD was diagnosed in 8/101 (8%) patients, including 7/8 with *PTPN11* gene mutations, 1/8 with a *RAF1* gene mutation. The only recurrent mutation was *PTPN11* c.124A>G (Thr42Ala). AVCD was partial in 6 cases, complete in one; in 4 was associated with other cardiac defects, including subvalvular aortic stenosis, mitral valve anomaly, PVS, and HCM. The associated cardiac defects were mutation-specific. Maternal segregation was observed in two families with *PTPN11* mutations and in one with *RAF1* mutation. CHDs in the affected relatives were discordant in families with *PTPN11* mutations, and concordant in the mother-sib pair with *RAF1* mutation. This study recommend to check Noonan syndrome features in syndromic patients with AVCD. Although mutations are heterogeneous, partial AVCD, and its association with left-sided obstructions, PVS and HCM must be considered clinical markers of RASopathies, often displaying genotype-phenotype correlation. Familial segregation of AVCD should be considered in the genetic counselling of families with RASopathies.

P02.185
Mutations in *PTPN11*, *SOS1* and *RAF1* genes and clinical characteristics of Noonan syndrome patients.

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Short stature, congenital heart defects, craniofacial dysmorphia and skeletal defects (thorax deformation) are the main clinical features typical for Noonan syndrome (NS). The disease is caused by mutations in genes encoding proteins of RAS/MAPK pathway, mainly in *PTPN11*, *SOS1* and *RAF1* genes. Together, 171 patients with clinical suspicion of NS were examined by an experienced clinical geneticist and the molecular analysis of *PTPN11*, *SOS1* and *RAF1* genes was performed. The pathogenic mutations in *PTPN11*, *SOS1* and *RAF1* genes were found in 65 (38.0%), 14 (8.2%) and 13 (7.6%) patients, respectively.

The clinical analysis of NS patients revealed that there is significant variation in disease phenotypic expression depending on the mutation presence in specific gene. The short stature was statistically more frequent in patients with mutations in *RAF1* and *PTPN11* (76.9% and 69.2%) than in *SOS1* (42.9%; $p<0.05$). Pulmonary valve stenosis was present in 30 (46.2%) patients with mutation in *PTPN11*, 7 (50.0%) in *SOS1* and 5 (38.5%) in *RAF1*, although the observed differences were not statistically significant. The hypertrophic cardiomyopathy and strabismus were more common in patients with mutated *RAF1* (46.2% and 38,5% vs. 2.7% and 12.3% for *PTPN11* and *SOS1* together, $p<0.05$). The delayed psychomotor development, speech delay and cryptorchidism were more frequently observed in patients with *PTPN11* and *SOS1* mutations (45.2%, 21.9% and 65.1% vs. 15.4%, 0% and 16.7% for *RAF1*, $p<0.05$). Our results confirm a significant correlation between the NS-causing mutation in specific gene and clinical symptoms of the disease.

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P02.186
Phenotype-genotype associations and significant differences between *PTPN11* and *SOS1* mutated patients with Noonan syndrome

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Noonan syndrome (NS) is an autosomal dominant disorder caused by heterozygous gain of function mutations in various genes (mainly *PTPN11*, *SOS1*, *RAF1* and *KRAS*) encoding proteins of the Ras-MAPK signaling pathway. Here we report genotype-phenotype correlation in a well-characterized cohort of 63 Polish patients affected by NS. We identified pathogenic mutations in *PTPN11* for 32 (51%), *SOS1* for 12 (19%), *RAF1* for 2 (3%) and *KRAS* for 1 (2%) unrelated patients. Our total mutation detection rate was 75%. All patients presented phenotype typical for NS, however we observed differences in the prevalence of some features depending on the mutated gene. Ptosis, macrocephaly, hypotonia, hyperkeratosis, sparse eyebrows, mitral valve anomalies and renal defects were significantly more prevalent among individuals with *SOS1* mutations. Whereas short stature, pulmonic stenosis, atrial septal defect, sparse hair and skin pigmentation were significantly more frequently associated with presence of *PTPN11* mutations Two characteristic NS features, such as mental retardation and hypertrophic cardiomyopathy were rarely associated with *PTPN11* or *SOS1* mutations, while were observed in our *RAF1* cases. Additionally, we revealed that 10 affected mothers and one affected father manifested a milder phenotype than their sick children.

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P02.187
Extreme clinical variability in Noonan-like syndrome with loose anagen hair due to the Ser2Gly SHOC2 mutation

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Noonan syndrome (NS) belongs to a group of developmental disorders due to deregulation of the RAS/MAPK pathway named RASopathies, including Cardiofaciocutaneous (CFCS), Costello (CS), LEOPARD (LS), Neurofibromatosis type I (NF1) and Legius syndrome. Some of these conditions, as CS, NF1 and Legius syndrome, are gene specific being caused by mutations in HRAS, NF1 and SPREAD1 genes respectively; the others syndromes are characterized by a considerable molecular overlapping. Two additional NS-like conditions have been recently described, caused by mutations in CBL and SHOC2 genes. In particular, a specific SHOC2 gene mutation (4 A>G predicting the Ser2Gly aminoacid substitution), is responsible for a distinctive condition "Noonan-like syndrome with loose anagen hair (NS-LAH)", characterized by a specific ectodermal phenotype. Here we report two cases of NS-LAH SHOC2 mutated patients revealing the extreme clinical variability of this condition. The first patient presented common NS features at birth with typical facial dysmorphisms and hypertrophic cardiomyopathy. However, he had a dramatic clinical evolution in the first months of life rather resembling that typical of CS. Central nervous system (CNS) involvement with drug-resistant epilepsy and severe developmental delay occurred associated with significant growth delay and dystrophyc appearance. The second patient presented a distinctive NS-LAH phenotype at birth, without CNS anomalies or cardiac defects documented in the 18 months clinical follow-up. These two cases represent the mild and the severe end of the same phenotypic spectrum, demonstrating that SHOC2 genotype-phenotype correlation is more complex than previously thought, and preventing the possibility of a genotype-based prognosis.

P02.188

Boy with Noonan syndrome with multiple giant cell lesions (NS/MGCL) and review of the literature

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Noonan syndrome with multiple giant cell lesions (NS/MGCL) was recently shown to be part of the phenotypic spectrum of the syndromes of the RAS/MAPK pathway. We report on a 13-year-old boy with a typical phenotype of Noonan syndrome including atrial septal defect, pulmonic stenosis, short stature, pectus excavatum, and multiple giant cell lesions of both jaws, and a *de novo* mutation in exon 3 of *PTPN11*, c.236A>G (predicting Q79R). *PTPN11* mutations are the most frequent cause of Noonan syndrome and Q79R is a well-described recurrent mutation. Including this patient, 24 subjects with molecularly confirmed NS, LEOPARD or CFC/MGCL syndrome have been reported to date. Of these, 21 subjects (87.5%) had *PTPN11*, *SOS1* or *RAF1* mutations and three (12.5%) had *BRAF* or *MAP2K1* mutations, confirming that MGCL is a rare complication of the deregulated RAS/MAPK pathway. The lesions of the mandible and to a lesser extent of the maxilla were first noted between ages 2 and 19 years (median 11 years) and were combined with facial asymmetry in 5/24 patients (21%). With one exception (mutation not reported), all 24 subjects demonstrated known mutations in the *PTPN11, SOS1, RAF1, BRAF,* and *MAP2K1* genes that were previously reported with RASopathies without MGCL.

P02.189

Widening the phenotypic spectrum of 5q35.3 microduplication encompassing *NSD1*: description of two more patients with the reversed Sotos syndrome phenotype

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Background: Loss-of-function mutations in *NSD1* and 5q35 microdeletions encompassing *NSD1* are a major cause of Sotos syndrome which is characterized by overgrowth, macrocephaly, characteristic facies and other features. Among several patients with partial trisomy 5q, five patients with confirmed microduplication of the 5q35.3 region including *NSD1* have been described. They show a 'reversed phenotype' of Sotos syndrome with microcephaly, short stature and mental retardation. We here report on two siblings with interstitial duplication 5q35, widening the phenotypic spectrum.

Patients: Both siblings had microcephaly, behavioral problems with agitation and lack of social distance, and a distinctive facial phenotype with thin upper lip, flat philtrum, short palpebral fissures and epicanthic folds. The 13-year-old girl showed mild to moderate mental retardation, short stature and cataracts. Her 15-year-old brother had learning problems with an IQ of 78. His length was within the lower normal range. The biological parents were neither available for clinical examination nor for testing.

Methods: Besides chromosomal analysis (with a resolution of about 550 bands) we performed SNP-array analysis (Affymetrix® Cytogenetics Whole-Genome 2.7M) and FISH (using a specific probe for NSD1).

Results: Chromosomal analysis was normal in both siblings. Molecular karyotyping in the sister revealed a 1.6 Mb interstitial duplication of 5q35.2-q35.3 containing 40 RefSeq genes including *NSD1* and the duplication could be confirmed in both siblings by FISH analysis. The siblings illustrate intrafamilial variability of the reversed Sotos syndrome phenotype.

P02.190

22q11.2 microduplication in a patient with bladder extrophy and delayed psychomotor development

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Bladder extrophy (BE) is a complex congenital anomaly,part of the clinical spectrum of the bladder extrophy-epispadias complex (BEEC).The BEEC represents a spectrum of urological abnormalities in which part of all the distal urinary tradt fails to close and is exposed to the outer abdominal wall.Previously,nine cases of classical extrophy of the bladder with underlying microduplication 22q11.2 have been reported (Lundin et al.2010;Draaken et al.,2010;Ludwig et al.,2011).A 10-year-old boy was referred for genetic evaluation for psychomotor retardation.He had a bladder extrophy at birth.He was adopted.He showed short stature,scar of repair of bladder extrophy,micropenis.Multiple ligation-dependent probe amplification(MLPA) analysis performed using the SALSA MLPA KIT P250 DiGeorge (MRC-Holland,Amsterdam,Netherlands)detected a micro-duplication 22q11.2.The duplication was confirmed in FISH and array-CGH.The array-CGH (Affymetrix Cytogenetics Whole-Genome,2,7 M Array) identified a duplication of 2419 kb in the 22q11.2 regionIn conclusion,this report extends the phenotypic spectrum of bladder extrophy in microduplication 22q11.2 and may point to possible gene(e) located in 22q11.2 playing a putative role in urogenital development.It provides further evidence of genotype-phenotype correlation

P02.191

Two years experience of Fibular Aplasia, Tibial Campomelia, and Oligosyndactyly

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Fibular Aplasia, Tibial Campomelia, and Oligosyndactyly (FATCO) syndrome (MIM#246570) is an extremely rare syndrome related with shortening and anterior bowing of the lower limb at the distal third of the tibia overlying soft tissue dimpling, oligodactyly of the foot, and oligosyndactyly of the hand, is associated with autosomal dominant inheritance/sporadic. So far no disease gene/genes were identified. According to our knowledge this is the 11th case of FATCO described in literature and 3rd case in Turkey. We presented two years of experiences of FATCO syndrome.

The patient was born at term with uneventful pregnancy and delivery. He was the first child of healthy parents, 17 years old mother and 25 years old father. They were non-consanguineous, both born in same small town.

The pregnancy was follow-up regularly. During follow-up pregnancy of mother triple screening test risk was low. Fetal ultrasonography showed echogenicity of the tibial osseous and shortening of both tibias during 21st, 25th and 27th weeks, respectively. The right upper extremity had evaluated in normal range and the left upper extremity could not be evaluated due to fetal position. His birth weight and head circumference was normal but length was short, 46 cm (<3 percentile). Our case demonstrated major common findings of FATCO syndrome.

We will discuss the perinatal and postnatal futures of the propositus during gestation, newborn and two years old period with previous patients in the literature.

P02.192

Ohdo syndrome Maat-Kievit-Brunner type is caused by mutations in *MED12*

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Ohdo syndrome [MIM 249620] is characterized by intellectual disability and the typical facial features including blepharophimosis. Clinically the blepharophimosis-intellectual disability syndromes have been classified in five distinct subgroups: del(3)(pter) type, Ohdo type, Say-Barber-Biesecker-Young-Simpson (SBBYS) type, Verloes type, and Maat-Kievit-Brunner (MKB) type. Here, we performed exome sequencing in two families with two affected males with Ohdo syndrome MKB type. Two novel missense mutations were identified in Mediator of RNA polymerase II transcription subunit 12 (*MED12*; NM_005120.2), p.(Arg1148His) and p.(Ser1165Pro), that segregated with the disease phenotype. Upon subsequent analysis of an additional cohort of seven single male patients with the broad definition of Ohdo syndrome, we detected one additional *de novo* missense change in *MED12*: p.(His1729Asn) in a patient with the MKB type. The occurrence of three different hemizygous missense mutations in three unrelated families with Ohdo syndrome MKB type, suggests that *MED12* is the causative gene for this Ohdo syndrome subtype.

Previously, three other specific missense mutations in *MED12* have been described in patients with FG syndrome and Lujan-Fryns syndrome. Our patients clearly differ from these two syndromes as they have the classical Ohdo features ptosis and blepharophimosis, while the high palate and high, prominent forehead as seen in Lujan-Fryns and FG syndrome and the typical ears in FG syndrome are less apparent. However, in adulthood the facial appearance of Ohdo patients type MKB becomes more coarse, and the distinction from FG syndrome becomes less apparent. Further studies are necessary to determine the exact genotype-phenotype correlations in *MED12* mutations.

P02.193

Two novel GJA1 missense mutations in patients presenting with oculodentodigital dysplasia

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Oculodentodigital dysplasia (ODDD) also known as oculodentoosseus syndrome (OMIM #164200) is a rare congenital, predominantly autosomal dominant disorder that comprises craniofacial, ocular, dental, and digital abnormalities. The syndrome is caused by GJA1 mutations. The clinical phenotype of ODDD involves characteristic facial dysmorphic features, ocular findings (microphthalmia, microcornea, glaucoma), syndactyly type III of the hands (fusion between fingers 4 and 5), phalangeal abnormalities, diffuse skeletal dysplasia, enamel dysplasia, and hypotrichosis. In two unrelated patients presenting with the typical clinical symptoms of ODDD, we demonstrated two different novel missense mutations of the GJA1 gene: c. 257C>A (p.S86Y) and c.317T>G (p. L106R). Our report expands the known mutational spectrum of the GJA1 gene and provides evidence on the importance of this highly conserved amino acid residues for the native functioning of the corresponding protein.

P02.194

Familial Osteofibrous Dysplasia with Pectus Excavatum: A Phenotypic Evaluation

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Four generations of a New Zealand family have autosomal dominant inheritance of bilateral congenital bowing of the tibia, hypoplastic fibula and pectus excavatum. The first three generations (reported in 1978) had several incidences of radiolucent lesions and pseudoathrosis of the tibia following fracture that required surgical intervention. The two affected members of the fourth generation have not sustained either fractures of the tibia or radiolucent lesions, but anterolateral bowing of the tibia was a persistent feature. One of these two individuals presented with thoracic scoliosis in addition to the tibial and pectus malformation. Congenital bowing of the tibia with the presence of radiolucent lesions or pseudoathrosis in this family is reminiscent of osteofibrous dysplasia (OFD). OFD can occasionally exhibit familial aggregation as in this family but is usually a unilateral, sporadic condition. Sporadic OFD presents with the same phenotype of anterolateral bowing, radiolucent lesions, pseudoathrosis upon fracture and apparent healing of bowing and/or lesions as skeletal maturity proceeds. The additional phenotypic features are suggestive of a mild anomaly in skeletal development that is especially apparent in the tibia. This family may inform the genetic basis of OFD and allied bone neoplasms such as adamantinoma and non-NF1 tibial pseudoarthosis.

P02.195

A novel C-terminal stop mutation in *COL1A2* in a family with moderate osteogenesis imperfecta

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Osteogenesis imperfecta is a connective tissue disorder, the vast majority caused by mutations in *COL1A1* or *COL1A2*-genes. These genes encode for collagen 1, which consists of two α1- and one α2-chains forming a triple-helical structure. The spectrum of clinical severity is wide, ranging from mild predisposition to fractures to perinatal lethality. We describe a 24-year-old female patient with postnatal femur fracture, blue sclerae, and approximately 30 fractures until her 16th year of life. Her father had blue sclerae and about 25 fractures between his 8th and 20th year of life. Molecular analysis revealed a heterozygous nonsense mutation c.4060C>T (p.Gln1354X) within the last exon of the *COL1A2*-gene in both patients. Such mutations are rare and the clinical phenotype has so far not been characterized. Nonsense or frameshift mutations in *COL1A1* typically lead to mRNA-decay and haploinsufficiency of the affected protein, resulting in mild forms of the disease. Nonsense mutations in *COL1A2* which lead to mRNA decay do not cause osteogenesis imperfecta. Missense mutations in *COL1A1* or *COL1A2* that affect triple helix formation lead to severe forms of the disease, attributed to a dominant negative effect of the dysfunctional protein. Nonsense mutations in the last exon, as it is found in our patients, are predicted not to result in mRNA decay but in the generation of a truncated protein. The respective domain is considered to be important for the assembly of the three chains. A dominant negative effect affecting a domain beyond the triple helix could account for the moderate clinical phenotype.

P02.196

Recurrence of osteogenesis imperfecta: a case report

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Perinatal lethal osteogenesis imperfect (OI) is the result of heterozygous mutations of the COL1A1 and COL1A2 genes. Point mutations resulting in the substitution of Gly residues in Gly-X-Y amino acid triplets of the triple helical domain of the alpha 1(I) or alpha 2(I) chains are the most frequent mutations. They interrupt the repetitive Gly-X-Y structure that is mandatory for the formation of a stable triple helix. Most babies have their own private de novo mutation. However, the recurrence rate is about 7% owing to germline mosaicism in one parent.

29y/o lady had recurrence of OI. Her first fetus was diagnosed as OI at 21 weeks gestation and she declined. She referred to us for her second pregnancy because of ultrasound findings. Short long bones with fractures, small chests and soft skulls were significant and the fetus was also suspected OI at 19 weeks gestation. Type II OI was suspected with these findings.

Her previous doctor explained her it would not recur because most cases of OI type II represent autosomal recessive traits. However, there is another possibility with germline mosaicism and this case could be in this group. In addition, similar extremely severe types of OI, Types VII and VIII, can be caused by recessive mutations to other genes.

It is very important to make an accurate diagnosis to plan future pregnancy. Hence genetic test for OI patients is recommended even if it is difficult to perform genetic test for every patients with OI in Japan.

P02.197

A new overgrowth syndrome with cranio-facial dysmorphisms, complex cardiac abnormalities, progressive scoliosis and mild cognitive impairment

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We report on two sisters of 14y6m and 11y old, born from healthy non consanguineous parents, presenting strictly overlapping clinical features. All auxological parameters were above 97th centile in both siblings. Facial dysmorphisms included flat face, palpebral fissures slant down, hypertelorism, deep set eyes, thick eyebrows, flat nasal bridge, prominent naso-labial folds, long philtrum, low set ears, open mouth appearance and multiple oral frenula. Cardiac evaluation showed DIA II with left-right shunt in both sisters while the younger also presents restrictive interventricular septal defect, right branch block, left ventricular hypertrophy and at 9 years of age she was diagnosed with mixoma which was surgically removed. Skeletal abnormalities included short neck, progressive severe scoliosis that requires the use of corset, brachymetacarpia of digit 5 and hallux valgus. Brain MRI displayed abnormality of white matter and asymmetry of the lateral ventricles. Psychomotor development was mildly delay. High-resolution array- comparative genomic hybridization (CGH) analyses was performed on the older sibling and did not disclose any disease-causing genomic rearrangement. According to our knowledge the siblings don't fit into any known disorder. The clinical history of the siblings was uploaded to the the web-based Dysmorphology Diagnostic System (DDS) developed by DYSCERNE, a European network of Centres of Expertise in dysmorphology (DG Sanco- Project:

2006122). The DDS experts' panel discussed over differential diagnosis and we concluded that the present siblings are likely affected by a previously undescribed autosomal recessive disorder. Exome sequencing and filtering the results based on a hypothesis-driven strategy is currently undergoing.

P02.198

Papillon-Lefevre syndrome and *GJB2* associated hearing loss in two sibs

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Papillon-Lefevre syndrome (PLS, OMIM #245000) is an extremely rare autosomal recessive disorder associated with mutations in *CTSC* (chromosome 11q14.2). Palmo-plantar hyperkeratosis beginning in early childhood is the hallmark of severe disease. The disorder is most often diagnosed by dentists, however, because of severe painful progressive periodontal disease with premature loss of teeth which affects both primary and secondary dentition. Pyogenic liver abscesses, presumably a consequence of neutrophil dysfunction, have been reported. Systemic retinoids, especially acitretin, can be very effective in treating the skin lesions and may have some effect on the periodontal manifestations. Dental implants may be an option.

Mutations in *GJB2* (chromosome 13q12.11) are a cause of autosomal recessive non-syndromic mild to profound sensorineural hearing impairment which is usually congenital (OMIM #220290). Estimated prevalence in the general population is 14:100,000.

We present a brother and sister who both have molecularly confirmed PLS as well as *GJB2* associated hearing loss. Seven of nine surviving sibs have neither disorder. Three sibs died of unknown cause in infancy. The parents, originally from Somalia, are not consanguineous by history. Both teenagers presented with severe periodontal disease and palmo-plantar hyperkeratosis interfering with mobility and sleep. The boy is deaf; the girl has minimal residual hearing

This case reminds us that treatment and follow-up can be significantly influenced by making the correct diagnosis of a rare disorder and that two monogenetic conditions occasionally co-occur

P02.199

CYP2C9 and VKORC1 polymorphisms influence the warfarin dose adjustment during initial anticoagulation and follow-up of 360 days

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Background: Warfarin is an anticoagulant that has been the standard to prevent and treat thromboembolism and, genotypic variations in the CYP2C9 and VKORC1 have been reported to predict dosing. Objectives:As an initial step towards clinical pharmacogenetic implementation, the main aim of this study was to determine whether CYP2C9 and VKORC1 polymorphisms influence the warfarin dose adjustment during initial anticoagulation and follow-up of 360 days. Methods: Two hundred six patients who were beginning warfarin therapy were selected. They were assessed with general and clinical characteristics, response to therapy followed on days 7-10, 30, 60, 180, 360, and adverse events. Results: During 360 days, the total dose variation was associated with predicted metabolic phenotypes according to CYP2C9*2 and CYP2C9*3 (extensive metabolizer (EM): +1.7±1.5 mg/week and intermediate or poor metabolizers (IM+PM): -5.5±2.5 mg/week; p= 0.03, adjusted for covariates). Dose variation during first month was also associated. Patients carrying VKORC1 and CYP2C9 variants presented lower required dose compared to patients carrying wild-type genotypes (p= 0.04 and p= 0.03, respectively). Conclusions: This genetic information is important in the initial anticoagulation dosing and during treatment maintenance. In this scenario, the present study could help to design programs towards individualization of warfarin therapy in the Brazilian population.

P02.200

Dosage-sensitive network in polycystic kidney and liver disease: Multiple mutations cause severe hepatic and neurological complications

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Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common genetic disorders. Most elderly patients also show liver cysts. Polycystic liver disease (ADPLD) can also occur isolated, but may also encompass kidney cysts. Variable disease expression even in the same family is incompletely understood. ADPKD and ADPLD overlap not only clinically, but also genetically and functionally. Both genes known for ADPLD, *PRKCSH* and *SEC63,* encode proteins that are involved in posttranslational translocation and quality control of proteins (such as the ADPKD proteins). We describe a family with liver and kidney cysts in which the much more severely affected index patient harbours a total of four mutant alleles in genes for ADPKD and ADPLD with massive hepatic and neurovascular complications leading to stroke at the age of 38. All other affected family members displayed a mild phenotype with practically no disease burden and a few liver and kidney cysts to varying degrees. In line with recent functional data, we postulate a dosage-sensitive, tissue-dependent network for polycystic liver and kidney disease in which additional mutational hits exert an aggravating effect and contribute to earlier and more severe disease expression. This concept may describe a general principle for the modification of disease expression and demonstrates how trafficking and quality control of proteins matter in human disease.

P02.201

Polycystic kidney disease (ARPKD/ADPKD) gets complex: Genetic network and mutations in multiple cilia-related genes

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Polycystic kidneys paved the way for elucidation of cilia-related disorders and notably most ciliopathies have a renal cystogenic component. Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common Mendelian disorders with a prevalence of 1:500-1000 and typically a late-onset disease caused by mutations in *PKD1* or *PKD2*. About 2% of ADPKD patients show an early and severe phenotype with considerable perinatal morbidity and mortality that can be clinically indistinguishable from the recessive form of polycystic kidney disease (ARPKD) caused by *PKHD1* mutations. We demonstrate severely affected PKD patients who carry, in addition to their expected familial germline defect, further mutations that are likely to aggravate the phenotype. We also show that polycystic kidney disease may also be mimicked by mutations in *HNF1ß* and genes typically causing other ciliopathies, such as Nephronophthisis and Meckel syndrome. Due to these aspects, we established a novel genetic testing approach based on Next-Generation Sequencing (NGS) that allows simultaneous investigation of all PKD and other ciliopathy genes.

P02.202

Pre and post-axial polydactyly caused by a novel dominant GLI3 mutation in a large kindred

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A large Jewish Moroccan kindred presented with 12 cases of isolated polydactyly. Affected individuals had either pre-axial, post-axial or combined polydactyly. Most had also syndactyly. Using polymorphic markers adjacent to candidate genes, linkage to SHH, GJA1, HOXD13, LMBR1, FBLN1 was ruled out. Analysis using markers D7S691 and D7S1526 near GLI3 followed by sequencing of the coding sequence of the gene, demonstrated that the phenotype in this family was due to a novel c.A1802G (p.H601A) mutation in exon 12 of GLI3 abrogating one of the C2H2 type zinc fingers of the encoded protein.

The study demonstrates the significant phenotypic variability of GLI3 mutations, with the same mutation leading to pre or post-axial polydactyly.

P02.203

Candidate genes and CNVs in patients with polymicrogyria.

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Polymicrogyria (PMG) represents a common cortical malformation and is characterized by an excessive number of small gyri as a result of the abnormal neuronal migration. Up to date mutations in 35 genes are related with various neuronal migration disorders. 13 chromosomal loci were associated with PMG.

Here we present the results of high resolution array CGH analysis (Agilent 2x400k) in 21 patients with PMG.

In one patient we identified de novo deletion 15q26.3 (24 Mb). Although known from the literature in association with developmental delay/multiple malformations this locus was not previously associated with PMG.

11 patients showed copy number variations (CNVs) neither listed as benign nor previously described in the literature. 7 patients had a single aberration (4 losses and 3 gains). In 3 patients a combination of 2 CNVs and in one patient 4 CNVs (gains) were seen. The size of the copy number losses and gains varied from 25 kb to 250 kb and from 36 kb to 250 kb, respectively. In six patients a segregation analysis was possible. A de novo event could be confirmed in one patient (loss). Four patients showed maternal inheritance (one mother partially affected) of the CNVs and one patient had two CNVs, one inherited from either parent. Two discovered CNVs reside within the known PMG loci 4q22.1 and 13q22.1.

In 9/21 patients only CNVs listed in Database of Genomic Variance were found.

The phenotype, candidate genes, possible causative role of the rare CNVs and further studies using NGS will be discussed.

P02.204

Polymicrogyria, schizencephaly, and eye anomalies in a girl with *COL4A1* mutation

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Mutations in *COL4A1* are associated with autosomal-dominant type 1 porencephaly; brain small-vessel disease; and hereditary angiopathy with nephropathy, aneurysms, and muscle cramps (HANAC) syndrome. Recently, mutations in *COL4A1* have also been identified in patients with presumed Walker-Warburg syndrome. Schizencephaly and abnormal gyration (such as polymicrogyria) have not been described as primary features.

We describe a girl with a novel mutation in *COL4A1* (c.2716+2T>C, IVS33+2T>C, het.). She was presented at the age of 4 months with complex brain (schizencephaly, polymicrogyria, hypoplastic corpus callosum, and ventriculomegaly) and eye (congenital cataract, microphthalmia) abnormalities. Her development was markedly delayed. In the further course, she developed West syndrome.

Prenatally she was diagnosed with bilateral cataracts and transient ventriculomegaly. Within the first month of life, the girl suffered from bilateral fulminant orbital phlegmona; consecutively, both orbits became markedly microphtalmic. When reassessing the consecutive MRI and CT scans that had been performed since birth, multifocal bleeding episodes at different stages of development and organization as well as porencephalic changes were detected in the early brain imaging studies. Polymicrogyria and schizencephaly most likely developed secondary to the vascular changes and bleeding episodes caused by the mutation during early stages of fetal development. Her parents and the dizygotic twin sister are healthy and do not carry the mutation.

This case highlights, that the brain phenotype may change over time in patients with *COL4A1* mutations and may mask the primary defects expected in these patients.

P02.205

New insights into Potocki-Lupski syndrome by characterisation of the duplicated region 17p11.2 with self-designed two-colour FISH probes

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The Potocki-Lupski syndrome (PTLS, MIM 610883) is a microduplication syndrome caused by a common duplication of about 3,7Mb in 17p11.2. The PTLS-associated duplication is reciprocal to the common 17p11.2 deletion syndrome (Smith-Magenis syndrome). Both, deletion and duplication rearrangements are caused by nonallelic homologous recombination between flanking repeat gene clusters (Zhang et al. 2010). Clinical features of PTLS include infantile hypotonia, failure to thrive, mental retardation, autism, behavioural abnormalities and speech delay.

Here we report about two patients with PTLS who were ascertained by karyotyping and array-CGH analyses. Patient 1, a 5 year old boy, showed an uncommon duplication of 4,7Mb. He demonstrated classical features like hypotonia, failure to thrive, mental retardation, speech delay, auto aggressive behaviour and facial dysmorphisms, but no congenital malformations. Patient 2, a 2 month old boy, presented the common duplication of 3,7Mb with hypotonia and additional club feet.

Our study was established to design new self-designed two-colour FISH probes for investigation the duplication region more precisely. Therefore, we amplified two genes from proximal and distal part of the duplication by Long Range PCR with a size in total of 30kb. After fluorescent labelling with different colours we hybridised the FISH probes on patients metaphase spreads. Both revealed an inverted duplication due to specific signal pattern.

Using our self-generated protocol to establish PTLS specific FISH probes it is possible to detect how the duplicated region is arranged. Furthermore, we demonstrate patients from the literature with similar duplication segments in comparison of their phenotype for phenotype-genotype correlation.

P02.206

The frequency and exact karyotypes of complex chromosomal aberrations in Prader-Willi syndrome

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The pathogenesis of Prader-Willi syndrome (PWS) includes deletion chromosome 15q11-13, maternal uniparental disomy (matUPD), and imprinting center abnormality. Some complex chromosomal aberrations are also known to cause PWS. But the frequency and exact karyotypes of chromosomal aberrations have not been reported. All 217 patients followed by us were diagnosed either by G-banding analysis, FISH, or methylation test. Six of them (2.7%) showed complex chromosomal aberrations (shown below). Four (case 1-4) had an extrachromosome originating from chromosome 15, one Robertsonian translocation between chromosome 14q and 15q (case 5), and one translocation between chromosome 15q12 and 16q24 (case 6). All of the 4 patients with extrachromosome had matUPD. On the extrachromosome, 3 of these 4 (case 1, 3, 4) showed no SNRPN. The other one (case 2) had SNRPN on the paternally-originated extrachromosome. The patient with Robertsonian translocation (case 5) had matUPD and his mother had the same karyotype with normal phenotype. One with translocation (case 6) showed no SNRPN on translocated chromosome 15.

In summary the frequency of complex chromosomal aberration was around 2.7% and many of them had matUPD with extrachromosome 15.

Karyotypes of cases

1. 47,XX,inv(13)(q22q34), idic(15)(q11.2) [4]/46,XX, inv(13)(q22q34) [56] .ish der (15)(D15Z1+, SNRPN-)
2. 47,XX, +idic(15) [3]/46,XX [17]. ish der (15)(D15Z1++, SNRPN++)
3. 46,XY,+mar [25]/46,XY [5]. ish der (15)(D15Z1+, SNRPN-)
4. 47,XX, der(15) [26]/46,XX [4] . ish der (15)(D15Z1+, SNRPN-)
5. 45,XY, t(14q;15q)
6. 45,XY, -15-16, +der (16)t(15;16)(q12;q24)

P02.207

Oro-dental abnormalities in a group of patients with Prader-Willi syndrome

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Prader-Willi syndrome is characterized by obesity, short stature, facial dysmorphism and behavior problems. The main features in the oral area include enamel hypoplasia, delayed tooth eruption, tooth decay and poor oral hygiene. Other reports mention micrognathia, xerostomia, hypodontia, supernumerary teeth, teeth fusions and dental erosion. The aim of this study is to evaluate oro-dental phenotype in Prader-Willi patients and recommending strategies for the dental management of cases with this syndrome. Due to very low incidence of the disease in general population, the study group included 12 patients, aged between 4 and 25 years. Evaluation of dental caries was carried out according to WHO criteria. Orthopantomographies were performed. Based on the radiological appearance of 7 teeth in the left hemimandibula, a dental maturity score was calculated, which was used to determine dental age. Enamel hypoplasia was revealed in 9 cases (75%). Cavities were recorded in all cases, causing massive crown destructions in adults, with early loss of a variable number of teeth, between 4 and 7, after the age of 20. Xerostomia was noticed in all, anodontia of one canine, microdontia and supernumerary tooth-odontom type occured each in one case, poor oral hygiene in 5 cases. An interesting feature was the finding of premature eruption of permanent teeth, 3 patients had early second molar eruption. Accelerated dental age was recorded in patients who were treated with growth hormone. Regular dental examination in Prader-Willi patients is necessary for correct management and justifies its inclusion in the panel of their mandatory investigations.

P02.208

Primordial Dwarfism: A case report of two South African patients

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Primordial dwarfism (PD) is the term used for a group of genetic disorders, which result in severe short stature and growth failure. „Primordial" has been defined as belonging to or being characteristic of the earliest stages of development. Thus, PD is a class of disorders where growth delay occurs at the earliest stages of embryonic development. Unlike some of the other forms of dwarfism where neonates may have normal growth parameters, children with PD are born small. Here we report on two South African girls with PD, aged four and six years. Short stature was evident at birth. Currently their growth parameters fall below the -8SD and -10SD curves, respectively. Both girls have clinical features suggestive of a diagnosis of Majewski Osteodysplastic Primordial Dwarfism Type II (MOPD II), and molecular testing has confirmed that the four year old is homozygous for a mutation in the causative gene, pericentrin (*PCNT*). Apart from severe intrauterine and postnatal growth failure, patients with MOPD II have microcephaly, skeletal dysplasia and a distinctive facial appearance. Individuals with MOPD II are at increased risk for several significant complications, which include insulin resistance, diabetes mellitus and central nervous system vascular malformations. As adults, their average height approaches 100cm, making them among the smallest of human beings. We compare the physical features and radiological findings of these two patients, followed by a brief discussion on the associated co-morbidities and current research on the genetic basis and natural history of PD.

P02.209

Primrose syndrome with testicular cancer: case report and review of the literature

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We report on a male patient with the rare finding of enlarged calcified external ear auricles combined with macrocephaly, mental retardation, dysmorphic facial features, bilateral cataracts, hearing impairment, sparse body hair, progredient muscle wasting, severe kyphoscoliosis and behavioural problems. All findings are consistent with the clinical diagnosis of Primrose syndrome. At the age of 28 years the patient developed a germ cell tumor and a seminome of his testicles, which was effectively treated by orchiectomy and chemotherapy. The patient died at the age of 31 years because of pneumonia. Array-CGH analysis performed by us on asservated DNA from testicular tissue, revealed no microdeletion or microduplication.

Primrose syndrome is an extremely rare neurodegenerative disorder of unknown cause. The first patient diagnosed with Primrose syndrome was described in 1982 by Dr. D. A. Primrose. Between 1982 and 2011 there have been published about seven cases of Primrose syndrome (OMIM #259050). To our knowledge our case is the second reported case of Primrose syndrome with testicular cancer; the first case was reported by Mathijssen et al., 2006. This new case of testicular cancer confirms an increased risk to malignancies, especially testicular tumors, as a part of Primrose syndrome.

We compare the phenotype and findings of our case to previously published cases of Primrose syndrome in order to expand the phenotypic spectrum. Up to now all cases of Primrose syndrome are mentioned to be sporadic without familial occurrence or consanguinity and probably related to a novel autosomal dominant mutation of a yet unknown gene.

P02.210

Identification of mosaic AKT1 mutations in two patients affected with Proteus syndrome but not in three patients affected with an asymmetric and disproportionate overgrowth not fulfilling the Proteus syndrome criteria

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Proteus syndrome (PS) [OMIM#176920] is a highly variable disorder characterized by asymmetric and disproportionate overgrowth of body parts, including bone overgrowth. The concept of a dominant lethal gene defect surviving by mosaicism was proposed by Happle over twenty years ago to explain the mosaic distribution of lesions and the sporadic occurrence. Recently, an activating missense mutation in the *AKT1* gene has been found by Lindhurst *et al.* (2011) to be associated with the PS. The AKT1 serine-threonine protein kinase is a central mediator of PI3-Kinase signaling which influence cell proliferation and apoptosis (Lee *et al.* 2011). Here, we screened affected tissues from two patients with classical PS and three patients with an asymmetric and disproportionate overgrowth not fulfilling the PS criteria; one of the latter with unilateral lower extremity overgrowth and two with cranial bone overgrowth. We used restriction-enzyme assay described by Lindhurst *et al.*. To test our method we created mutated PCR fragments and showed a sensitivity up to ~5% mutated PCR-fragments. We detected the *AKT1* mutation in the affected bone, fat and cartilage but not in the blood sample of two Proteus syndrome affected patients. The mutated allele frequency ranged between 4 and 20 percent. We did not detect *AKT1* mutation in affected tissues of the patients with cranial bone overgrowth and unilateral extremity overgrowth. This data suggest a different molecular mechanism accounts for the overgrowth in these distinct from PS cases.

P02.211

Causal mutations of *PRSS1* gene and association with hereditary chronic pancreatitis

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Hereditary chronic pancreatitis (HCP) belongs into the group of rare diseases. HCP follows an autosomal dominant inheritance pattern with a penetrance of approximately 80%. The incidence is expected to be 3,5-10 cases/100 000 inhabitants in Europe and USA. It was shown that in approximately 50% of families affected with HCP, mutation of *PRSS1* gene was present.

PRSS1 gene encodes cationic trypsinogen and some mutations described in this gene lead to higher stability of prematurely activated trypsin in pancreas or higher autocatalytic activation of inactive trypsinogen to trypsin. Another gene associated with HCP is *SPINK1*. This gene encodes serin protease inhibitor Kazal type I, which is markedly upregulated in the pancreas during active inflammation. Variants present in this gene may interrupt the specific inhibition of active trypsin in pancreas.

Recently we have started the analysis of mutations in *PRSS1* and *SPINK1* genes in Slovak HCP families. Although, we have so far tested only few families, several mutation carriers were already identified. One unreported variant, the p.Ile141Asn that we detected, presumably has a negative effect on the protein function. This novel variant may be specific for our population and will be analyzed further.The genetic testing resulting in discovery of the mutations in mentioned genes associated with HCP may help to enroll the patients into special preventive programs. Here we discuss the importance of genetic testing of *PRSS1* and *SPINK1* in patient with pancreatitis as the risk of pancreatic cancer is elevated in patients with diagnosed HCP to more than 50% .

P02.212

A large deletion in the GNAS gene in a patient with pseudohypoparathyroidism type 1a (PHP-1a)

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Albright hereditary osteodystrophy (AHO) is a rare autosomal dominantly inherited condition associated with short stature, obesity, subcutaneous ossifications and shortening of the long bones of the hands and feet. Some degree of development delay, generally mild, is common. The underlying molecular cause is reduced activity of the alpha subunit of the stimulatory guanine nucleotide-binding protein (Gsalpha). Gsalpha is encoded by GNAS and heterozygous mutations including missense, nonsense, small insertions and deletions in GNAS exons 1-13 have been reported to cause AHO. Submicroscopic deletion detected by array CGH, involving the whole GNAS locus, has also been reported. GNAS is subjected to maternal imprinting in some human tissues, including the thyroid gland, and hormone resistance (particularly for parathyroid hormone and thyroid-stimulating hormone) is associated with AHO, only when the genetic defect is maternally inherited.

AHO with hormone resistance (pseudohypoparathyroidism type 1a (PHP-1a)) was diagnosed clinically in an adopted boy with subcutaneous ossification on the left leg, short stature (2.5 percentile), brachydactyly, obesity (weight for height 97.5 percentile), a round face with a short neck and mild development delay. He had PTH resistance with hypocalcemia and mild TSH resistance.

MLPA analysis revealed heterozygosity for a deletion of exon 7-13 in GNAS. The clinical feature of PTH resistance indicates that the deletion was located at the maternal allele. This case underscores the usefulness of MLPA in the diagnosis of AHO. To the best of our knowledge, a similar partial GNAS deletion has not been described previously.

P02.213
Genetic study of PTEN mutations among individuals with ASDs / MR and macrocephaly

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Autism spectrum disorders (ASDs) are a group of severe neurodevelopmental conditions among which pervasive developmental disorder (not otherwise specified) and autistic disorder are the most common. The prevalence of ASDs is currently estimated at 60 and 13 per 10,000 for ASDs and autism, respectively. Autistic disorder is often associated with macrocephaly; 24% of patients have head circumference (HC) at >98th centile. Mutations in the PTEN gene have been reported in patients with ASDs and significant macrocephaly (HC ranging from +2.5 SD to +8 SD). Germline PTEN mutations also cause a variety of inherited cancer predispositions like the Cowden, Bannayan-Riley-Ruvalcalba, Proteus and Proteus-like syndromes. These conditions may also have neurobehavioural features resembling autism as well as overgrowth and macrocephaly. On the contrary, most macrocephalic autistic patients with confirmed PTEN mutations were lacking the typical signs of these syndromes, at least at the time of testing. We have selected 53 autistic individuals with HC ranging from +2 SD to +4.8 SD (including their relatives) for PTEN mutation analysis. Three novel (p.Asp331Thrfs*11, p.Thr321Glnfs*23, p.Glu242*) and two known germline mutations (p.Pro246Leu, p.Arg130*) have been found in 8 of the 53 probands (15%). We discuss possible genotype/phenotype correlation in our group of patients. Our data support former findings that PTEN mutations are frequent in patients with ASDs and macrocephaly. Therefore PTEN testing should be considered in such patients. The findings may impact the assessment of the recurrence risk and medical management of the families including early cancer prevention. Supported by CZ.2.16/3.1.00/24022 and MZ0FNM2012.

P02.214
Ptosis, arched eyebrows, hypernasal speech, obesity & mild learning disability- a clinical & mapping study

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We report 15 members of a three generation pedigree with ptosis, velopharyngeal incompetence, dysmorphism and a learning disability. The index case presented with nasal regurgitation, ptosis, obesity and developmental delay. His mother has similar features. She has 6 children, 4 of whom are affected. The maternal grandfather had ptosis & cannot read or write. He had 8 children, 5 affected & 3 unaffected. Two aunts of the index case have ptosis, obesity & learning difficulties. One has a son with ptosis. Mapping analysis was performed on an Illumina Human-1M array on 15 samples including 8 affected & 7 unaffected individuals. Three regions of interest on Chromosome 2, 10 & 18 were identified. A number of genes within these regions are of interest including: NRXN1, NLGN1, PIK3CA, FXR1. However, one gene, FOXI2, stands out. Many of the forkhead genes have important biological functions in multiple species. Mutations in FOXL2 cause Blepharophimosis-ptosis-epicanthic inversus, a dominant disorder with ptosis as a prominent feature. Mutations in FOXC2 cause Lymphoedema-Distiachiasis, a dominant disorder also causing ptosis & cleft palate. FoxO proteins play a critical role in cellular differentiation, proliferation, apoptosis and stress resistance. Therefore there is evidence that mutations in forkhead proteins can result in many of the clinical findings seen in our patients (ptosis, velopharyngeal insufficiency, speech & language difficulties & obesity). We are currently performing exomic sequencing on 5 members of the family (4 affected & 1 unaffected) & will perform Sanger sequencing on any variant of interest in the other family members.

P02.215
Hemodynamic and genetic analysis in children with idiopathic/heritable and congenital heart disease associated pulmonary arterial hypertension

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Background: Idiopathic (I) pulmonary arterial hypertension (PAH) is rare in childhood and can be heritable (HPAH) caused by defects of genes participating in the TGFß signaling pathway. The genetic background of PAH associated with congenital heart defects (CHD-APAH) is less clear. The aim of this study was to compare hemodynamic and genetic findings in children with I/HPAH and APAH-CHD.

Methods: Prospectively included were consecutive children with invasively confirmed diagnosis of I/HPAH or CHD-APAH. Assessment of family members, pedigree analysis and systematic screening for mutations in the genes BMPR2, ACVRL1, ENG, SMAD1, SMAD5 and SMAD9 was performed.

Results: 19 children with I/HPAH (6.3±4.7 years) and 11 with CHD-APAH (7.2±4.5 years) were included. Four mutations (BMPR2 n=2; ACVRL1 n=2), and 3 not yet described unclassified sequence variants (ACVRL1 n=1; SMAD9 n=2) were found in I/HPAH children. One ACVRL1 mutation has not been described before. In CHD-APAH patients 1 BMPR2 mutation and 2 unclassified sequence variants (ENG n=1, BMPR2 n=1) were identified.

Conclusion: Mutations and unclassified variants with functional impact in different TGFß signalling genes occurred in 21% of I/HPAH patients and 27.3 of patients with CHD-APAH and may influence the clinical status of the disease. Therefore, genetic analysis in children with various forms of PAH is important, may be of clinical and prognostic relevance, and shows the complexity of the genetic background.

P02.216
Associated malformations in patients with radial ray deficiency

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Infants with radial ray deficiency (RRD) often have other associated congenital malformations. The purpose of this investigation was to assess the prevalence and the types of associated malformations in a defined population. Each affected child was examined by a geneticist, all elective terminations were ascertained, and the surveillance for malformations was continued until 1 year of age. The associated malformations in infants with RRD were collected in all livebirths, stillbirths and terminations of pregnancy during 26 years in 346,831 consecutive births in the area covered by our population based registry of congenital malformations. Of the 67 infants with RRD born during this period (prevalence at birth of 1.93 per 10,000), 60 (89.6 %) had associated malformations. There were 18 (26.9%) patients with chromosomal abnormalities including 15 trisomies 18, and 32 (47.8%) nonchromosomal recognized dysmorphic conditions. There were no predominant recognised dysmorphic conditions, but VA(C)TER(L) association. However, other recognised dysmorphic conditions were registered including, Fanconi, thrombocytopenia absent radius, and Holt-Oram. Thirty two (47.8 %) of the patients were multiply, non syndromic, non chromosomal malformed infants (MCA). Malformations in the cardiovascular system, the urogenital system, the central nervous system, and the digestive system were the most common other malformations. Prenatal diagnosis was performed in 47.8 % of dysmorphic syndromes with RRD. The overall prevalence of associated malformations, which was more than one in two infants, emphasizes the need for a thorough investigation of infants with RRD. A routine screening for other malformations may be considered in infants and in fetuses with RRD.

P02.217
'Noonan Spectrum Test' - comprehensive screening for RASopathies

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The Ras/MAPK signal transduction pathway is critical for the regulation of proliferation and differentiation of multiple cell types. Deregulation of the pathway results in a number of disorders with overlapping physical manifestations. These include postnatal growth reduction; skeletal, ectodermic and haematologic anomalies; congenital heart defects and hypertrophic cardiomyopathy, and variable cognitive deficit. With an incidence of 1:100-2500 in all live births, Noonan syndrome (NS) is the most common RASopathy. To date, pathogenic mutations in twelve genes (*PTPN11, SOS1, RAF1, SHOC2, BRAF, MAP2K1, MAP2K2, SPRED1, CBL, KRAS, NRAS* and *HRAS*) have been shown to underlie NS and other Ras/MAPK-related conditions that include Cardio-facial-cutaneous (CFC), Costello, LEOPARD and Legius syndromes, enabling a molecular diagnosis in up to 75% of affected patients.

In a joint collaboration between SW Thames Molecular Genetics Diagnostic Laboratory and NewGene, we have developed a single diagnostic test to screen for all the Noonan spectrum disorders, based on next generation sequencing (NGS) technology. The NGS assay, using the Roche amplicon approach with multiplex PCR, has been validated using samples from patients referred for Noonan related syndromes that were previously tested by a 3-stage approach at St George's. We compare the cost and turn-around-times of this 'Noonan Spectrum Test' with similar assays currently available in the EU and the US and outline a new service strategy for molecular diagnosis of RASopathies. We include some case studies to demonstrate the benefits of this approach to testing.

P02.219

De novo mosaic ring chromosome 18 in a child with a Fallot type double outlet right ventricle, developmental delay, growth retardation, and polysplenia with right isomerism

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We report on a 9 months old child with Fallot type double outlet right ventricle, developmental delay, growth retardation, microcephaly, polysplenia with right isomerism, latent hypothyroidism, absent 12th rib, strabismus and dysmorphic features. Karyotyping and FISH studies revealed two cell lines, one with monosomy 18 (13 %) and one with r(18) (87 %). Ring chromosome 18 is a rare chromosome disorder with highly variable phenotype, which probably depends on the extent of the terminal 18q and 18p deletions. A clear genotype-phenotype correlation though, was hampered by the lack of exact breakpoint mapping in previously published cases. The majority of reported patients showed mild features of the 18q- syndrome. Only a minority of patients with ring chromosome 18 presented with clinical features of 18p- syndrome or a combination of these two syndromes.The phenotype of the 18q- syndrome is characterized by characteristic face, mental retardation, short stature, microcephaly, abnormal male genitalia, congenital heart defect, congenital aural atresia/stonosis, hypotonia, foot deformity, and white matter abnormalities. Common features in 18p- syndrome patients are dysmorphic features, growth and mental retardation, immunological problems, skeletal abnormalities, cardiac defects, brain malformations, alopecia and dystonia. High-resolution SNP-array molecular karyotyping in our patient revealed a 14.7 Mb deletion and an adjacent 277 kb duplication at chromosome 18p and a 3,8 Mb deletion at 18q. This finding explains the clinical overlap with 18p- syndrome in our patient. We just identified a second patient who is currently under further investigation.

P02.220

Is Roferon-A (Interferon alpha-2a) Teratogenic Risk Factor-X?

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BACKGROUND Roferon-A (Interferon alpha-2a) is a pregnancy risk category C drug, which has been shown to demonstrate a statistically significant increase in abortifacient activity in rhesus monkeys when given at approximately 20 to 500 times the human dose. A particular birth defect may have its origins through multiple mechanisms and possible exposures, including medications. A specific pathogenic process may result in different outcomes depending upon factors such as embryonic age at which a drug is administered, duration, dose of exposure and genetic susceptibility. This case report discusses the teratogenic effect of Roferon A.

CASE An infant was exposed to 1x1 1000000IU /day of Roferon-A from the beginning of pregnancy until the 28th gestational week. Preterm newborn (28th week) showed the following abnormalities: Cliteromegaly, hydranencephaly, microcephaly, hypotelorism, frontal bossing, asymmetry of head , big right ear, cleft plate, absence of nasal bridge and single alae nasi. Chromosomal analysis of proband revealed 46,XX karyotype.

CONCLUSIONS Identifying teratogenic mechanisms may not only be relevant for etiologic and post-marketing research, but may also have implications for drug development and prescribing approach for women of reproductive age. Six teratogenic mechanisms are associated with medication use. Many medications classified as class X are associated with at least one of these teratogenic mechanisms. Our patient may be the first case which is possible to be related with one of these mechanisms of Roferon-A. Moreover, in the present case, teratogenic effects were observed even though the dose was lower than in the previously reported Roferon-A embryopathy cases.

P02.221

Rubinstein-Taybi-like syndrome: clinical and molecular genetics delineation

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In 1963, Rubinstein and Taybi reported a syndrome (RTS) characterized by mental retardation, broad thumbs and toes, and recognizable facial abnormalities with a high propensity to neoplasms. RTS is caused by submicroscopic 16p13.3 deletions in approximately 10% of patients. Mutations in the *CREBBP* gene (16p13.3) explain about 30% of the cases. Recently, mutations in the *EP300* gene (22q13) were reported in 5 patients. *EP300* gene can be considered as a rare cause of RTS.

We report here, three patients presenting clinically with a RTS. These are two Belgian patients, a male and a female, aged respectively 29 and 11 years, followed in the genetics and paediatric neurology clinics in Leuven and a Tunisian patient followed in the oncology clinics of Sousse (Tunisia). The conventional karyotype was normal. We found no deletion on 16p13 by FISH. By aCGH we detected in the first patient a terminal deletion on chromosome 6q and a terminal duplication on 7q. We confirmed by FISH that these submicroscopic chromosomal abnormalities were the product of an unbalanced reciprocal translocation (6;7). The second patient has a *de novo* microduplication of 500 kb on chromosome 5q11.2. We found no abnormality in the third patient by aCGH at a resolution of 1 Mb.

In this report, we will discuss the pathogenic relevance of these abnormalities and their possible contribution to the clinical phenotype. In conclusion, we show that aCGH is a powerful tool for investigating the genetic aetiologies of Rubinstein-Taybi-like syndrome.

P02.222

Midline defect with single central incisor masks facial phenotype of Rubinstein-Taybi syndrome

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We report on a patient followed from one to 11 years of age. She presented with intellectual disability and facial dysmorphism. She was born to healthy unrelated German parents and has a healthy elder sister. Pregnancy was complicated by polyhydramnios. Caesarean section was performed at 38th week of gestation. Birth measurements were normal, but the patient needed ventilation and showed brachycardia. A naevus of the glabella, choanal atresia, a fleshy nose, a high arched palate and ovarian cysts were diagnosed after birth. At the age of one year a single central incisor and right sided aortic arch and left sided vena cava superior were observed. MRI scan showed a thin corpus callosum and mild frontal brain atrophy. The patient walked and talked at the age of 3 years. She is short and obese and has normal head circumference. She has myopia and had mucotympanon during childhood. Endocrine follow up showed elevated oestriol levels and premature thelarche. She has a single central incisor of her permanent teeth, but molecular analyses in *SHH* and *SIX3* genes gave normal results, as did numerous other investigations. At the age of 10 years broad thumbs and toes became more obvious and talon cusps were observed, leading to the tentative diagnosis of Rubinstein-Taybi syndrome (RTS). Detection of a deletion of exons 24 to 31 of the *CREBBP* gene confirmed RTS. Although holoprosencephaly is described in RTS the alteration of the facial gestalt by the midline defect postponed the final diagnosis for 10 years.

P02.223

Defect initiation of proteoglycan synthesis in patients with joint dislocations,bicuspid aortic valve and other heart defects

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Bicuspid aortic valve (BAV) is the most frequent inborn heart defect. It is associated with insufficiency and/or stenosis of the heart and results in the pathologic changes in left-ventricular structure and function as well as a dilatation of the ascending aorta. BAV increases the risk of cardiac death. We studied a family with inherited joint dislocations and a congenital heart disease with multiple defects (BAV with aortic root dilatation, mitral valve prolaps, VSD, ASD). We mapped the disease to chromosome 11 and subsequently identified hypomorphic mutations in glucuronyltransferase-I (GlcAT-I), the enzyme catalyzing the synthesis of the last step in the linker region tetrasaccharide formation, which connects glycosaminoglycans and the core protein of a proteoglycan. We showed that the mutations reduce enzymatic activity and decrease the levels of all three lines of O-glycanated proteoglycans, namely dermatan sulfate, chondroitin sulfate and heparan sulfate proteoglycans. Real-time PCR showed that GlcAT-I is expressed in heart, aorta, bone, and osteoblasts - tissues affected in the patients. Further, GlcAT-I protein was present in the medial tissue of the human aorta, indicating that altered GlcAT-I function may be involved in developmental or degenerative aortic root dilation and aortic aneurysm. Our results point to the proteoglycan synthesis as a candidate gene pathway for several cardiovascular malformations as well as for congenital joint dislocations.

P02.224

Deletion of a long-range cis-regulatory element for the TWIST1 gene in a family with mental retardation and mild craniofacial dysmorphism

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Disruption of the normal *cis*-regulatory architecture of disease gene loci has been described as a special cause of different genetic disorders. The *TWIST1* gene locus might be one of those. Mutations within the coding region of *TWIST1* are associated with the Saethre-Chotzen syndrome. Large deletions encompassing *TWIST1* and its neighbouring genes also contribute to this disorder, but some patients have additional significant learning difficulties, which led to the suggestion of a novel microdeletion syndrome 7p21.1. Here we describe a three generation family, in which several members have a slight to moderate mental retardation and mild craniofacial dysmorphism. On array-CGH analysis variable constellations of two different copy-number changes flanking the *TWIST1* gene at 7p21.1 could be detected in the affected and non-affected probands. One is a duplication of ~800 kb nearly 270 kb upstream of *TWIST1*, which contains 3 genes of so far unknown function (*TWISTNB*, *MIR3146*, *TMEM1*). The other, a small deletion of ~150 kb, is located approximately 2.9 Mb downstream of *TWIST1* and encloses the gene LOC729920. Both changes are not yet known as pathogenic or benign CNVs. Based on the clinical and molecular findings in our family and considering the current knowledge about '*cis*-ruption disorders' we suggest - in particular for the smaller deletion - disease causing effects on the function of *TWIST1* and/or other genes nearby.

P02.225

A syndromic condition with scalp defects, developmental delay and dysmorphic features maps to chromosome 20.

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In 2007, Al-Gazali et al. reported on an apparently new autosomal recessive condition comprising hypotonia, developmental delay, cutis aplasia of the vertex and dysmorphic features in an inbred Arab family. It was proposed that this could represent a severe recessive form of scalp-ear-nipple syndrome (Clin Dysmorphol 2007).

We performed homozygosity mapping in this family and found clear evidence of linkage to a single large region on chromosome 20 encompassing an interval of approximately 35 Mb (15,200,000 - 51,300,000). The locus was confirmed but not further refined by another affected child born to this family more recently. In a sporadic patient with scalp defects, mild-to-moderate cognitive impairment and facial dysmorphism, we discovered a significant stretch of homozygosity overlapping with the above mentioned region. This girl was born to healthy parents originating from a remote area in Romania but who denied consanguinity.

We hypothesize that the affected individuals in these two families share the same very rare autosomal recessive condition, the hallmarks of which are congenital scalp defects and intellectual disability. The gene for this condition is probably located on chromosome 20. Further investigations are on the way to identify the causative gene for this syndrome.

P02.226

A *de novo* mutation in the *SETBP1* leading to Schinzel-Giedion syndrome and juvenile myelomocytic leukemia

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Schinzel-Giedion syndrome (SGS; MIM #269150) is a rare multiple congenital malformation syndrome. A clinical diagnosis of SGS may be made by identifying the dysmorphic features, including prominent forehead, midface retraction, and short, upturned nose plus typical skeletal anomalies or hydronephrosis. Typical skeletal anomalies of SGS include a sclerotic skull base, wide supraoccipital-exoccipital synchondrosis, inreased cortical density or thickness and broad ribs. In addition, there is an increased prevalence of neoplasia in SGS.

We present a 3-month-old girl patient with both SGS and juvenile myelomonocytic leukemia. She had craniofacial abnormalities, hypertrichosis over forehead, bilateral grade 3 degree hydronephrosis, secundum atrial septal defect, skeletal abnormalities, hydrocephaly and severely hypotonia. She was the first child of the parent and her family history was unremarkable. Her karyotype was 46, XX. In her follow-up, she had high white blood cells (WBC: 55.040/mm^3) and high level liver enzymes activity. Her bone marrow biopsy consisted with juvenile myelomonocytic leukemia. She died at the age of 10 month since untreatable bronchopneumonia. Mutation analysis for the mutation hotspot in the *SETBP1* gene showed a *de novo* mutation (p.Asp868Asn (c.2602G>A)). In the literature, overexpression of *SETBP1* had been related with growth advantage in hematopoietic progenitor cells. Our case had both juvenile myelomonocytic leukemia and SGS. To our knowledge, leukemia in SGS was not described previously. This *de novo* mutation may be associated with leukemia in SGS.

P02.227

Sertoli cell tumor and gonadoblastoma in an untreated 29-year-old 46,XY phenotypic male with Frasier syndrome carrying a *WT1 IVS9+4C>T* mutation

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Objective: Frasier Syndrome (FS) phenotype in 46,XY patients usually consists of female external genitalia, gonadal dysgenesis, high risk of gonadoblastoma, and development of end stage renal failure usually in the second decade of life. FS is caused by heterozygous de novo intronic splice site mutations of the Wilm's tumor suppressor gene 1 (*WT1*), although few cases with typical exonic *WT1* Denys-Drash mutations that resemble an FS phenotype have been described. The aim of this study was to present further data on the spectrum of FS phenotypes, through the evaluation of a 29-year-old patient with a predominantly male phenotype and coexistence of Sertoli cell tumor and gonadoblastoma.

Results Genetic analysis using standard methods for DNA sequencing confirmed FS due to a *WT1* gene mutation, IVS9+4C>T.

Conclusions This very rare case illustrates the natural course of FS over many years, due to neglect by the patient to address his need for follow-up and adds further data on the spectrum of FS phenotypes associated with IVS9+4 C>T mutations. The coexistence of the rare Sertoli cell tumor and gonadoblastoma, emphasizes that the early clinical recognition and molecular identification supports appropriate patient management, especially with respect to the high risk of gonadal malignancy.

P02.228

Lobar holoprosencephaly and eye anomalies in a patient with a deletion in the *SHH* regulatory region

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HPE is one of the most common congenital malformations in humans and it is characterized by the incomplete separation of the cerebral hemispheres into distinct right and left halves. Clinical expression is extremely variable ranging from minor facial signs to complexes craniofacial anomalies such as ciclopia. Main genes involved include *SHH, GLI2, PTCH1, TGIF, ZIC2, TDGF1, SIX3, GAS1, ZIC3.* We report a Brazilian patient presenting a mild form of lobar HPE associated with eye anomalies who presented a deletion in the SHH. Brain CT Scan showed hypotelorism, hypoplastic nasal structures, the premaxilla is highly placed with a single central maxillary incisor, midline clefting extending from the premaxilla to hard palate, lateral ventricles are hypoplasic and fused at the midline. Array-CGH analysis showed a loss at the chromosome 7q36.3 - 155,452,499-155,992,499bp; (size 540 kb) in the *SHH* regulatory region. In mice, the *Shh* gene has six enhancers that regulate *Shh* transcription in the embryonic forebrain. The *Shh* floor-plate enhancers (SFPE2) and *Shh* brain enhancer (SBE1) are localized approximated 200 kb downstream from *Shh* promoter, the other enhancers, SFPE1, SBE4, SBE2 and SBE3 are approximated 400 kb upstream from *Shh* promoter, and three of these enhancers are highly conserved in human. The microdeletion found in our study affected at least three of these enhancer elements (SBE2, SBE3 and SBE4) and suggests that the HPE phenotype is probably casual for loss of *SHH* enhancers.

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P02.229

Deletion causing SHH disruption in a family with severe hydrocephalus

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A couple presented to the SACGS with a history of two previous TOP for severe hydrocephalus. The gender of the first fetus was uncertain, and the second was male. Given the likelihood of X-linked recessive inheritance, the L1CAM gene was tested in fetus 2 and returned a normal result. An array CGH test was also performed. This showed a 7q36 terminal duplication in fetus 2, no DNA was available from fetus 1. Parents were tested and the duplication was identified in the father who was phenotypically normal. An MRI scan was then arranged in the father which showed a small tectal hamartoma not causing obstruction.

The 7q36 duplication in this case was 562kb in length, with one breakpoint disrupting the SHH (sonic hedgehog) gene. Heterozygous deletions of the SHH gene are known to cause holoprosencephaly and the maldevelopment of midline brain structures. Duplication of 7q36, has been reported to cause hydrocephalus, hypotonia and cleft palate, with variable severity. It has previously been postulated that overexpression of SHH could contribute to this phenotype.

It was explained to the parents that the SHH gene disruption was a likely but uncertain cause of the hydrocephalus. They embarked on a third pregnancy. CVS and FISH showed that the fetus did not carry the duplication. A normal male was subsequently born.

P02.230

Case report of Shwachman-Bodian-Diamond syndrome (SBDS) with a combined point mutation and large deletion

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Shwachman-Bodian-Diamond syndrome is a rare autosomal recessive disorder characterized by exocrine pancreatic insufficiency, hematologic defects, short stature and skeletal malformations. Here we describe one patient with features of a skeletal dysplasia. Initial analysis with 180k oligo-aCGH detected a 220 kb deletion harbouring the complete SBDS gene and part of the neighboring gene TYW1 indicating a possible cause for clinical sign of a skeletal dysplasia. Array-CGH in both parents demonstrated the same deletion in the healthy father. Sequencing of the SBDS gene in the patient detected an already published donor splice site mutation in intron 2 of the SBDS gene demonstrating the second mutation on the patients non deleted allele.

Additional sequence analysis of the respective mutation in both parents detected the heterozygous mutation in the healthy mother giving the couple a 25% recurrence risk for following new pregnancies.

This is to our knowledge the first case with a combination of a classical small point mutation combined with a large deletion in SBDS. Initial detection started from aCGH and thus demonstrates the usefulness of initial screening by aCGH, because small proportions of already known monogenic disorders should be due to large deletions not detectable by conventional sequencing. Up to date 25% of SBDS-patients described in the literature lack mutations in the SBDS-gene. Due to the detected mutations prenatal testing could be offered for the new pregnancy.

P02.231

Molecular investigations of Estonian patients with Silver-Russell and Beckwith-Wiedemann syndrome

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Silver-Russell (SRS) and Beckwith-Wiedemann (BWS) syndromes are both clinically heterogeneous disorders. Due to variable presentation molecular confirmation of these diseases is necessary. SRS is mainly characterized by severe pre-and postnatal growth restriction and typical triangular face. BWS is an overgrowth syndrome involving predisposition of tumor development. Defective expression of imprinted genes (IGF2, H19, CDNK1C, KCNQ1, KCNQ1OT1) at 11p15 is implicated in etiology of both syndromes. IRC1 hypomethylation is the major cause of SRS and IRC2 hypomethylation mostly found in BWS. Both disorders occur sporadically, but familial inheritance is also described.

Altogether 28 patients were enrolled in BWS and 20 patients in SRS group. All patients` clinical symptoms were re-evaluated by one investigator. In BWS patient's group 19/28 and in SRS patients 14/20 fulfilled the minimal diagnostic criteria. Molecular analysis was performed by methylation-specific MLPA (MRC-Holland).

In SRS group hypomethylation in IRC1 region was found in 4 SRS patients including 2 siblings. One SRS patient had maternal duplication in 11p15 involving both IRC1 and IRC2 regions. She has inherited 11p15 region duplication from her mother who shows overgrowth since the birth and clinical features of BWS. Patients´ mother has inherited duplication from father. Therefore 38.5% (5/13) of SRS patients exhibited an epimutation at the 11p15 region, which is consistent with other investigations. Interestingly, two familial SRS cases were found in the group.

BWS was confirmed in one patient with hypomethylation in IRC2. In almost 95% of BWS patients we could not confirm the clinical diagnosis, therefore molecular investigations should continue.

P02.232

Monozygotic male twins with ICR1 and ICR2 hypomethylation on chromosome 11p15

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Silver-Russell syndrome (SRS) is a congenital imprinting disorder mainly characterized by intrauterine as well as postnatal growth restriction, body asymmetry, relative macrocephaly and a typical triangular face. SRS is mostly caused by dysregulation of imprinted genes controlled by ICR1 and ICR2 located on chromosome 11p15.5 or maternal uniparental disomy of chromosome 7.

Here we report on a pair of male monozygotic twins discordant for SRS. They were delivered by caesarean section at 34 weeks of gestation. Birth weight of the twin A (2400 g; 25th-50th centile), as well as other auxologic parameters were appropriate for gestational age. Birth weight of the twin B was 1150g (<5th centile) and the difference between head and chest circumference amounted to 9 cm. He experienced severe failure to thrive requiring feeding by gastrostomy. At the age of 4.5 years, the brothers presented height and weight diversity of 13 cm and 6.1 kg, respectively.

Genomic DNA extracted from peripheral blood of both twins was analysed at the differentially methylated regions of *H19* and *KCNQ1OT1* genes. Methylation specific MLPA analysis revealed ICR1 and ICR2 hypomethylation in both brothers. Second analysis performed on DNA isolated from buccal swabs also indicated decreased level of methylation in both ICRs.

So far, in SRS, one concordant and four discordant monozygotic pairs carrying the ICR1 epimutation have been observed. This is the first report on monozygotic twins discordant for SRS with both ICR1 and ICR2 hypomethylation.

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P02.233

Silver-Russell Syndrome - correlations between the phenotype and (epi)genetic alterations

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Silver-Russell syndrome (SRS) is a well known congenital imprinting disorder with hypomethylation of ICR1 on chromosome 11p15.5 or maternal uniparental disomy of chromosome 7 (mUPD7) identified in about 60% and 10% of patients, respectively. The disease is characterized mainly by severe pre- and postnatal growth retardation, relative macrocephaly and a small triangular face. Several scoring systems have been proposed but making diagnosis may be still challenging due to wide spectrum of clinical features. Thus studies of patients with confirmed diagnosis of SRS may provide additional valuable information. We performed retrospective and prospective study of a group of 55 SRS patients diagnosed and followed at a single centre (CMHI). Among them there were 45 with ICR1 hypomethylation and 10 with mUPD7. Maternal hetero-, isodisomy and a mixture of hetero- and isodisomy were demonstrated. Growth parameters, phenotype characteristics and developmental anomalies were analyzed and scored. Mean birth weight was lower (-3.1±0.156 SD vs. -2.8±0.306 SD) and head circumference was significantly larger (-0.638±0.174 SD vs. -2.042±0.421 SD; p=0.002) in ICR1 hypomethylation than in UPD7 patients. Asymmetry (face/body/limb) and major congenital anomalies were also more frequent in patients with ICR1 hypomethylation; three individuals manifested gonadal dysgenesia and ambiguous genitalia, 2 males and 1 phenotypically female with male karyotype (46,XY CGD). Cesarean sections were exceptionally frequent in both groups (~50% in ICR1 hypomethylation and in 70% mUPD7). Follow-up of somatic development in both SRS groups enabled analysis of the phenotype depending on epigenetic modulations.

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P02.234

Submicroscopic chromosomal imbalances significantly contribute to the phenotype of SRS and SRS-like patients

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Application of microarray-based molecular karyotyping has lead to a more than two-fold increase in the detection rate in comparison to conventional cytogenetics in patients with mental retardation (MR) and further congenital anomalies. However, it is not routinely implemented in molecular diagnosis for syndromes not typically associated with MR, including Silver-Russell syndrome (SRS). SRS is a clinically and genetically heterogeneous disorder characterized by growth retardation, relative macrocephaly, a triangular face and asymmetry. The clinical spectrum is broad and the diagnosis is rather subjective. Up to now, in nearly half of the SRS patients (epi)genetic alterations on chromosomes 7 and 11 can be detected while ~50% remain without molecular diagnosis. Recently, submicroscopic imbalances have been reported in single cases but systematic studies are missing.

To determine the contribution of submicroscopic imbalances to the aetiology of SRS, we performed molecular karyotyping in 41 patients referred as SRS without (epi)genetic alterations on chromosomes 7 and 11 using the Affymetrix SNP Array 6.0.

Thereby we identified pathogenic de-novo copy number variations with sizes ranging from 672 kb to 9.158 Mb in eight patients. Some of them were associated with known microdeletion syndromes with overlapping features with SRS. In 5 further patients imbalances with so far unknown clinical significance were detected.

In conclusion, as pathogenic submicroscopic imbalances were detectable in a significant proportion of our patients molecular karyotyping should generally be implemented in routine diagnostics for growth retarded patients with even slight dysmorphisms suggestive of SRS.

P02.235

The mutation in KRT5 gene in Iranian EB patients

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INTRODUCTON: Epidermolysis Bullosa (EB) is a group of inherited disorders in which skin blisters develop in response to minor injury. There are four main types of EB Including: SIMPLEX, JUNCTIONAL, HEMIDESMOSAL and DYSTROPHIC. Because of many overlapping clinical manifestations, identifying the exact type of EB is complicated. The most severe and common form of EB is SIMPLEX that caused by a mutation in either of the keratin genes, KRT5 or KRT14.The purpose of this study is to investigate of KRT5 gene common mutation in Iranian affected patients.

MATERIALS and METHODS: Eighty clinically diagnosed patients as EB evaluated and their DNA extracted from blood sample.PCR reaction fallowed by direct sequencing were done using designed primers for hotspot exons of KRT5 gene including exon 1, 4, 5 and 7.

RESULTS: A novel mutation was detected in codon 308 and about 18 % of patients illustrated a kind of mutation in selected regions. The most hotspots were exon 4 and 5. Interestingly the most of mutations were diagnosed in compound heterozygous form.

P02.236

Identification of a novel deletion in the *DHCR7* gene in a patient with SLOS

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Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive defect of cholesterol biosynthesis with characteristic dysmorphism, mental and growth retardation, and multiple congenital malformations. Mutations in the human Δ7-sterol reductase (*DHCR7*) gene are the genetic cause of this syndrome. In most of the analyzed patients with distinct phenotypic features and/or biochemical findings two mutations can be identified by sequencing of coding exons 3 to 9 and adjacent intron boundaries of the *DHCR7* gene (detection rate approx. 96%). In a small number of clinically and/or biochemically positive patients, only a single heterozygous mutation can be identified. We analyzed 9 of these patients by self-made multiplex ligation-dependent probe amplification (MLPA) of exons 3 to 8 of the *DHCR7* gene. In one patient, we could identify a heterozygous deletion of exons 3 to 6, in addition to the heterozygous common mutation p.Arg352Trp (c.1054C>T) in exon 9 of the *DHCR7* gene. The deletion leads to an almost complete loss of the gene which is presumably disease causing. At birth the patient was small for gestational age, had short proximal limbs, syndactyly of toes 2 and 3, an atrial septal defect, horseshoe kidney, and typical facial features. During the first year psychomotor retardation, muscular hypotonia, and feeding difficulties evolved. Plasma sterol analysis showed elevated 7- and 8-dehydrocholesterol and decreased cholesterol. Exon deletions in the *DHCR7* gene have only been reported once in a SLOS patient with holoprosencephaly. Therefore, we recommend MLPA analysis in patients suspected of SLOS harbouring only one mutation identified by exon sequencing.

P02.237

In frame deletion and missense mutations of the C-terminal helicase domain of SMARCA2 in three patients with Nicolaides-Baraitser syndrome

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Using high resolution molecular karyotyping with SNP arrays to identify candidate genes for etiologically unexplained intellectual disability, we identified a 32 kb *de novo* in frame deletion of the C-terminal helicase domain of the *SMARCA2* gene in a patient with severe intellectual disability, epilepsy, sparse hair, prominent joints and distinct facial anomalies. Sequencing of the gene in patients with a similar phenotype revealed *de novo* missense mutations in this domain in two further patients, pointing to a crucial role of the SMARCA2 C-terminal helicase domain. The clinical features observed in all three patients are typical of Nicolaides-Baraitser syndrome, an only rarely reported syndrome with mainly moderate to severe intellectual disability and other typical aspects like a recognizable facial gestalt, sparse hair, epilepsy, wrinkling of the skin, prominent interphalangeal joints and broad distal phalanges. Notably, one of our patients with a p.Gly1132Asp mutation showed typical morphological features but an exceptional good development with borderline overall IQ and learning difficulties, thus expanding the phenotypic spectrum of Nicolaides-Baraitser syndrome.

P02.238

Co-occurrence of the SMMCI syndrome and a 5q21 deletion in a young girl patient

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Solitary median maxillary central incisor (SMMCI) or single central incisor is a rare dental anomaly. It is estimated to occur in 1:50,000 live births. SMMCI syndrome is a complex, autosomal dominant developmental disorder in which an SMMCI is seen in association with midline nasal cavity defects (choanal atresia, mid-nasal stenosis, nasal pyriform aperture stenosis) and variably holoprosencephaly.

We report a 21month-old girl, who was the second child of unrelated parents. She was referred to our genetic counseling service for evaluation of dysmorphic features suggesting the SMMCI syndrome and psychomotor development delay. On physical examination, she presented a single central incisor, choanal atresia, nasal pyriform aperture stenosis, ocular hypotelorism, short stature and microcephaly. No brain anomalies were identified by cerebral CT-scan.

A de novo heterozygote missense mutation 494C>T was identified within SHH gene, leading to the replacement of Alanine at amino acid position 165 with Valine. Affymetrix Whole-Genome 2.7M Array Chip revealed a deletion of 695kb of the 5q21.1-q21.2 region.

In conclusion, it difficult to establish genotype-phenotype correlations of the 5q21 deletion because of the simultaneous presence of the SMMCI syndrome.

P02.239

Two Portuguese families with recurrent episodes of pain and different SCN9A mutations - Primary Erythermalgia or Paroxysmal Extreme Pain Disorder?

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Background: Mutations in *SCN9A* gene can cause three very different pain phenotypes: Primary Erythermalgia (PE), Paroxysmal Extreme Pain Disorder (PEPD) and Channelopathy-associated Insensitivity to Pain (CIP). The first two disorders result from gain-of-function mutations and are characterized by recurrent episodes of pain, but the accompanying manifestations are usually distinct.

Objectives: The authors aim to present two Portuguese families with recurrent episodes of pain, in which the diagnosis between PE and PEPD was difficult to establish due to overlapping clinical manifestations. The first family is one large kindred with 26 affected subjects and the second family has 3 affected members.

Methods: After characterizing the clinical manifestations, we performed mutation analysis of *SCN9A* gene in 17 of the 26 affected and in 2 non affected members of family 1, as well as in all the affected subjects of family 2.

Results: In all the affected individuals of family 1, except for one female subject, a heterozygous missense mutation c.4835T>C (p.Leu1612Pro) was identified. In family 2 complete sequencing of *SCN9A* gene revealed a heterozygous unclassified mutation c.4880T>G (p.Met1627Arg), which is most likely pathogenic and is present in all the affected family members.

Conclusion: We believe that these two families are the first Portuguese families reported to have mutations in *SCN9A* gene, in which the molecular diagnosis was determining to correctly classify the clinical phenotype. This stresses the importance of performing mutation analysis in affected individuals to confirm the clinical diagnosis and to support pharmacological treatment, which can reduce the frequency of pain episodes.

P02.240

Clinical variability of Sotos syndrome patients with no NSD1 deletion identified

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Sotos syndrome is an overgrowth syndrome characterized by increased birth length and weight, excessive growth during the first years of life, advanced bone age, learning disability, and distinctive facial features (including macrocephaly, ocular hypertelorism and prominent mandible). Other findings associated with Sotos syndrome are: behavioral problems, cardiac anomalies, cranial MRI/CT abnormalities, scoliosis and seizures. Sotos syndrome is estimated to occur in 1:14,000 live births, and most cases are sporadic. Even though *NSD1* is the only gene associated with the Sotos syndrome, in nearly 20% of the cases no genic abnormality can be found. In 5 affected unrelated patients who are currently being treated at Hospital Santa Marcelina, in Sao Paulo, Brazil, no *NSD1* deletion or duplication was identified by the MLPA method. The purpose of this report is to describe the phenotypic spectrum of these patients. Molecular genetic testing is important not only to confirm the diagnosis, but it also may help determining genotype-phenotype correlations and monitoring the affected patients in order to identify medical complications that may arise from their condition.

P02.241

Partial deletion of SOX6 in a boy with mental retardation, extrapyramidal motor disorder and skeletal anomalies

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We report on a 17-year old boy who was referred to us with a seemingly progressive neuropsychiatric disorder of unclear aetiology. He presented with mild mental retardation, developmental delay, skeletal malformations with sternum abnormalities and L-DOPA responsive extrapyramidal motor disorder including ataxia, dysarthria, apraxia, tremor and mimic tics. Array CGH analysis (Affymterix 2.7M array) revealed a monoallelic de novo 84 kb deletion on chromosome 11p15.2 encompassing exon 14 to 16 of the SOX6 gene. The deletion was confirmed by MLPA (multiplex ligation dependent probe amplification). SOX6 belongs to the family of Sry-related HMG box transcription factors with regulatory functions during embryonic development. It plays important roles during early chondrogenesis, muscle development, erythropoiesis and development of the central nervous system. While Sox6 knockout mice show an early lethality, conditional Sox6-knockouts (Sox6fl-) revealed skeletal anomalies with short sternum, including fusion of the fourth and fifth sternebrae (Dumitriu et al., 2006). Due to the fundamental role of SOX6 in a variety of developmental processes and the skeletal similarities between the patient and the mouse model we suggest the partial deletion of SOX6 as apparently disease causing in this patient.

P02.242

Molecular Genetic Confirmation of the Diagnosis Spastic Paraplegia 31 in a Teenage Boy

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Spastic paraplegia (SPG) 31 (MIM #610250) is an autosomal dominant neurodegenerative disease. Typical signs are proximal weakness of the lower extremities and progressive spasticity with gait abnormalities, the upper extremities and the sensory system are usually not affected. The age of onset is during the first or second decade in 70 % of affected individuals, in the remainder after the age of 30 years. The genetic cause of SPG31 are mutations in the *REEP1* gene (MIM *609139) on chromosome 2p11.2, which were described for the first time in 2006 in six unrelated families. Variable expressivity and incomplete penetrance have been noted. Recent studies indicate that *REEP1* mutations are the third most frequent cause of hereditary spastic paraplegia, being responsible for 6-8% of cases.

We present a 14-year-old boy from an otherwise neurologically unremarkable family showing abnormal statomotoric and language development. At first muscular dystrophy was suspected until he showed signs of spastic paraplegia at the age of 7 years. Muscle biopsy and neuroradiologic imaging were normal. The boy is receiving regular treatment by physiotherapy, surgery (elongation of Achilles tendons) and medication (botox injections, baclofen). Now at age 14 years he is in a stable condition, being able to ambulate using several auxiliary devices, and an excellent swimmer. Various genetic investigations (genes *SPG3*, *SPG4*, *SPG7*, *SPG20*, karyotyping) did not disclose a cause for his disease. After seven years of diagnostic attempts we found a novel heterozygous truncating mutation in the *REEP1* gene, c.550C>T (p.Gln184X), which confirmed the diagnosis in this patient.

P02.243

Hereditary Spastic Paraplegia Type 8 (SPG8): A novel mutation in a German family

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Hereditary Spastic Paraplegia (HSP) is caused by progressive upper motor neuron axons degeneration. Central-motor-system deficits lead to lower limb paraparesis. HSP can be classified in pure HSP with lower limb spasticity only and complicated HSP with other neurological and non-neurological symptoms. SPG8 is one of the more aggressive subtypes of autosomal dominant pure HSP. The age of onset varies from the twenties to the sixties and there is relatively little interfamilial variability. The *KIAA0196* gene, consisting of 29 exons coding for the strumpellin protein with 1150 amino acids, has been identified as the SPG8 locus mapped to chromosome 8q24.12. There have been only three mutations reported in six families until now.

Here we report on a male patient, aged 27 years, and his mother, aged 50 years. Both suffer from spastic-atactic gait disorder, pes cavus, muscle atrophy at lower limbs ("stork-legs"), muscle hypertonia, brisk reflexes, wide reflex zones, and cloni.

DNA analysis of *KIAA0196* gene revealed the novel missense mutation c.1859T>C; p.Val620Ala in both patients. We did not find this mutation in 598 control chromosomes of German origin. It affects the same α-helix motife (amino acids 619-628) like two mutations in five of the six reported families and resides within the 75 amino acid domain well conserved among mammals.

The novel heterozygous missense mutation in exon 15 of the *KIAA0196* gene co-segregates with SPG8 in this family and was not detected in the mother's elder healthy son, her healthy brother and her mother.

P02.244

Subtelomeric 10q deletion: A new case with vertebral anomalies

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10q terminal deletion is characterised by psychomotor and growth retardation, congenital heart defect, genital and urinary tract anomalies, microcephaly and facial dysmorphic features consisting of hypertelorism, strabismus, broad prominent nasal bridge and low-set malformed ears. Some authors observed behavioral disorders. We report on a boy with psychomotor and growth retardation. He was born from second bigeminal pregnancy with unilateral cryptorchidism. Twin brother healthy. Clinical evaluation revealed microcephaly, widow's peak, palpebral fissures slanted up, epicanthic folds, strabismus, hypermetropia, malformed ears, thin upper lip, low posterior hairline, webbed and short neck, asymmetric thorax, scoliosis, clinodactyly. Radiogram of cervical spine showed abnormal lordosis, fusion of vertebre, C5-C7 spina bifida. A standard karyotype was normal. Some of dysmorphic features, especially webbed and short neck with chest deformity, suggested Noonan syndrome. Molecular analyses of PTPN11 and SOS1 genes did not confirm this diagnosis. Further clinical evaluation showed behavioural problems consisting of hyperactivity, hyperkinesis with destructive tendency. Some behavioural anomalies are frequently observed in chromosomal microaberrations. MLPA studies with a set of subtelomeric probes identified subtelomeric deletion of chromosome 10q. Subsequent FISH analysis with specific 10q probes confirmed this deletion. Parent's studies to asses the presence of cryptic translocation are in progress.

P02.245

Evaluation after sudden unexplained death in young patients

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Introduction: Hereditary arrhythmia syndromes are found in more than half of initially unexplained cases of sudden cardiac death (SUD) in young persons. Additionally to autopsy findings, examination of relatives and molecular testing if appropriate enables establishing the diagnosis.

Methods: Family members of 29 SUD patients consulted our clinic. Mean age of death was 24±15 years. Pedigree analysis and clinical examination in relatives was performed, followed by phenotype guided genetic testing in the deceased, if appropriate. In case of no clinical suspicion in relatives but death related to specific triggers (e.g. swimming) sequencing of the RYR2-gene resp. the 3 mostly affected LQTS genes was performed. If a mutation was found cosegregation within the family was verified before establishing the diagnosis.

Results: In n=16 cases (55%) a hereditary arrhythmia syndrome could be found. In n=12 (75%) of these cases clinical examination of relatives led to the diagnosis. In 3 families two arrhythmia syndromes (LQTS and CPVT, resp. HCM) were present.

Conclusion: In case of SUD in a significant percentage of cases a postmortem diagnosis can be performed. When there are only unspecific findings clinical screening of relatives leads to a proper diagnosis in a large proportion. Genetic evaluation should follow clinical family examination because: 1. therapy should be started without waiting for the genetic result if indicated. 2. in 3 families (18%) two hereditary disorders were present and 3. in 2 families the phenotype of the deceased was untypical so that the proper diagnosis would not have been made without family screening.

P02.246

Male with mosaicism for a supernumerary derivative X chromosome lacking the *XIST* gene and phenotypic features of craniofrontonasal syndrome

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Background: Craniofrontonasal syndrome (CFNS, OMIM 304110) is an X-linked disorder with typical craniofacial dysmorphism (brachycephaly, facial asymmetry, coronal craniosynostosis, hypertelorism, broad nasal root, bifid nasal tip), syndactyly, broad halluces, and hypoplastic corpus callosum. Mutations in the gene for ephrin-B1 (EFNB1) located at Xq13.1 have been identified as the primary cause of CFNS which paradoxically shows a more severe phenotype in heterozygous females than in hemizygous males. In rare cases, CFNS can be caused by X-chromosome anomalies.

Case report: We describe a five month old boy with severe dysmorphic features including a broad face, hypertelorism, broad nasal root, bifid nasal tip and multiple congenital anomalies (agenesis of the corpus callosum, patent ductus arteriosus, VSD and hypospadias).

Cytogenetics including FISH analysis revealed mosaicism for a supernumerary derivative X chromosome (mos 47,XY,+der(X)del(X)(p11.1)del(X)(q13) [7]/46,XY[23] lacking XIST. Parental cytogenetic studies were normal.

Discussion: The severe phenotype of CFNS in females with a heterozygous EFNB1 mutation is hypothesized to result from inequalities in gene dosage for EFNB1 due to X inactivation. A patchy ephrin-B1 defect leads to disturbed closure of cranial sutures by a process termed cellular interference. Mosaicism for a derivative chromosome expressing EFNB1 in one cell line due to a lack of XIST may explain similar phenotypic features in the present male patient and one other previously published case.

P02.247

Cryptic deletion in chromosome 1q43 associated with syndactylies detected by arrayCGH - a different critical region for syndactylies?

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We report a case of a woman with a known familial history of syndactylies probably associated with a cytogenetically balanced translocation t (1;9)(q43;q32). At the age of 19 years she came for genetic counseling while being pregnant. She herself was analyzed by cytogenetic karyotyping where a translocation was detected which she inherited from her father. As her father, her brother and her half-brother from father's side also had syndactylies of hands and feet as well as a skeletal dysplasia and were carriers of this specific translocation, we performed arrayCGH analysis on all of them. ArrayCGH analysis revealed a microdeletion in 1q43 which spans over 528 kb of size in all three members of this family who bear the translocation. The deletion includes partly the CHRM3 (cholinergic receptor, muscarinic 3) and the FMN2 (formin-2) gene, two genes which are hardly described in literature until now. Formin-1 is the founding member of a family that share specific domains of homology and are classified together as the formin homology proteins. Deficiency mutations in formin-1 lead to profound developmental defects in limb and kidney formation. To date variations in formin-2, a gene which seems to have a high degree of similarity to formin-1, are rarely detected and understood.

In order to elucidate this specific region, we intend to perform next generation sequencing (NGS) to correlate the clinical phenotype to this deleted region.

P02.248

CGH-array detection of a "de novo" chromosome 19p13.3 deletion: case report.

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Introduction: the development of high-resolution array-CGH has allowed the identification of genomic alterations not previously detectable with routine techniques. We present a patient with a "de novo" genomic imbalance of 19p13.3, not detected by routine techniques (high resolution G-banded chromosome analysis and MLPA).

Patients and Methods: five members of the same Spanish family: the index patient, a 23 year old male who presented athonic epileptic crisis, mild mental retardation and dysmorphic phenotype, the unaffected parents and two unaffected sisters.

Oligonucleotide comparative genome hybridisation-based microarray analysis (array-CGH; 105A or 180K, Agilent Technologies) was performed on each DNA sample of each family member.

Results: the proband showed three deletions not previously described in the general population, two of them potentially pathogenic, deletion in 19p13.3 and 22q21.3. He also presented eighteen copy number variations (CNVs) previously described in the general population,

The deletion in 22q21.3 was also present in the sisters and in the mother.

The 19p13.3 monosomy covers 821kb (from position 982,017 to position1,803,579) which includes 33 genes, four of them pathological (STK11, NDUFS7, GAMT and TCF3).

Conclusions:

The "de novo" 19p13.3 monosomy seems to be responsible for the pathology of the proband.

This is the first case of 19p13.3 monosomy described in Spanish population (two cases have been previously reported among other populations, Siggberg, 2010 and Smith, 2010).

CGH-arrays allows for the genetic diagnosis of new syndromes, but further studies are needed to establish genotype-phenotype correlation.

P02.249

A rare combination of Syntelencephaly, Wormian bone and Split Metopic suture

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Syntelencephaly is a rare anomaly characterized by fusion of the hemispheres in the posterior frontal and parietal regions and is considered a new variant of holoprosencephaly.Wormian bones are accessory bones that occur within cranial suture and fontanelles, most commonly within the posterior sutures. They occur more frequently in disorders that have reduced cranial ossification, hypotonia or decreased movement, thereby resulting in deformational brachycephaly.Here, we report a one-week- old male who presented with abnormal head shape and concern for craniosynostosis. He had rachycephaly, alopecia, right hemiparesis and closed anterior fontanelle. Three dimentional CT scan revealed absence of the anterior fontanelle, sagittal wormian bone and split metopic suture. Alopecia was noted in the skin overlying the wormian bone. MRI scanning showed

deficient formation of the interhemispheric fissure with fusion of parietal lobes and agenesis of corpus callosum. We used a custom array with a 44K whole genome and extra 14,217 additional probes for all known 226 genes of skeletal dysplasia. We detected no significant copy number changes in our patient. To our knowledge, this is the first reported case with syntelencephaly, wormian bone and split metopic suture.

P02.250

Identification of TAZ mutation in a family with X-linked dilated cardiomyopathy by Next Generation Sequencing

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Familial dilated cardiomyopathy (DCM) is defined as DCM of unknown cause in 2 or more closely related family members. We reported a family with 2 male siblings both presented with heart failure in infancy and subsequently confirmed to have DCM without conduction abnormalities. Endo-myocardial biopsy (in the elder sibling), extensive metabolic workup, viral studies and NimbleGen CGX-12 array were all normal. Oligonucleotide-based target capture (Sureselect, Agilent) followed by next generation sequencing (Illumina HiSeq2000) was used to capture variants of 46 genes implicated in the causation of cardiomyopathy (Partners Healthcare Center for Personalized Genetic Medicine). Clinically significant /novel variants are confirmed by independent Sanger sequencing. A hemizygous variant c.718G>C (p.Gly240Arg) in exon 10 of TAZ gene is identified. This variant is likely pathogenic as it has been reported in 5 individuals with X-linked infantile DCM and was described in a family with endocardial fibroelastosis. So far, the 2 siblings did not show evidence of skeletal myopathy, stunted growth, neutropenia or abnormal urine organic acid analysis. Next generation sequencing (NGS) allows efficient screening of a panel of genes in complex disorders like DCM, in which there is substantial overlap among phenotypes, multiple causative genes, and some mutations associated with > 1 phenotype. The identification of TAZ mutation has major impact in the medical surveillance of our patients as they need to be monitored for symptoms of Barth syndrome in addition to DCM. (Funding support from Children's Heart Foundation, Hong Kong)

P02.251

Novel insertion in exon 5 of the *TCOF1* gene in Twin Sisters with Treacher Collins syndrome

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Treacher Collins Syndrome (TCS) is an autosomal dominant disorder, associated with an abnormal differentiation of the first and the second pharyngeal arches during fetal development. It cause craniofacial deformities with typical clinical symptoms: downward slanting of the eyelids, hypoplasia of the zygomatic bone, mandibular hypoplasia. The estimated incidence is 1/50000 live births, with 60% of the cases resulting from a *de novo* mutations. The syndrome is mostly caused by mutations in the *TCOF1* gene, which encodes the serine/alanine-rich protein named Treacle. TCS can be also caused by mutations in the *POLR1C* and *POLR1D* genes encoding subunits of RNA polymerases I and III. Over hundred mutations of the *TCOF1* gene responsible for TCS have been described, which about 70% are deletions.

Investigated DNA fragments were amplified by PCR and were subsequently subjected to multitemperature single-stranded conformation polymorphism analysis. Fragment of the allele, which exhibited an abnormal MSSCP pattern were eluted from the gel and used as template for reamplification of single band by PCR method. The PCR products were purified followed by direct sequencing.

In the patients - two monozygotic twin sisters a novel, heterozygotic insertion c.483_484ins185 was detected. It is the longest discovered in *TCOF1*. This mutation was absent in the patients' father, brother and uncle, which probably indicates a *de novo* origin. The c.483_484ins185 insertion causes a reading-frame shift and premature termination of translation at 167aa.

We believe that these findings facilitated a precise diagnosis of both patients and extended our knowledge on the pathogenesis of TCS.

P02.252

Beta-globin deletion in a transfusion dependent child with parents showing no elevated HbF

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The molecular investigation of beta-globin gene cluster deletions is usually performed in the presence of fetal hemoglobin (HbF). In this study, a family showing no elevated HbF (0.6% and 0%), the mean corpuscular value (MCV: 59.9 fL and 64.7 fL), and HbA2 (3% and 2.9%) for the maternal and paternal sides respectively has referred to our laboratory having a 3 months old child with severe anemia undergoing blood transfusion every 15 days.

Firstly, the most common alpha thalassemia deletions were investigated by gap-PCR and the paternal side revealed homozygote alpha gene deletion -α3.7and consequently the child has inherited the -α3.7in heterozygous form. The maternal side did not show any alpha gene mutation even by alpha-globin DNA sequencing. Direct sequencing for beta- globin gene was also performed and no mutation was detected for the family. Finally, multiplex ligation probe amplification (MLPA) was subjected to detect alpha- and beta- globin large deletions in the affected child and the results indicate a huge homozygote deletion in beta-globin gene cluster with the same pattern as Indian GγAγ (δβ)° or Belgian (50 Kb) deletions. The deletion was confirmed in both paternal and maternal sides in heterozygous form.

This study shows that MLPA can effectively identify different and unknown types of beta-globin gene deletions, to allow characterizing previously unsolved thalassemia intermedia genotypes, and remarks the probability of presence of beta deletions in cases that do not show significant elevations of HbF.

P02.253

Mutations in WNT10A are present in more than half of isolated hypodontia cases

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Hypodontia or the congenital absence of one or more permanent teeth is the most common congenital anomaly in man. The disorder is highly heritable and occurs as an isolated anomaly or as a symptom of a large variety of syndromes, including Ectodermal Dysplasia (ED). The recent finding of hypodontia in carriers of the autosomal recessive disorder odonto-oncychodermal dysplasia due to mutations in *WNT10A* (OMIM 257980; OODD) made *WNT10A* an interesting candidate gene for dental agenesis. This motivated us to study the contribution of *WNT10A* mutations in comparison with mutations in other genes that are associated with hypodontia in isolated hypodontia patients. We tested a panel of 34 probands that show variable severity of isolated tooth agenesis for mutations in the candidate gene *WNT10A* and the genes *MSX1*, *PAX9*, *IRF6* and *AXIN2*. The probands all had agenesis with a range of 6 - 28 teeth. Nineteen cases with non-syndromic probands (56%) showed alterations in the WNT10A gene: 8 probands were homozygous, 4 probands were compound heterozygous and 7 probands were heterozygous for a single WNT10A mutation. In individuals, tested as heterozygote for a WNT10A mutation, tooth agenesis was comparable in males (6/9; 67%) and females (7/12; 58%). In conclusion, we identified *WNT10A* as a major gene in the aetiology of isolated hypodontia. By including this gene, the yield of molecular diagnostics in isolated tooth agenesis has increased significantly from 15% to 71%. This approach will be of help in a more optimal counselling of patients with hypodontia and their family members.

P02.254

Live-born Child with Trisomy 22

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Chromosomal abnormalities represent a major cause of spontaneous abortions (SABs). Trisomy 22 has been identified as the third most common trisomy in SABs, representing 16% of cases. Due to severe organ malformations associated with trisomy 22 a live-born child is a very rare event. Here, we report on a male infant with complete, non-mosaic trisomy 22 in peripheral blood lymphocytes (PBLs) and skin fibroblasts.

The patient was born at 35+5 weeks by caesarean section as the second child of a 44-year-old female and a 40-year-old male. The parents are healthy and unrelated. Prenatal sonographic examinations had revealed IUGR, dolichocephalus, SUA, absent right kidney, and hypospadia. Due to the parents' beliefs they did not opt for additional prenatal diagnostic procedures and chose to continue the pregnancy.

Birth weight and length were below the 3rd percentile; APGAR 5/6*/8* (*CPAP). GTG-banded chromosomes from PBLs and fibroblasts showed an additional chromosome 22 in all metaphases analyzed (47,XY,+22). SNP array and aCGH demonstrated a complete trisomy 22. Clinical features included dolichocephalus, hypertelorism, flattened nasal bridge, dysplastic ears with preauricular sinuses and tags, medial cleft palate, anal atresia, and coronary hypospadia with scrotum bipartitum. Echocardiography showed persistent foramen ovale and ductus Botalli. Essential therapy was implemented in close coordination with the parents. The child died 29 days after birth due to respiratory insufficiency and deterioration of renal function; an autopsy was not performed. This patient's history complements other reports illustrating that children with complete trisomy 22 can survive until birth and even some time beyond.

P02.255

Recurrent hypoglycemia due to growth hormone deficiency and resistance in a preterm with Turner syndrome

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Objectives: i) To report a case of infantile hypoglycemia in Turner syndrome (TS) patient with growth hormone (GH) deficiency and resistance, and ii) to compare conventional cytogenetics and array CGH in the diagnosis of a complex TS karyotype. *Case report:* In a preterm (32 weeks) with prolonged and cholestatic jaundice, recurrent hypoglycemia occurred at the age of 1.5 months and related to GH deficiency. There were no other endocrine or syndromic features. GH therapy was started at a usual dose of 25-30 µg/kg/d but hypoglycemia recurred. Hepatopathy and hypogammaglobulinemia suggested X-recessive GH deficiency type 3 with non-random X-inactivation but resolved spontaneously. Nonetheless, a TS karyotype 45,X[75]/46,X,i(Xq)[21]/47,X,i(Xq)x2[4] was diagnosed with an apparent isochromosome fusion at the centromere. Upon this diagnosis, GH dose was doubled (50 µg/kg/d) and blood glucose normalized consistently. In array CGH, the signal of Xp deviated more strongly than that of Xq but the relation of the signals differed substantially from what the karyotype predicted. The isochromosome fusion point was relocated to Xp11.22, distal to a block of mental retardation genes that escape X-inactivation. *Conclusions:* i) TS with GH deficiency or resistance is a differential diagnosis of hypoglycaemia in infants. ii) Array CGH is useful in precisely delineating isochromosome structure but may be erroneous in quantification of TS mocaicism.

P02.256

Maternal isodisomy for chromosome 9 in a patient with IgA nephropathy, short stature and intellectual disability

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Descriptions of patients with maternal isodisomy 9 are rare in the literature (10 cases of maternal UPD 9 and 2 cases of paternal UPD 9). The possibility of hidden mosaicism or homozygosity of mutations in autosomal recessively inherited diseases hampers the delineation of a clear UPD 9 phenotype.

Here we report a 34 years-old man with maternal isodisomy for chromosome 9 detected by genome-wide combined copy number and genotype profiling using a high-resolution CN/SNP array. The major problems in this patient were renal failure because of IgA nephropathy, hypothyroidism, short stature with overweight, hypercholesterinemia, hypertriglyceridemia and hyperuricemia. Other features were dislocation of the patella, atopic eczema, eye problems (strabism, nystagmus, myopic astigmatism), inguinal hernias and umbilical hernia as an infant. Since infancy a disproportional large distended abdomen was noted with unidentified cause. At the age of about 15 years some hearing problems on the left side were noted.

The young man was able to live alone and was working 50% at a protected workplace.

No evidence of trisomy 9 mosaicism could be detected in blood and buccal smears.

P02.257

Can we prevent ELST related hearing loss through early audiological ELST diagnosis? - A case of deafness due to microscopic ELST and an international collaborative study

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Background

Endolymphatic sac tumours (ELSTs) occur in up to 16% of von Hippel-

Lindau (vHL) patients. Timely diagnosis and surgical excision of tumors is essential to prevent severe audio-vestibular morbidity as even microscopic ELSTs can cause irreversible hearing loss. We present a case in which deafness caused by a microscopic ELST possibly could have been prevented by early pre-symptomatic audiometric diagnosis.

Methods

Full medical records of the patient's subjective audio-vestibular symptoms, audiological examinations, and Magnetic Resonance Imagings (MRIs) of the inner ear from 1995-2011 were collected and evaluated.

Results

A 42-year-old male *VHL* mutation carrier with initial normal hearing was followed for twenty years with audiometry and MRI of the brain and inner ear as part of a surveillance program. He reported occasional bilateral tinnitus, but no subjective hearing-loss until 2009 when in his right ear hearing began to deteriorate and progress to total deafness.

Despite annual MRIs, a right-sided ELST was not visible until 4 months after onset of deafness in 2010, when it appeared as 4 x 3 mm tumor mass. Although his hearing was objectively within normal limits until 2009, a distinct audiometric pattern of low-frequency hearing loss could retrospectively be seen from first audiometry.

Conclusions

Previous reports suggest that certain audiometric patterns as seen in our patient may indicate early ELST development. Accordingly, audiometry may be an important diagnostic tool to detect non-symptomatic ELSTs. To investigate the use of audiometry for this purpose we have initiated an international collaborative study and hope to attract new collaborators: http://icmm.ku.dk/english/icmm-staff/marie_luise_bisgaard/vhl_collaborative_research/

P02.258

A 725 kb deletion within the 22q13.1 chromosomal region detected by array CGH associated with Waardenburg syndrome

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Waardenburg syndrome (WS) is a rare (1/40,000) autosomal dominant disorder resulting from melanocyte defects, with varying combinations of sensorineural hearing loss and abnormal pigmentation of the hair, skin, and inner ear. Due to the variety of additional clinical symptoms and genetic heterogeneity, WS is classified into four clinical subtypes (WS1-S4). Mutations in six genes have been identified to be associated with the different subtypes of WS, among which *SOX10* (SRY bOX10 transcription factor) gene, which is localized within the region 22q13.1. Whole gene *SOX10* deletions have been described by Bondurand et al. [2007] suggesting that haploinsufficiency due to *SOX10* gene deletions should be encountered when testing for WS. *SOX10* is a member of the *SOX* family transcription factors and is a key transcription factor of neural crest development. In this study we report a case of a 13 year old male with a unique de novo 725 kb deletion within the 22q13.1 chromosomal region, encompassing *SOX10* and another 13 OMIM listed genes and presenting clinical features of a neurologic variant of WS. **Very few patients have been documented with whole gene deletions of *SOX10* and also very few data is known regarding deletions within 22q13.1 and of neurologic defect candidate genes such as** *PLA2G6*, *KCNJ4* and *PICK1*, **which are localised within the deleted region.** In this study **we compare the clinical features of our patient to other reported cases with analogous 22q13.1 deletions and look into** genotype-phenotype correlations**.**

P02.259

WAGRO syndrome caused by deletion 11p14.2p11.2

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WAGR is a contiguous gene deletion syndrome caused by loss of distal portion of chromosome 11p13 band. Its subphenotype WAGRO including obesity is associated with additional haploinsufficiency of BDNF gene with locus 11p14.1.

We present a case where diagnosis of the WAGRO was delayed because of the absence of predictable tumor in a mentally retarded female with aniridia and severe obesity with onset at 10 years of age. She had craniofacial dysmorphism, nasal cleft, and large anterior fontanelle. Diagnosis of aniridia and cataracts was confirmed early after birth. Multiple renal cysts spontaneously disappeared in later life. Menarche started at the age of eleven, but secondary amenorrhea ensued early. Enuresis nocturna and epilepsy have been a constant problem. Neither gonadoblastoma nor Wilms tumor were diagnosed. She was referred to geneticist to specify the diagnosis at the age of 24.

We have used effective genetic diagnosis work-up: a routine G- banded karyotyping and SNP array (Illumina HumanCytoSNP-12v2.1) analysis. The deletion has been detected by classical karyotype and further updated by SNP array. The 18 Mb haploinsufficiency of 11p11.2 -14.2 has been confirmed containing over 70 genes including PAX6 and WT1 associated with WAGR. Haploinsufficiency of BDNF in this deleted region can explain the WAGRO phenotypic features.

The observed disease phenotype depends on gene dosage and specific sequences involved in hemizygous deletion. It is necessary to determine the extent of each 11p deletion. The early diagnosis may provide a possibility of more specific health supervision which could be essential for the patient's prognosis.

P02.260

The mitochondrial ND1 m.3337G>A mutation associated to multiple mitochondrial DNA deletions in a patient with Wolfram syndrome and cardiomyopathy

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Wolfram syndrome (WFS) is a rare hereditary disorder also known as DIDMOAD (diabetes insipidus, diabetes mellitus, optic atrophy, and deafness). It is a heterogeneous disease and full characterization of all clinical and biological features of this disorder is difficult. The wide spectrum of clinical expression, affecting several organs and tissues, and the similarity in phenotype between patients with Wolfram syndrome and those with certain types of respiratory chain diseases suggests mitochondrial DNA (mtDNA) involvement in Wolfram syndrome patients. We report a Tunisian patient with clinical features of moderate Wolfram syndrome including diabetes, dilated cardiomyopathy and neurological complications.

The results showed the presence of the mitochondrial ND1 m.3337G>A mutation in almost homoplasmic form in 3 tested tissues of the proband (blood leukocytes, buccal mucosa and skeletal muscle). In addition, the long-range PCR amplifications revealed the presence of multiple deletions of the mitochondrial DNA extracted from the patient's skeletal muscle removing several tRNA and protein-coding genes.

Our study reported a Tunisian patient with clinical features of moderate Wolfram syndrome associated with cardiomyopathy, in whom we detected the ND1 m.3337G>A mutation with mitochondrial multiple deletions.

P02.261

Array-CGH detection of cryptic genomic rearrangements in children with X-Linked Intellectual Disability

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Clinical and genetic observations have shown that X-Linked Intellectual Disability (XLID) is a very heterogeneous entity, involving syndromic and non-syndromic indistinguishable forms with more than 100 causative genes implicated and an estimated prevalence of ~10% in males. Underlying X chromosome defects are not always visible with conventional cytogenetic techniques as a first-line approach, making the genetic evaluation and diagnosis difficult. Array Comparative Genomic Hybridization (Array-CGH) has proven a high throughput method, allowing genome wide XLID gene screening in the diagnostic workup. Overall 198 male patients with a large spectrum of phenotypic variability (developmental delay, seizures, dysmorphic feautures, congenital anomalies), normal previous conventional karyotype and negative genetic tests (FRAX, RETT, FISH tests or metabolic screens) were analyzed with Agilent platform 4x180K and 1x244K oligoarrays(>170.000 probes and >236.000 probes respectively with a high resolution of 8.9 Kb). 14 X-chromosome cryptic rearrangements containing clinically relevant genes were detected in 21 patients, explaining partially or completely the pathological phenotype (Table below).The remarkable percentage of positive patients is probably due to the strict criteria of patient selection. Array-CGH offers a higher diagnostic yield for the identification of previously unreported X-linked aberrations of unclear significance, elucidating novel non-syndromic/syndromic forms and permitting better dinstiction and correct reclassification of specific XLID cases.

Aberration (size in Mb)	Proximal->Distal (UCSC hg18), Mb	No of Pat.	Genes	Inheritance	Relevant Phenotype
DUP Xp22.33 (0.026)	1.68->1.71	1	*ASMT*		Autism Spectrum Disorder
DUP Xp22.32 (0.1)	5.8-5.9	1	*NLGN4X*		Autism Spectrum Disorder
DUP Xp22.31 (1.61-1.65)	6.47-8.12	2	*HDHD1A,STS, VCX2,hsa-mir-651*	1 *maternal*	Syndromic XLID
DUP Xp22.13 (0.058)	18.33->18.39	1	*CDKL5*	*De novo*	Syndromic XLID
DEL Xp22.12 (0.023)	21.67->21.69	1	*SMPX*	*De novo*	Non-syndromic XLID
DEL Xp11.3 (0.56)	43.21->43.8	1	*MAOA,MAOB*	*maternal*	Non-syndromic XLID
DUP Xp11.22 (0.013)	53.23->53.25	1	*JARID1C*	*De novo*	Non-syndromic XLID
DEL Xq13.2 (0.058-0.264)	73.4->73.7	2	*SLC16A2,ZCCHC13*		Syndromic XLID
DEL Xq13.3 (0.041-0.1)	74.47->74.52	2	*ZDHHC15*	1 *De novo*	Non-syndromic XLID
DEL Xq21.1 (1.83)	81.1->82.9	1	*POU3F4*		Non-syndromic XLID
DEL Xq21.31 (0.103-0.38)	90.6>91.2	5	*PCDH11X*	1 *De novo,* 1 *maternal*	Non-syndromic XLID
DUP Xq27.1 (0.069)	139.38->139.44	1	*SOX3*		Syndromic XLID
DEL Xq27.3-q28 (0.62)	146.5->147.1	1	*ASFMR1,FMR1, FMR1NB*	*De novo*	FRAX region(mosaic)
DUP Xq28 (5.03)	149.7->154.7	1	*GABRA3,MECP2, L1CAM,IRAK1*	*De novo*	RETT syndrome

P02.262
Interstitial Xq duplication in a male patient - clinical, cytogenetic and arrayCGH characterization of a new case

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Prevalence of isolated Xq duplications is presently unknown. At least 30 male patients with this aberration have been reported, with the majority localized within Xq12-q24. Large, cytogentically visible Xq duplication are rare. However, application of microarray-based technique gives the chance for detection of smaller aberrations, as well as defined genes influencing the causative phenotype.

Clinical manifestations in described cases vary depending on the gender of the patient and on the size of duplication, hence gene content of the duplicated segment. Consequences of over-expression of X-linked genes are not well known. In most male cases the consistent phenotype includes profound muscle hypotonia accompanied by severe psychomotor and growth failure, hypoplastic genitalia, seizures and craniofacial dysmorphism. Such phenotype seems to be quite specific, however, due to rarity of this disease, it is difficult to suspect it based on clinical symptoms.

In our presentation we report the clinical and laboratory data of 3-year-old boy with profound generalized hypotonia, growth failure resulting from Xq duplication identified cytogenetically as 46, XY,dup(X)(q21q22?)mat. Further delineation of the duplicated region by arrayCGH refined the breakpoints to Xq13.1-q22.11 and showed the duplication size of 32 Mb. This is only the second case with similar aberration characterized molecularly.

By comparison of our patient's phenotype with the previously reported male with overlapping duplicated region, we hope that presented detailed results give insight into the genotype-phenotype correlation of Xq-linked genes duplication.

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P02.263
A 91kbp interstitial deletion of Xq24 encopassing the UBE2A gene, in a boy presenting with intellectual disability, impaired speech, microcephaly, growth retardation VSD and hirsutism.

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Interstitial deletions of chromosome Xq24 are rare with only five reported cases so far. In all of these cases the deletions included the *UBE2A* gene and the size of the deletion was almost identical, ranging from 275kb to 371kb and encompassing between 5 and 6 genes in addition to the *UBE2A* gene.

We describe a 2y and 3m old boy with a 91 kb deletion in the Xq24 region detected by array comparative genomic hybridization (array CGH). His phenotype includes: severe intellectual disability with absent speech, hypotonia, feeding problems, mild conductive hearing loss, recurrent aspiration pneumonia and prolonged neonatal hypoglycemia. He has microcephaly (-4.5 SD) and growth retardation (his weight is -4.5 SD and height -5 SD). His dysmorphic features include: large open fontanel, hypertelorism, up-slanted palpebral fissures, synophrys, depressed nasal bridge, marked general hirsutism a ventricular septal defect and normal genitalia. The mother carries the same deletion. The deleted X chromosome in her blood lymphocytes is completely inactivated (0:100).

This is the smallest deletion encompassing the *UBE2A* gene reported so far. Only three genes are located in the deleted Xq24 region found in this boy: *NKRF*, *UBE2A* and *CXorf56*. *UBE2A* deficiency syndrome that is caused by point mutations in the *UBE2A* gene, has been shown to cause syndromic X-linked intellectual disability (XLID) with dysmorphic features, severely impaired speech, small penis and hirsutism. Our case further supports previous suggestion that deletion of *UBE2A* is sufficient to cause the *UBE2A* deficiency syndrome.

P02.264
Interstitial duplication in the case of Yunis Varon Syndrome

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Brief Introduction: The aim was to evaluate the relationship between abnormality in the region of 5p15.31-5p15.2 represented on the microarray analysis, classified as an interstitial duplication and the subsequent clinical presentation of Yunis Varon Syndrome.**Materials and Methods**: Neonate with dysmorphic, typical phenotypic and radiologic features of Yunis Varon Syndrome, born as 37 weeker, 2300g, Apgar score 1/2/3/3, a first child to a healthy couple. Prenatal ultrasound scans, fetal biometry and fetal MRI were performed showing malformations in central nervous system, hypertelorism, micrognathia, abnormal views of extremities. Chromosomal caryotyping from amniotic fluid cells cultures was done - 46, XX.Postnatally the baby was growth restricted, hypotonic and dysmorphic. Microcephaly, hypertelorism, microtia, narrow high-arched palate, wide fontanelles, bilaterally absent thumbs and great toes were observed. Radiologically - aplasia of both clavicles. ASD II in echocardiogram. No abnormalities in abdominal ultrasound scans. Chromosomal karyotyping from peripheral blood lymphocytes was correct female karotype.Autopsy showed agenesis of corpus callosum with atrophic subcortical nuclei and hydrocephalus, middle and external ear anomalies, bilateral dysplasia of eye bulbs, hyperaemia of kidneys and lungs and focal liver necrosis.Microarray analysis was performed using NimbleGen CGX-12, revealing abnormality in the region of 5p15.31-5-p15.2, covering ADCY2, MTRR, SEMA5A, TAS2R1, CCT5, CMBL genes, which is classified an interstitial duplication.**Conclusions**: Microarray analysis using NimbleGen 135k CGX-12 enables to identify submicroscopic changes, localized in genome regions bigger than 30-100 kb and therefore, abnormality in the region of 5p15.31-5p15.2 classified as an interstitial duplication can highly be a pathogenic one.

P02.265
Expanding the clinical phenotype of patients with *ZDHHC9* mutation

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In 2007, four families with a mutation in the *ZDHHC9* gene have been described, out of the screening of 250 families with X-linked intellectual deficiency (ID). ID was either isolated or associated with a marfanoid habitus. ZDHHC9 encodes for a palmitoyl transferase that catalyses the posttranslational modification of NRAS and HRAS. Since this first description, no additional patient has been described. A new family has been identified in France from the systematic screening of X-linked ID genes in 95 families, carrying the unreported *p.R298X* variant leading to a stop codon, cosegregating with the disease. A 18-year-old patient and his 40-year-old maternal uncle have been evaluated. Clinical examination revealed normal growth parameters, lingual fasciculations, a limited extension of the elbows and metacarpophalangian joints, and acrocyanosis. There was no facial dysmorphism or marfanoid habitus. Brain MRI revealed dysplastic corpus callosum. Neuropsychological testing demonstrated a mild to moderate mental retardation. The younger suffers of significant impairment of behaviour requiring attention or treatment, and the older a generalised anxiety disorder (ICD 10). Speech evaluation revealed a satisfactory oral language since both were able to provide information and to understand conversations of everyday life. Occupational therapy examination revealed impaired visuospatial and visuomotor performance with poor drawing/graphic skills. These manifestations appear not enough specific to permit to define specific criteria justifying *ZDHHC9* screening in patient with ID, and emphasized the value of next generation sequencing for genetic counselling in families with X-linked ID.

P03. Cytogenetics

P03.002

A clinical study of patients with pericentromeric deletion and duplication within 16p11.2-p12.2

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The pericentromeric region on 16p is susceptible to chromosomal rearrangements. There are many reports that deletions of 16p11.2 are observed about 1% among patients with autism. The 16p11.2-p12.2 deletion syndrome is characterized by developmental disabilities and dysmorphic features. We have identified pericetromeric deletion and duplication, within 16p11.2-p12.2 in three patients by Cytogenetics 2.7M array. Patient 1 was a 10-year-old girl with autism and obesity. She had a heterozygous 593kb deletion in 16p11.2. Her mother with the same deletion was not autistic, but was obese. Patient 2 was a 2-year-old boy with VSD, hypotonia, developmental delay and frequent ear infections. He had.dysmorphic features including flat face, down slanting palpebral fissures, low-set posteriorly rotated ears and thin upper lip. Autistic features were not seen. Microarray analysis revealed a 7.7Mb deletion at 16p11.2-p12.2. He showed common clinical features to the 16p11.2-p12.2 deletion syndrome. Patient 3 was an 8-year-old girl with developmental delay, autism and dysmophic features including hypertrichosis, wide mouth and macrocephaly. She showed poor communication skills and ritualized patterns of interests and behavior. Microarray analysis revealed a 6.7Mb duplication at 16p11.2-p12.2. The duplicated region of patient 3 was very similar with the deleted region of the patient 2. We suggest that 16p11.2-p12.2 duplication may be a new syndrome with autism.

P03.004

17q21.31 microdeletion syndrome in monozygotic twins

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Chromosome 17q21.31 microdeletion syndrome is a genomic disorder caused by recurrent ~600 kb deletion of the region containing the common 900 kb inversion. The inversion is associated with the H2 haplotype present in ~20% of Europeans. H2 carries additional low-copy repeats susceptible to NAHR which can lead to the deletion. The syndrome is characterised by intellectual disability, hypotonia, long face, tubular or pear-shaped nose, bulbous nasal tip and friendly behaviour.

We present monozygotic twin sisters carrying the microdeletion and showing only slightly different phenotypes. Both had disproportionate short stature, short upper and lower limbs, thoracic hyperkyphosis, low-pitched voice and similar long, thin and coarse face, coarse hair, thick eyebrows, pear-shaped nose, smooth broad philtrum, thick lips, mandibular prognathism, and hirsutism. Twin A had high palate and Twin B had wide-spaced teeth, diastema, more severe intellectual disability, more coarse facial features, strabismus, and horizontal nystagmus.

The microdeletion was identified in Twin A using BAC array CGH and confirmed in both twins but not in the parents using FISH. Potential genomic differences were subsequently searched using Illumina Human CytoSNP-12 SNP arrays (~300K) and Nimblegen 2.1M whole-genome CGH array. These analyses identified no differences potentially responsible for the phenotypic differences, which could possibly be related to a more severe perinatal history of Twin B or the variable expressivity of the disorder. The father and mother were homozygous for H1 and H2, respectively, and the maternal 17q21.31 allele was missing in the twins.

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P03.005

De novo ring chromosome 21 with a complex 21q interstitial and terminal deletion with array-CGH, in patient with several congenital anomalies, mental retardation and thrombocytopenia

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We present a 20 months old infant with a de novo ring chromosome 21, detected in prenatal kariotype, with breaking point in p11.1 and q22.3, in which postnatal array-CGH demonstrated an almost complete loss of the q arm in the affected chromosome, with little microduplication fragments within four deleted fragments (1.2 Mb to 23 Mb) between 21q11.2-q21.1 and the chromosome end. Phenotypically, the infant presents moderate to severe growth and developmental delay, structural cardiac defects, bilateral hip dysplasia, microcephaly with cerebral atrophy, mild thrombocytopenia and facial dysmorphism, with similar features that those described in patients with partial or complete 21 monosomy. There are multiple affected genes, one of them, RUNX1, has been reported to pedisponse, when in haploinsufficiency, to thrombocytopenia and acute myelogenus leukemia. In this case, the array-CGH has contributed to a more accurate diagnosis of the chromosome disorder, which with at first seemd just a terminal 21q deletion instead of an almost complete 21 monosomy.

P03.006

Homozygous 2p21 deletion syndrome due to maternal uniparental disomy 2

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The 2p21 deletion syndrome (MIM 606407) is a contiguous gene syndrome caused by homozygous deletions on chromosome 2p21. Depending on the size of the deletion different clinical symptoms have been described. Deletions of the SLC3A1 and PREPL genes are responsible for the hypotonia-cystinuria syndrome. Homozygous mutations in the SLC3A1 gene were found in patients with isolated cystinuria (MIM 220100). We report on a girl born preterm to non consanguineous healthy parents. At delivery, the mother was 44 years old. After birth, meconium ileus and a persistent Ductus arteriosus Botalli had to be corrected surgically. Profound muscular hypotonia was present and psychomotor development was delayed. Failure to thrive was so severe that at 2 years age tube feeding was still necessary. Karyotype on conventional cytogenetic analysis was apparently normal (46,XX). Array-CGH analysis uncovered a small homozygous deletion of approximately 28.6 kb that disrupts the PREPL and the CAMKMT genes but not the SLC3A1 gene. Quantitative PCR analysis of both parents demonstrated a heterozygous deletion of the same size in the maternal blood only. We hypothesized that a maternal uniparental disomy could be responsible for the homozygosity of the deletion in the daughter. A microsatellite analysis showed maternal isodisomy 2 in the critical region 2p21. Possibly meiotic non-disjunction followed by trisomy rescue led to homozygosity for the small maternal deletion. This case illustrates a complex multistep process leading to a disease causing genomic imbalance. Moreover, it underlines the necessity of investigations in the parents for a correct interpretation of array-CGH results.

P03.007

De novo chromosome (2)(q24.2q24.3) deletion in a 17-months old developmentally delayed girl

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We present a 17 months old female patient, first examined at the age of 11 months for developmental delay. The girl is the second child of a healthy and non-consanguineous couple. The family history was unremarkable. She was delivered by caesarian section at 38 weeks of gestation with a birth weight of 2900 g (pc=25th), length of 50 cm (pc=50th) and cranial circumference of 32.5 cm (pc=10th). Clinical evaluation revealed: hypotonia, microcephaly, high forehead, hypertelorism, palpebral ptosis, strabismus, arched mouth, low set ears, pectus excavatum, coxofemoral dysplasia. Development milestones were delayed, she held her head at 6 months, sat at 11 months and started to walk sustained at 17 months. For lack of eye contact and repetitive hand movements we performed FISH analysis using Abbott probes for Angelman syndrome, but result was negative. Cerebral MRI evaluation was normal. Upon last evaluation at age of 17 months, the patient had length of 80 cm (pc=50th), head circumference 44 cm (<pc3rd), weight of 8400 g (<pc3rd). 180k Agilent aCGH detected a deletion on chr2: 162261555-164571028 bp hg19, chromosome(2)(q24.2q24.3). The deletion was confirmed using qPCR. Parents were checked and deletion occurred de novo. Nine genes are located in the deleted region including TBR1, SLC4A10, KCNH7 and FIGN, which may be good candidates in generating the phenotype TBR1 is involved in cortical development SLC4A10 has been previously associated to epilepsy. KCNH7, is involved in the regulation of NMDA receptor level in cortical neurons. FIGN are molecular chaperones with a role in embrionic development.

P03.008

Same genotype, different phenotype in a mother and son with genomic imbalance

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With the broad application of molecular techniques like aCGH, findings of inherited chromosomal imbalances from unaffected or mildly affected parents have become more frequent. The phenotypical variability in these cases is often attributed to genetic, epigenetic and environmental modifiers.

Here we report a 33 years-old male with cognitive impairment, speech delay, epilepsy and hearing deficiency but no dysmorphic features. Subtelomeric FISH studies detected a 5p monosomy and a 18p trisomy consistent with the presence of a derivative chromosome from a (5;18) translocation. The same rearrangement was found on the unaffected mother. Subsequent aCGH analysis further delineated the rearrangement as a 1,7 Mb terminal deletion of 5p and a 2,1 Mb terminal duplication of 18p.

The patient's phenotype is consistent with other reported 5p distal deletions upstream from cri-du-chat syndrome critical region. The 18p duplication doesn't seem to contribute to the phenotype, as reported patients with larger duplications, in some cases encompassing the whole short arm, were clinically normal.

Absence of clinical phenotype in the mother may be due to a second hit on the patient, for example, a recessive mutation within the hemizygotic region, or a mutation in its vicinity that alters expression pattern of contained genes.

P03.009

First case of 5q13.2 duplication revealed by array comparative genomic hybridization

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We report on a case of 5q13.2 duplication uncovered by array comparative genome hybridization (array CGH). The proposita (age 9 months) presented with brain malformations (cerebellar hypoplasia and atrophy of frontal and temporal lobes), congenital heart and eye abnormalities, muscle weakness/atrophy, brachycephaly, broad nasal bridge, short nose, high palate, low-set and deformed ears, short neck, distal digital and nail hypoplasia. Array CGH revealed an interstitial duplication of 5q13.2 (approximately 3.8 Mb) spanning chromosome 5 region from 68.9 to 72.7 Mb according to NCBI Build 37.3. The region contains 74 genes, from which 14 are indexed in OMIM. Among notable disease-associated genes of this region are SMN1(*600354), SMN2(*601627), NAIP(*600355), GTF2H2(*601748), MCCC2(*609014). In addition, a deletion of 2q37.3 (subtelomeric 2q) encompassing 3 genes, two of which are also indexed in OMIM, was detected. Although 2q37.3 deletion represents a CNV, its impact on clinical manifestations in the index case cannot be excluded. It is to note that mapping of spinal muscular atrophy (SMA) genes (i.e. SMN1 and SMN2) to 5q13 have suggested these loci to be susceptible to genetic instabilities and DNA duplications. However, no cases of large constitutional duplications within 5q13.2 have ever been reported. One can hypothesize that genomic organization of SMA region predisposes both to small intragenic and large constitutional chromosomal duplications. Our case report supports current trends in molecular cytogenetics, which postulates molecular karyotyping (array CGH) as the most effective way to reveal previously unreported cases of constitutional chromosomal and genomic rearrangements.

P03.010

Morbid obesity, hypogonadism, minor facial dysmorphism and mild mental retardation in a patient with a 2.7 Mb duplication on chromosome (6)(q14.1) detected by arrayCGH

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The association of obesity and developmental delay as presenting clinical features was the subject of many reports. Several gene mutations and genomic imbalances of specific loci scattered on different chromosomes have been reported to be involved in the pathogenesis of human obesity. Here we present a 29 years-old male, with hypogonadism, morbid obesity, minor facial dysmorphism and mild mental retardation evaluated for a suspected diagnosis of Prader-Willi syndrome. His weight was 180 kg (>95th percentile), height was 172 cm (25th percentile) and BMI was 60.84 kg/m2. His deceased father and paternal grandparents presented morbid obesity. As PWS was suspected, FISH analysis using specific probes was performed and didn't reveal the microdeletion. Next we performed a 180k aCGH which detected a duplication on the chromosome (6)(q14.1) (chr6: 76096298-78763228 bp hg19). Imbalances of the long arm of chromosome 6 were reported in several patients with Prader-Willi-like phenotype and SIM1 has often been considered the possible causes of nonsyndromic and PWS-like monogenic obesity. However SIM1 is not involved in the duplication detected in the patient. A recent study found 2 patients with comparable clinical features to our patient, having interstitial deletions at 6q14.1q15, partially overlapping our patient's duplicated region. Thus it is possible that alteration of dosage-sensitive genes in the 6q14.1q15 region may represent the cause of a novel clinically recognizable syndrome. The identification of the specific mechanisms of genetic predispositions for morbid obesity is important for the adequate management of individuals with morbid obesity and may improve the outcome of these patients.

P03.011

Gene regulatory variation in Cynomolgus monkeys

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Macaque monkeys are a key model species for various fields of biomedical research such as simian immunodeficiency virus pathogenesis, transplantation biology, drug development and safety testing. Cynomolgus monkeys (*Macaca fascicularis*) are the most widely used non-human primate species for drug safety testing in pharmaceutical companies and experimental results might be influenced by variation in biological processes among the individuals sampled. Knowledge of genetic factors contributing to variability with respect to biological drug responses could help to design better experimental approaches, which in turn would help to reduce, refine or even replace animal experiments. We attempted to investigate the importance and implications of genetic variation on cellular processes using genome-wide information on copy number variation (CNV) and gene expression from Cynomolgus monkeys originating from three different populations used in pharmaceutical research (Mauritius, China and the Philippines). Using aCGH data and a CNV calling pipeline combining different methods for CNV calling, we quantified copy number variation among our cohorts, which we then correlated with tissue specific gene expression data from five different tissues (heart, kidney, liver, lung, spleen). We identified several loci where copy number variation is associated with changes in gene expression levels of several genes in a tissue specific manner. Of interest is, that many of these changes occur in the kidney, which is also involved in drug metabolism. Using further downstream analyses, we will attempt to get information on the cellular processes possibly affected by these gene regulatory changes and make statements on potential implications for drug safety testing.

P03.012

Clinical utility of molecular karyotyping using high resolution array comparative genomic hybridization (aCGH)

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Array comparative genomic hybridization (aCGH) is used to detect small copy number variants (CNVs) within the genome that are not visible by conventional karyotyping. The clinical application of aCGH has helped the genetic diagnosis of patients with unexplained developmental delay (DD)/ intellectual disability (ID), autism spectrum disorders (ASD), with or without multiple congenital anomalies (MCA) and/ or nonspecific features. We have implemented aCGH (244K & 4x180K Agilent aCGH platform, theoretical resolution <50Kb), since 2008 on 334 patients with various degrees of DD/ID, seizures, ASD, MCA and normal previous conventional karyotype. The patients had also received other genetic tests (FRAX, RETT, single FISH tests or metabolic screens), which were normal. Clinically significant submicroscopic imbalances were detected in 84 (~25.15%) patients. From the total of 103 pathological CNVs detected, 38 were *de novo,* 5 were maternally inherited (one mosaic) while 4 were paternally inherited. In 66 patients 1 pathological CNV was detected, 51 were deletions (range: 0.03-18.4Mb) and 15 were duplications (range: 0.071-34.2Mb). More specifically 30 had one of the previously well known microdeletion/ microduplication syndromes, while six others had an additional smaller aberration modifying the final phenotype. The remaining had recently recognized syndromes or novel pathogenic imbalances some of them overlapping with reported cases in DECIPHER and ISCA databases. 18 patients had more than 1 pathological aberration, six had a combination of 2 deletions, 10 a combination of a deletion/duplication, one 3 deletions, and finally one had a combination of 2 duplications and 1 deletion with sizes ranging from 0.025-19.8Mb.

P03.013

Back to the karyotype: a case of mosaic marker chromosome 11 detected by aCGH

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Array comparative genomic hybridization (aCGH) is currently being used in many cytogenetic laboratories as a first-line approach to detect chromosome imbalances associated with development delay, mental retardation and congenital anomalies. However some abnormalities are better elucidated by conventional techniques such as standard karyotyping, namely when the presence of a marker chromosome or chromosomal mosaicism is suspected.

Here we report the clinical and cytogenetic findings of a 4-year old girl with intellectually disability, mild dysmorphism and macrocephaly with a 5,9 Mb gain in chromosome 11 detected by aCGH and a previous normal prenatal karyotype.

The size and pattern of the aCGH showing a three and four copies profile suggested a complex rearrangement involving the 11q11-q12.2 region, compatible with a supernumerary marker chromosome (SMC). The case was then reevaluated by karyotyping revealing a de novo mosaic SMC in approximately 70% of the cells analyzed.

Although aCGH accurately identified the chromosome and gene content of the SMC in the patient presented here, karyotype was necessary to determine the presence and structure of the marker and to established the associated abnormal cell line.

We compare our patient with other reported cases of SMC(11), to determine the respective contributions of this rearrangement to the phenotype.

P03.014

Genomic instability in the Alzheimer's disease brain: cancer-like cellular behavior mediates neurodegeneration via non-malignant aneuploidization

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Uncovering relationship between genomic instability and neuronal cell cycle and death is required for identification of the Alzheimer's disease (AD) neurodegeneration pathway. A hypothesis considering structural and functional alterations to genome landscape was proposed to link ectopic cell cycle events and aneuploidy. Previous experimental evidences suggest that genomic landscape in the AD brain is featured by of genomic or chromosomal instability manifesting as tetraploidy and aneuploidy. Aberrant DNA replication, leading to intracellular replication stress in the AD brain is likely to produce the accumulation of genomic instabilities in vulnerable neuronal cells and to promote neurodegeneration. Here, we report on molecular cytogenetic analysis of aneuploidy, tetraploidy, and DNA replication events in the AD hippocampus to define the role of neuronal genome instability in AD pathogenesis. Increased aneuploidy rates (4-21%) (monosomy and trisomy) affecting chromosome 21 and chromosome X was observed in the AD brain in contrast to 1.2-3.6% rates in the unaffected brain. However, increased rates of tetraploidy and DNA replication activity in AD were not observed. We were able to demonstrate that the incidence of aneuploid neuronal cells affected by aneuploidy was significantly higher in degenerating brain areas (hippocampus, prefrontal cortex) as to less affected areas (cerebellum). This suggests that cancer-like cellular behavior in the AD brain mediates neurodegeneration via non-malignant aneuploidization and represents the leading genetic factor contributing to AD pathogenesis. Thus, neurocytogenomics provides for a new molecular/cellular mechanism underlying somatic neural genome diversity in brain diseases. Supported by DLR/BMBF (BLR 11/002).

P03.015

Assessment of genotoxic risks in Tunisian health care workers occupationally exposed to antineoplastic drugs

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Antineoplastic drugs (AND) constitute a heterogeneous group of chemicals known for their mutagenic, carcinogenic and teratogenic effects. They include alkylating agents, mitotic spindle inhibitors, free radical generators. Hospital personnel handling AND are potentially exposed to health risk; however, investigations on their genotoxicity are inconsistent, and little information in Tunisian medical staff was available. Hence, our aim was to evaluate cellular DNA damage of medical staff occupationally exposed to AND under routine working conditions. The level of cellular DNA damage was determined in lymphocytes with chromosomal aberration (CA) and sister chromatid exchange (SCE) assays of 26 exposed subjects and 30 controls, matched for age and sex. An individual DNA repair capacity was determined by a mutagen sensitivity assay with Bleomycin (BLM) treatment in the late S-G2 phase of the cell cycle. Statistical analysis was performed using Mann-Whitney U test and Chi-square test. The results showed a significant increase in CA frequency ($p<0.01$) and SCE frequency ($p<0.05$) in exposed subjects compared with controls. The mutagen sensitivity assay showed a significant increase breaks/cell (b/c) frequency in exposed subjects ($p<0.05$). Our study has shown that increased genetic damage was evident in medical staff due to AND occupational exposure. We suggest that cytogenetic follow up studies should be included in regular health examination for this population, at least in cases of accidental exposure.

P03.016

Are apparently balanced chromosome rearrangements associated to a higher risk of congenital anomalies due to cryptic genomic imbalances?

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Genomic imbalances detectable by array-CGH have been postulated as the underlying cause of developmental delay and/or congenital abnormalities (DD/MCA) in 10-15% of patients with normal karyotype. Similarly, over 40% of apparently balanced chromosome rearrangement (ABCR) carriers with abnormal phenotype present copy number variants (CNVs), both at the breakpoints and elsewhere in the genome, implying a potential higher risk for DD/MCA.

We present the preliminary results of an on-going genome-wide arrayCGH analysis (Agilent 180K) in three populations: G-1) Individuals DD/MCA and normal karyotype (n=5), G-2) ABCR carriers with DD/MCA (n=9), G-3) ABCR carriers with normal phenotype (n=6).

A similar total number of CNVs per individual was present in all three groups (Table 1). However, potentially pathogenic imbalances were significantly more frequent among DD/MCA patients, independently of their karyotype (10,3% and 14,4%, in G-1 and G-2 groups, respectively), than in phenotypically normal ABCR carriers (1,5%). All imbalances among ABCR carriers were located outside of the breakpoint regions. Pathogenic imbalances were present in 80%-G1 and 77.8%-G2 of DD/MCA patients and in 16,7% in phenotypically normal group.

Genomic imbalances have an important role in the pathogenesis of phenotypic abnormalities. However, simple ABCR do not seem to confer a significantly higher risk for DD/MCA associated to the presence of cryptic genomic imbalances. Further systematic and comparative studies will be needed to provide adequate prenatal genetic counselling in pregnancy with ABCR.

Table 1.Summary of patients and imbalances detected by genome-wide array-CGH analysis

Patients	Karyotype	Total Nº CNV	Nº CNVs potentially pathogenic	Imbalance	Size
G1-1	46,XX	12	0		
G-1.2	46,XY	10	1	dup Yq11.22	296Kb
G-1.3	46,XY	14	2	del 8q24.12 del 17p12	227Kb 150Kb
G-1.4	46,XX	10	2	dup19p12 del7q36.2-q36.3	762Kb 20Mb
G-1.5	46,XY	12	1	del 7p21.1	66 Kb
Total G-1 (n=5)		***58***	***6 (10,3%)***		
G-2.1	46,XY,t(3;5)	9	1	del 15q22.31	50 Kb
G-2.2	46,XX,inv(15)	10	2	del4q13.1 del10p15.3	193Kb 128Kb
G-2.3	46,XY,inv(15)	12	1	del10p15.3	128 Kb
G-2.4	46,XY,t(16;17)	10	4	dup4p16.1 dup9p24.1 dup16p11.2 dupXp22.12	218Kb 308Kb 206Kb 629Kb
G-2.5	46,XY,t(1;13)	18	1	del 9p23	155 Kb
G-2.6	46,XY,t(3;6;13)	13	3	del6q13q14 del6q14.1 del13q31.3	873Kb 126Kb 1 Mb
G-2.7	46,XX,t(15;18)	8	0		
G-2.8	46,XY,inv(11)	11	2	del1p31.1 del1q21.1	122Kb 2.3Mb
G-2.9	46,XY,t(8;17)	6	0		
Total G-2 (n=9)		***97***	***14 (14,4%)***		
G-3.1	46,XX,t(3;5)	10	0		
G-3.2	46,XX,t(16;17)	9	1	dupXp22.12	629Kb
G-3.3	46,XX,t(19;21)	12	0		
G-3.4	46,XY,inv(2),t(2;12)	13	0		
G-3.5	46,XY,t(3;7)	11	0		
G-3.6	45,XY,rob(14;21)	11	0		
Total G3 (n=6)		***66***	***1 (1,5%)***		

P03.017

CASK gene heterozygous deletion in a female patient with microcephaly and cerebellar hypoplasia

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CASK gene (OMIM 300172) has an important function during neural development, having a crucial role during synaptogenesis and cerebellar and forebrain development. It encodes a member of the membrane-associated guanylate kinase (MAGUK) protein family, highly expressed in the nervous system of both adult and fetuses. *CASK* gene deletions, duplications and mutations have been recently reported to be associated with mental retardation and microcephaly with pontine and cerebellar hypoplasia (MICPCH - OMIM 300749). Deletions have only been reported in female patients, while mutations have been reported in both males and females. Missense mutations can cause a milder phenotypic spectrum while inactivating mutations and deletions can be associated with reduced male viability or even *in utero* lethality.

We report a female patient with mental retardation, ataxia, microcephaly, cerebellar hypoplasia, ventricular septal defect and scoliosis that was analyzed with a 4x180K Agilent oligonucleotide array-CGH. Array analysis revealed a 900 Kb *de novo* deletion at Xp11.4 between positions 41,342,834-42,241,039 involving *CASK* gene. This report, together with published data, reinforces the hypothesis that haploinsufficiency of *CASK* gene is responsible for mental retardation associated with MICPCH. In the presence of a clinical phenotype with these characteristics, *CASK* gene mutations but also genomic copy number changes should be considered.

P03.018

ArrayCGH analysis of constitutional cryptic deletions of 1q43-q44 and 3p26.3 in a boy with microcephaly and developmental delay

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Mental retardation is a distressing disorder affecting approximately 3% of the population. Among these, cytogenetic anomalies explain about 30% of patients with more severe mental retardation. Using conventional methods, detection of subtle structural aberrations is limited to 6-10Mb. We observed a boy in which developmental delay and mild dysmorphic phenotype in spite of a normal karyotype remained highly suggestive for a chromosomal aberration. The most impressive features were microcephaly, absent speach and epilepsy. MLPA analysis for deletions / duplications of subtelomeric chromosome regions using commercial kits P070 and P036 (MRC, Holland) detected a deletion of 3p. Array CGH analysis was performed using custom designed whole-genome oligonucleotide arrays (OGT, UK), covering the human genome at a median density of 2.5 kb. Except the deletion of 3p26.3 encompassing 1.054 Mb arrayCGH revealed a constitutional interstitial deletion of 2.64 Mb at 1q43-q44. Possible impact of both cryptic chromosomal aberrations on the patient's phenotype is discussed.

P03.019

Outcome of Array-CGH analysis in 197 Spanish patients with idiopathic mental retardation, dysmorphic features and/or congenital anomalies.

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INTRODUCTION: Array genomic hybridization (aCGH) is being used clinically to detect pathogenic copy number variants (CNVs) in individuals with intellectual disability (ID), dysmorphic features (DF) and/or congenital anomalies (CA). It is now widely adopted as a first-tier clinical diagnostic test. Our aim is to review the diagnostic yield in our unit.
MATERIAL AND METHODS: We performed a retrospective review of aCGH data (180-400k) of 197 patients with ID, DF and/or CA, with normal karyotype and subtelomeric MLPA, between july 2009 and july 2011.
RESULTS: We found pathogenic genomic imbalance in 28 (14.2%) of these 197 patients. Within this group, 4 patients (14.28%) had previous balanced rearrangement in karyotype. The size of the pathogenic structural aberrations found varied from 17 Kb to 14.1 Mb. In 90 patients (45.68%) polymorphic CNVs were detected, non-clinically significant CNVs in 39 (19.8%) and variants of uncertain clinical significance (VOUS) in 40 (20.3%). Parental complementary aCGH analysis was done in 39 patients.
CONCLUSION: 1) The diagnostic yield of our study (14.2%) was consistent with prior reports (11-20%). 2) aCGH is a valuable tool in patients with mental retardation, dysmorphic features and/or congenital anomalies of unknown etiology. 3) Further investigations are needed in order to clarify the role of VOUS in the pathogenesis since they account for a significant proportion of our results. 4) Effective clinical interpretation of these studies requires considerable skill and experience.

P03.020

Additional evidence to support the role of the 20q13.33 region in susceptibility to autism

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Autism spectrum disorders (ASD) are a group of highly heritable complex neurodevelopmental disorders and identifying its genetic bases has been challenging. The susceptibility genes so far identified seem to be involved in the proper establishment of the synaptic cleft, the secretion of surface proteins, the excitation/inhibition balance, or the overall cellular translation processes, suggesting that impacting translation-dependent processes like synaptic plasticity or cell-to-cell connectivity may lead to an ASD phenotype. Chromosomal imbalances identified by conventional or molecular cytogenetic techniques account for 10-15% of patients with autism. Here, we report a third case of pure *de novo* 20q13.33 deletion in a boy presenting with autism. Metabolic evaluation and standard karyotype were reported as normal, as was *FMR1* molecular analysis. The Human Genome Microarray CGH 180K from Agilent® used for array-CGH analyses revealed an interstitial deletion of at least 556 kb in the 20q13.33 region (arr20q13.33(61,229,038-61,785,825)x1, hg18). This deletion encompassed 21 genes including *CHRNA4* and *KCNQ2*. These genes are interesting candidate genes in the autistic phenotype since they have been reported in association with entities with exclusive neurological manifestations such as epilepsy. The frequent association between epileptic and autistic phenotypes suggests that these diseases may share common predisposing genes. Interestingly, a *de novo* 20q13.33 duplication encompassing *CHRNA4* and *KCNQ2* in a girl with autism has also been reported. This study underlines that copy number variations of the 20q13.33 region can cause autism and emphasizes the importance of array-CGH in the genetic work-up of patients with autism.

P03.021
A de novo balanced translocation that affects chromosomal bands Xp21.2 and 11q13.1 is associated with an autism spectrum disorder.

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An autism spectrum disorder was diagnosed in a 3 1/2 year old boy and consecutive cytogenetic analysis revealed a balanced translocation involving the X chromosome and chromosome 11 leading to the following karyotype: 46,Y,t(X;11)(p21.2;q13.1).ish t(X;11)(wcpX+,wcp11+;wcpX+,wcp11+) dn. No additional obvious phenotype anomalies or dysmorphic features could be recognized. A cryptic genomic unbalance up the achieved resolution was be ruled out by application of a 60k Agilent array CGH analysis which showed normal results. Balanced chromosomal aberrations in patients with a particular phenotype represent a valuable resource to identify causative genes involved in such cytogenetic rearrangements. Besides conventional but tedious approaches like FISH analysis using tiling path clones to finally identify chromosome breakpoint spanning clones and the gene(s) affected, recently more straightforward methods were demonstrated to be successful. These techniques include so called array painting procedures as well as breakpoint analysis of balanced chromosome rearrangements by next-generation paired-end sequencing. The particular chromosomal breakpoints involved in this specific case are close to or nearly identical to chromosomal bands in similar rearrangements reported in a very few patients with autistic traits. However the number of potential candidate genes at these loci is still to high for a classical Sanger sequencing strategy to characterize the involved gene. Since our group has already successfully applied the array painting methodology in comparable settings we have now also started to evaluate the next-generation paired-end sequencing approach to compare the efficiacy of these quite different techniques in the described patient as well as similar cases.

P03.022
Brain-specific X chromosome aneuploidy is likely to contribute to the pathogenesis of autism and can explain the unsolved paradox of male susceptibility

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Autism is a common childhood psychiatric disorder characterized by impaired social interaction and communication, repetitive/stereotypic behavior. Numerous studies indicate that chromosomal and genomic imbalances play a role in the etiology and pathogenesis of autism. However, the incidence and role of genomic imbalances in the autistic brain - the prime target of the disease - have not been addressed. Here, we report on the first evaluation of mosaic aneuplody in the autistic brain. Postmortem brain tissue samples (cerebral cortex and cerebellum) of 12 autistic patients and 12 age-/sex-matched controls were provided by the Brain and Tissue Bank for Developmental disorders, University of Maryland. In the male autistic brain, we observed statistically significant increase of chromosome X aneuploidy rates in the cerebral cortex and cerebellum (p=0.0166) as compared to control samples. Autistic spectrum disorders currently affect four times as many males as females. Mosaic chromosome X aneuploidy in the brain may help to explain the preponderance of autism among males in addition to specific alterations of the X chromosome genes. We conclude that intercellular genomic variation manifesting as brain-specific low-level mosaic aneuploidy is one of the possible genetic factors likely contributing to autism neuropathology. Our findings support the hypothesis that somatic genome instability could affect as homeostasis of aneuploid neurons as functioning of the neuronal network in the whole autistic brain, playing, therefore, an important role in the pathogenesis. Supported by BMBF/DLR (RUS 09/006).

P03.023
Evaluation of genomic imbalances in apparently balanced rearrangements

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Apparently balanced rearrangements are generally associated with a normal phenotype. In some cases, however, phenotype alterations are observed, which have been attributed to different pathogenic mechanisms including intragenic rupture, deletions, duplications, position effect, or may even be a chance association. We investigated 11 patients with altered phenotype and apparently balanced rearrangements: eight *de novo* translocations, two inherited inversions and one complex *de novo* rearrangement. Cytogenetic and molecular techniques, including SNP-array and fluorescent *in situ* hybridization (FISH) detected genomic imbalances in two patients. Using 200 kb filter in array analysis, one female patient with an apparently balanced translocation between chromosomes 6 and 14 was found to have a 1.1 Mb deletion in 6p. In another female patient, who presented a complex rearrangement involving chromosomes 2, 10, 13 and 21, two deletions of 10q were detected, one of them next to the breakpoint measuring 1 Mb, and the other one, more distal, approximately 7 Mb in size, probably related to the phenotype. Two of the patients had genomic imbalances smaller than 200 kb, involving genes which might be related to their phenotype. We concluded that molecular techniques such as arrays, associated to cytogenetic methods can help in detecting genomic imbalances which are invisible under the microscope and unveiling the role of genes involved in the phenotype variability of patients with apparently balanced rearrangements. Furthermore, the molecular characterization of the alterations found provides information for the follow-up of the patients and genetic counseling of their families (Financial support: FAPESP, Brazil).

P03.024
Two balanced chromosomal modifications transmitted from parents to their offspring

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A 33 years old pregnant woman was investigated by amniocentesis.

Prenatal karyotype from cultured amniocytes was performed due to increased risk for trisomy 21 at serological test without ultrasonographic abnormalities.

Fetus' karyotype established by GTG banding was: 46,XX,t(3;6)(q28;q13);inv(5)(p14q11.2). We performed chromosome analysis in the parents for checking if chromosome modifications in fetus are „de novo" or are inherited from parents. Mother's karyotype revealed the inversion on chromosome 5 [46,XX,inv(5)(p14q11.2)] and father's karyotype showed the other modification, the translocation t(3;6)(q28;q13).

We informed the family about results and we found out that the mother's brother has the same modification as her and this man was cytogenetic investigated because his infertility.

Conclusion: Kariotypes of fetus and his parents were performed for correct genetic advices.

P03.025
Cytogenetic effect in vivo and in vitro of the antihypertensive drugs ARBs on human lymphocytes

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Hypertension is the most prevalent, treatable risk factor for diseases of the heart, brain and kidneys. The majority of hypertensive patients need long-term administration of antihypertensive agents and that is why the duration of the pharmacological treatment requires documentation of long-term safety and efficacy, including sensitive indices of genotoxic damage.

Angiotensin II receptor blockers (ARBs) are a widely used class of drugs that are growing in popularity due to the excellent blood pressure control and tolerability. However, recent concerns have surfaced about possible links between ARBs and increased cancer risk.

Chromosomal aberrations (CAs) represent the most extensively used and validated biomarker in populations exposed to genotoxic agents and it is also associated with a higher cancer risk. This study aimed to evaluate the genotoxic potential of five kinds of ARBs (candesartan, valsartan, eprosartan, telmisartan and olmesartan), assessed *in vivo* and *in vitro* for their capacity of inducing CAs on lymphocytes of 55 patients and 10 controls.

Results revealed that total number of CAs as well as the mean frequency of CA/cel and % of aberrant cell were increased for the patients and at the therapeutic doses tested *in vitro* compared to the controls. Chromatid type aberrations were the predominant CA. Most of the chromosomal breakpoints locations coincided with specific *loci* known as fragile sites, which are preferential targets for mutagens and carcinogens.

The results are consistent with our previous studies of beta-blockers genotoxicity and provide evidence for an association between antihypertensive therapy and DNA damage in human lymphocytes.

P03.026

Step by Step, Formation of Complex Chromosomal Rearrangements

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Complex Chromosome Rearrangements (CCR) are defined for the aberrations involving three or more breakpoints on two or more chromosomes. The occurrence is extremely rare. There is no available classification according to the formation mechanism.

We report here a new familial case of CCR involving chromosomes 3, 6 and 10 with 4 breakpoints

Proband, 33 years old male, was referred to our cytogenetic laboratory for chromosome analysis due to the history of repeated miscarriages.

Chromosome analysis revealed a balanced CCR in the proband (Karyotype: 46,XY,t(3;6;10) (3pter->3q12::6q24->6qter;6pter->6q14.2::10p11.2->10pter;3qter->3q12::6q24->6q14.2::10p11.2>10qter). The mother with a history of two alive sibs and four miscarriages, was the carrier of the same CCR. Surprisingly the brother of the proband was a carrier of a simple reciprocal translocation, (46,XY,t(6;10)(q14;p12)).

CCR was confirmed with FISH study by using Chromoprobe Multiprobe System (Cytocell) and centromeric probes of 3, 6, and 10.

We suggest that the CCR in the family most likely have arisen in two consecutive steps; the first step was the translocation t(6;10)(q14;p12) and the second step was the new translocation occurred between the chromosomes 3 and derivative 10.

We concluded that the mother was hidden mosaic for both of the cell lines, simple translocation and complex rearrangement. This family was interesting to understand the formation mechanisms of CCR.

P03.027

3D position of constitutive heterochromatin regions within the nucleus of chorionic villi and embryonic tissues.

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Nuclear architecture and chromatin organisation during interphase are known to play crucial roles in the regulation of gene expression. Constitutive heterochromatin regions (CHR) in chromosomes of embryonic tissues are characterized by tight condensation, late replication and methylation. Meanwhile decondensation, hypomethylation, early replication and DNAse 1 hypersensitivity CHR in human trophoblast cells were registered. These data could be indicated CHR in chromosomes of chorionic villi has unusual functional state.

The aim of study was analysis of 3D position of CHR of chromosome 1 (1q12) in human trophoblast and embryonic cells. It is known that CHRs are associated with nuclear periphery and form chromocenter. Our 3D-FISH results showed a significant repositioning of 1q12 towards the centre of the nucleus and near of chromocenter in chorionic villi sample from early pregnancy (4-5 week). We found no change in the position of 1q12 in human embryonic tissues and chorionic villi from 5-6 week to 36 week pregnancy. Almost all of FISH-signals (correspond to 1q12) were closer to the nuclear periphery and in chromocenter.

In conclusion, today little is known about the nuclear organization in extraembryonic and embryonic tissues. Data of 3-D FISH analysis of this work have indicated in possible functional role of CHR in embryogenesis of human.

P03.030

Frequency of chromosomal aberrations among general human population

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Genetic monitoring is used for surveillance of professionally exposed people to ionizing radiation, chemicals and medical treatments and rarely to investigate the state of general population exposed to environmental mutagens that include all mentioned and life-style factors and habits. We have performed cytogentic analyzes of chromosomal aberrations (CA) in blood lymphocytes among healthy population, including the data of their age, gender and smoking habits (none of them was professionally exposed to ionizing radiation).

Analyzes were done for 200 examinees, 200 cells were counted for each of them for CA. Laboratory used standard method of 48h cultivation of peripheral blood lymphocytes.

We concluded that there is no significant difference in CA frequency among gender ($p > 0,05$); that there is highly significant difference in frequency of acentric fragments among age groups (20-29y; 30-39y; 40-49y; 50-59y and 60-70y)($p<0,001$); and that there is no significant difference in frequency of acentric fragments and ring chromosomes depending of smoking habits ($p>0,05$) but there is significant difference in frequency of minute fragments depending of smoking habits($p<0,05$). These suggest that smoking can cause genetic instability in humans and potential micro mutations that eventually may play a role in cancerogenesis.

P03.032

Submicroscopical duplication of the Wolf-Hirschhorn critical region. Case report.

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Chromosomal rearrangements in the short arm of chromosome 4 can result in two different clinical entities: deletion causes Wolf-Hirschhorn syndrom (WHS) - characterized by severe growth delay, mental retardation, facial dysmorphism and congenital anomalies. Genotype-phenotype correlations of patients with WHS point to a critical locus (4p16.3) to be responsible for the main characteristics of this disorder. In addition it was shown that not only deletions but also duplications of the WHS critical region cause mental retardation and anomalies. The duplication phenotype overlaps partially with the deletion phenotype, but facial dysmorphism is different.

We report clinical and laboratory data of one month old patient. He was born to young unrelated healthy parents, 1G/1P, pedigree unremarkable. Patient was macrosomic, head circumference compared to body was microcephalic, he had dysmorphism- periorbital fullness, large hands and feet, macroglossia, umbilical hernia, developmental delay, but no congenital anomalies. Cytogenetic investigation using Giemsa banding revealed karyotype 46,X,add(Y)(q12)dn. Using HumanCytoSNP-12 array (Illumina Inc) a 6,8 Mb duplication was found in the region 4p16.3-16.1. The result was confirmed with FISH-analysis using WHS Region Probe (Cytocell, UK).

The patient had pathological unbalanced translocation and his final karyotype was 46,X,der(Y)t(Y;4)(q12;p16)dn.ish der(Y)t(Y;4)(Yqter-,WHSCR+).arr 4q16.3q16.1(1-6,806,837)x3.

Conclusion: using different laboratory cytogenetic methods allowed to get a correct diagnosis, the family got appropriate genetic consultation and prenatal invasive diagnostics during following pregnancy.

P03.033

Impact of different chromosomal inversions on infertility

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Objective: One of the frequent occurrences in chromosome rearrangements is inversion of different Chromosomes. An inversion does not usually have phenotypic effect in the majority of inversion heterozygote carriers, when it is a balanced rearrangement. However, infertility, miscarriages and/or chromosomally unbalanced offspring can be observed in carriers of either type of inversions especially per centric inversions.

Material and Methods: We investigated the karyotypes of 13017 infertile individuals being referred to Genetic laboratory of Royan infertility institute between 2005 and 2011, using standard GTG banding. We attend to report the variety of heterozygous par centric and per centric inversions. C- Banding for par centric inversions of the long arm of Y chromosome used for

more precise detection.
Results: A total of 252 cases (1.93%) showed these chromosomal alterations. Twenty one (0.16%) were inv(Y)(p11.2q11.2), fifteen (0.11%) inv(Y)(q11.2q12), one hundred thirty nine (1.06%) inv(9)(p11q12), forty four (0.34%) inv(3)(p11q11.2), nine (0.07%) inv(2)(p11.2q13), and twenty four (0.18%) inversions of other chromosomes.
Conclusion: Human inversion mutations occur at a low but detectable frequency. Although the number of inversions has been considered normal variants such as inv (9)(p11q12), we are trying to discuss the importance of this chromosomal rearrangement and its role on infertility or spontaneously abortions. Carriers of such inversions are at risk of producing abnormal gametes during meiosis that may lead to unbalanced offspring. Our data suggests that it's better for all couples with the same symptoms to have karyotype analysis on a parallel with their routine tests.

P03.034

Mosaic trisomy 1q and Fryns-like phenotype

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Fryns Syndrome is a lethal condition characterized by diaphragmatic hernia, coarse facial features, lung hypoplasia, cardiac defects, and the characteristic distal limb defects. Autosomal recessive inheritance has been suggested on the basis of occurrence in both sexes and recurrence in siblings with healthy parents. Cases with chromosomal abnormality have been reported with clinical findings very similar to Fryns syndrome. Duplication and or deletion of long arm of chromosome 1, and anomalies of chromosomes 15, 6 and 22 have been reported in cases with Fryns-like syndrome.
Herein, we reported a case of midtrimester fetus with multiple congenital anomalies. Prenatal ultra-sound at 21 weeks of gestation demonstrated congenital hernia of diaphragm and hydrocephaly. Pregnancy was terminated and fetus was sent for autopsy, karyotyping and aCGH. Autopsy examination showed microphthalmia of left eye, hydrocephaly, hypoplasia of corpus callosum, left optic nerve hypoplasia, congenital hernia of diaphragm, micrognathia, dysplastic ears, lung hypoplasia (left lung), malformed uterus and right club foot. Chromosomal study showed mosaic 46,XX/47,XX+1(q21-q41). Array comparative genomic hybridization (a-CGH) confirmed mosaic duplication of long arm of chromosome 1q21 to 1q41. Our fetus has many of the clinical features of Fryns syndrome. Our case gives further evidence that 1q duplications are associated with a Fryns-like phenotype including congenital hernia of diaphragm, pulmonary hypoplasia, micrognathia, long philtrum and joint contractures.

P03.035

Mosaic interstitial duplication of the long arm of chromosome 20 associated with vertebral malformations as the only major phenotypic manifestation

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Duplication of the long arm of chromosome 20 as the only chromosomal aberration is rarely described. Better known are reports on patients with a mosaic trisomy 20 or a rearranged chromosome 20 with additional imbalances.
We report on a girl born in the 35th week of gestation to a 31 year old mother. The patient is the second child of healthy parents. Birth weight, length and head circumference were within the normal range according to the gestational week. The girl presented with some mild dysmorphic features, e.g. low set and dysplastic ears, short nose with depressed nasal root, simple philtrum and thin lips. Ultrasounds of heart, abdomen and kidneys were normal while the corpus callosum was shortened and thicker than usually. Multiple vertebrae anomalies, e.g. hemi as well as cleft vertebrae and rip fusion were identified by radiography. Cytogenetic and molecular cytogenetic analysis revealed a mosaic female karyotype with a duplication of part of the long arm of chromosome 20 in 8% of blood cells. The karyotype is described as 46,XX,dup(20)(q11.2q13.3)/46,XX. ish dup(20)(D20S1157+,20QTEL14+,wcp20+). The parents have normal karyotypes.
Hemi and cleft vertebrae as well as rip fusion have been described in cases with duplication 20 associated with other chromosomal imbalances. The present case helps to further establish the correlation of these malformations with duplication of a part of the long arm of chromosome 20.

P03.036

Chromosome kissing in association with the ATR-X syndrome

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ATR-X (X-linked α-thalassemia / mental retardation) syndrome is one of the syndromes associated with abnormal epigenetic gene regulation, which appears males with X-linked mental retardation, HbH disease, skeletal abnormalities, and autistic behavior. ATR-X syndrome is caused by a mutation in the ATRX gene localized on the X chromosome (Xq21.1), which encodes ATRX protein, one of the chromatin-remodeling proteins. However, the details of molecular mechanism with symptoms of this syndrome are still unknown. Here to learn more about the relationships between nuclear architecture and failure of epigenetic regulation in the ATR-X syndrome, we examined characteristics of spatial positioning of following three chromosome arm specific regions by 3D-FISH technique; 1) Xq (ATRX gene has mapped on), 2) 16p (HBA has mapped on), and 3) 11p (HBB has mapped on). After image acquisition by confocal laser scaning microscope, analysis of relative spatial positioning of three painted regions was performed. The results showed that neighborhood association of particular two chromosome territory regions called as chromosome kissing was observed with high frequency between Xq and 16p and between 11p and 16p in cell nuclei from the ATR-X syndrome patients, respectively. The frequency of the same combination from the normal individual is approximately halves of them, respectively. Thus we considered that the spatial arrangement of nuclear architecture has been affected after one has been attacked with the ATR-X syndrome.

P03.037

Use of customised array CGH to investigate the sequence composition around the breakpoints of *de novo* CNVs according to their parental origin

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Among a large series of *de novo* CNVs identified by array CGH, we found the proportion of LCR-mediated imbalances (formed by non-allelic homologous recombination) to be significantly higher among maternally- compared to paternally-derived CNVs. To investigate the contribution of repetitive sequences other than LCRs to the formation of CNVs, we refined the breakpoint intervals (BPIs) of 37 patients with *de novo* non-LCR mediated CNVs (18 maternal and 19 paternal) using an Oxford Gene Technology customised oligonucleotide array and screened the BPIs for the presence of homologous and/or repetitive sequences. Twelve BPIs (in 10 patients) could not be refined further due to the high repetitive sequence content. For the remaining BPI the average size was reduced from 113kb (range 14 - 391kb) to 2.6kb (121bp - 34kb). At least 17/36 maternal and 18/38 paternal breakpoints occurred within the intron of a gene. The majority (76%) of BPI contained at least one repetitive sequence element and for 8/18 maternal CNVs and 5/19 paternal CNVs, the same or similar repetitive sequence element was present at both BPI. However, for those CNVs where both breakpoints were mapped to intervals below 1kb, only 1/9 showed significant homology between the BPI. Therefore, although we have demonstrated the utility of large scale breakpoint mapping using customised array CGH, further work to try and determine the exact breakpoint site by junction fragment cloning will be required to assess the contribution of repetitive sequences other than LCRs to the formation of CNVs.

P03.038

A case of *de novo* complex chromosomal abnormality involving a t(8;10) and an interstitial deletion 5q(q33.1→q34) characterized by GTG banding, FISH and cCGH

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Interstitial deletions of the long arm of chromosome 5 involving the region 5q33.1→q34 are rare occurrences. The clinical features of patients carrying similar deletions include dysmorphic facial features, such as epicanthus, retrognatia, protruding left ear and asymmetric mouth, high-arched palate, four finger lines and clinodactyly of digits II and V on both hands.
We report on a female child aged 13 presenting with development delay, agenesis of the corpus callosum, hallux diverted into, clinodactyly of 3rd, 4th and 5th fingers, obesity, hepatic steatosis, vesicular lithyasis and bilateral macular changes. Classical karyotyping using high resolution GTG banding revealed a *de novo* complex rearrangement including three abnormal chromosomes: 5, 8 and 10; apparently there was an inversion in the long arm

of chromosome 5 and a t(8;10). FISH whole chromosome painting probes confirmed an apparently balanced t(8;10), a deleted chromosome 5 and confirmed the inexistence of any other chromosomal involvement.
To define the deletion breakpoints and the extent of the deletion, cCGH techniques were performed and revealed an interstitial deletion 5(q33.1→q34). The final karyotype was: 46,XX,der(5)inv(5)(q21q33.1)del(5)(q33.1q34) t(8;10)(q13;q21.2)dn. ish cgh del(5)(q33.1q34).
The authors enhance the importance of using high resolution banding combined with molecular cytogenetic techniques for more precise definition of complex chromosomal rearrangements in patients with uncharacteristic phenotypic features and compare the present case findings with previously published data.

P03.039

A de novo complex chromosomal rearrangement involving four chromosomes in an infertile male with oligospermia: Case report

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Purpose: Complex chromosomal rearrangements (CCR) are rare events involving more than two chromosomes and more than two breakpoints. They are usually associated with infertility or sub fertility in male carriers. We examined a 29 year oligosperm man with a history of Varicocelectomy, normal testes size and normal endocrinology profile,who referred for chromosome analysis to genetic laboratory of Royan infertility institute.
Method: Chromosomal analysis was performed from peripheral blood lymphocyte cultures and analyzed by GTG banding. Additional tests such as C-banding and multicolor fluorescence in situ hybridization (FISH) procedure for each of the involved chromosomes were performed to determine the patterns of the segregations. Y chromosome micro deletions in the azoospermia factor (AZF) region were analyzed with multiplex polymerase chain reaction. To identify the history and origin of this CCR, all the family members were analyzed.
Result: The case was a complex chromosomal translocation; 46,XY,t(13;16;14;18) (q31.2;p13.2;q24.2;q21.2). No micro deletion in Y chromosome was detected. Just his monozygous twin brother has the same de novo reciprocal exchanges. The other siblings and parents were normal.
Conclusion: CCR are associated with male infertility as a result of the disruption of spermatogenesis due to complex meiotic configurations and the production of chromosomally abnormal sperm. In other words, it is likely that these chromosomal rearrangements might have influence in decreasing the number of sperms. To have a chance of healthy offspring**,** preimplantation genetic diagnosis (PGD) method is suggested.

P03.040

Complex translocation involving 13, 15 and 16 chromosomes: a case report

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Complex chromosomal rearrangements (CCR) occurring in phenotypically normal persons are rare. CCR are usually considered to include severe reproductive impairment by disturbing the meiotic process and producing unbalanced gametes responsible for high reproductive risk. Most of CCR are reported to be de novo.
We report a case of a complex translocation in a 38 years old female with menopause praecox and sterility. Conventional chromosomal analysis revealed an apparently balanced translocation involving 13, 15 and 16 chromosomes. This balanced complex translocation (BCT) involves three chromosomes and three different breakpoints. Molecular cytogenetic analysis with whole chromosome probes , centromeric and locus specific FISH probes showed breaks at 13q21.2, 15q26 and 16q23. Carriers of balanced complex translocation have a high risk of having spontaneous abortions or a child with an unbalanced karyotype. Our patient was informed by genetic counsellor.

P03.041

High frequency of copy number abnormalities in adult patients with mental disabilities and psychiatric disorders

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Background: Copy number variants (CNV) are associated with a significance increased risk of diseases for the individual and/or their family members. They are contributing to the development of congenital anomalies, intellectual disabilities, spectrum autism disorder and other psychiatric disorders. It is known that the prevalence of psychiatric disorders among adults with intellectual disability is higher than in control population.
Methods: We analyzed a cohort of 100 adult patients affected by mild/moderate intellectual disability associated with psychiatric disorders and minor dysmorphic features. Genetic analysis included array comparative genomic hybridization (aCGH) (Agilent 400K), performed in 45 cases at present.
Results: We detected 89 rare and potentially pathogenic CNVs in 37 cases, with an average of 2,4 CNV/case (1-8). These CNVs include 184 genes (2,07 genes/CNV). At present, we can correlate known CNVs with intellectual disability and/or psychiatric disorders in 13 patients (31,1%). Deletions and duplications found in these cases are: del2p12, del2p16.3, dup3q29, del12p12.1, dup15q11q13, del15q13.1q13.3, dup15q25.2, del15q26.2, dup15qter, dup17q24.1q24.2, del22qter, and dupXq22.1. Genes responsible of psychiatric disorders and some of them also with intellectual disability are: *CTNNA2, NRXN1, PAK2, SOX5, GABRB3, CHRNA7, ADAMTSL3, MCTP2, APOH* and *SHANK3.* Del2p16.3 is present in three patients and is the only recurrent CNV associated with psychiatric disorders. *NRXN1* gene is related with susceptibility to autism, schizophrenia and mental retardation.
Conclusion: We most emphasise the high frequency of rare CNVs associated specially with psychiatric disorder in patients with mild/moderate intellectual disability.
This work was supported by a grant of FIS (PI080778).

P03.042

Detection of cryptic chromosome rearrangements by BAC Genome Array-CGH in five patients, with normal and/or abnormal karyotypes, associated with Mental Retardation, Autism and/or Epilepsy: new insights for genotype/phenotype correlation

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We re-examined ten patients with normal and/or abnormal karyotypes and dysmorphic features, associated with Mental Retardation, Autism and/or Epilepsy. We applied a fast BAC Genome Array- CGH platform (Cytochips Blue-gnome, Techno-genetics - Bouty). Cyto-Chips are high quality BAC microarrays (4898 BAC Clones spotted in quadruplicate 0.6 Mb). This approach led us to discover further cryptic chromosomal rearrangements, previously undetected by conventional cytogenetic procedures. We identified two genes: **SLC8A3** (human gene for member 3 of solute carrier family 8), a sodium-calcium exchanger electively expressed in the brain, and a possible candidate gene for Epilepsy (Nucaro et al 2010) and the **CSMD1** gene (Cub and sushi multiple domains 1) a candidate gene for Autism associated with Mental Retardation (MR) and Epilepsy (Nucaro et al 2011). This approach allows us to better delineate the genotype/phenotype correlation in our patients. Our experience shows the validity of the BAC platform as a reliable method for genome-wide screening of chromosomal aberrations, as well as oligonucleotide-based Array CGH, in patients with idiopathic Mental Retardation and/or in association with Autism and Epilepsy.

P03.043

A de novo interstitial deletion at 1p36.11 in a patient presenting with severe psychomotor delay, sensoneural hearing loss, congenital heart defect and dysmorphic features

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We report on a patient with severe psychomotor delay, sensoneural hearing loss, absent speech, hydrocephaly, congenital heart defect, broad thumbs, dysmorphic features (flat nasal bridge, pointed chin, low-set, abnormal ears) and *de novo* interstitial deletion at 1p36.11 detected by arrayCGH (400K). The deleted segment at 1p36.11 is 1 Mb in size and involves 20 protein coding genes. Based on the clinical and molecular data analysis we suspect *PIGV* gene as one of the strongest candidate genes responsible for severe psychomotor delay, deafness and limb anomalies in our patient. Horn et al. (2011) reported two cases with homozygous and compound heterozygous missense mutations of *PIGV* and intelectual disability, hearing loss, muscular hypotonia and dysmorphic features, which are observed in our patient either. Besides, the main clinical features of our patient are also common for 1p36 monosomy syndrome. The critical region for 1p36 monosomy syndrome is located at 1p36.33 and is 25 Mb proximally from the interstitial deletion at 1p36.11 detected in our patient. We predict that the overlapping phenotype of nonoverlapping deletions within 1p36 region could be caused both by haploinsufficiency of one or more genes because of their deletion and because of the disturbance of their expression by disruption of an essential regulatory element or position effect as the juxtaposition of a euchromatic gene with a region of heterochromatin. The expression studies of candidate genes, especially *PIGV* gene in cases of 1p36 deletions are indicated.

P03.044

A 3.8 Mb deletion in 10q11.21-q11.22 in a 7-year-old Iranian boy with mild dysmorphic features and developmental delay

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Here we report on a 7-year-old Iranian boy with mild dysmorphic features including high nasal bridge, low-set prominent ears. The patient also had developmental delay, mild intellectual disability, increased deep tendon reflex, spasticity and ataxia. Brain MRI performed at ages 5 and 6 showed fluid collections in temporal lobe beside of sylvian fissure compatible with arachnoid cyst. Whole genome BAC Array CGH was performed using CYTOCHIP genomic BAC array and showed a 3.8 Mb interstitial deletion on long arm of chromosome 10 encompassing bands 10q11.21-q11.22. Thirteen OMIM genes are situated in this region. To date, ten patients with deletion of 10q11.2 documented with routine karyotyping, and an additional 19 patients with deletion of 10q11, overlapping 10q11.21-q11.22 region, confirmed by array comparative genomic hybridization (aCHG) have been reported. Intellectual disability and developmental delay were the only clinical features common to all cases. Ataxia and increased deep tendon reflex, additional findings in our patient, were reported in only a few of the aCGH confirmed patients. Our case is of interest in that it is the first case of pure deletion of 10q11.21-q11.22 region.

P03.045

Difficulties in the molecular diagnosis of a patient with features of 1p36 deletion

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Deletion 1p36 is one of the most common subtelomeric aberration with an estimated incidence of 1 in 5 000 to 1 in 10 000 live births. It is characterized by severe developmental delay, microcephaly with distinct facial phenotype which is often accompanied by internal organs malformations and seizures.

We present a case of 8-year-old boy with multiple congenital anomalies/ mental retardation in whom neither classical G-binding karyotype nor FISH for 1p36 deletion and subtelomeric MLPA test revealed any aberration. He presents with aggressive behavior, craniofacial features consisting of microbrachycephaly, straight eyebrows, deep-set eyes, epicanthal folds, midface hypoplasia, broad and flat nasal bridge, long philtrum, pointed chin, low-set, abnormal ears. Besides, absence of the septum pellucidum, Ebstein anomaly and club foot were noted.

Despite the normal results of FISH and MLPA subtelomeric screening and because of either facial dysmorphism predictive for deletion 1p36 (midfacial hypoplasia and horizontal eyebrows) or heart defect (Ebstein anomaly) further molecular analysis - arrayCGH - was performed. This only allowed confirm the clinical diagnosis and let to recognition of deletion. Interestingly, the Agilent SurePrint G3 Human CGH and SNP Microarray 4x180K revealed 2 aberrations: within 1p36.22-p36.32 (including 8.43 Mb, 101 genes) and 1p36.12 (of 0.44 Mb, 12 genes).

In this presentation we like to underline the importance of detailed analysis of clinical features which may lead to the diagnosis, even with the correct result of screening studies.

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P03.046

Pure 1q43q44 deletion characterized by array CGH - a case report

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Deletions involving chromosome 1q43q44 generate a recognizable phenotype, including psychomotor retardation, characteristic dysmorphic features, microcephaly, hypoplasia or agenesis of the corpus callosum and various other anomalies.

We present the clinical and molecular findings of a 3 year-old girl, referred for genetic investigations due to phenotypic features suggestive of 1q43q44 deletion.

Classical and molecular cytogenetic investigations were performed on peripheral blood lymphocytes by GTG-banded karyotyping, FISH and array CGH. Karyotype analysis was performed for both parents.

Cytogenetic analysis showed a de novo terminal deletion of chromosome 1, 46,XX,del(1)(1q43q44). Array CGH (105K, Agilent) was performed for accurate characterization of 1q deletion and for exclusion of other imbalances at 1q or in other genomic regions. A 10,98 Mb deletion of chromosome 1, spanning from 238,224,320-249,212,668 (hg19), was the only aberration detected. The results were confirmed by FISH.

Our case add data to the current body of knowledge regarding the pure deletion of 1q43q44; detailed molecular characterization allows for better genotype-phenotype correlations and contributes to the further delineation of the phenotypic spectrum in this syndrome.

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P03.047

A de novo interstitial deletion of 20q11.21q11.23 in a boy with prenatal diagnosed megacystis and cystic renal dysplasia, analatresia, ventricular septal defect (VSD), dysmorphic features and abnormal feet

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Background: Interstitial deletions involving 20q11 are very rare. Only 3 cases with similar clinical features have been reported (Hiraki Y et al, Am J Med Genet 2011,155A(2):409-14).

Case report: We describe a boy with de novo 20q11.21q11.23 deletion. Molecular characterization of del(20)(q11.21q11.23) by high-resolution SNP microarray showed an approximately 5,7 Mbp deletion. A megacystis was diagnosed in early pregnancy which led to hydronephrosis IV and oligo/anhydramnios. Postnatal the boy showed multiple congenital anomalies such as analatresia, a ventricular septal defect, hypoplastic aortic arch, hyospadia, deformity of the thorax, hipluxation on both sides, talipes calcaneus and flatfeet as well as dysmorphic facial features including microcephalia, retro-/micrognathia, deep set ears, broad nasal bridge. Furthermore feeding difficultiers were reported. Due to the renal disorder the boy died because of renal failure a few weeks after birth.

Discussion: Compared to the other three cases with deletion 20q11-q12 there is a similarity between our patient and the patient described by Hiraki Y et al. Because of our patient´s early death we will not know if there would have been a mental retardation or behavior abnormality. The 5,7 Mbp deleted segment identified in our patient encompasses at least 81 genes and 9 miRNAs.

Our case adds new information to characterize the phenotype of patients with rare proximal microdeletion (20q).

P03.048

A 25 Mb Deletion of Chromosome 4q13.2 -q22.3 in a Male Fetus with severe skeletal abnormalities

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We report on a 20-week-male fetus with interstitial deletion of the long arm of chromosome 4q13.2-q22.3. Ultra-sound examination at 19 weeks revealed multiple congenital anomalies and therapeutic abortion was performed at 20 gestational weeks. Fetus was sent for autopsy examination and aCGH study. Autopsy examination revealed micromelia of upper and lower limbs, long philtrum, micrognathia, small low-set ears, hypertelorism, abnormal segmentation of right lung, adrenal agenesis. Radiology showed triangular shaped hypoplastic ossification representing the humerus. There was absent ossification of the radius and ulna with marked micromelia of all those 3 bones. In the lower extremities, a thin single ossification of the femur with absence of a severely hypoplastic tibial anlage was seen. The pelvis was tiny, high, narrow ileum and only ischial ossification was present. The skull showed a posterior ossification defect. The thorax showed elongated clavicles and 12 ribs. Array comparative genomic hybridization was performed, revealing a 25 Mb deletion of chromosome 4q13.2-q22.3. Even though skeletal abnormality and limb shortening has been previously reported in 4q deletion cases, none of them have such severe skeletal anomalies as seen in our case.

P03.049

Clinical consequences of 8pter deletion - new case of a girl with subtle facial dysmorfism and moderate mental retardation

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Microscopically visible 8pter deletions are associated with growth and mental impairment, minor facial anomalies, congenital heart defect and behavioral problems. Submicroscopic subtelomeric 8p deletion is fairly uncommon. The patients either with microcephaly, normal facial appearance, mild mental retardation or clinical phenotypes of autism were described. One could speculate that there is strong correlation between the clinical phenotypes and the size of 8pter deletion.

We present a case of pure 8pter deletion found in a 17-year-old girl with microcephaly, minor dysmorphic features, developmental delay, moderate mental retardation, dyslalia and the features of depression, born as 5th child of healthy parents. In her family history there were no cases of neither miscarriages nor mental retardation. Cytogenetic classic study revealed no visible aberrations, but no high resolution analysis was performed. The diagnosis of deletion of 8pter region was establish by MLPA, confirmed by FISH and delineated by array-CGH. Our patient has the terminal deletion of the 8p with the proximal breakpoint at 10075000 bp in band p23.1. The deleted region has the size of ~ 10,1 Mb and contains 154 genes possibly involved in the phenotype of our patient. Examination of the mother did not show this deletion, the father was unavailable for this study. Our report shows that the clinical phenotype of 8p deletion is determined not only by the size and location of the deleted region but also by other factors.

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P03.050

Multiple Genomic Rearrangements in a consanguineous family

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Introduction

The birth prevalence of congenital disorders in children of first-cousin parents is about double of the general population (4-5%). Some cases can be explained by the cumulative inheritance of susceptibility genetic variation, including unbalanced genomic rearrangements.

Clinical Report

The proband, a 2 year-old boy, born to consanguineous parents (r=1/8), was referred to the medical genetics clinic due to global psychomotor development delay, unspecific facial dysmorphisms, oesophageal atresia and failure to thrive. Since there was a family history of learning difficulties, the parents and the siblings were also studied. The etiologic investigation revealed a heterozygotic 19q13deletion on subtelomeric MLPA analysis in the proband, in both affected parents and in the two healthy sisters, and a 19q13nullisomy in the older brother. As this was the more severely affected member an arrayCGH was performed which revealed a 1p33tetrasomy and a 3p21.31duplication. The parents' arrayCGH identified a 1p33duplication in both parents and a 3p21.31duplication in the mother.

Proper cognitive assessments were performed to all members and array-CGH analysis is being carried out to the proband and his sisters.

Discussion/Conclusion

Co-inheritance of genomic abnormalities may explain the developmental disorders and cognitive deficits in this family. The affected genomic regions include putative relevant genes, such as *CHMP2A,* which participates in cell cycle progression regulation, *NSUN4*, which interferes with DNA methylation and *TREX1* that interferes in cell apoptosis. It is likely that the cumulative effect of aberrations contributes to more pronounced clinical phenotypes. Genotype-phenotype relation is under investigation and will be discussed in the presentation.

P03.051

ArrayCGH characterization of a deletion on 2q13 associated with developmental delay and facial dysmorphism (case report)

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Genomic imbalances play a major role in the pathogenesis of many human genetic diseases. The modern genome-wide analysis tools (such as array-CGH) have led to the discovery of novel copy-number variations (CNVs); many of them are known to be associated with diseases. Recently, CNVs at 2q13 have been described in literature and the DECIPHER database as increasing risk for developmental delay and cranial facial dysmorphism.

We present a 5-year-old girl, who was born with microcephaly, navel cord hernia, and mild dysmorphic features (deep-set short nose, small chin, low hairline, and low-set ears). A neuropsychological examination revealed mild psychomotor retardation and speech delay. The proband suffers from frequent febrile convulsion, dental caries and frequent respiration infections.

G-banded karyotyping at 550 bands resolution defined the karyotype of the proband as normal.

An approximately 1.79 Mb deletion in chromosome band 2q13 (chr2:111415137-113194067 bp, hg19) was identified by arrayCGH and verified by real-time quantitative PCR (qPCR). The patient's karyotype was therefore revised as 46,XX.arr 2q13(111,415,137-113,194,067)x1 mat. The deletion on 2q13 was also detected in the maternal grandmother using a specially designed FISH probe.

Inherited chromosomal aberrations from parents in whom the variant was insufficient to cause such disease might be still causative for a pathological phenotype in the next generation. The mother and the grandmother had normal phenotype (except a mother's speech delay in childhood). Distinguishing pathogenic and benign CNVs is still challenging in the clinical practice.

P03.052

Trisomy 8p11.23 as a result of a dicentric chromosome 8

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Here we present on a case with de novo dic(8;8)(p11.2;q11). An eight years old boy was referred for genetic studies. He had microcephaly, autism, developmental delay, mental retardation, a dysmorphic face, joint problems and chest deformation. GTG banding analyses of the index patient and his parents were done according to standard protocols. The parents karyotypes were normal. To characterise the aberrant chromosome 8 found in the patient fluorescence in situ hybridization (FISH) was performed using centromeric probe for chromosome 8 and subcentromeric probes set (partial chromosome paint probes for 8p and 8q, BAC probes RP11-503A24 located on 8p11.21, RP13-116A4 located on 8q11.21 and centromeric probe). The dicentric chromosome 8 with duplication of a small region 8p11.23 was discovered: dic(8)(pter->q11::p11.23->qter).

Partial trisomy 8p is a relatively frequent anomaly as this can be result of an inversion-duplication or be found in the offspring of balanced translocation carriers; different breakpoints related to 8p have been reported, even though genes from the olfactory receptor gene family are involved more frequently. Still, there are controversial information about clinical features of these patient. In the sSMC database (http://www.fish.uniklinikum-jena.de/sSMC.html) cases with duplication of the same region with and without clinical findings are described. Our report provides one more case with features which shares some common clinical descriptions of partial trisomy 8p syndrome. Supported in parts be DFG (LI 820/38-1) and the Else Kröner-Fresenius-Stiftung (2011_A42).

P03.053

Atypical chromosomal rearrangements in Down syndrome - clinical and cytogenetic study

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Down syndrome (DS) is one of the most common genetic disorder, and is caused by complete or partial 21 chromosome trisomy. The incidence of DS has a wide geographic variation, and is related directly to prenatal medical care assistance (country economic status). A variety of chromosomal abnormalities can be associated with this disorder.

In the last 10 years in the Cytogenetic laboratory of University of Medicine and Pharmacy „Grigore T. Popa" Iasi were performed 2491 constitutional karyotypes with G banding and were confirmed cytogenetically 571 cases with DS. From the number of diagnosed patients we discriminate: (i) 510 cases (89.00%) with homogenous trisomy of chromosome 21 (from these five cases also present chromosome 9 inversion); (ii) 31 cases (5.41%) with chromosome 21 trisomy in mosaic without translocations; (iii) 25 cases (4.36%) with unbalanced Robertsonian translocations between the following groups of chromosomes: 14 and 21 (14 cases), 21 and 21 (6 cases), 13 and 21 (3 cases), 15 and 21 (one case), 22 and 21 (one case); (iv) 5 cases (0.87%) with different abnormalities other than chromosomal 21 trisomy: one case of chromosome 21 inverted duplication, one case of chromosome 21 dicentricity, one case of chromosome 21 insertion into chromosome 18, and two cases involving one or more translocations of chromosomes other than chromosome 21.

We focus our study on the latter 5 cases that present atypical cytogenetic DS, in order to illustrate some rare variants of chromosomal abnormalities and cytogenetic particularities. Each individual case is compared to literature data.

P03.054

Mosaic interstitial duplication 14q

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Interstitial duplications in the long arm of chromosome 14 are rarely encountered. We report a male patient who was born with multiple congenital anomalies. The patient had microcephaly with structural brain abnormalities on MRI, polycystic kidneys, a VSD and an ASD. Newborn hearing screening showed mild-moderate hearing loss. He had low-set, posteriorly rotated dysplastic ears and a cleft of the soft palate. Furthermore a sacral dimple was present, a small penis and overlapping toes of the left foot with of proximal placement of the 5th toe.

Conventional karyotyping demonstrated a mosaic (95%) interstitial duplication in the long arm of chromosome 14. SNP array analysis was performed to characterize the duplication in more detail. Both parents had a normal karyotype. Detailed molecular characterizations and reports of chromosomal imbalances in combination with clinical phenotypes are important for accurate genotype-phenotype correlations in genetic counseling.

P03.055

A new case with de novo proximal duplication of 10q11.2q21.3

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Here we report on a case with de novo duplication of proximal 10q11.2 to 10q21.3. An eleven months old boy with developmental delay was referred for genetic studies. The patient was the first child of a healthy, non-consanguineous parent. He presented with microcephaly, deep set eyes, arched palate, hypotelorism, strabismus, down slanted corners of the mouth, hypotrophy and motor development delay. Cardiac problems included coarctation of aorta and open arterial duct. His right hand showed an absence of the middle metacarpal bones.

The patient karyotype was 46,XY,dup(10)?(q24.3-qter)dn. To characterize the der(10) fluorescence in situ hybridization (FISH) was applied using whole chromosome painting, multicolor banding (MCB) and subcentromere specific probe sets for chromosome 10. Finally, the aberration was described as dup(10)(q11.2q21.3)dn. To the best of our knowledge only seventeen cases with proximal duplication 10q11-q22 were reported, yet. All of them had common dysmorphic features, cardiac or renal defects. Our patient shares some of these features but others as kidney defects, muscle hypo- or hypertonia, feedings difficulties are absent. In conclusion, only molecular cytogenetics allows us to precisely describe aberrant chromosomes; here we report a new case with proximal duplication 10q11 to q21.3 without involving chromosomal band q22 as in other previously found cases. Supported in parts be DFG (LI 820/38-1).

P03.056

Severe phenotype in a child with inverted duplication 10q24.1>q26.3: array-based evidence for the inverted low copy repeat model

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Background. Duplication of the distal long arm of chromosome 10 causes a clinically recognizable dysmorphic syndrome. Most patients are severely mentally retarded. Congenital malformations including microphthalmia and heart defects are suggested to occur only in larger duplications including q24. Generally the aneuploidy is caused by a translocation inherited in an unbalanced fashion. Here we report on microarray analysis of a large de novo inverted 10q duplication & subtelomer deletion.

Case report. The female patient was term-born, small for gestational age, referred because of growth and psychomotor delay, microcephaly, severely handicapped vision due to right-sided microphthalmia, coloboma of the iris and choroidea, small palpebral fissures and bilateral ptosis, ventricular and atrial septum defect.

Lab results. GTG studies with a lymphocyte culture showed an extended long arm of chromosome 10. FISH using wcp 10 and subtelomeric probe 10q revealed the extra material to be exclusively derived from chromosome 10 and lack of the subtelomeric region 10q, respectively. Microarray investigation affirmed the 10q24.1>q26.3 duplication and adjacent subtelomeric deletion including a 6kb single copy stretch within the duplicated segment.

Conclusion. The clinical severity of chromosome 10q duplication seems to depend particularily on the involvement of 10q24 suggesting a dosis effect in the respective genes. The subtelomeric deletion most likely does not influence the phenotype. This report adds to the still few patients with 10q duplication delineated by array methods and the increasing understanding of genotype-phenotype correlations. Also, our results provide molecular evidence for the inverted low copy repeat model postulated by Bonaglia (2000).

P03.057

A de novo interstitial duplication 14q32.11-q32.32

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The 2- year- old patient presented with minor clinical features, as epicanthus, microcephaly, full lips and developmental delay. The child was born at 39 weeks of gestation with a birth weight of 3200g and length 50cm after the pregnancy with polyhydramnios.

Additional material in the distal part of chromosome 14q was identified by GTG-banding. The parents karyotypes were normal. Standard fluorescence in situ hybridization (FISH) was performed using whole chromosome painting, multicolour banding (MCB), subtelomeric and different bacterial artificial chromosome (BAC) probes for chromosome 14. FISH analysis showed duplication of distal part of chromosome 14. Subtelomeric probe 14q and BAC probes in 14q24.3 (73.0-73.2Mb) , 14q32.33 (105.7-105.8 Mb), 14q32.33 (106.0-106.1 Mb), and 14q32.33 (106.1-106.2 Mb) showed no duplication, while the probes RP11-1A3 (14q32.11; 89.4-89.5 Mb) and RP11-65M20 (14q32.11, 91.8-92.0 Mb) demonstrated two signals, each on the der(14). Finally, based on GTG-banding and FISH results the derivative was described as dup(14)(q32.11q32.32)dn. Only few cases with "pure" duplication of this distal region of chromosome 14q were described (Decipher). In most of them mental retardation, developmental delay, microcephaly were described. Here we report on a new case with de novo duplication of 14q32.11q32.32 (located possibly between 89.4-92.0 Mb). Supported in parts be DFG (LI 820/38-1).

P03.058

De novo 2p21-p16.3 duplication in a patient with severe growth retardation and significant delayed bone age.

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Herein, we report on the phenotype of a 12-year-old girl with partial trisomy 2p. She is the second child of healthy non-consanguineous parents of Belgian origin. She was born at term after a pregnancy complicated by intrauterine growth restriction and oligohydramnios. The growth retardation persisted postnatally. In addition, she presented diaphragmatic hernia, moderate psychomotor development, feeding difficulties, and recurrent respiratory infections. She had prominent forehead, relative macrocephaly, marked hypertelorism, thin upper lip and delayed closure of the anterior fontanelle. The veins were visible on trunk and extremities. She had lumbar hyperlordosis. At the age of 12, her growth parameters were: height 114.0cm (-5.6 SD), weight 18.5kg (-5.8 SD), and OFC 49.4cm (-2.2 SD) (father's height: 156.5cm (-3.6 SD), mother's height: 154cm (-2.1 SD)). According to Greulich and Pyle, bone age was 6 years and 10 months at the age of 10 years and 6 months. The IGF-1 levels were relatively low (64 ng/ml; normal: 78-405 ng/ml) and she had been treated by growth hormone therapy for 19 months without any clear benefits on growth.

Genetic workup identified a *de novo* tandem dup(2)(p21~22p16) by high-resolution conventional karyotyping, followed by FISH. SNP-array revealed a 9.6 Mb interstitial duplication, arr 2p21p16.3(42,295,716-51,981,249)x3, encompassing 45 genes.

To our knowledge, this is the first case of 2p21-p16.3 duplication. Pure 2p trisomy, encompassing this region, is very rare, and was mostly identified by standard karyotyping. Molecular characterization of additional patients will allow a better determination of the breakpoints and the genes involved in the phenotype.

P03.059

A de Novo 5 Mb duplication of Xp11.23-p11.3 with non-random X inactivation: Clinical report of female twins and molecular cytogenetic characterization

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Introduction:

Partial duplications of the short arm of the X chromosome (Xp) in males result in intellectual disability, facial dysmorphism and variable malformations. In females, a normal phenotype should be expected if the abnormal X chromosome is preferentially inactivated. Recently, duplications of Xp encompassing band p11.2 were found in females with developmental delay and other anomalies. Unexpectedly, most of them showed preferential activation of the duplicated X chromosome.

Clinical Report and Genetic Findings:

We describe female twins of twentyfive months of age with developmental delay, seizures, EEG and MRT abnormalities, and facial dysmorphism. Both children were sociable and smiled frequently. The first child had a small atrial septum defect, the second had a patent foramen ovale and a dilated renal pelvis. SNP-Array analysis in one child and both parents showed a *de novo* ~5 Mb duplication of Xp11.23-p11.3 in the child. Subsequent FISH analysis confirmed this duplication in both children. The twins showed selective inactivation of the normal X chromosome in more than 80% of blood cells.

Discussion:

The described twins have phenotypic features in common with other males and females carrying a partially overlapping Xp duplication. These individuals have developmental delay, seizures, EEG abnormalies, similar behavioral phenotypes and facial dysmorphism. The described twins as well as almost all reported affected females show selective inactivation of the normal X chromosome, suggesting that increased expression of a gene within the common duplicated region leads to skewed X-inactivation. Functional disomy for genes within the duplication results in the described phenotype.

P03.060

Latest innovations in oligo FISH enable high resolution detection

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The discovery of complex structural variations that exist within individual genomes has prompted a need to visualize chromosomes at a higher resolution than previously possible. In response to the need for such high resolution visualization, we have developed a new generation of fluorescent *in situ* hybridization probes targeting specific regions of the genome. These new probes, Agilent SureFISH probes, are designed using an *in silico* design strategy that specifically avoids placing oligonucleotides in repetitive regions of the genome, allowing for highly specific detection of the region of interest. Each SureFISH probe is designed to a specific region of the genome and is generated from complex libraries containing hundreds to thousands of unique high quality long oligonucleotides. The resulting probes provide high specificity and enable users to detect aberrations in targeted regions of the genome as well as aberrations near highly repetitive elements. Because Agilent SureFISH probes are designed for specific, non-repetitive, regions of the genome, they provide superior resolution as compared to other available technologies.

P03.061

How to narrow down chromosomal breakpoints in small and large derivative chromosomes - a new probe set

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Here a new fluorescence in situ hybridization (FISH-) based probe set is presented and its possible applications are highlighted in thirty-four exemplary clinical cases. The so-called pericentric-ladder-FISH (PCL-FISH) probe set enables a characterization of chromosomal breakpoints especially in small supernumerary marker chromosomes (sSMC), but can also be applied in large inborn or acquired derivative chromosomes. PCL-FISH was established as 24 different chromosome-specific probe sets and can be used in two- up multicolor-FISH approaches. PCL-FISH enables the determination of a chromosomal breakpoint with a resolution between 1 and ~10 megabasepairs and is based on locus-specific bacterial artificial chromosome (BAC) probes. Thus, PCL-FISH leads to a better resolution than most FISH-banding approaches. To approve the result eight of the in 29 sSMC cases were studied by array-CGH; the used sSMC-specific DNA was obtained by glass-needle based microdissection and DOP-PCR-amplification. Results obtained on 29 sSMC cases and 5 larger derivative chromosomes are presented and discussed. Finally, application perspectives of the probe set in tumor as well as in evolutionary cytogenetic studies are given. Supported in parts by Else Kröner-Fresenius-Stiftung (2011_A42), the Deutscher Akademischer Austauschdienst (DAAD), the Monika-Kutzner-Stiftung and the Stefan-Morsch-Stiftung.

P03.062

Genotype phenotype correlation in 15 patients with deletion of chromosome 1q24-q25

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Patients with deletions of 1q24q25 share common features of growth deficiency, microcephaly, small hands and feet, dysmorphic face and severe cognitive deficits. We report fifteen patients with 1q24q25 deletions, who show distinctive features of a clinically recognizable 1q24q25 microdeletion syndrome: prenatal and postnatal growth deficiency, microcephaly, mental retardation, small hands and feet with distinctive brachydactyly and fifth finger clinodactyly, single transverse palmar flexion creases and a distinctive dysmorphy (hypertelorism, small and low set ears, short nose with bulbous nasal tip and micrognathia). Radiographs demonstrate disharmonic osseous maturation with markedly delayed bone age. Occasional features include cleft lip and/or palate, genital, cardiac and renal abnormalities.

Using array comparative genomic hybridization, we defined the critical deletion region as 717kb at 1q25.1 (chr1 :173,710,633-174,427,602, hg19 coordinates), containing 7 genes and including CENPL (Centromere protein L).

CENPL encodes centromeric protein L, a protein essential for proper kinetochore function and mitotic progression. The growth deficiency in this syndrome is similar to what is seen in other types of primordial short stature with microcephaly, such as Majewski osteodysplastic primordial dwarfism, type II (MOPD2) and Seckel syndrome, which result from loss-of-function mutations in genes coding for centrosomal proteins. Therefore, CENPL is a candidate for microcephaly and for growth deficiency in 1q24q25 microdeletion syndrome.

P03.063

A new multicolor-fluorescence in situ hybridization probe set directed against human heterochromatin: HCM-FISH

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Here a new multicolor fluorescence in situ hybridization (mFISH-) probe set is presented and its possible applications are highlighted in fifteen clinical cases. The so-called heterochromatin-M-FISH (HCM-FISH) probe set enables a one-step characterization of the large heterochromatic regions within the human genome. HCM-FISH closes a gap in the up to now available mFISH probe sets as those do normally not cover the acrocentric short arms, the large pericentric regions of chromosomes 1, 9 and 16, as well as, the band Yq12. Still, these regions can be involved in different kinds of chromosomal rearrangements like translocations, insertions, inversions, amplifications and marker chromosome formation. Here examples are given for all these kinds of chromosomal aberrations, detected as constitutional rearrangements in clinical cases. Application perspectives of the probe set in tumor as well as in evolutionary cytogenetic studies are given. Supported in parts by BMBF/DLR (BLR 08/004 and BRA 09/020), DFG (LI 820/19-1, LI 820/32-1), Else Kröner-Fresenius-Stiftung (2011_A42) and Dr. Robert Pfleger Stiftung.

P03.064

Hyperspectral Imaging of Chromosomes: a New Approach for Label Free Karyotyping

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Staining techniques are routinely used to identify metaphase chromosomes based on their unique banding pattern. Advanced molecular cytogenetic techniques like Fluorescence-In-Situ-Hybridization (FISH) provide a more sensitive tool for complex and small structural aberrations. In both cases a broad expert knowledge is necessary to understand and diagnose diseases.
We have developed a new technique for fast and label free karyotyping using a Hyperspectral Imaging System (HSI) which can easily be integrated into a standard light microscope. With this system we measure the stray light interference pattern of an unstained chromosome or substructures of the chromosome with a diode array spectrometer. The complex spectra can be interpreted as spectra of "nanostructured particle arrays" of different size and refractive indexes. The signature of each spectrum is due to the superposition of the interference pattern of the different layer thicknesses, the spectral interference of the band pattern and changes in refractive indexes along the chromosome axis. The hyperspectral data can be analyzed using multivariate data analysis and the chromosomes can be classified by their individual spectral features.
The results are confirmed with model particle array measurements. These measurements show strong correlation with calculated Mie interference spectra. Furthermore, FDTD (Finite Difference Time Domain) simulations of model chromosomes confirm the photon diffusion pattern as well. Substructures of chromosomes can also be analyzed by Near Field Spectroscopy (imaging beyond diffraction limit) measuring the stray light pattern of sub structures of chromosomes as small as 50 nanometers.

P03.065

Origin of genomic imbalances detected by array-CGH in children with intellectual disability.

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We present the data from the genetic screening of 14 children with intellectual disability (ID), dysmorphic features and congenital malformations. 28 parents were also included to this study. The diagnostic algorithm included classical cytogenetic analysis (CC), performed according to the standard procedures, array-CGH performed with Agilent SurePrint 3G Human CGH Microarray Kit (4x180K platform) and subtelomeric FISH experiments performed with Vysis ToTel subtelomeric probes.
All children presented normal karyotype. Subtelomeric FISH showed normal hybridization pattern in all children. In 14 children 16 different imbalances were found, of which 8 were duplications and 8 deletions. All parents were analysed with CC and a-CGH, which helped to establish the origin of aberrations found in children. 8 aberrations were de novo, 3 of maternal and 3 of paternal origin. In one patient presence of 2 aberrations was the result of paternal balanced translocation.
The use of array-CGH technique helped in direct genotype-phenotype correlation in our patients. In only 4 de novo cases the size and gene content of aberration strongly correlated with patients phenotype. In 3 de novo cases the conclusion of pathogenic influence of the imbalance still need more evidence. In familial cases it was not possible to exclude the potential pathogenic role of inherited changes - further molecular analysis is required.

P03.066

Molecular cytogenetic analysis of a new case of inv dup chromosome 15 syndrome .

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The chromosome region 15q11q13 is known for its instability, and many rearrangements may occur in this imprinted segment. Supernumerary marker chromosomes formed by the inverted duplication of proximal chromosome 15q region was identified as one of the must frequent human marker chromosomes. We report here, the clinic finding and cytogenetic analysis of a girl with mental retardation. The child has a clinic who should suspect a chromosomic aberration because she has a developmental delay, hypotonia and dysmorphic features. Standard cytogenetic techniques and FISH analysis using painting and locus specific probes were performed to identify and characterize the chromosome anomaly. A supernumerary marker chromosomes formed by the inverted duplication of proximal chromosome 15 was identified. FISH using chromosome 15 specific centromeric probes (Spectrum Green,CEP 15q11.2,D15Z1) and SNRPN, UBE3A and PML genes confirm the duplication of 15q11-13 region in the dicentric marker chromosome. The critical region involved in duplications is a gene-rich region that comprises the three γ-amino butyric acid (GABA)-A receptor subunit genes (GABRB3, GABRA5, and GABRG3). These subunit genes may contribute to the clinical picture. In fact, even if it is still unclear how the GABAergic neuromodulators influence the developing brain, alterations of this system may cause both epilepsy and behavior problems to occur. Therefore, unexpected prenatal diagnosis, for maternal age or serum screening, of such a chromosomal abnormality would certainly lead to difficulties in genetic counselling and prognosis.

P03.067

Low level mosaicism of chromosomal aneuploidies in intellectual disability

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It has been shown that oligonucleotide array comparative genomic hybridization (array-CGH) allows the identification of mosaic aberrations. The detection sensitivity was reported to correlate with aberration size. We used a high resolution array to molecular karyotype 73 patients with intellectual disability. In 4/73 (5.5%) patients, mosaicism was found. The first one for chromosome 18 of 21 Mb, with a copy number variant of 1.7, indicating a deletion mosaicism. The second and the third one for chromosomes X and 3, of 18 Mb and 19 Mb, respectively. The copy number level was for both 2.3, pointing to a trisomy mosaicism. In the last case, a trisomy 14 in mosaic form was detected, with a copy number level of 2.4. Importantly, this finding matched, perfectly the phenotype, which reflected the mosaicism on the skin. Despite the size of this aneuplody, it had gone unrecognized with classical karyotyping due to the low mosaic rate and possibly due to selection that might occur during culturing the lymphocytes. For the first three cases, we performed a fluorescence-in-situ-hybridization (FISH) and we confirm the mosaic aneuploidy. The abnormality was detected in 4/26 (15%), 1/31 (3%) and 6/41(15%) metaphases, respectively.
Our findings demonstrate the power of molecular karyotyping using high resolution arrays to detect low level mosaicism, which would go undetected using classical karyotyping. Moreover, they raise the question whether low level mosaicism of certain chromosomal regions are indeed the cause of some cases of intellectual disability and warrant further investigation.

P03.068

Evaluation of the SNP 6.0 Array for molecular karyotyping in patients with intellectual disability

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In the last years molecular karyotyping has evolved into a common technique for diagnostic testing in patients with intellectual disability (ID). It has been shown that high resolution array systems uncover apparently pathogenic copy number variations (CNVs) in up to 15 % of patients with ID and normal conventional karyotype.
In this study we analyzed 79 patients with intellectual disability with the Affymetrix 6.0 SNP array and scored CNVs with a minimum size of 100 kb and 5 markers. We detected a total of 855 aberrations with a mean of 10.82 CNVs per patient. A group of 820 molecularly karyotyped controls and the Database of Genomic Variants were used to rule out common genomic variants and artefacts, resulting in 56 remaining rare individual CNVs in 40 patients with sizes from 114 kb to 11.350 Mb. The first 23 of these CNVs, including the smallest one, were systematically validated by an independent method (QPCR, MLPA, FISH) and could all be confirmed. Segregation analy-

sis of 54 CNVs in 38 patients was performed in the respective families (13 de novo, 20 maternal, 21 paternal). In case of parental inherited CNVs the phenotype of the parents and the affected region/gene content were re-evaluated. In summary, 20 CNVs in 18 affected individuals were assumed to be potentially pathogenic, varying from large de novo aberrations and frequent recurrent microaberration syndromes to single gene defects (NRXN1, CDKL5). In conclusion, we identified potentially disease causing CNVs in 22.8 % of affected individuals, exceeding the prognosticated rate.

P03.069

A 7 Mb microdeletion in chromosome 2 band p13.3p15 associated with devolopmental delay, heart abnormalities and facial dysmorphisms

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We report on a patient with a de novo 7 Mb deletion in the short arm of chromosome 2, from band 13.3 to 15. The proband, a girl, presented congenital heart defects (atrial septal defect and muscular ventricular septal defect), facial dysmorphisms, poor growth, intellectual disability, interstitial lung disease and severe gastroesophageal reflux.

The rearrangement encompassed 47 genes, 2 of which are known disease gene (C2ORF86 and ANTXR1).

Three cases with partially overlapping deletions were reported in literature and database. Wohlleber and co-workers (2011) described two patients with a de novo 2p14p15 deletions. One patient with a de novo 2p14 deletion was reported in ECARUCA database.

Our patient presented more severe clinical features and a deletion larger in size than patients reported to date.

We suggest that the complex phenotype of our patient is caused by the haploinsufficiency of several genes in the region or by different molecular mechanisms described in chromosomal rearrangements, such as impaired expression patterns.

P03.070

A new intersticial deletion of the long arm of chromosome 4 (q13.2:q13.3) in a girl with growth hormone deficiency

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Intersticial deletions of chromosome 4 long arm are relatively rare, with less than 70 cases reported. The majority of cases are terminal 4q deletions with loss of the bands q31-q33, resulting in a characteristic phenotype. In contrast, deletions within the proximal long arm of chromosome 4 have rarely been reported. There is a wide clinical presentation among patients who share similar breakpoints. We report one patient with proximal intersticial deletion of chromosome 4q, defined at the molecular level by array-CGH. The proband had a 7.7 Mb deletion, spanning from band q13.2 to q13.3.

The patient, a 11 year-old female was found to have craniofacial dysmorphic features, hiperextensible joints, severe short stature with growth hormone deficiency , developmental delay and seizures. No signs of puberty were observed.

Pure intersticial deletions of 4q have been rarely reported in the literature and none of them was accurately mapped by last generation molecular techniques. Intersticial deletions of 4q are associated with a variable phenotype, including growth retardation, dysmorphic features, upper and lower limbs malformations, developmental delay (speech delay is usually more severe than motor delay) and seizures. The deleted region in our patient contains several genes (*MUC7, ENAM, SLC4A, GC)*, including the *GNRHR (Gonadotropin-Releasing Hormone Receptor)* gene*,* associated with hypogonadotropic hypogonadism.

This is the first report of a patient with a 4q13.2- q13.3 deletion and growth hormone deficiency.

P03.071

How common are the cryptic inverted duplication deletion rearrangements among cytogenetically visible terminal deletions?

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Inverted duplication deletions (inv dup dels) are rare chromosomal rearrangements characterized by the presence of terminal deletions with contiguous interstitial duplications. They originate through asymmetrical breakage of a dicentric intermediate, formed after the repair of a double strand break. Some, like the inv dup 8p, are recurrent and mediated by either parental paracentric inversions or low copy repeats. Non-recurrent inv dup dels have been described for many chromosome arms, usually with the cytogenetically visible duplicated region longer than the deleted one. Inv dup dels associated with large deletions and small duplications can be misdiagnosed cytogenetically for pure terminal deletions, with consequences for genotype - phenotype correlations and patient management.

We used microarray (Affymetrix) to define 12 cases of cytogenetically diagnosed terminal deletions (with breakpoints at 3p25.3, 5p15.3, 5p15, 6q25.3, 7q34, 9p23, 10p13, 10q26.3, 10q26.12, 15q26.1, 18p11.2, Xp22.31). Pure terminal deletions were confirmed in 9 cases. Inv dup del was diagnosed in one patient, with a terminal deletion of 13.4 Mb at 9p23-p24.3 accompanied by a contiguous, 822 kb duplication at 9p22.3-p23. FISH analysis confirmed the inverted nature of this duplication. Two other patients showed discordant results on the array: one with mosaicism for cell lines with two different 5p terminal deletions and the other with an interstitial deletion of 15q25.3-q26.2.

Our study of terminal deletions revealed a discordant array result in 3/12 (25%) of cases. One inv dup del was observed (8% of the group), which suggests that inv dup del rearrangements are not infrequent among the cytogenetic terminal deletions.

P03.072

Paracentric Inversion of Chromosome 5 detected in Prenatal Diagnosis

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Inversions are a fairly uncommonly detected chromosome rearrangement, not considering the variant forms, with a estimated frequency range 0.12‰ to 0,7‰ (pericentric) and 0,1‰ to 0,5‰ (paracentric). Usually they are balanced with no clinical significance unless the breakpoint occurs within a gene. Between 85-90% of inversions are inherited and the risk of having a phenotypic consequences depends of the type (para- or pericentric) and size of the inversion.

The authors report a case of a paracentric chromosome 5 inversion detected on prenatal diagnosis. Amniotic fluid and blood samples (parent's) cultures were performed; high resolution GTL-banded metaphases were analyzed according laboratory routine protocols. Fluorescence *in situ* Hybridization (FISH) was done with painting, subtelomeric and unique sequence for Cri-du-Chat probes to confirm the cytogenetic analysis. A small paracentric inversion, between the bands p13.1 and p15, was detected and it was inherited from the mother. Since the region involved was very small (band p13.1 and p15), this inversion was difficult to detect, and only with high resolution chromosomes it was possible to visualize it.

Inversions involving the chromosome 5 are rare and is important that the new cases detected should be reported for determining the genotype-phenotype correlation to be used for genetic counseling and risk evaluation.

P03.073

A case with isochromosome18p

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Isochromosome 18p (i(18p)), is a rare chromosomal disorder that occurs once in about every 140.000 live births, affects males and females equally and results in tetrasomy 18p. Most of the cases are due to a de novo formation but in the literature familial cases were reported. The phenotype of tetrasomy 18p has been primarily delineated by published case series and reports. Findings reported in more than 25% of these cases include neonatal feeding

problems, growth retardation, microcephaly, strabismus, muscle tone abnormalities, scoliosis/kyphosis, and variants on brain MRI. Developmental delays and cognitive impairment are universally present.

Marker chromosomes are seen in the 0.06% of the population and are small chromosomes that are additional to the normal chromosome count, the origin of which cannot be determined by standard chromosome analysis. Molecular cytogenetic testing as M-FISH and CGH array techniques can be used to determine the origin of the marker chromosome and help reaching exact diagnosis in such patients.

Here, we report a young female with dysmorphic features as microcephaly, dolichocephaly, high arched palate, low-set ears, open mouth appearance, high palate and long philtrum, clinodactily, presenting a small metacentric chromosome at the routine chromosomal analysis. Besides the dysmorphic features she also has muscle hypotonia, spasticity, strabismus growth and intellectual retardation.

The performed CGH array revealed the presence of chromosome 18p tetrasomy in this patient. The diagnosis of tetrasomic 18p syndrome is consistent with the complex clinical features in our patient.

P03.074

High resolution array CGH study in newborns with isolated cleft/lip palate

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Clefting is a common condition, found in 1/700-1/1000 births, with a complex etiology. Although most cases are isolated, a subset is associated with other anomalies and linked to already known syndromes involving cleft lip or palate (CL/P), such as van der Woude syndrome. Until now, a number of genes have been suggested to be involved in clefting events, but they account for small proportion of the recognized etiology. With the implementation of array CGH for diagnostics purposes, among the mechanisms leading to clefting, microdeletions have been hypothesized to play a significant role.

Herein we present a study of 33 patients with isolated CL/P with the use of whole genome Agilent 180k microarray with mean resolution of 16kb. We have found 10 copy number variations (CNV) overall (9 deletions and 1 duplication), ranging in size from 27 kb up to ~8Mb. Microaberrations found in the study are not covering any known microdeletion/microduplication syndrome regions. The summary of all cases and genotype-phenotype correlation will be presented. The most interesting ones will be presented in details, i.e del 17q12 where only *SLFN12* is deleted.

Our results demonstrate that a high resolution array CGH is an efficient tool in diagnostics of patients with patients with isolated cleft lip/palate. Furthermore characterization of the novel pathogenic CNVs identified in our study can help in understanding the role of defined genes in clefting.

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P03.075

Chromosomal aberrations and Micronuclei frequency in patients treated with J-^{131} for therapeutic causes

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Patients with thyroid diseases have been treated with different doses of J-^{131} in therapeutic causes. Using standard method (cultivation 48 hours of peripheral blood lymphocytes) 12 patients have been cytogenetically analyzed two times. First culture was set before treatment with J-^{131}, and another was set 7 days after application of J-^{131}. Chromosomal aberrations (CA) were analyzed - 200 cells per patient and micronuclei frequency (MN) 1000 cells per patient. Micronuclei appear during cell division as result of acentric fragment or whole chromosomes condensation left in anaphases (it is considered as marker of structural or/and numerical chromosomal aberrations existence).

Applied doses of J-^{131} were 10mCi; 15 mCi and 20 mCi for 4 patients per each dose. At first set of analyzes as initial no significant CA or MN‘s were found. At second set of analyzes for patients who received doses of 10 mCi and 15 mCi it is apparent slow increase of CA and MN frequency (small aberrations); while for those who received dose of 20 mCi there is apparent increase of CA (even bicentric chromosomes) as well as increase of MN‘s frequency alone or with 2 micronuclei per cell.

This is initial phase of research that is going to be further investigated on large number of patients.

P03.076

Comparative array-CGH platform analysis in a clinical setting for diagnosing individuals with intellectual disability and developmental delay

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We investigate the practical performance of three different microarray platforms for their implementation in our diagnostic setting in two hundred cases of Spanish DD/ID in 2 hospital centers.The total cohort consisted of 200 patients, 100 of who were analyzed with KaryoArray®v3.0, 32 on the Agilent Human Genome CGH 244K and for the remaining 68 patients, on the Agilent Human Genome CGH 44K.As we expected targeted array revealed less common CNVs than did the whole-genome arrays, which has a clear advantage in clinical use. Nowadays it is straighter forward to recognize alterations against background of CNVs.These data support that higher yields mainly depend on patient inclusion clinical criteria and the microarray design. When non-strict criteria are followed, higher yields are also found using Karryoarray. The frequency of VOUS was similar in all three platforms (around 5% of cases). Although more studies are required in order to asses the real significance of these CNVs with unclear clinical relevance, we speculate that some of them are likely to be pathogenic.The classification and interpretation of all the CNVs detected in both groups showed that CMA is a clinical useful tool for genetic diagnosis of ID/DD, with an overall diagnostic yield of around 15%.However and considering the resolution of each of the array platforms, all pathogenic imbalances, except one case, would have been identified despite the platform used.

	Agilent 4x44K	Agilent 244K	KaryoArray®v3.0
Pathogenic	3%	18.75%	25%
VOUS	4.4%	6.25%	5%
Benign	75%	75%	42%
None CNV detected	17.6%	0%	31%

P03.077

A rare variant of Klinefelter syndrome patient with a mosaic 48,XXXY/47,XXY/46,XX/45,X/46,XY karyotype studied by GTG-banding and fluorescence in situ hybridization.

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Klinefelter syndrome is the first human sex chromosomal abnormality to be reported. The majority of Klinefelter syndrome patients have the XXY karyotype. Approximately 15% of Klinefelter patients, however, are mosaics with variable phenotypes.

A 39-year-old male was investigated for primary infertility. Clinical examination showed an intelligent man with normal facial appearance and small firm testes. Testicular histopathology revealed marked atrophy of the testes with no spermatogenesis and absence of germ cells. Hormonal profile showed elevated levels of FSH, LH and low levels of testosterone. Chromosome analysis from whole blood culture showed cells with 48,XXXY/47,XXY/46,XX/45,X/46,XY mosaicism. The predominant cell line was 47,XXY (86.66%). This was confirmed by fluorescence in situ hybridization (FISH) using a dual-color X/Y probe. In our case, FISH also detected the presence of a small population of cells with the 48,XXXY and 45,X karyotypes not previously detected in the initial 30-cell GTG-banding analysis. 46,XY/47,XXY mosaicism is not uncommon. However, mosaicism of multiple sex chromosome aneuploidy is rarely observed. Thus, this case illustrates the utility of FISH as an adjunct to conventional cytogenetics in assessing the chromosome copy number in each cell line of a mosaic. Because most infertile individuals with Klinefelter syndrome may wish to reproduce with the aid of modern reproductive technology, it is important that accurate estimation of the frequency of abnormal cells be obtained for accurate risk estimation and genetic counseling.

P03.078

Genomic imbalances in infertile men detected by array analysis

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Array analysis frequently reveals the cases of multiple independent structural abnormalities with more complex phenotypes than expected. We present results of two infertile men. They were studied cytogenetically using GTG banding and FISH methods, and whole-genome array analysis (Illumina).

Case 1: 46,XY,t(10;15)(p11.1;q11.1) in an infertile man with glaucoma. FISH (Oncor) and array analysis showed that translocation was reciprocal (recT) and apparently balanced. Array analysis revealed two uncommon novel deletions in 2q22.1 and 18q22.1, which contribute to the genomic instability. Infertility might be caused by several factors including both genomic instability and a high frequency of sperm aneuploidies reported in a male carriers of a recT. Glaucoma was probably caused by the mutation(s) of *LRP1B* gene, located in the breakpoint region 2q22.1.

Case 2: 46,XY,t(5;13)(q33;q12.1) in an infertile man with allergy. It was recT confirmed by FISH (Cytocell). Array analysis showed that it was balanced, but also revealed a novel 681-kb microduplication at 9q31.1 (arr 9q31.1(102,352,111-103,033,172)x3). Infertility might be caused by the haploinsufficiency of tubulin (*TUBA3C*) gene located at breakpoint region 13q12.1 but also by arised genomic instability. He also had allergy, unlike his non-allergic parents with normal karyotypes but with microduplication in the same region, where allergy-related quantitative trait locus (QTL) 12 is localized. In our patient, probably both QTL 12 and balanced translocation gave rise of genetic over threshold, and disorder.

These findings show that one aberration can often predispose to the formation of others with phenotypic consequences.

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P03.079

Molecular-cytogenetic analysis of marker chromosomes - clinical importance and diagnostic possibilities

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Supernumerary marker chromosomes (SMCs) are structurally abnormal chromosomes unidentifiable by classical cytogenetic methods. Their general incidence is about 1:2,000 (regardless of gender, ethnicity, etc.). SMCs represent a highly heterogeneous group of chromosomal aberrations associated with different clinical consequences: The majority of SMC-carriers have no clinical symptoms, but some SMC could be related to fertility problems (particularly in males), to mental retardation or to congenital defects. To determine the clinical importance, it is essential to identify an original chromosome, from which the SMC was derived and determine as accurately as possible the genetic material that is present in the SMC.

We demonstrate four cases of predominantly mosaic non-acrocentric SMCs and diagnostic procedures which enabled their determination. These patients represent all groups with higher SMC frequency mentioned above. All cases were examined postnatally, but one of them is closely related to ongoing pregnancy (previous pregnancy of this female with primary finding of SMC was terminated due to multiple foetal defects). Our report discusses diagnostic possibilities, reliability, and limitation of some common molecular cytogenetic techniques, especially standard fluorescence in situ hybridisation (FISH) using satellite probes, whole chromosome painting probes and/or locus-specific probes, and multicolour FISH (painting and centromeric one). Some SMC samples were submitted for further examination by array-CGH, but this analysis failed in cases with low frequency mosaics.

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P03.080

SNP array evaluation of a mosaic supernummery marker chromosome in a girl with developmental delay

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Clinical report

The propositus is a 7-year-old female, who is the second child of non-consanguineous parents. Pregnancy was uneventful with an uncomplicated delivery at term. Births weight 2.7 kilos and birth length 50 centimeters. In infancy she had problems feeding and showed delayed motor milestones and speech development. She has no malformations or dysmorphic features.

Materials and methods

G-band chromosome analysis was performed on PHA-stimulated peripheral blood lymphocytes; 20 metaphases were analyzed revealing 5 metaphases with a small supernumerary marker chromosome and 15 metaphases with a normal karyotype. The identity of the marker was investigated by FISH analysis using a chromosome 15 and a chromosome 14/22 centromere probe. None of these FISH analysis revealed the chromosomal origin of the marker chromosome.

SNP-array detected a mosaic gain of chromosome 1 material. The genotype data clearly showed the presence of a third allele but the intensity data were only slightly differed from normal.

FISH using a chromosome 1 centromeric probe subsequently confirmed the chromosome 1 origin of the marker chromosome. In order to estimate the mosaic level, 300 metaphases were scanned detecting 44 metaphases with the marker chromosome.

Both parents had a normal SNP-array.

Discussion

SNP array provides a high-resolution method to detect mosaic gains and loses and can be used as a first choice to characterize a marker chromosome. This strategy is both cost and time efficient.

Marker chromosomes of chromosomal 1 origin without phenotypic consequences have been described. We believe that this marker chromosome explains the phenotype of the patient.

P03.081

Rare case of three small supernumerary marker chromosomes originated from chromosomes 1, 12 and 18 in a girl with congenital abnormalities

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The genetic relevance of small supernumerary marker chromosomes depends on their amount and type of additional euchromatin. If they are present as mosaics the phenotype of the carrier depends on the amount of pathologic cells and their equal or unequal distribution in the patient. Three different SMCs are therefore an extreme rare finding.

We present the case of a patient showing 3 different autosomal markers as a mosaic combined with a normal cell line.

Two of the markers were identified as derivates of chromosome 12 and 18 which are classified as frequent aberrations, the third was originated from chromosome 1. The extra chromosomes were analysed by a combination of SNP array and FISH (cen and wcp probes). The size and the frequency were striking different. Besides, we observed an unequal combination of the 3 derivates.

We report on a four years old girl. She revealed a mosaic karyotype in her lymphocytes:

mos48,XX,+mar1,+mar2[18]/47,XX,+mar1[8]/49,XX,+mar1,+mar2,+mar3[2]/46,XX[2].ish der(1)(wcp1+,D1Z1+), der(12)(wcp12+,D12Z1+), der(18)(wcp18+,D18Z1+). By SNP array investigation (Affymetrix 6.0 SNP array) we identified gains of 1p12→p11.2, 12p13.1→q13.11, 18p11.21. Patenal origin could be delineated for der(1) and der(18).

Clinical evaluation revealed severe mental retardation, absent speech, prominent forehead, epicanthus folds, hypertelorism, large ears, depressed broad nasal bridge, long smooth philtrum and a wide mouth with thin upper lip. She still walks on the tips of her toes.

A karyotype-phenotype correlation was set up and the clinical findings of our patient were compared with the patients features of patients with isolated duplications of the three regions mentioned above.

P03.082

Genomic imbalances in a cohort of Iranian patients affected by multiple congenital anomalies (MCA)

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Congenital anomalies (CA) affect 2-3 % of live births and are seen in 25% of deaths in perinatal period and the first year of life. Cryptic genomic imbalances might be an important cause of CA. New high resolution oligo array platforms have been shown increased detection rate as well as potential to discover new regions involved in CA. In this study genomic imbalances were studied in a cohort of MCA patients using conventional and newer molecular cytogenetic techniques and compared their cost effectiveness in routine clinical perspective.

Eighty five Iranian patients affected by MCA were studied for chromosomal aberrations using G-banding. Three MLPA kits were used to screen for genomic imbalances in subtelomeric and 21 microdeletion syndromes. Nimblegen Human CGH 3x720K Whole-Genome Tiling v3.1 Array was used to interrogate genomic imbalances through the genome.

In G-banding 8 patients showed aneuploidies. Two patients diagnosed with marker chromosomes and 1 patient with an additional segment on 4q. The last three patients and other patients with normal results in karyotype were further analyzed with MLPA and array CGH. MLPA detected 8 and array CGH detected additional 4 clinically significant genomic imbalances. The overall detection rate was 28.2%.

In conclusion, array CGH detects all genomic imbalances detected by karyotype and MLPA. Array CGH recommended as the first line test in MCA patients and karyotyping just if be necessary. The exception is for those who are suspected to aneuploidies according to their phenotypes that karyotype is suggested first then array CGH if karyotype be normal.

P03.083

Mental retardation, speech delay, attention-deficiency/hyperactivity disorder, and delicate microangiopathy in a boy with 11p13 deletion

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Deletions of the 11p14-p12 region has been previously described in WAGR and Potocki-Shaffer syndrome both accompanied by mental retardation (MR). *SLC1A2, PRRG4,* and *BDNF* were hypothesized to contribute to the abnormal mental development in patients with 11p deletion. Here we report on a 5.9-year-old boy with MR, speech delay, attention-deficiency/hyperactivity disorder, delicate microangiopathy, and 11p13 deletion. Parental DNA was not available for analysis. A 1.155 Mb deletion was detected by array CGH and confirmed by real-time PCR. Deleted region includes *CD44, SLC1A2, PAMR1, FJX1, TRIM44, LDLRAD3, MIR3973, COMMD9,* and *PRR5L*. Some of these genes are expressed in brain. In particular, SLC1A2 protein is responsible for glutamate transport. Accumulation of extracellular glutamate causes calcium homeostasis dysfunction, increased production of NO, free radicals, and cytotoxic transcription factors, proteases activation, and, as a consequence, neuronal damage leading to neurodegenerative disease, inflammation or ischemic events. Yet although the function of *FJX1* in human remains unknown, in rodent it regulates dendrite extension. TRIM44 may play a role in neuronal differentiation and maturation. LDLRAD3 participates in amyloid precursor protein proteolysis leading to beta amyloid formation which fibrillar form is the primary component of amyloid plaques found in the brain of patients with Alzheimer disease. COMMD9 presumably regulates the ubiquitin pathway and homeostasis. Based on the brain specific functions reported for these genes, the detected 11p13 deletion can be considered to be pathogenic. This study was supported by European Community's Seventh Framework Programme, CHERISH project no. 223692.

P03.084

Submicroscopic chromosomal rearrangements in Ukrainian families with severe syndromic mental retardation

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Upon the identification of de novo genomic/chromosomal rearrangements, the recurrence risk is considered very low.

We report on two Ukrainian mental retardation (MR) families with paternal origin unbalanced translocation grown out of balanced ones in their healthy fathers.

The first proband is a 16 years old girl with severe MR, trigonocephaly, dystrophic features. The proband's aunt has the same MR phenotype. The karyotyping results are: father - 46,XY,t(2;10)(q35;q26),9ph and proband - 46,XX,der(10)t(2;10)(q35;q26)pat. 44K array-CGH of proband showed del10q26.3-qter (2257 kb) together with dup2q35-qter (24378 kb). The CNVs are pathogenic with many MR candidate genes involved in. The same rearrangements have been confirmed in the aunt on 400K array-CGH. Thus proband has derivative chr10 of paternal origin generated by translocation, involving the chr2 long arm. This unbalanced translocation in the aunt and balanced one in proband's father had the same origin from one of their parents.

The second proband is a 20 years old woman with severe MR, hypertelorism, generalized hirsutism, dystrophic features. Two pathogenic CNVs have been identified by 400K CGH-analysis: del5p15.2 (10 Mb) and dup10q25.3-26.3 (18 Mb). Karyotype analysis showed: mother - 46, XX, father - 46,XY,t(5;10)(p15.2;q25.3) and proband - 46,XX,der(5)t(5;10)(p15.2;q25.3)pat. Proband has paternal origin unbalanced translocation involving the short arm of chr5 and the chr10 long arm.

These data demonstrate that the identification of such pathogenic CNV by CGH accompanied by FISH is important not only for diagnosis but also for recurrence risk to family members' estimation.

This research is a part of CHERISH project (n° 223692).

P03.085

Identification of recurrent chromosomal syndromes in patients with mental retardation using 44K array-CGH: report of two cases.

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Mental retardation (MR) is a condition of incomplete development of the brain with the onset occurring before 18 years and is estimated to affect 1-3% of the population. The etiology of MR is very heterogeneous and in about half of cases the cause still elusive. Chromosome imbalances are one of the most important causes. The advance of cytogenetic technologies has improved the diagnostic rate of small chromosome abnormalities such as microarray-based comparative genomic hybridization. The complementarity of cytogenetic tools (Karyotype, Fluorescent in Situ Hybridization and array-CGH) is still needed to characterize the cryptic chromosomal imbalances.

In this study, genomic DNA's from 13 patients with unexplained MR were analyzed by genome wide high-resolution 44K Agilent® oligonucleotides arrays. Pathogenic microdeletions have been detected in two patients presenting MR and congenital malformations, encompassing regions of Xp22.3 and 8p23.1 containing dosage sensitive genes critical for normal development. These results were in accordance with those observed in previous studies: the detection rate of our pathogenic CNV's was 15,4 % (14,4% in other studies). The causality of these rearrangements were determined as well as their parental origin.

It is true that whole genome arrays have significantly succeeded in revealing recurrent chromosomal syndromes but in other way, these high sensitive technologies have complicated the clinical interpretation of many copy number variants of unclear significance. This emphasizes the need of conventional and molecular cytogenetic combination to better clarify the phenotype-genotype correlation in patients with unexplained MR.

P03.088

Microcephaly and Blepharophimosis in a girl with 46,XX,ins(6;3)(q23;q27q21)

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This clinical report describes a one year old girl with severe microcephaly, moderate developmental delay and blepharofimosis. She had no internal organ malformations and structural brain abnormalities. Frequent upper respiratory infections were noted. The chromosome analysis revealed 46,XX,ins(6;3)(q23;q27q21) de novo. 44k array was also performed and showed no abnormalities.

The Blepharophimosis phenotype is known to be associated with the FOXL2 gene which is located at 3q22.3. The ATR gene that is responsible for the Seckel Syndrome phenotype is located at 3q23. We are awaiting targeted array analysis results, which will show us the etiology of overlapping microcephaly and blepharophimosis phenotypes.

P03.089

A patient with moderate intellectual disability and a deletion of 2p14-p15 overlapping with deletions of previously published cases

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Microdeletions spanning 2p14-p15 have been described in two patients with developmental and speech delay and intellectual disability (ID) but no congenital malformations or severe dysmorphy. One additional patient with a similar deletion has been identified in the ISCA study of developmental delay. We report a prematurely born boy with a de novo deletion encompassing the deletion overlap of these three cases. He had clinical features partly consistent with the first two cases from whom detailed description is available including absent speech, severe microcephaly, long face, bulbous nasal tip and thin upper lip. He had thin short stature and moderate ID, and his overall clinical picture was more severe compared to the first two cases. His karyotype was normal but Illumina HumanCytoSNP-12 BeadChip analysis revealed a 3.7 Mb long deletion of 2p14-p15 between (and including) the COMMD1 and SPRED2 genes. FISH confirmed the deletion in the patient but not in the parents. The deletion affected 17 protein-coding RefSeq genes and 3 non-coding RNA genes. The shortest region of overlap of the four deletions contained 10 genes. Some of them including SLC1A4 and CEP68 could be candidates for ID. The Decipher database and two recent studies of large ID cohorts (ISCA and WashU/Signature) list additional patients with deletions extending proximally into the region of the 2p15-p16.1 microdeletion syndrome. Multiple genes from this region may thus be associated with ID, and some patients may show composite phenotypes. Analysis of additional cases is needed to clarify this complexity. Supported by CHERISH, CZ.2.16/3.1.00/24022 and MZ0FNM2012.

P03.090

Complex chromosomal aberration in a boy conceived after intracytoplasmic sperm injection predicts adult onset leucodystrophy

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We report on a *de novo* interstitial del/dup aberration consisting of a 13.3 Mb deletion of 5q15-5q21.3 (92.1-105.4 Mb, hg19) in tandem with a 23.6 Mb direct duplication of 5q21.3-5q23.3 (106.1-129.7 Mb, hg19). Although the aberration covered a total of 60.6 Mb, it was cryptic, i.e., not detectable by karyotyping at a resolution level of 500 bands. Array CGH indicated a diploid region of 0.6 Mb between deletion and duplication. The aberration affected a 14-month-old boy conceived after intracytoplasmic sperm injection who presented with developmental delay, muscular hypotonia, partial agenesis of the corpus callosum, prominent forehead, low set ears, hypertelorism, wide-bridged nose, retrognathia, high palate, and cryptorchidism. The duplicated segment comprised the *LMNB1* gene, thus predicting adult-onset autosomal-dominant leukodystrophy and revealing a temporal dimension of the phenotype. Counselling problems implicated by this prediction include "the right not to know" that the patient might want to use when coming of age.

P03.091

Microdeletion and Microduplication Syndromes

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The widespread use of whole genome analysis based on array comparative genomic hybridization (aCGH) in diagnostics and research has led to a continuously growing number of microdeletion and/or microduplication syndromes (MMSs) connected to certain phenotypes. These MMSs also include increasing instances in which the critical region can be reciprocally deleted or duplicated. This review catalogues the currently known MMSs and the corresponding critical regions including phenotypic consequences. Besides the pathogenic pathways leading to such rearrangements, the different detection methods and their limitations are discussed. Finally, the databases available to for distinguishing between reported benign or pathogenic copy number alterations are highlighted. Overall, a review of MMSs that previously were also denoted 'genomic disorders' or 'contiguous gene syndromes' (CGS) is given.

P03.092

A further case of microdeletion 15q26.1 encompassing CHD2 and RGMA: clinical description and review of the literature

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Chromosomal abnormalities including microdeletions and microduplications are known to cause severe developmental disorders, mental retardation, dysmorphism and seizures. We report a further case of microdeletion 15q26.1 in a 25 year old man, presenting with mental retardation (IQ 48), generalized seizures with onset at 2 years of age, severe kyphoscoliosis, truncal obesity, growth retardation (height on the 3rd centile), psychiatric disorder with anxious and aggressive behavior, astigmatism, delayed puberty, normal head circumference and dysmorphism (upslanting palpebral fissures, long eyelashes, hypoplastic alae nasi, short philtrum, narrow hands and feet, tapering fingers). He was the first child born to non-consanguineous Swiss parents, family history was unremarkable. Array-CGH revealed a 415kb microdeletion at 15q26.1 encompassing only two genes: CHD2 and RGMA. The deletion was confirmed by qPCR and found to be *de novo*. To our knowledge this is the third case reported with microdeletion 15q26.1 encompassing only these two genes. So far CHD2 haploinsufficiency has been associated with lordokyphosis, reduced body fat and growth retardation in mouse model. RGMA seems to perform several functions in the developing and adult nervous system and could be a candidate gene for mental deficiency and seizure disorder. We review the clinical features of the reported cases and discuss the role of CHD2 and RGMA as critical genes in microdeletion syndrome 15q26.1.

P03.093

3q26.33-3q27.2 microdeletion: a new microdeletion syndrome?

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We describe two unrelated patients carrying the same 3q26.33-3q27.2 microdeletion.

Patient 1, 6 years old, was initially seen at age 2 years. Parents reported IUGR since the first month. Cesarean section was performed at 32 gw due to growth arrest; BW 1380 g, L 39.5 cm, OFC 29.5 cm. Array-CGH: de-novo 4 Mb deletion.

Patient 2, 17 years old, was initially seen at 5 months. Pregnancy was unremarkable; emergency Caesarean section was performed at 37 wg because of maternal hypertension. BW 1500 g, OFC 29.5cm; he required resuscitation. Tonic seizures developed at three hours of age. Array-CGH: 4.28 Mb deletion.

Both presented with neonatal hypotonia, muscular hypotrophy, severe feeding problems (gavage feeding), recurrent upper airways infections, developmental delay (both sat at 18 months, Pt1: at 6 years has no language, nor sphincter control, and does not walk independently; Pt2: walked at 4 years and has language delay), severe growth impairment (all measures below 3rd centile).

Both patients share common dysmorphic features: thin skin, flat facial profile, medially sparse eyebrows, epicanthal folds, flat nasal bridge and tip, short philtrum, downturned corners of mouth.

Patient 1 had also oral aversion, gastroesophageal reflux, bladder diverticula and vesicoureteral reflux, retractable lift right testicle, markedly delayed teeth eruption. Patient 2 had also micropenis, which required testosterone replacement, and mirror movements.

P03.094

A comparative cytogenetic analysis of miscarriages following natural conception and assisted reproductive technologies

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Chromosomal abnormalities are the most common cause of spontaneous miscarriage during the first trimester. We performed a comparative study of abnormal karyotype frequency and type in miscarriages following natural conception (group I, n=558) and assisted reproductive technologies (ART) (group II, n=121). Standard karyotyping was made on QFH/AcD banded metaphase chromosomes, obtained from chorionic villi samples. The rate of abnormal karyotypes was 66,5% in group I vs. 50,4% in group II. The difference is explained by the lower percentage of abnormal karyotypes in miscarriages from patients under 35 in group II compared to group I (36,5% vs. 63,1%). In miscarriages from patients over 35 the frequency of abnormal karyotypes was higher compared to normal in both groups: 76,7% vs. 23,3% in group I and 65,5% vs. 34,5% in group II). This tendency was registered when the terms of miscarriages were analyzed: in miscarriages under 7 weeks of gestation from group II the frequency of chromosomal pathology was lower, than in their counterparts from group I (45,6% vs. 66,2%). In miscarriages over 7 weeks from group II the frequency of abnormal karyotypes increased up to 57,7%. These results demonstrate a leading role of non-genetic factors in early pregnancy loss for ART clinic patients under 35. A wide spectrum of aberrations, including trisomies, monosomy X, polyploidy and structural chromosomal rearrangements, detected in miscarriages did not differ between groups, indicating no increased risk of chromosomal pathology, associated with ART.

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P03.095

Microduplication 22q11 in two patients with learning disabilities

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Susceptibility of the chromosome 22q11 region rearrangements has been recognized in clinical disorders as DiGeorge/Velocardiofacial syndrome. The 22q11.2 microdeletion syndrome is the most common of these conditions, representing a spectrum of clinical anomalies affecting multiple organ systems. 22q11.2 duplication syndrome has also been recently characterized as a different clinical entity with features overlapping 22q11.2 deletion syndrome. Evidence has implicated low-copy repeats (LCRs) on 22q as mediator of nonallelic homologous recombination that result in rearrangements of 22q. We performed Multiplex ligation-dependent probe amplification (MLPA) using SALSA MLPA kit P250 DiGeorge in two patients presented with learning disabilities and detected variable microduplication of 22q11 region. Patient 1 showed a microduplication including the region between LRC22-F and LCR22-H involving the SMARCB1 and SNRPD3 genes. Patient 2 showed microduplication at LCR22-D, involving the TOP3B gene. Chromosomal rearrangements in distal 22q11 region, as well as microduplications, are less common than rearrangements in the proximal region. One possible explanation is that LCR22E-H is smaller than the proximal LCRs are thus less susceptible to rearrangements. Our preliminary results do no support a correlation between the size of the duplication and the severity of the phenotypic presentation.

P03.096

MLPA as screening method in detection of submicroscopic rearrangemets detected in patients with developmental delay/intellectual disability

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Chromosomal rearrangements represent a significant cause of developmental delay/intellectual disability (DD/ID). The implementation of Multiplex Ligation-Dependent Probe Amplification (MLPA) has enabled the analysis of chromosomal aberrations beyond the range of routine karyotyping. In this study we prospectively screened 150 patients with DD/ID with or without dismorphic features or additional congenital abnormalities using SALSA MLPA P036, SALSA MLPA P070 and SALSA MLPA P245 kits, which are specifically designed to detect subtelomeric chromosome imbalances and 21 microdeletion syndromes respectively. The aim of the study was to determine the ability to detect chromosomal abnormalities in patients with DD/ID using a combination of MLPA kits and to analyze the feasibility of the use of additional MLPA specific telomere and microdeletion probe mix as an additional confirmatory test. The MLPA screening revealed chromosome aberrations in 21 (14%) cases: 11 subtelomeric rearrangements (3 deletions: del4p, del15q and del22q, 4 duplications: dup 9p, dupX/Yp, 3 deletions and duplications: dup3p/del8q, dup8p/del18q, del 12p/dup22q and one del/dup19p) and 10 microdeletions (5 DiGeorge syndrome, two 17q21.31 microdeletions, one 15q24 microdeletion and one Prader-Willi/Angelman syndrome). The use of two subtelomeric kits per patient has reduced the rate of false positive and negative results and improved diagnostic yield. MLPA specific telomere and microdeletion probe mix have proven to be suitable for confirmation and better characterization of selected aberrations. Conclusion: MLPA is a fast, sensitive and cost-effective technique for screening DD/ID patients. Use of combination of appropriate kits improves diagnostics and is now used in our routine work.

P03.097

NRXN1 deletions identified by array comparative genome hybridisation in a clinical case series - further understanding of the functional relevance to neurodevelopmental disorders.

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Microdeletions in the NRXN1 gene have been associated with a range of neurodevelopmental disorders, including autism spectrum disorders, schizophrenia, intellectual disability, speech and language delay, epilepsy and hypotonia. We carried out array CGH analysis on 10,397 individuals referred for diagnostic cytogenetic testing, using a custom oligonucleotide array, which included 215 NRXN1 probes (median spacing 4.9kb). We found 34 NRXN1 deletions (0.33% of referrals) ranging from 9 to 942kb in size, of which 18 were exonic (0.17%), and predominantly affected the alpha isoform of NRXN1. No NRXN1 duplications were found. Several patients had exonic deletions in both NRXN1 and other loci implicated in neurodevelopmental disorders (CNTNAP2, CSMD3 and the Williams-Beuren syndrome locus) and two patients had duplications of the 22q11.2 locus. Patients with NRXN1 deletions had a range of phenotypes including developmental delay, learning difficulties, ADHD, autism, speech delay, social communication difficulties, epilepsy, behaviour problems and microcephaly. The targeting of dense oligonucleotide probes to the NRXN1 locus on array comparative hybridisation platforms provides detailed characterisation of deletions in this gene, and is likely to add to understanding of the function and mechanism of action of NRXN1 in neural development.

P03.098

Clinical phenotypes and genotype-phenotype analysis of intragenic *NRXN1* deletions

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Neurexin1 (*NRXN1*) is a presynaptic neural cell adhesion molecule and receptor which functions in the stabilization of the synapse by interaction with postsynaptic neuroligin proteins. The *NRXN1* gene is 1.1MB in size and codes for two protein isoforms *NRXN1a* and *NRXN1b*, each of which has multiple promoters and collectively may code for thousands of different transcripts. Previous reports have demonstrated a significant association of copy number variation within this gene to both autism spectrum disorder and schizophrenia, as well as addiction, intellectual disability and vertebral anomalies. We have identified 11 patients with *NRXN1* intragenic deletions by chromosomal microarray. Deletions ranged in size from 8kb to 352 kb. Family study was conducted for six cases and identified three as *de novo* changes and three as maternally transmitted. Cognitive or behavioral reasons for referral were given for 10 of the 11 patients (90%) ranging from profound intellectual disability and encephalopathy to developmental delay. Five of 11 patients had a diagnosis of autism or autism spectrum disorder (45%). The characterization of these patients will expand the clinical phenotype associated with *NRXN1* deletions, and aid in the genotype phenotype correlation of deletions within this structurally complex gene.

P03.099

Structural chromosomal aberrations diagnosed by FISH

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GTG-banded karyotyping provides gold standard in clinical cytogenetics which is widely used in medical-genetic consultations, despite of development and adoption of up-to-date molecular methods. However, GTG-method often is limited in sensitivity. Accuracy of cytogenetic diagnostics of chromosomal rearrangements increases with using FISH, which is an excellent approach to this aim. We used FISH-method with different DNA-probes and have developed the algorithm of investigations. We analyzed the karyotypes of 31 patients with dysmorphic features and congenital malformations. When the analysis of GTG-karyotypes at level 550 bands revealed presence of chromosomal structural rearrangements (deletions or duplications), we investigated parents' karyotypes to establish the origin of aberrations. In 27 cases these aberrations arised de novo, and in other cases were non-balanced variants of parents' translocations. For identification of chromosomal rearrangements we used FISH with different DNA-probes: WCP, PCP, CEP, subtelomeric probes, LSI, and also multicolor technologies - mFISH, mBAND, m-cenFISH. In cases of deletions we used appropriate chromosome-specific subtelomeric probes. In cases of additional material of unknown origin we used mFISH, subtelomeric probes then mBAND. When derivate chromosomes were revealed chromosome-specific CEP and then mFISH was used. In cases sex chromosome abnormalities we used PCP X long and shot and LSI SRY. In cases supernumerary chromosomal rearrangements - m-cenFISH, WCP, then subtelomeric probes. In result of the application of listed FISH-probes the patients' karyotypes were verified and breakpoints were estimated. The FISH-tests developed algorithm utilization allows to determine the origin of rearrangements and to reduce research time and costs.

P03.100

The cytokinesis-blocked micronucleus (CBMN) assay in workers at stone-crushing units

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The stone-crushing industry is a labour -intensive sector where most of the operations are performed in a highly dust-polluted area often violating the pollution control board guidelines. Since the cytokinesis-blocked micronucleus assay (CBMN) assay provides a deeper insight into the mechanisms contributing to genome damage events that could increase risk of developmental and degenerative diseases, in the present study chromosomal damage was assessed by CBMN in peripheral lymphocytes of 23 stone-crushing unit workers(32.69±1.42 y) employed for more than six years(7.74±0.25y) with a daily work schedule of 8-12h/day, in comparison with 09(33.00±0.50 y) controls matched for gender, age, and socio-economic status and smoking habits with no past/present history of any exposures. The study was cleared by the Institutional Ethics Committee. Voluntary written informed consent was obtained from all study participants and a face- to -face interview was conducted using a pre-designed questionnaire. The results of the assay reveal a statistically significant ($p \leq 0.000$) two fold elevated percent frequency of MNd cells in the workers (0.55±0.02) compared to the control group (0.26±0.03). As these workers are continuously being exposed to workplace genotoxicants (causing structural alterations to chromosomes which can lead to altered gene dosage and expression), the evaluation of chromosomal damage in these occupationally- exposed workers can be an important pathogenetic and prognostic predictor of future disease-related changes.

P03.101

Cytogenetic abnormalities in peripheral blood lymphocytes of patients with malignant salivary gland tumors during neutron therapy

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Neutron therapy is used in more than 25 leading radiology centers in the world, however, cytogenetic monitoring of cancer patients during this type of therapy have not been performed. The frequency and spectrum of cytogenetic damages in peripheral blood lymphocytes were investigated in 9 patients with malignant neoplasms of the parotid salivary glands during the treatment by fast neutrons in the cyclotron U-120. There were three time points: before treatment, 24 h after the first fraction and at the end of the neutron therapy. Mode of exposure included: single focal dose - 1.6-2.4 Gy, 3-4 sessions, and the total tumor dose - 5.5-8.4 Gy (equivalent to 23-44 Gy of photon radiation). Chromosomal aberration analysis was performed according to standard protocol in the first mitosis of PHA-stimulated lymphocytes. Cytokinesis-blocked micronucleus test was performed in combination with FISH using a pancentromeric DNA probe. It was shown that chromosome-type aberrations were prevalent among all cytogenetic abnormalities both before and after neutron therapy. The frequencies of chromosome-type aberrations and all micronuclei increased significantly after both the first fraction and the whole therapy comparing with the levels before the therapy ($p<0.05$). The predominant chromosome-type aberrations were paired (acentric) fragments (57 % of all chromosome-type aberrations). The observed mutagenic effect could be considered to optimize the neutron therapy in patients with tumors of the salivary glands. This research is supported by the target Federal Program of „Research and development on priority directions of scientific-technological complex of Russia for 2007-2013 years" No. 16.512.11.2063.

P03.102

Clinical and molecular characterization of a patient with de novo 0.45 Mb deletion of 2p16.1

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The widespread use of microarray methods has contributed to the identification of several new rare microdeletion syndromes including that associated with deletions of 2p15-p16.1. The 2p15-p16.1 microdeletion syndrome is characterized by developmental delay, intellectual disability, autism, microcephaly, growth retardation, facial abnormalities, disturbed vision and other symptoms. We report here an 11-year-old autistic girl showing clinical features consistent with the syndrome. Conventional cytogenetic analysis of the patient showed a normal female karyotype. Illumina HumanCytoSNP-12 BeadChip analysis revealed a 0.45 Mb long deletion of the paternal allele of 2p16.1. FISH analysis confirmed the deletion in the patient but not in any of her parents. The deleted region contains only 3 protein-coding RefSeq genes, BCLA11, PAPOLG and REL, and 1 long non-coding RNA gene FLJ16341. Based on high phenotype similarity of our patient with 6 reported patients showing the typical phenotype of the 2p15-p16.1 microdeletion syndrome we propose that the critical region of the syndrome can be narrowed down and that these brain expressed genes can be considered candidates for the clinical symptoms. However, multiple deletions of variable length within the interval between 2p14 and 2p16.1 have been described in patients with intellectual disability but not necessarily the other typical symptoms of the syndrome and some of these deletions do not overlap. This observation indicates that also other genes located in this broader unstable region are associated with cognitive functioning. Supported by CHERISH 223692, SF0180027s10, CZ.2.16/3.1.00/24022 and MZ0FNM2012.

P03.103

A *de novo* 3.8 Mb duplication of chromosome 14q22.3q23.1, including *OTX2* and *ARID4A*, in a developmentally delayed boy

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We present a three year old developmentally delayed boy with a vocabulary of less than twenty words, who started walking two years of age. He has thin, fine hair and a preauricular tag, but otherwise no dysmorphic features. Magnetic resonance imaging (MRI) of the brain revealed a subarachnoidal cyst. A *de novo* 3.8 Mb duplication involving chromosome 14q22.3q23.1 was detected by aCGH analysis (chr14:55331483-59107556 bp, hg19). There are no previous reports of patients carrying a duplication of similar size in this region, which in addition to the relatively high number of genes involved make genotype-phenotype correlations challenging. Among the 32 RefSeq genes affected by the duplication, genes of potential interest are *OTX2*, *ARID4A, GCH1* and *DACT*. *OTX2,* orthodonticle homeobox 2, is a homeodomain-containing transcription factor expressed in brain, whose haploinsufficiency is linked to ocular developmental anomalies and developmental delay. *ARID4A,* AT rich interactive domain 4A, is a gene involved in chromatin remodeling, therefore likely to have pleiotropic effect. *GCH1*, GTP cyclohydrolase 1, partially duplicated at the proximal border of the imbalance, causes dopa responsive dystonia and malignant hyperphenylalaninemia with autosomal recessive inheritance, although the patient's phenotype is not consistent with this syndrome. *DACT,* dapper, antagonist of beta-catenin, homolog 1, partially duplicated at the distal border, is involved in the Wnt- mediated developmental processes.

We suggest that increased dosage of *OTX2* and ARID4A might have a relevant role in the emergency of the clinical phenotype in our patient.

P03.104

Complex X-chromosome rearrangement. How could it happen?

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We report the SNP-array finding of a complex X-chromosome rearrangement in a now 4 year old girl with delayed psychomotor development and failure to thrive.

We found on the short arm of the X-chromosomes neighbouring regions with different aberrations, namely a duplication (Xp22.2), a loss of heterozygosity (LOH) segment (Xp22.11-Xp22.2) with normal allele frequency and a deletion (Xp21.1-Xp22.11). There were no other findings on SNP-array to suggest different reasons for the girl's symptoms. SNP-arrays on both parents were also performed. The mother had a duplication (Xp22.11-Xp22.2) matching the duplication and LOH segment in the girl. The father had no deletions or duplications. G-band karyotypes from the girl showed visible aberrations in both X-chromosomes; one with a duplication in Xp and one with a deletion in Xp (46,XX,dup(X)(p22.1p22.2)mat,del(X)(p21p22.2)dn)

Results of additional FISH analyses, using probes corresponding to the duplicated segment Xp22.2, the segment containing LOH (Xp22.11-Xp22.2) and the deleted segment (Xp21.1-Xp22.11), showed that the paternally derived X-chromosome did not harbour any of these three segments, suggesting that the unbalanced complex duplication/LOH was a result of inheritance of two derivative X-chromosomes. A maternal derivative X-chromosome with a duplication segment corresponding to the LOH region combined with insertion of the paternal duplication segment and a derivative paternal X-chromosome with a deleted segment encompassing all three regions.

We speculate that the reason for this complex rearrangement could be a post zygotic mitotic recombination, perhaps caused by an inverted duplication on the maternal X-chromosome, although this could not be verified.

P03.105

Chromosome evolution 180 degrees backwards - a case report

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We present molecular cytogenetic evidence that the carrier of a paracentric inversion inv(7)(q11.23q22.1) has reversed the evolutionary inversion that distinguishes the chromosome 7 homologs of human and gorilla. The inversion was observed in a 41 years old patient with inconspicuous phenotype after routine cytogenetic analysis because of recurrent abortions in the partner of the patient. FISH experiments with a panel of BAC probes localized the inversion breakpoints at 76,5-76,9 Mb and 102,2-102,4 Mb (GRCh37.p5, Feb 2009), respectively. The proximal breakpoint maps approximately two Mb distal of the Williams-Beuren Syndrome critical region. Importantly, both breakpoints reside in large clusters of primate specific segmental duplications, which by non-homologous allelic recombination (NAHR) may have facilitated both the evolutionary inversion in the human/chimpanzee common ancestor and the inversion in the case presented here, and possibly also in several other cases described in the literature as inv(7)(q11q22). In summary, this example adds to the mounting body of evidence that some structural chromosomal aberrations in humans can be caused by inherent instability of genomic regions that were already prone to break during evolution, thus demonstrating that evolutionary genomic changes and human chromosome pathology may be two sides of the same coin.

P03.106

A novel report of partial trisomy of distal 7q and partial monosomy of distal 13q in a child with mental retardation,dysmorphism and ambiguous genitalia

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A four year old boy was hospitalized with neurodevelopment delay, growth delay, mental retardation, brachycephaly, neuromuscular abnormality, several dysmorphic features and ambiguous genitalia. Cytogenetics investigation using high resolution GTG banding technique showed an abnormal chromosome 13 described as 46, der(13)t(7;13)(q32;q32)mat. The mother had a balanced reciprocal translocation between chromosomes 7 and 13. FISH technique using subtelomeric probes for 7q and 13q confirmed the translocation and the der13 in the child. For Further characterization of the breakpoints aCGH was used.whole genome oligoarray was performed using CYTOCHIP ISCA 4X44K version1.1.In the involved chromosomes using array CGH showed a 7.7 Mb deletion at 13q33.3 to qter and a 22Mb gain at 7q33 to q36.3. The breakpoints using conventional Cytogenetics technique were furtherer refined with array CGH from 7q22 to 7q33 and from 13q32 to 13q33.The concomitant occurrence of partial monosomy 13q with other chromosomal abnormalities is uncommon. The patient's intellectual disability seems to be in accordance with 13q deletion syndrome and his ambiguous genitalia is more likely to be due to 7q partial trisomy. Specific neurological and neuromuscular problems like seizure and hypo/hypertonia have been described in most 7q partial trisomy patients but not in 13q partial monosomy. Dysmorphic features like low set ears, frontal bossing and positional abnormalities in teeth,epicantic fold and hypertelorisum, Low hair line and Retro/micrognathia , present in our patient, have been described in other cases of partial trisomy 7q and 13q deletion.

P03.107

Partial Trisomy 1q associated to Partial Monosomy 11q: Cytogenomic and Clinical findings

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Citogenomics methods have provided significant improvement in the diagnosis of rare diseases in individuals with developmental delay, multiple congenital anomalies and autism spectrum disorders. We describe a patient with partial trisomy 1q and partial monosomy 11q and a 46,XY,der(11)t(1;11)(q41;q24)pat karyotype. Further investigations using FISH-BACs and SNP-array (6.0 Affymetrix) disclosed a ~37 Mb duplication of 1q32.3q44 associated to a ~2 Mb deletion of 11q25. The patient, a 13 year-old boy, has short stature, facial and corporal dysmorphisms. Cardiac evaluation showed atrial septal defect corrected by surgical treatment. He presents intellectual disability, aggressive and hyperactive behavior, limited verbal language repertoire and dysarthria. To the best of our knowledge, this is the first report of a partial trisomy 1q32 associated to a partial monosomy 11q25 in the literature. Thus, the patient revise karyotype is 46,XY.ish der(11)t(1;11)(q32.3;q25)pat (RP11-663C5-,RP11-262H5+;RP11-15J5+,RP11-265F9-).arr 1q32.3q44(212,508,954-249,224,376)×3,11q25(132,927,027-134,944,770)×1. Among the duplicated genes at 1q region, DISC1 and TRAX are crucial in neural development and TBCE and RAB3GAP are associated with neurodevelopmental disorders and mental retardation. Also the genes RYR2, VTSIP, ARVD2, ARVC2 are associated to cardiac abnormalities. Comparing the molecular karyotype and the phenotype of our patient to few similar cases, the clinical features of our patient are more likely due to partial trisomy 1q than to partial monosomy 11q. Although one of the critical regions for conotruncal heart defects, that include JAM3 gene, is within 129.0-130.6 Mb at 11q25. Cytogenomic methods extended the scope of molecular diagnosis thus making possible a more comprehensive approach to identify pathogenic genomic imbalances.

P03.108

A girl with partial trisomy 2p and monosomy 9p syndromes and sex reversal

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Partial trisomy 2p is associated with multiple distinctive findings including psychomotor delay and dysmorfic facial features. The deletion 9p syndrome is characterized by mental retardation, trigonocephaly, midface hypoplasia and a long philtrum. Distal 9p deletions have also been reported in patients XY and sex reversal, with or without 9p deletion syndrome. Our patient presented low birth-weight, developmental delay, generalized hypotonia and seizures. Facial dysmorphisms included high forehead, wide nasal root, anteverted nares, short neck, and normal external female genitalia. Conventional cytogenetic analysis was performed. Array-CGH was carried out using the Constitutional Chip 4.0 BAC Array platform. The karyotype was 46,XY,der(9)t(2;9)(p21;p24).arr 2p25.3p21(366,137-42,681,415)x3,9p24.3p24.2(97,018-2,299,539)x1mat. She inherited the chromosome 9 derivative from her mother who had the karyotype 46,XX,t(2;9)(p21;p24). Our patient did not present with trigonocephaly, but presented with other features characteristic for 9p deletion phenotype. Considering the extent of the 9p deletion in this patient (~2 Mb from the telomere), our results support the observations made by some authors, suggesting a more distal critical region for 9p deletion syndrome phenotypes. Our patient had also a 2p duplicated segment an average of 42 Mb. This segment was larger than those described by some authors (in general, 2p23→2pter), but presented many features in common with those described for them. The differences could be explained due the different breakpoints and genes involved in each patient. The present study could contribute to the description of unusual chromosomal aberrations affecting chromosomes 2 and 9 with sex reversal.

P03.109

A boy with partial trisomy 3p and monosomy 10q due to an unbalanced 3p:10q translocation

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Trisomy 3p syndrome is a rare syndrome characterized by psychomotor and mental retardation, decreased muscle tone, seizures, short neck, hypertelorism/telecanthus, dysmorphic ears and congenital heart defects. Partial deletion of the long arm of chromosome 10 is a relatively frequent cytogenetic abnormality and exists considerable heterogeneity in the clinical presentation even among family members who share the same deletion boundaries. Common facial appearance, cardiac and urogenital anomalies, and a high incidence of neurodevelopmental deficits are relatively consistent features for deletion 10q syndrome.

We report a 6 years old boy presented with dysmorphic features such as microcephaly, hypertelorism, narrow palpebral fissures, epicanthal folds, flat nasal bridge, deep philtrum, prognatism, malocclusion of teeth, large-protruding ears, uplifted earlobes, short neck, neck webbing, wide spaced nipples, umblical hernia, camptodactyly of third fingers, clinodactyly of fifth fingers, sandal gap, overriding toes, clinodactyly of fourth toes, cryptorchidism, micropenis. The patient also had seizures, growth retardation and mental motor retardation. Abdominal USG showed hydronephrosis and a cranial MR examination revealed enlarged posterior fossa. After conventional cytogenetic screening the karyotype of the proband was described as 46,XY,der(10)t(3;10)(p24;q26). This karyotype confirmed by microarray analysis. This analysis showed a 9,45 Mb gain in chromosome 3 and 5,07 Mb loss in chromosome 10. His father is a carrier of a balanced translocation between chromosomes 3 and 10 [46,XY,t(3;10)(p24;q26)]. So our patient has a partial duplication of 3p and partial deletion of 10q.

This case presented to contribute the literature owing to rarity of the trisomy 3p and distal monosomy 10q syndrome.

P03.110

Partial trisomy of 7q34 with loss of the heterochromatic region of Y chromosome

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We present a male infant with partial trisomy of the long arm of chromosome 7. The patient is the first child of healthy and unrelated parents, born on term, birth weight 3750 g (50 to 75 centile), length 58 cm (+4 SD). Because of dismorphia, cardiac defect and delayed psychomotor development the cytogenetic study was performed at the age of 20 months.

The karyotype 46,X,der(Y)t(Y;7)(q12;q34)dn revealed de novo partial trisomy 7q34 and loss of heterochromatic region of chromosome Y. High resolution GTG banding revealed de novo derivate Y chromosome from translocation (Y;7)(q12;q34) in all methapases and normal karyotype in the patient's father. FISH with wcp from chromosome 7 showed two normal signals, and an extra signal in the long arm of chromosome Y. Chromosome Y showed presence of SRY, centromere and loss of heterochromatic region.

Cases with a 7q34 pure partial trisomies are uncommon. Patient shares most of the common findings with previously described patients such as post natal growth retardation, hypertelorism, epicanthus, low-set ears, micrognathia, short neck, hypotonia, skeletal anomalies, cardiac defect and developmental retardation with IQ 47. He is also characterized by hyperflexible fingers, Sydney line on and right testicular retention, a feature that has previously not been described.

Cases like these are useful, given clinical manifestation are only due to pure 7q trisomy: however, further molecular studies are needed to determine genes located in this region of the long arm of chromosome 7, and to elucidate the phenotypic correlation of the regions of this chromosome partial trisomy.

P03.111

Two distinct phenotypes in 11 individuals, demonatrating alternate unbalanced recombinants derived from a cryptic paternal balanced translocation between chromosomes 10 and 14

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Background: Two syndromes, each characterized by a distinct cluster of clinical features, segregated in 11 individuals from one kindred. All affected children were the products of non-consanguineous matings. However, all affected individuals shared a common progenitor. The diagnosis of unbalanced chromosomal abnormalities was sought. Yet, cytogenetic studies were reported to be normal. Molecular cytogenetics tools were undertaken to further investigate this prototype of abnormalities.

Methods: Following signed informed consent (parental), blood samples were drawn, for DNA extraction, from all available patients (n=10) and parents (n=6), pertaining to three nuclear families, from one kindred. Clinical, neurological and developmental assessments were undertaken in selected patients and two distinct phenotypes, A and B were deliniated, marked by mental retadation, either moderate or severe, respectively, and salient dysmorphic features associated with early scenescence (phenotype B). Whole genome SNP array analysis using the „HumanCytoSNP-12v2.1 DNA Analysis BeadChip Kit (Illumina)" was undertaken on two affected individuals demonstrating distinct phenotypes.

Results: Whole genome SNP array analysis identified an unbalanced cryptic translocation involving a terminal 5 Mb deletion (100273988-106353482) of 14q32.2-14Q32.3 and a terminal 5 Mb duplication (125708-5329074) of 10p15.3 -10P15.1 in patient with phenotype A. An alternate unbalanced recombinant, namely terminal 5 Mb deletion (125708-5329074) of 10p15.3 -10p15 and terminal 5 Mb duplication (100273988-106353482) of 14q32.2-14q32.3, was shown in patient with phenotype B. **Conclusions**: Investigations of apparently balanced chromosomal rearrangements in patients with abnormal phenotype by molecular cytogenetics tools, especially by array CGH, has become the gold standard for deciphering cryptic chromosomal abnormalities.

P03.112

Application of array-CGH in prenatal diagnosis and aborted fetuses

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Array -CGH, in postnatal diagnosis for intellectual disability is currently being used as a first-tier diagnostic test. However, in prenatal diagnosis, chromosomal analysis remains the method of choice. Its introduction in routine prenatal diagnosis is still in its infancy and further studies are needed prior to its implementation. Here we present our results from application of array-CGH in selected amniotic fluid and CVS samples as well as in first and second trimester samples from aborted fetuses (POC-Products Of Conception or skin biopsies). Fifty-one prenatal cases were referred for array-CGH for ultrasound abnormalities (N=37) or for further investigation of chromosomal abnormalities (N=14). The 105K Cytochip array, (BlueGnome Ltd.) was applied and two *de novo* and one inherited, from an affected mother, deletions (6%) ranging from 2.5-14Mb, were detected. In addition the origin of two marker chromosomes was identified. One of the abnormalities detected, would have been missed with conventional cytogenetics highlighting the importance of array-CGH in prenatal diagnosis. In addition the characterization of chromosomal abnormalities with array-CGH offers valuable information for the pregnancy outcome. Forty-six samples from aborted fetuses which failed to grow in vitro were analyzed using the Cytochip BAC array and six (13%) autosomal full trisomies were detected. No cases were detected with submicroscopic copy number changes that could have been missed with conventional cytogenetics. However since BAC arrays were used further studies are necessary with higher resolution arrays in order to evaluate the importance and the value of array-CGH in miscarriages.

P03.113

Prenatal diagnosis of common chromosome disorders by QF-PCR.

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The quantitative fluorescent PCR assay, implemented during the last few years, opens the way for common chromosome disorders prenatal diagnoses within a few hours after sampling. Study summarizes 3 years prenatal diagnosis for chromosome abnormalities by QF-PCR experience. 17 STR markers (D21S11, D21S1437 D21S1411, D21S226, D13S628, D13S634, D13S742, D18S380, D18S386, D18S391, D18S535, AMXY, DXS981, DXS6854, X22, P39, XHPRT) were used throughout the study. Altogether 53 aneuploidies (34 trisomy 21 cases, 11- trisomy 18, 3 47 XXY, 2 45X and 2 69 XXY) out of total 1105 fetuses were picked up. Submicroscopic polymorphic microsatellites duplications were observed in 12 cases as clear trisomic triallelic or diallelic patterns for one chromosome-specific STRs. Duplications were detected in two sample for one STR on chromosome 21 (D21S1437), in six cases for one of the markers selected on chromosome 13 (4 cases for D13S634 and 2 for D13S742), in three sample for STRs on sex chromosome (2 cases for X22 and 1 for P39) and in the remaining case with D18S51 marker. The maternal duplication origin have been demonstrated in 5 cases by QF-PCR analysis of the same marker in both parents, in one sample polymorphism was found as inherited from the father. In three cases *de novo* origin of duplication was proved.

The submicroscopic duplications in microsatellites should be treated with caution as it needs further discrimination for both partial trisomy or full autosomal trisomy detected with single informative STR. The analysis of both parents with the same marker enables rare inherited polymorphism identification.

P03.114

De novo pure subtelomeric microduplications as a cause of dysmorphic syndromes of unexplained etiology

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Unbalanced chromosomal rearrangements in gene-rich subtelomeric regions lead to mental retardation (MR), dysmorphy, multiple congenital anomalies (MCA) and/or abnormal growth or behavior. The number of reported deletions is much higher than the number of duplications, which likely reflects both ascertainment bias and limitations of available diagnostic methods. Duplications may result in milder phenotypes, or certain characteristic features that are different to those of the corresponding deletions, and consequently, patients may not present for clinical evaluation. New molecular techniques (MLPA or array-CGH) allow easier identification of subtelomeric microduplications, but their frequency and clinical significance are still largely unknown. Previous studies gave a frequency of subtelomeric duplications of 0-2%; most have been described in sporadic cases with their genomic sizes poorly determined.

We estimated the frequency of pure subtelomeric microduplications in a group of 491 patients with MR, dysmorphy and/or MCA and normal karyotype using MLPA and delineated the identified microduplications using array-CGH. In 3 patients (prevalence of ~0,61%), MLPA revealed a subtelomeric duplication without a concurrent deletion: duplication of 4p15.2pter (24.91 Mb), 9p13.1pter (38.55 Mb) and 17p13.2pter (5.77 Mb); all had occurred de novo.

Based on our findings and literature data, pure subtelomeric microduplications are an infrequent cause of dysmorphic syndromes. However, further studies are needed to clinically and molecularly define novel duplication syndromes, what is particularly important for dysmorphology and genetic counseling.

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P03.115
Submicroscopic Xq28 deletions are frequent in "MECP2-mutation-negative" Rett syndrome girls

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MECP2 mutations are a well-recognized cause of Rett syndrome (RTT). From 60 to 90% cases of RTT usually demonstrate point or frameshift mutations of MECP2. In the remainder, the cause of the disease is usually unidentified. Occasionally reported cases of Xq28 deletions encompassing MECP2 suggest that at least a small proportion of MECP2-mutation-negative RTT cases might result from submicroscopic subtelomeric Xq losses. Using array comparative genome hybridization (array CGH) with a higher coverage of chromosome X, we have tested whether submicroscopic Xq28 deletions (encompassing MECP2) contribute to the etiology of RTT in "MECP2-mutation-negative" cases. We have found that 4 girls among 28 RTT females without MECP2 mutations (addressed by direct sequencing) exhibited submicroscopic Xq28 deletions. The size of the deletions was estimated to be approximately 603kb. It is noteworthy that all the deletion cases demonstrated almost exactly the same breakpoints located at 153.25 and 153.86 Mb of chromosome X according to NCBI Build 37.3. Clinical manifestations in these cases resembled to classical RTT with additional clinical manifestations such as atypical facial dysmorphisms, congenital heart malformation, intrauterine growth retardation, congenital eye malformations probably due to losses of other genes located at Xq28. Two deletion cases were associated with late-onset regression (at 24 and 38 months). Our data indicate that about 14% of MECP2-mutation-negative RTT cases can arise from submicroscopic Xq28 deletions. This suggests that array CGH (chromosomal microarray) analysis is warranted in MECP2-mutation-negative RTT cases, regardless of monogenic nature of the disease.

P03.116
The ring 14 syndrome: phenotypic map

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The ring 14 syndrome is a rare condition, whose precise clinical and genetic characterization is still limited. Pathogenic mechanisms are unknown, with particular regard to the severe seizure disorder. We analyzed 27 patients with ring 14 syndrome and 9 patients with linear 14q deletions, affecting the proximal (n=3) or the distal (n=6) segment. Clinically, the ring14 syndrome was characterized by a recognizable phenotype of shortness of stature, distinctive facial appearance, microcephaly, scoliosis, and ocular abnormalities, consisting mainly of abnormal retinal pigmentation, but also retinitis pigmentosa, strabismus, glaucoma, and abnormal macula. Nearly all patients presented with severe intellectually disability, and some had aggressive and hyperactive behavior. Drug-resistant epilepsy was another consistent finding. Genetically, the ring was complete in 6/27 cases, while it showed a small terminal deletion, varying in size from 0.3 to 5 Mb, in the other 21. In two of these a cryptic 14q duplication of 2.5 and 9.7 Mb, respectively, proximal to the deleted segment, was also identified. Deleted rings were 75% paternal and 25% maternal in origin. UPD (14) was excluded in all cases. Our observations, along with literature review of additional 39 ring 14 cases and 42 linear deletions, led us to map retinal abnormalities and epilepsy to the proximal 14q11.2- q12 region, and behavior disorders, susceptibility to infections and typical facial characteristics to the 14q32 region. We consider that haploinsufficiency is the most likely underlying mechanism for facial dysmorphisms, susceptibility to infections and behavior disorder, and gene silencing for seizures and retinal abnormalities.

P03.117
ArrayCGH characterisation of ring chromosome 9 formation due to inverted duplication and terminal deletion in a patient with sex-reversal

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We present the molecular characterisation of the case of ring (9) chromosome arisen as a healing mechanism in a patient with inverted duplication and terminal deletion. Ring chromosome 9 was initially diagnosed by high resolution karyotyping and shown to have an additional duplication of band p23. Fluorescent in situ hybridisation (FISH), multiplex ligation-dependent probe amplification (MLPA) and quantitative fluorescent-polymerase chain reaction (QF-PCR) maker analysis were used to characterise additionally the aberration and to establish parental origin. The final karyotype was designated as 46,XY,r(9)(p24.3;q34.3) inv dup(9)(p24.3p22.3)mat. Array-CGH was performed to further map the aberration. The result revealed a complex rearrangement involving deletion of 841,839 bp the at band 9p24.3, followed by an intact segment approximately 926,106 bp in size, and a large duplication of 12.73 Mb extending from band p24.3 through p22.3. The patient presented overlapping clinical features of the terminal deletion and associated duplication. The deletion involved sex reversal critical region which resulted in ambiguous external genitalia and bilateral ovotestes. In addition, she presented with growth retardation, dysmorphic features, cerebellar hypoplasia, a small atrial septal defect and low-normal intellectual development. There is only one report of a patient with ring chromosome 9 containing an inverted 9p22.3-p24.3 duplication, but without terminal deletion. Phenotypic characteristics are similar to our patient, confirming the hypothesis of a separate dup9p22.3-p24.3 phenotype, distinct from the well described 9 p duplication syndrome, thus confining its critical region to 9p22.1-p22.2.

P03.118
Clinical consequences resulting of the ring chromosome 13 configuration

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Ring chromosomes usually result from two terminal breaks in both chromosome arms followed by fusion but can also be formed by different mechanisms. We studied three patients with r(13) karyotyping with G-banding, array (platform Illumina Quad610) and FISH with bacterial artificial chromosome probes, as follows. **Patient I.** Three year-old boy, preterm, IUGR, microcephaly, narrow and oblique forehead, upslanting palpebral fissures, ocular hypertelorism, prominent nasal bridge, high palate, prominent incisors, large and dysmorphic ears, peno-scrotal inversion, scrotal hypoplasia, prominent and large halluces, renal ectopia, hypotonia and severe neuro-psychomotor delay. Cytomolecular results: 46,XY,r(13)(p13q33.1).arr 13q33.1(101,543,509-103, 001,462)x3,13q33.1q34(103,003,268-114,142,980)x1 **Patient II.** One year-old boy, IUGR, microsomia, microcephaly, micrognathism, bilateral epicanthic folds, long eyelashes, small nose, prominent nasal bridge, long philtrum, broad helices, low set dysmorphic ears, high palate, thin upper lip, thoraco-lombar scoliosis, right feet pos-axial polydactyly, hypotonia and neuro-psychomotor development delay: 46,XY,r(13)(p13q34). arr 13q21.33q34(70,141,036-113, 656,958)x3,13q34(113,759,040-114,123,122)x1. **Patient III. 5** year-old boy, preterm, IGRU, microcephaly, ocular hypertelorism, proeminent nasal bridge, long nose, spaced nipples, otitis, leucopenia, speech delay, hypotonia and neuro-psychomotor development delay: 46,XY,r(13)(p13q34).arr 13q34(110,304,519-114,123,122)x1. The patients I and II present a duplicated segment associated with the terminal deletion that was inverted in patient II, while the patient III showed simple terminal deletion. We can observe the three patients present clinical features usually found in del(13q), partial duplication 13q and r(13) considering their phenotypes are influenced by many factors such as the size of the deletion, presence or not of interstitial duplication, ring instability and epigenetic factors, showing the difficulty in defining a specific phenotype r(13) patients. (FAPESP)

P03.119

Robertsonian translocation and consanguinity

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Robertsonian translocation, which occur with a prevalence of ~1 in 1000 in the general population, result from the rearrangement of two acrocentric chromosomes. The most common Robertsonian translocation is between chromosomes 13 and 14. This translocation can occur de novo or be transmitted by one of the parents. The rearrangement form trivalents at meiosis may result in unbalanced gametes. Zygotes carrying monosomy are not compatible with life and most translocation trisomy conceptuses are expected to result in the First trimester loss. However, some of them survive beyond the second trimester and to term. There may be infertility problems and miscarriage in couples carrying these translocations. In the present report, a couple was referred to our clinic due to an 8 years history of infertility. There was a first cousin marriage between couple. Their families also present numerous consanguineous marriage. Cytogenetic analyses were done in the couple and their families. Both couples have a 45,XX,t(13;14)(q10;q10) and 45,XY,t(13;14)(q10;q10) karyotypes. Cytogenetic analyses were also extended to their parents. Female proband's mother and male proband's father were found to be 44,XX,t(13;14),t(13;14) and 44,XY,t(13;14),t(13;14), respectively, indicating double Robertsonian translocation while their couples have normal karyotypes. All other possible carriers of Robertsonian translocation in the family were analysed. Each couple in this family was given genetic counselling who has been seeking pregnancy and healthy child. Thus, they were taken under preimplantation genetic diagnosis programme.

P03.120

De novo and inherited copy number variants are a common cause of short stature

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Shortness of stature is one of the most common paediatric concerns. 3 % of the general population present with a body height below -2 SD score. In the majority of cases the underlying cause remains unknown. Recent GWAS found significant evidence for both single nucleotide and copy number polymorphisms associated with height variation in the general population. These associations explain only a small fraction of the overall variability of human height. To identify novel genetic causes of growth retardation under a "rare variant - frequent disease" hypothesis we performed molecular karyotyping in 121 families with idiopathic short stature using Affymetrix SNP 6.0 array and scored copy number variants (CNVs) with a minimum size of 10 kb and 5 markers. A total of 4,432 aberrations with an average of 36 copy number changes per individual were identified. After exclusion of common polymorphisms using 820 healthy control individuals and comparison with known pathogenic CNVs, we carried out a gene-centric analysis by investigating known gene functions, tissue expression and murine knock-out phenotypes. We found 14 potentially pathogenic CNVs (11.6 %) in 14 patients, including 6 duplications and 8 deletions. All aberrations were larger than 100 kb. 6 were de novo with 1 overlapping the 22q11.1 microdeletion region, and 8 inherited from the affected parent, including the 1q21 region in 2 cases and 3q29 in 1 case. In conclusion, our data indicate that CNVs are one of the main causes of growth retardation in a frequency comparable to other conditions as e.g. intellectual disability.

P03.121

Genetic complexity in a girl with short stature and mental retardation - case report

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SHOX (short stature homeobox-containing gene) is a member of the highly conserved paired homeobox (HOX) family and is known to control important aspects of growth and development. This gene, located in the PAR1 regions of chromosomes X and Y, is the first gene shown to be involved in the development of characteristic features of Turner syndrome.

We present the case of a female patient, the first child of a Caucasian unrelated family with mild dysmorphic features, microcephaly, growth and mental retardation. MLPA analysis of the telomeres revealed an Xpter duplication, later confirmed with FISH analysis using commercially available SHOX probes. Also, the blood karyotype investigation showed a deletion on the long arm of chromosome 13, region q12q14, which includes CDX2, HMGB1, BRCA2, KL and TNFSF11 genes.

The implications of SHOX duplication and interstitial deletion of 13q for the patient's phenotype individually and in combination are discussed, along with a short review of the literature.

P03.122

High resolution oligo array-CGH analysis of single cells

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Genomic imbalances are a major cause of constitutional and acquired disorders. The ability to characterize single cells isolated from solid tumors or pre-implantation samples represents an important advancement. FISH and PCR-based methods have been used to analyze chromosomes of a single cell. However, these approaches can only analyze a limited number of genetic loci simultaneously. By contrast, analysis of genome-wide copy number changes at the single-cell level can be performed by comparative genomic hybridization (CGH). Bacterial artificial chromosome (BAC) arrays have been used for this purpose. However, typically BAC arrays only contain a few thousand probes and are prone to batch-to-batch variation in performance.

Here we describe a method for researchers that combines single-cell whole genome amplification (WGA) with copy number analysis employing high-resolution *in situ* synthesized Agilent SurePrint G3 8x60K oligo CGH microarrays. As a proof-of-principle experiment, we assayed the copy number difference between a reference sample and a test sample with a known aberration, each using amplified DNA that was diluted to single cell levels. We visualized the expected aberrations in Agilent CytoGenomics software. We then assayed the genomic aberrations in single cells biopsied from embryos, in which not only we detected whole chromosome losses or gains, but also found smaller aberrations of portions of chromosome arms.

The ability to detect abnormalities involving any of the 24 chromosomes represents a major advantage over FISH and PCR-based methods. The high reproducibility of high-resolution oligo CGH microarrays offers new possibilities for research on genetic analysis of single cells.

P03.123

SNParray-detected seemingly neutral familial CNVs as causative pathogenic events

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SNP array represents a unique technique for the identification of cytogenetically undetectable submicroscopic alterations (microdeletions, microduplications) and copy number neutral events (LOH, UPD). Routine usage of the whole genome genotyping by the Illumina HumanCytoSNP-12v2.1 generates both types of results; the definite pathogenic/benign CNVs and CNVs or LOH regions of uncertain clinical significance.

In 686 analysed samples during 2 year period (10/11) the total of 2699 CNVs was detected; 913 CNVs in fetuses with abnormal ultrasound findings and 1756 CNVs in children with psychomotoric retardation and/or genetic stigmatisation: 4.4% (41/913) and 6.6% (116/1756) of clinically relevant pathogenic CNVs in prenatal and postnatal samples, respectively; 3.9% (36/913) and 4.1% (72/1756) CNVs of uncertain clinical significance in prenatal and postnatal samples, respectively.

85 parent samples were analysed to clarify the CNV relevance and 18 of CNVs were confirmed as de novo and likely pathogenic microdeletion/ microduplications. Out of the total of 49 maternally or paternally inherited CNVs, 26 were assessed as the genomic variants. Interestingly, 23 of the parental CNVs are likely pathogenic as different mechanisms of the variable expressivity or incomplete penetrance of each of the familial alterations were documented: mosaics, X-linked CNV, discreet parental phenotype, second hit model, different aberration size and copy number and uncovered

mutation on the second chromosome. Therefore, we conclude that the proof of inheritance of seemingly neutral genetic aberration does not exclude its pathogenic role.

P03.124

Characterization of a postnatal "de novo" sSMC derived from chromosome 20

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A genetic imbalance induced by the presence of a sSMC (S*mall Supernumerary Marker Chromosomes)* is the major reason for clinical symptoms in sSMC carriers, although the 44% of prenatally ascertained cases with sSMC are familial cases without clinical effect. Nevertheless, the risk of an abnormal phenotype associated with a *de novo* sSMC is 7%-28%.

sSMCs cannot be easily characterized by conventional cytogenetic banding techniques generating a lot of diagnostic problems, nevertheless, the recent application of new molecular techniques have resolved the limitations of those techniques and therefore increased the right prognosis for all patients.

Here we report a 2 months old girl who showed at birth a weight of 2050 gr p(<3), an OFC of 32cm p(<3), and a length of 43,5 cm p(<3), together with hypotonia. She was the second child of a healthy couple with a previous healthy child and 3 previous miscarriages. Her evolution at home was with no gain weight and after 2 months, an intrathoracic stomach with duodenal bulb infradiaphragmatic, a light colpocephaly and corpus callosum hypoplasia were diagnosed. After surgery she is having good evolution, getting oral alimentation and at 4 months old she weights 4110 gr. She had a normal prenatally karyotype: 46,XX but a postnatal karyotype showed a "de novo" small marker in all cells: 46, XX + mar. The application of an Array-CGH showed a **gain in the gene dosage [min(20)(:p11.1→q11.21:)] which was confirmed by FISH to be present in th sSMC.**

Array-CGH and FISH are essential for diagnosis of sSMC.

P03.125

Structural abnormalities of the sex chromosomes in gonadal dysgenesis

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Introduction: Gonadal dysgenesis are characterized by absence or underdevelopment of the gonads which produce sterility (infertility) and sexual characteristics remain underdeveloped. They are caused by numerical and structural abnormalities of the sex chromosomes.

Material and methods: Our group is formed by 23 patients with structural abnormalities of the sex chromosomes diagnosed in Medical Genetics Center Iasi, Romania, between January 2001-December 2010 from a number of 2362 karyotypes. Chromosomal analysis was done using cultures of lymphocytes and GTG bands. In selected cases we used fluorescence in situ hybridization (FISH) postnatal and in one case antenatal FISH discover the abnormality.

Results: There were 20 cases with X chromosome structural abnormalities and just 3 of the Y chromosome, in all cells or in mosaic form. For X chromosome, the abnormalities were: isochromosome (12 cases of q arm isochromosome), ring (5 cases) and deletions (2 for p arm and 1 for q arm). For Y chromosome there were three abnormalities: dicentric, ring and marker chromosome.

Conclusions: Structural abnormalities of X and Y chromosome are less common than aneuploidy in gonadal dysgenesis and the most frequent clinical picture for female gonadal dysgenesis is Turner syndrome. The karyotype and FISH are useful for cytogenetic characterization of these abnormalities.

P03.126

Subtelomere deletion and additional chromosomal segments in mental retardation

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The prevalence of mental retardation is estimated to be 1 to 3% of the general population. Mental retardation has many etiologies which can be broadly classified into genetic and environmental causes. The condition is common and the cause of MR is still largely unknown in 30-50% of cases. We reported on three female cases with mental retardation and multiple congenital anomalies and have additional (add) chromosomal segments. The parents of the three cases have normal karyotype. Karyotype in the 1st case was 46,X,add(X)(q), the second 46,XX,dd(2)(q), the third case 46,XX,add(21)(q). Using the total subtelomere (Vysis), case 1 has an additional segmene of chromosome 7(q31-36) and deletion of chromosome Xq subtelomere. Case 2 has additional segment of chromosome 15(q25-26) and del of chromosome 2q subtelomere. Case 3 has additional segment of chromosome 17q(22-25) and deletion of 21 subtelomere. Deletion of the subtelomere may be the underlying mechanism of these abnormalities. The FISH technique can identify to a certain limits the sites and extent of deletion and duplication. We recommend array CGH to detect the exact copy number of deletion and duplication and this can explain the relation of genotype/phenotype.

P03.127

Molecular screening for subtelomeric aberrations in Thai patients with idiopathic mental retardation and autism by multiplex ligation-dependent probe amplification (MLPA)

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Chromosomal rearrangements involving telomeres have been identified to account for approximately 5-10% causes of mental retardation (MR). This finding leads to the suggestion that all cases of undiagnosed MR should be screened for subtelomeric aberration. Nevertheless, resolution of standard karyotyping using G-banding is limited and cannot detect this anomaly. Therefore, in this study, multiplex ligation-dependent probe amplification (MLPA) technique, was used to screen 129 patients with idiopathic MR. Twelve of them were also diagnosed with autistic disorder. We identified 5 patients (3.87%) with subtelomeric aberration. All have MR with normal karyotypes. One patient has a submicroscopic deletion at 1p36.33 which was confirmed by real-time PCR. There are two patients with subtelomeric duplication at 15q11.2 and 11p15.5 subsequently. Two patients have the same duplication at Xp22.33. Results were confirmed by using additional MLPA probes. Parental samples were also examined when available. Interestingly the deletion at 1p36.33 and duplication at Xp22.33 fall into the regions where copy number variation (CNV) have been reported. However the pathologic effect of these aberrations is still inconclusive. Further characterization of these aberration boundaries and screening in normal population should be performed. Nonetheless this study shows that MLPA technique is able to detect subtelomeric aberration in patients with idiopathic MR and may increase diagnostic yield especially where array facility is unavailable.

P03.128

De novo supernumerary dicentric marker chromosome 15 with contained Prader-Willi Angelman Critical Regions in a girl with a subtle phenotype

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Background: Supernumerary marker chromosomes (SMC) are structurally abnormal chromosomes that most often are derived from the acrocentric chromosomes and especially chromosome 15.

Large SMC (15) which include the Prader-Willi Angelman Critical Region (PWACR) are nearly always sporadic and maternally derived when parental origin have been established.

Most cases with large SMC(15) have a severe phenotype typically including hypotonia, motor and speech delay, seizures, moderate to severe learning disability and autism while dysmorphic features are absent or subtle and growth is usually normal. Hence chromosome analysis may not be thought of. Cases are most easily ascertained through chromosome and fluorescence in situ hybridisation (FISH) studies.

Method: A three year old girl from a bilingual family was investigated. She had hypotonia, a modest speech delay and unsteady gait due to a foot deformity. Different genetic methods such as chromosomal analysis, FISH, Multiplex Ligation-dependent Probe Amplification (MLPA), array comparative genomic hybridization (ACGH) and single nucleotide polymorphism array (SNP array) were used to elucidate her phenotype.

Results: The results will be presented at the meeting.

P03.129

Deletion of the 3q26 region including the *Evi1 and MDS1-gene* in a neonate with congenital thrombocytopenia and subsequent aplastic anemia

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Gene-targeting studies in mice showed a key role of EVI-1 protein in maintaining hematopoiesis and argue for gene dosage requirement of EVI-1 in the regulation of hematopoietic stem cells. Furthermore, a fusion transcript of *Mds1* and *Evi1* was shown to play a critical role in maintaining long-term hematopoietic stem cell function. Inappropriate activation, usually due to a translocation, of EVI1 is a well known andunfavorable change in several myeloid malignancies. We report for the first time a constitutional deletion encompassing the *Evi1 and Mds1* in a human, and argue that this is causative for the congenital bone marrow failure in this patient.

P03.130

A phenotypically normal male carrying an unbalanced translocation 47,XY,+der(22)t(X;22)(q13;q11.2)

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Background We have carried out genetic counselling of a pregnant couple expecting their first child. The mother was nine weeks pregnant at first contact. A known translocation, t(X;22)(q13;q11), runs in the father's family. The father has been told, based on chromosome analysis on a CVS from his mother, that he carries an unbalanced translocation, which may result in a Klinefelter syndrome phenotype. He is phenotypically normal, also as regards stigmata consistent with Klinefelter syndrome, but has never been karyotyped postnatally. **Methods** In light of this, we performed chromosome analysis of the father using standard karyotyping, whole-chromosome painting (WCP) with chromosome X and 22 centromeric probes and finally SNP-array analysis. CVS taken from the mother was analysed using routine rapid qPCR, MLPA subtelomere analysis and standard karyotyping. **Results** The results showed that the father carries two normal chromosomes 22, a normal X chromosome and an additional chromosome consisting of X and 22 material. His karyotype is 47,XY,+der(22)t(X;22)(q13;q11.2). The derivative chromosome harbours the X-inactivation center (XIC) at Xq13, which undoubtedly explains its lack of severe phenotypic impact. The results of the CVS analyses were normal, showing that the parents were expecting a child with a normal 46,XY karyotype.

P03.131

Triploidy masaicism in a six -year-old dysmorphic girl with mild mental retardation

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A 6 year-old dysmorphic girl with mild mental retardation and dysmorphism is described. Clinical features of the child included patchy hyper pigmented skin, high nasal bridge, short philtrum , microgonathia , simple ear, simian crease, brachydactyly and syndactyly in all foot fingers.

Chromosomal study was performed using GTG banding technique on peripheral blood sample and skin biopsy to rule out chromosomal mosaicism. Interphase FISH investigation on Peripheral blood and skin biopsy, using X and Y chromosome centromeric probes, was carried out.

The child's peripheral blood karyotype was triploid with 69, XXX chromosome complements. Metaphase spreads obtained from skin biopsy revealed two cell lines: the majority of cells (42 cells, 84%) were 69,XXX and 8 cells(16%) were 46,XX. FISH result was as follows: interphase cells of cultured peripheral blood revealed 3 signals indicating X chromosomes and none for Y signal, in all studied cells and skin biopsy culture showed 34% of cells with 2 X chromosome signals and 66% with 3 signals confirming mosaicism for triploidy. Parent's karyotypes were normal.

Triploidy is usually an unviable situation unless a normal diploid cell line is present. The peripheral blood karyotype was pure triploidy while the skin cells demonstrated a mosaic pattern. The interphase FISH showed a higher percentage of normal karyotype. This study reiterates the use of cytogenetic studies on skin biopsy and interphase FISH for the evaluation of mosaicism. To our knowledge, this is one of the rare reported cases of triploidy in a patient surviving to the age of 6 year.

P03.132

A boy with partial trisomy 10q due to an unbalanced 10q:22p translocation

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Distal trisomy 10q syndrome is a rare syndrome characterized by microcephaly, facial dysmorphism, hypotonia, joint laxitiy, scoliosis, short neck, growth retardation, mental motor retardation, cardiac, ocular and renal abnormalities. Most of the cases are diagnosed in infancy or in childhood and rarely include prenatal findings.

We report a 15 years old boy presented with dysmorphic features such as microcephaly, round face, facial hirsutism, frontal upsweep, low frontal hairline, hypertelorism, epicanthal folds, blepharophimosis, thick eyebrows, long curved eyelashes, flat nasal bridge, hypoplastic alae nasi, macrostomia, bow-shaped mouth, malocclusion of teeth, prominent incisors, high palate, gum hypertrophy, micrognathia, simple ear, short neck, kyphosis, scoliosis, hypogonadism, fusiform digits, bilateral hallux valgus, short third toes on right foot, syndactyly 2-3 of toes on left foot. The patient also had hypotonisity, growth retardation and mental motor retardation. Echocardiography showed pulmonary stenosis and a cranial MR examination revealed thin corpus callosum, small arachnoid cyst on anterior temporal lobe but hearing test was normal. After conventional cytogenetic screening the karyotype of the proband was described as 46,XY,der(22)t(10;22)(q24;p11). His mother is a carrier of a balanced translocation between chromosomes 10 and 22 [46,XX,t(10;22)(q24;p11)]. So our patient has a partial duplication of 10q and partial deletion of 22p. Rearrangement of 22p11->pter does not have clinical implications. So clinical features of the proband suggested as a result of trisomy 10q24->qter.

This case presented to contribute the literature owing to rarity of the partial trisomy 10q syndrome.

P03.133

Increased risk of trisomy 21 (T21) in offspring of carriers of balanced non-contributing autosomal rearrangement (Rea) is not accounted for by interchromosomal effect (ICE). A post fertilization effect of paternal Rea on segregation of maternal chromosomes is suspected

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Recent studies showed that chromosomal rearrangement could affect the behavior at meiosis of other chromosomes (interchromosomal effect). If ICE makes any appreciable contribution to etiology of aneuploidy, one may expect an incidence of balanced Rea in parents of aneuploid offspring is increased over population figure.

Objectives: Meta analysis of own and published data on incidence of autosomal balanced Rea in patients with T21, in parents of offspring with T21, and in newborn controls. Meta analysis of own and published data on parental and cell origin of T21 in offspring of carriers of autosomal balanced Rea.

Results: An increase in incidence of reciprocal translocations and inversions (but not of robertsonian translocations) in parents of offspring with T21 (7.1‰ and 2.7‰) compared to controls (1.5‰ and 0.4 ‰), $p<0.001$ and $p<0.01$, correspondingly. Absence of typical female predominance among carrier parents (12 females/13 males) and a predominance of paternally derived Rea in patients with T21 (5 maternal/9 paternal). Similar proportion of T21 of maternal origin in the affected progeny of both maternal and paternal Rea carriers. However when the Rea was maternal, a typical prevalence of errors in the 1st meiotic division of oogenesis was observed while in cases where the Rea was paternal, the profile of errors in oogenesis was different, with a high proportion of errors in 2nd meiotic division.

Conclusion: We did not find evidence of paternal ICE. Instead, the data obtained allow us speculating about a post fertilization effect of paternal Rea on segregation of maternal chromosomes.

P03.134

A prenatal diagnosis case with trisomy 4 confined to placenta

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Confined placental mosaicism (CPM) is diagnosed when some trisomic cells are detected on chorionic villus sampling (CVS) and only normal cells are found on a prenatal test, such as amniocentesis. CPM is detected in approximately 1-2% of ongoing pregnancies that are studied by CVS at 10 to 12 weeks of pregnancy.

Most pregnancies that are diagnosed with CPM continue to term with no complications and the children develop normally. However, some pregnancies with CPM have complications, due to placental dysfunction, uniparental disomy of the fetus and undetected mosaic trisomy of the fetus. The risks for these settings depend of the origin of error, level of mosaicism and chromosome involved.

The study of CVS in normal karyotype fetus with ultrasound abnormalities may be considered, since in some cases, can help in the diagnosis.

Case report:

A 31 years-old primigravida was referred to amniocentesis at 25 weeks of gestation after the detection of ultrasound abnormalities and intrauterine fetal death (IUFD). The karyotype was 46, XY. The post mortem examination showed early fetal death with a maturity corresponding to 24 weeks, IUGR and short femur, coexisting micrognathia, nuchal edema (2.1mm), mild hydrocephalus, cerebellar vermis hypoplasia, bilateral pyelectasis and pulmonary hypoplasia. Cytogenetic analysis of placenta sampling showed a karyotype of 47, XY, +4 in all cells examined.

Mosaicism of trisomy 4 is a rare condition, with 8 cases reported in the literature, and usually associated with anomalies. The authors compare the cytogenetic and the fetus's clinical findings with those described in the literature.

P03.135

Study of Turner Syndrome and presentation of unique cases within the Jordanian population

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Chromosomal number and structure determine the normal gender phenotype in humans. Carrying a copy of the X and Y chromosome determines the male phenotype and carrying two copies of the X chromosome determines the female phenotype. However, in some cases abnormalities in number and/or structure of the chromosomes are associated with a wide range of syndromes, including Turner syndrome. Turner syndrome affects approximately 1 in 2,500 liveborn females, with the most common karyotype in the affected individuals being 46, XO. The reminder of the patients, however, carry mosaic cell lines containing a second sex chromosome (either X or Y chromosome). In about 6% of the female Turner syndrome patients, the second cell line would contain a structurally abnormal Y chromosome. In the current study, we have reviewed 136 positive Turner syndrome cases that have been referred to the National Center for Diabetes, Endocrinology and Genetics, Amman, Jordan. We summarize the data obtained, with a special focus on 2 cases with unique karyotypes and 16 cases which present with structural abnormalities of the Y chromosome. Experimental approaches utilized include standard karyotyping, probe-specific FISH and *SRY* gene sequencing.

P03.136

Turner syndrome with a ring X chromosome and atypical manifestation

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Turner syndrome is a disease characterized by ovarian dysgenesia determinated by partial or complete absence of one X chromosome. Approximately 6% of patients have ring X chromosome. Clinical features are heterogeneous and typical physical anomalies are often mild or absent.

The 3 years 10 months old girl was reffered to the genetic department due to myelomeningocele and severe developmental delay. The patient had short stature, a broad forehead, dysmorphic face Kabuki-like, myelomeningocele, bilateral hydronephrosis, possible cardiac anomalies (heart murmur 2/6 degree on left sternal border) and mental retardation. The karyotype showes mosaicism 45,X [51.06%]/ 46,X,r(X) [48.94%] . FISH test for XIST locus is in study.

Patients with r(X) are reported to have a higher incidence of a more severe phenotype and usually present mental retardation. Some studies have shown a correlation between the phenotype severity and the presence or absence of a functional XIST. Our patient showed a atypical clinical features with reminescent features of Kabuki syndrome. The prognosis of the patient is poor, is largely based on the severity of clinical condition.

P03.137

The power of SNP array: incidental diagnosis of paternal uniparental disomy of chromosome 15 in a child with a large chromosomal deletion of 11q21q22.3

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Array analysis is a first line diagnostic test used to search for copy number changes in children with developmental delay and/or congenital anomalies. SNP-based arrays provide additional information about copy number neutral large contiguous regions of homozygosity (LCSH), which can reflect parental relatedness or uniparental disomy (UPD). A proportion of Prader-Willi and Angelman syndromes are caused by maternal and paternal UPD of chromosome 15, respectively. Both syndromes have distinct phenotypes and are usually suspected clinically and confirmed with molecular studies.

Here we report a patient with prenatal onset of growth impairment, developmental delay, bilateral iris colobomas and dysmorphic features. SNP array (Affymetrix) analysis showed a 12.9 Mb deletion of chromosome 11q21q22.3, as well as a 25 Mb LCSH on chromosome 15q11.2q21.1. As a single LCSH detected on SNP array is suggestive of UPD, molecular analysis of the methylation pattern and parental inheritance was performed and confirmed the presence of paternal UPD of chromosome 15. On reevaluation at 20 months of age, the patient showed global developmental delay but overall good health including no seizures. In general, she was felt clinically to be doing better than expected for a child with both Angelman syndrome and additional large chromosomal imbalance.

This is a first report of a patient with a UPD syndrome and additional, apparently unrelated, chromosomal rearrangement. The diagnosis of Angelman syndrome was not suspected clinically in our patient and represents a fortuitous finding. This case highlights the power of SNP based arrays in providing relevant clinical diagnosis.

P03.138

Maternal segmental uniparental disomy 14 in an adult patient with typical upd(14)mat phenotyp

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We report on a 26 year old graduate student with short stature (161cm), low birth weight (2010g at term), feeding difficulties in the first 3 years of life followed by abiding hunger, truncal obesity, muscular hypotonia, small hands and feet, precocious puberty and hyperlipidemia. Chromosomal examination revealed a normal male karyotype 46,XY and methylation/deletion testing on 15q11-q13 gave no clue for Prader-Willi-Syndrome. In the following marker testing for upd 14 only 2 out of 4 markers were informative and the 2 informative markers showed biparental inheritance. Molecular karyotyping was performed (Affymetrix Cytogenetics 2.7M Array) and no known chromosomal aberration was found. However on chromosome 14 a very long homozygosity was suspicious. We repeated marker testing on chromosome 14 with 7 markers and could verify **segmental maternal uniparental disomie 14q24.2-qter**. The phenotype of our patient fits well for maternal uniparental disomy 14. We conclude upd in the region 14q24.2-qter being responsible for the full phenotype of upd (14) mat and emphazise the importance of testing several informative markers in this region in patients with suspected upd 14. Our results are confirmed by different reports in the literature about patients with epimutations at 14q32.2.

P03.139

Simultaneous occurrence of a duplication encompassing the Wolf-Hirschhorn-Syndrome critical region 2 and a terminal deletion of chromosome 4p in a patient with multiple congenital malformations and developmental delay.

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We report on a female patient with multiple congenital malformations (e.g. anal atresia, tetralogy of Fallot, club foot and cervical spine abnormalities), delayed motor skills and language impairment that shows a small duplication in the short arm of chromosome 4 and a terminal deletion of 4p.

Conventional cytogenetic analysis (GTG- banding), Fluorescence- in situ hybridization (FISH), multiplex ligation- dependent probe amplification analyses (MLPA) and array- based comparative genomic hybridization (aCGH) were performed.

We used an oligonucleotide- based array with an average probe distance of 100 kb (CytoChip Oligo 2x105k v.1.1, BlueGnome). The aCGH analysis re-

vealed a 0.5 Mb duplication, encompassing the Wolf- Hirschhorn- Syndrome critical region 2 (WHSCR-2), and a terminal deletion on 4p16.3 spanning 1.6 Mb.
According to our current knowledge there are only two cases given in the literature with simultaneous occurrence of a duplication of the WHSCR-2 and a terminal deletion 4p (reviewed by Roselló et al. 2009).
The data of our patient help to improve characterization of the phenotype caused by a duplication of the WHSCR-2 in combination with a terminal deletion of chromosome 4p.

P03.140

Wolf-Hirschhorn syndrome phenotype due to der(4)t(4;8)(p16.1;p23.1) caracterized by aCGH

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A partial 4p deletion causes a Wolf-Hirschhorn syndrome (WHS) phenotype. On the other hand, 8p23.1->pter duplication was reported without phenotypic effect. We report on 5-year-old female with deletion of 4p and duplication of 8p presenting WHS phenotype. GTW-banding, FISH and array-CGH were performed and the karyotype was 46,XX,der(4)t(4;8)(p16.1;p23.1)dn.arr 4p16.1(130,000-8,202,790)x1,8p23.1(304,160-6,396,578)x3. She had developmental delay, seizures and dysmorphisms as long eyelashes, synophrys, malrotated helices of ears, micrognatia, a posterior cleft palate, feeding difficulty, cardiac disease and hiper-rotated kidneys. At five years, she has no verbal comunication, and does not walk. The patient phenotype is likely due to 4p deletion and the small 8p duplication seen has no phenotypic effect as reported in the literature. A report of the same band region 8p23.1 is refered to interrupt on *GATA4* gene related to congenital heart defects, however our patient has a 8p duplication started at 5Mb from that gene, thus we thought that her cardiac disease could not related to *GATA4* gene. Furthermore, the breakpoint in 8p is related as recurred in the same region on patients presenting t(4;8) with about 8 Mb trisomic 8p segment. Differently, our patient has an average 6.09 Mb duplication 8p, and lead to smaller size duplication than refered in the related reports. Although the basic genomic defect and the phenotype in WHS is heterogeneous, most of the morphological traits of the hybrid phenotype in the child with der(4)t(4;8)(p16.1;q23.1) can be attributed to deletion of 4p16.1, since terminal duplication of 8p likely have no phenotypic effect.

P03.141

Delineation of syndromic Wolff-Parkinson-White due to a 20p12.3 microdeletion of the *BMP2* region in a boy, his sister and their mosaic mother

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OBJECTIVE:
We report on the clinical and genetic findings in a family with variable phenotypic expression of a 20p12.3 microdeletion. The proband was a young man with re-entrant tachycardia due to Wolff-Parkinson-White syndrome (WPW), dysmorphic features, skeletal findings and cognitive deficits.
METHODS:
DNA from the patients, the father and an older, healthy sister of the proband was analysed using a 105 k (G4425B-014698) Human Genome CGH Oligo Microarrays (Agilent Technologies, CA). An additional Affymetrix 2.7 SNP array analysis was performed on DNA from the mother to check for mosaicism. FISH-analysis of metaphase chromosomes (>50 metaphases) and interphase nuclei (>100 nuclei) were done using BAC FISH probes (RP11-184L8 (*BMP2*-gene) and a control probe RP11-977N11 (20q13.2)) were obtained from Empire Genomics (http://www.empiregenomics.com).
RESULTS:
Agilent 105k aCGH analysis revealed a 970 kb deletion, including the *BMP2*-gene, with genome position g.(6265313_6333051)_(7158672_7235395), at 20p12.3, in the proband, and in the mother. FISH-analysis of the proband and his younger sister detected a microdeletion of the *BMP2*-gene in all cells analyzed. Fifty seven percent of the mother´s blood cells manifested the deletion, revealing a mosaic state. aCGH and SNP array analysis provided no calls for mosaicism.
CONCLUSIONS:
We have reported three patients with 20p12.3 microdeletion, without complete penetrance for WPW syndrome and cognitive impairment. Mosaicism might escape detection by array-based karyotyping techniques. This underscores the importance of employing FISH analysis whenever mosaicism is suspected.

P03.142

Chromosome Xp deletions- Variable phenotypes within several families

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Background: The clinical expression of Xp deletions varies according to gender, deletion size and genomic content, X inactivation pattern and other factors. We describe our experience with female carriers of Xp deletions.
Method: Families of female Xp deletion carriers were assessed. Family history was obtained and clinical evaluation of family members was preformed. Karyotype was analyzed for available family members, as well as in the setting of prenatal diagnosis. Assessment of the X inactivation pattern was evaluated when possible
Results: Three families were assessed. Family 1 consisted of multiple female carriers of an Xp22.1 deletion. All had short stature and some had additional problems such as delayed motor & speech development, emotional oversensitivity, excessive weight gain and attention deficit disorder. Prenatal diagnosis was declined. CMA analysis is pending Family 2 was ascertained during pregnancy with a complex fetal heart malformation. The female fetus had a terminal deletion at Xp22. The pregnancy was terminated. Both mother and maternal grandmother, who had short stature, had the same deletion, as well as 2 female fetuses in subsequent pregnancies. All had a skewed X inactivation pattern. Family 3 presented in pregnancy with fetal short femurs at 22 weeks. Fetal karyotype showed a maternally inherited terminal deletion at Xp21.3. Pregnancy was terminated. The couple is in the midst of PGD, so far with low ovarian response
Conclusions: Xp deletions are associated with variable inter and intra-familial phenotypes. This difficulty in predicting clinical outcome is a challenging issue, especially in prenatal counseling.

P03.143

The breakpoint sequence of a 10,5Mb Xp22 duplication causing a developmental disorder in a male patient.

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Microscopically visible segmental duplications of the short arm of the X chromosome are relatively rare findings and only a few reports exist. All male patients reported show mental retardation combined with various additional dysmorphic features. Most cases are inherited from a heterozygous but otherwise asymptomatic mother. Here, we report on the breakpoint sequence of an Xp22 duplication found in a now 17 year old severely retarded patient. The duplication was initially seen by classical cytogenetic analysis and confirmed later by molecular karyotyping on Illumina SNP chips. The limits of the duplication could be determined this way to within a few kilobases. The duplicated region comprises of about 10.5 Mb on Xp22 and is containing more than 200 genes. The breakpoints of the duplication were determined by sequencing the 5.5 kb amplicon containing the breakpoints and found to lie in a region of Alu elements of about 300 bp and of very high homology.

P03.144

A report of a new case with Xq chromosome duplication

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Rare duplications can occur on the long arm of the X chromosome and may be familial or de novo. They may lead to different phenotypes, depending on the X chromosome region affected. Most dup (Xq) females appear phenotypically normal, or may manifest short stature, facial dysmorphy and gonadal dysgenesis.We present the phenotypic findings of one patient with Xq duplication: C.I, female, 1 year old who was reffered to our hospital for proportional dwarfism. Clinical evaluation also revealed craniofacial dysmorphy: down-slanted palpebral fissures, epichantus, broad nose and lowset ears. Other features were mild pectus excavatum, right hand with clinodactyly of the fifth finger and mild psychomotor retardation. Paraclinical evaluation:

growth hormone and thyroid hormone levels were normal.

The karyotype was abnormal and revelead a Xq duplication: 46,X,dup(X)(q13q22), who included “critical region” (Xq13-q21) involved in premature ovarian failure. The proximal region of Xq contains genes that normally escape to X chromosome inactivation. In this context we appreciate that the patient may have fertility problems such as primary or secondary amenorrhea.

We could not do the karyotypes of the parents, necessary to establish the origin of the chromosomal abnormality, because the child was placed in a institutional care setting.

P03.145

Familial interstitial direct duplication of chromosome (X)(q23q25) detected by aCGH associated with phenotypic variability

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Chromosomal rearrangements occur frequently in patients with mental retardation associated or not with other anomalies. Two brothers and their mother were investigated for mental retardation. Initially, a 32 years-old man was evaluated due to short stature, facial dysmorphism with prognathism, obesity, hypoplastic genitalia, developmental delay and behaviour problems, especially with regard to eating. His brother presented with mild facial dysmorphism, mental retardation and misbehaviour. The mother had milder phenotypic features and mild mental retardation. For this family the IQ varies from 40 for propositus, to 50 for his brother, and 60 for their mother.

Standard cytogenetic analysis for propositus was normal. Because the clinical signs were suggestive for Prader-Willi syndrome, FISH analysis was performed, but no deletion on 15q11q13 was found. Addional testing with MS-MLPA for Prader-Willi syndrome was also negative. Array CGH analysis (180k Agilent) on patient revealed a chromosome X duplication, chrX:115568872-126991548 bp, hg19. By quantitative Real-Time PCR the duplication was verified in the proband and also detected in his brother and mother. BAC FISH established that the duplication is in situ and has a direct orientation. Result of the X inactivation study in the mother showed 100% skewing in leukocytes.

In our case the duplicated region Xq23q25 seems be associated with phenotypic variability. Because the phenotypic variability of the two brothers couldn’t been explained we suspected a possible influence of other genes on the expression of the genes within Xq23q25 region, as well as epigenetic factors influence.

P04. Reproductive genetics

P04.02

Partial deletion of *DMRT1* causes 46,XY ovotesticular disorder of sexual development (DSD)

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Ovotesticular disorder of sexual development (DSD) is an unusual form of DSD, characterized by the presence of testicular and ovarian tissue in the same individual. In a subset of patients ovotesticular DSD is caused by 46,XX/46,XY chimerism or mosaicism. To date only few monogenetic causes are known to be associated with XX and XY ovotesticular DSD like mutations in *RSPO1*, *SRY* and *SOX9*.

We performed high-resolution array-CGH (comparative genomic hybridization) in a female patient with 46,XY ovotesticular DSD with testicular tissue at the one side and an ovary harbouring germ cells on the other. We identified in the patient a small deletion affecting exons 3 and 4 of the *DMRT1* gene. Results obtained by array-CGH were confirmed by RT-qPCR (quantative PCR).

To this point of time, deletions and missense mutations of *DMRT1* are associated only with XY gonadal dysgenesis. But according our present findings we conclude that haploinsufficiency of *DMRT1* can cause both, XY gonadal dysgenesis and XY ovotesticular DSD. Furthermore, to the best of our knowledge, this is the smallest deletion affecting *DMRT1* in a patient with DSD presented to this point of time

P04.03

High throughput copy number counting in single cells - a method for the detection of meiotic and mitotic errors

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Clinical background are the low pregnancy and baby take home rates after in vitro fertilisation techniques (IVF). Major reason is the high frequency of chromosomally abnormal (i.e. aneuploid) oocytes. Selection of euploid oocytes is thus an attractive strategy to increase the number of live births following IVF.

The ploidy status of oocytes can be indirectly investigated by analysing the chromosome content in polar bodies (PB) I and II which are results of the first and second meiotic division before and after fertilisation; errors in meiotic divisions are due to chromosome non-disjunction and early sister chromatid separation. Therefore investigation of the chromosome content of PB I and II requires techniques which allow investigation of all chromosomes at the resolution of chromatids.

In contrast to microarray formats we count chromatids directly - molecular copy number counting (MCC) applied to a single cell, i.e. polar body. MCC is based on limiting dilution of the DNA to a concentration of less than one molecule per PCR reaction and digital PCR. The number of chromatids per chromosome is analysed by counting the numbers of positive PCR reactions representing target sequences on all chromosomes. To investigate all chromosomes with several markers we run a multiplex PCR followed by single marker PCRs with the BioMark system from Fluidigm.

This method is simple and applicable to monitor not only meiotic but also mitotic cell divisions, copy number changes in general and to establish haplotypes for regions of interest in any given single cell.

P04.04

M2/ANXA5 is a risk factor for recurrent pregnancy loss (RPL) in a population undergoing in vitro fertilisation (IVF)

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Carriage of the M2 promoter haplotype of ANXA5 was verified as risk factor for RPL in various patient cohorts. M2/ANXA5 results in reduced expression levels of the protein in placenta leading to various thrombophilia related placental pathologies and ultimately RPL. We performed a risk stratification study in women undergoing IVF.

The IVF cohort of 695 women from the Hormone and Fertility Center, Munich, 500 fertile female controls from the Institute of Human Genetics, Münster and 533 population controls from the PopGen biobank, Kiel were genotyped via sequencing. Equal genetic backgrounds were confirmed through genome-wide SNP analysis.

Carriers of M2 faced a higher relative risk of 1.2 to belong to the IVF group compared to population controls and of 1.4 in comparison with fertile women. This overall elevated risk was contributed by a subgroup of women with previous pregnancy losses, where the appropriate relative risks amounted to 2.3 and 3.8 accordingly. Carriage of M1 or M2 was not associated with biochemical pregnancy loss, implantation rates, ovary reserve, hormone status, number and quality of egg cells and general embryonal development. Interestingly, successful pregnancy outcomes tended to be more frequent for M1 carriers with comparable losses rate. This would signify a possible protective function for M1 in pregnancy, as previoulsy suggested.

In conclusion, the effect of M2/ANXA5 on pregnancy losses was reconfirmed. This excludes biochemical pregnancy losses and implantation failures. There is a possible protective effect of M1 carriage on pregnancy outcomes that needs further evaluation in other patient cohorts.

P04.05

Y chromosome AZF deletions/duplications and spontaneous pregnancy loss

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Spontaneous abortion (SA) is the most common pathology in obstetrics with incidence of 10-15% among clinically recognized pregnancies. The occurrence in three or more consecutive pregnancies is defined as recurrent SA with incidence of 0.5-3% in women trying to conceive. The etiology is diverse and multifactorial (uterine abnormalities, autoimmune, infectious, endocrine, and genetic factors) but still the major part of it (50-60%) remains idiopathic. The male factor has been poorly evaluated in SA. The aim of this study was to determine the association of Y chromosome AZF deletions/duplications with spontaneous pregnancy loss. One hundred and seven men from couples experiencing two or more SA, and 116 fertile men were enrolled in this study. DNA extracted from whole blood was tested for presence of sex chromosome aneuploidies, AZF deletions, partial AZFc deletions and duplications using 13-plex quantitative fluorescent PCR and subsequent capillary electrophoresis on ABI 3130 Genetyc Analyzer. In total, three partial AZFc deletions (1 gr/gr and 2 b2/b3) and 21 AZFc duplications (12 b2/b4 and 9 b2/b3) were detected. Partial AZFc deletions were slightly more frequent among men with SA (1.87%) in comparison to controls (0.86%), while the AZFc partial duplications were more frequent among controls (12.07%) than in men with SA (6.54%). None of the men with SA and controls had any sex chromosome aneuploidy or complete AZF deletions. In conclusion, our study suggests that partial AZFc deletions might represent a male risk factor for SA, while partial AZFc duplications might have a protective role.

P04.07

Mutation analysis of CYP21A2 gene in couples with unexplained infertility problems

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Introduction. Infertility is a worldwide reproductive health problem that affects approximately 15% of married couples. Defects in the CYP21A2 gene cause steroid 21-hydroxylase deficiency, which is the most frequent cause of congenital adrenal hyperplasia (CAH). CAH is a genetic condition that can affect both men and women. In this study we have analyzed mutations of the CYP21A2 gene in couples with unexplained fertility problems and healthy controls.

Methods. DNA was extracted from peripheral blood samples. Allele specific PCR was performed for the detection of mutations IVS2-12 A/C>G and I172N. Gene deletion/conversion was detected with competitive PCR and capillary electrophoresis.

RESULTS. 160 couples with unexplained fertility problems and 200 healthy controls were included in the study. All three mutations were detected as presented in the table.

Cconclusions. The present finding indicates that no significant difference in the prevalence of CYP21A2 mutations can be found in probands with fertility problems when compared with controls without an infertility history. The results also imply that the presence of the most common mutations in the CYP21A2 gene by heterozygote carriers has no influence on their fertility.

Results of CYP21A2 mutation analysis

Mutations	Couples with unexplained fertility problems (n = 320)	Healthy controls (n = 200)
IVS2-12 A/C>G	4	3
Exon 4/I172N	1	0
deletion/conversion	3	3
Total CYP21A2 mutations	8	6
Chi square (all samples vs. Controls) 0.11, p = 0,739 (df = 1)		

P04.08

Spectrum of chromosomal heteromorphism variants of infertile patients undergoing for Assisted Reproductive Technology (ART)

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Cytogenetic studies of infertility patients, couples with recurrent pregnancy loss are important part of ART service. Variation in length of heterochromatin regions, stalks, satellites are described as phenomena without phenotypic effects, but relationships between chromosome polymorphism and reproduction are under investigation.

We present the data of chromosome heteromorphism variants (ChrHV) detected in couples, referred to ART.

Among 2148 patients, examined by standard karyotyping during 2010-2011 years, ChrHV were revealed in 460 cases (22%, sex ratio 1:1). The spectrum of involved chromosomes included: 1 (2.4%), 9 (6.1%), 13 (23.5%), 14 (26.3%), 15 (23.5%), 16 (2.2%), 21 (17.7%), 22 (11.5%), Y (3.7%). Heteromorphism of single chromosome was detected in 82.8%, two chromosomes in 14.6%, three chromosomes in 2.6% cases. Total number of ChrHV was 68. Single variant (ps+, pss, pstk+, ph, phqh, qh) was revealed in 80.4% patients. Association of 2 variants in the same (13pstk+ps+; 14pstk+ps+; 15pstk+ps+, etc.) or different chromosomes (1qh+,Yqh+; 13ps+,22ps+; 14pstk+, 15pstk+; 21pstk+,Yqh-, etc.) was found in 16.1% cases. 3% persons displayed 3 variants: 9ph,13pstk+,21pstk+; 9qh-,14pstk+,15pss; 13pstk+,14pstk+,15pstk+; 13pstk+,21pstk+,22pstk+; 14pstk+,15pstk+ps+; 14pstk+,15pstk+,15pstk+; 15pstk+,21pstk+,22pstk+; 21pstk+,22pstk+ps+,etc. One patient showed 4 variants: 46,XX,14pstk+,21pstk+,21pstk+ps+. The common ChHV were: 14pstk+ (17,4%), 15pstk+ (13%), 13pstk+ (12,2%), 21pstk+ (9,3%). Polymorphism of heterochromatic segments of chromosomes 1, 9, 16, Y identified in 13% cases. Variants 9phqh, 15pss, var15(q11.2q13) registered rarely.

Results demonstrate a wide spectrum of ChHV in infertile couples. For best understanding of relationships "ChHV-reproductive failure-outcome prognosis", especially in rare sporadic variants, the balance of karyotype is to be confirmed with suitable methods, including molecular cytogenetic analysis.

P04.09

Chromosomal outcome of 110 children born after Assisted Reproductive Technology (ART)

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There is a large population of children conceived via Assisted Reproductive Technology (ART), which continues to increase worldwide, without a clear understanding of associated long-term outcomes. There is growing evidence that ART children seems to be different from naturally conceived children. For routing through

these differences, we evaluated cytogenetic results occurring in children born after ART, using their cord blood lymphocytes.We investigated the karyotypes of 110 samples From January 2010 till January 2012, using standard GTG banding. 5 (4.5%) chromosomal alterations were detected among these children. 3(2.72%) of these

aberrations were diagnosed in one of the parents, and 2(1.81%) were de novo changes. In comparison with the frequency of these abnormalities in newborns of general population (0.5%-1%), our results shows a slightly higher incidence of chromosomal changes among children born after ART. There is no much higher risk

of chromosomal abnormalities due to ART. Therefore, these alterations could be correlated with the underlying parental

risk of abnormalities and not with the ART procedure itself. Although ART is now accepted as the treatment of choice for severe infertilities, concerns about its safety and the potential risks for the offspring remain.

P04.10

SEMA7A and *SEMA3A* in congenital hypogonadotropic hypogonadism

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Congenital hypogonadotropic hypogonadism (HH) is characterized by incomplete or absent puberty caused by the lack or deficient number of hypothalamic gonadotropin-releasing hormone (GnRH) neurons, disturbed secretion or action of GnRH, or both. Several genes are connected with the disorder, but in ~70% of the cases the genetic cause remains unresolved. *Sema3a* and *Sema7a* mutant mice have a reduced number of GnRH neurons in their brains, *Sema3a* being essential for the patterning of vomeronasal axons while in *Sema7a* mutants the olfactory system remains unaffected. Thus, *SEMA3A* is a good candidate gene for Kallmann syndrome (HH and hyposmia/anosmia) and *SEMA7A* for normosmic HH (nHH). Whole-exome sequencing of a patient with nHH revealed a heterozygous mutation, R148W, in *SEMA7A*. It was predicted probably damaging by PolyPhen2, and absent in 200 controls. Sanger-sequencing of *SEMA3A* and *SEMA7A* in 40 other patients revealed no other rare variants. However, the patient with the *SE-*

MA7A mutation also had a heterozygous mutation, C389X, in *KISS1R*, a gene implicated in autosomal recessive nHH and encoding kisspeptin receptor with an important role in regulating GnRH secretion. No other *KISS1R* mutation was found in the patient when genomic DNA and cDNA was sequenced. We hypothesize that the nHH of this patient could result from a combination of a reduced number of GnRH neurons and a reduced number of functional kisspeptin receptors in them. Our findings provide support for the role of *SEMA7A* mutations in the pathogenesis of HH in human, and for the proposed digenic or oligogenic inheritance in this disorder.

P04.11

Evidence for expression of *Cyp19A1* (cytochrome P450, family 19, subfamily A, polypeptide1) in human embryonal carcinoma cell line

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Introduction
Cyp19A1 is a single copy gene located on chromosome 15q21.2 in human genome which encodes aromatase, the key enzyme for estrogen biosynthesis. In the final step of mentioned process, this integral membrane enzyme catalyzes the removal of 19-methyl group of the A-ring of androgens in an irreversible manner, resulting in conversion of androgen to estrogen which named aromatization. In human, aromatase gene is expressed in a tissue specific manner, in the way that expression of *Cyp19A1* has been reported in organs such as gonads, brain, skin, placenta and adipose.
Embryonal carcinoma (EC) cells derived from testicular tumors are valuable tools for investigating embryogenesis and developmental biology processes. Since EC cells are malignant but their terminally differentiated derivatives are not, understanding the expression profile of these embryonal cells maybe be of value for diagnostic and maybe therapeutic purposes in embryology.
Material and methods
In the current work, the mRNA expression level of *Cyp19A1* gene was evaluated in a human EC cell line named NT2/NTERA2, using quantitative real-time PCR technique.
Result and discussion
Our results clearly showed the expression of *Cyp19A1* in NT2 cell line. Our finding implies a dynamic role of *Cyp19A1* aromatase gene in developmental processes and maybe in cancer.

P04.12

Performance Evaluation of the Celera Cystic Fibrosis Genotyping Assay with the 3500xL Genetic Analyzer (RUO*) and Three Nucleic Acid Isolation Technologies

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Cystic Fibrosis is a recessive inherited genetic disorder, resulting from an unregulated cystic fibrosis transmembrane conductance regulator (CFTR) gene. Detection of this disorder is crucial in deciding timely treatment, leading to a better quality of life. The Celera Cystic Fibrosis Genotyping Assay is a qualitative *in vitro* diagnostic device used to genotype a panel of 32 mutations (plus poly-T and exon 10 polymorphisms) in the CFTR gene from genomic DNA isolated from human whole blood. The assay provides information intended for use in the carrier screening of adults of reproductive age, as an aid in newborn screening, and in the confirmatory diagnostic testing of newborns and children.
Due to differences in sample preparation technologies, three different methods were used to assess the isolation of genomic DNA from EDTA human whole blood. Two operators independently processed 22 samples on two days using the MagNA Pure Compact Nucleic Acid Isolation Kit (Roche), the QIAamp DNA Blood Kit (Qiagen), and the Puregene Blood Core Kit (Qiagen). The extracted DNAs were characterized for concentration, purity, and performance in the detection of wild-type and mutant alleles using the Celera Cystic Fibrosis Genotyping Assay with the 3500xL genetic analyzer (Life Technologies; RUO*).
The results demonstrated that DNA isolated by these sample preparation methods were of sufficient concentration and purity to allow accurate genotyping by the Celera Cystic Fibrosis Genotyping Assay with the 3500xL genetic analyzer.
*Research Use Only

P04.13

The former annotated pseudogene DHFRL1 is expressed and functional

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Dihydrofolate reductase (DHFR) is a folate enzyme which reduces dihydrofolate into tetrahydrofolate in the presence of NADPH. DHFR was previously thought to be the only enzyme capable of this reaction however we show that humans have a second dihydrofolate reductase enzyme encoded by the former pseudogene DHFRL1 (dihydrofolate reductase like - 1), located on chromosome 3.We demonstrate that the DHFRL1 gene is expressed and shares some commonalities with DHFR. Recombinant DHFRL1 can complement a DHFR negative phenotype in both bacterial and mammalian cells. Enzyme kinetics shows that the Km for NADPH is similar for both enzymes but DHFRL1 has a higher Km for dihydrofolate when compared to DHFR, indicating a lower affinity for the substrate. Localization of DHFRL1, visualized using confocal microscopy, shows that DHFRL1 has a strong presence in the mitochondria, where it is proposed by Anderson et al (2011) to participate in de novo thymidylate synthesis to support mitochondrial DNA replication. We also found that DHFRL1 has the ability to bind its own mRNA in the same translational auto-regulation method as DHFR; with both enzymes capable of replacing each other. Methotrexate (MTX), a potent inhibitor of DHFR, is known to disrupt this regulation mechanism. We demonstrate that DHFRL1, which has a lower binding affinity for MTX, requires a higher concentration of the drug to disrupt the protein: RNA binding complex. The identification of a second dihydrofolate reductase enzyme encoded by a previously unrecognised retrogene will have a major impact on previous research surrounding DHFR.

P04.14

Maternal stress factors associated with Down syndrome birth

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Down syndrome due to trisomy 21 is the most common human chromosomal abnormality. In order to gain insight into maternal stress factors responsible for nondisjunction, we genotyped 12 microsatellite markers spanning along 21q from centromere to telomere in 138 individuals with free trisomy 21 and in their parents and analyzed the association among reduced recombination, maternal age and nondisjunction. The approach was informative for 119 families in determining parental origin with 89.91% being maternal and 10.09% is paternal. The distribution of nondisjunction in maternal meiotic I and meiotic II stages were 81.19% and 19.81% respectively. The mean maternal age of nondisjunction in our Indian population is 27.58±6.4 years which is significantly lower than that of Caucasians. We created a genetic map of long arm (21q) in maternal meiosis I nondisjoined chromosome 21. The distribution of chiasma shows a difference throughout the length of 21q with more recombination towards telomeric end in comparison to control data. The telomeric exchange is found to be a significant risk factor for meiotic I nondisjunction, irrespective of the age of the mother. An increase in both zero- and one exchange events in younger mother (< 29) suggests reduction of recombination. The linkage map of 21q (39.58cM) was significantly shorter than the control female linkage map, indicating an overall reduction of recombination. Telomere length estimation indicates that telomere length attrition may be associated in some way with meiosis I and meiosis II nondisjunctions of chromosome 21.Reduced recombination & telomeric exchange are important maternal stress factors .

P04.15

FMR1Genotype Repeat Size Analysis as a Genetic Test Necessary Prior Fertility Treatments.

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Premature ovarian failure [POF (MIM 311360)] is an early ovarian dysfunction characterized by cessation of menstruation before the age of 40 years. The aetiology of this disorder is complex and the underlying genetic defects are largely unknown. It has been estimated that ~21% of POF cases are associated with expanded alleles of the *Fragile X mental retardation* [*FMR1,* FXS (MIM 309550)] gene. Intermediate and pre-mutated *FMR1* alleles may become unstable generating a full mutation with further expansion in the following generations, when passed from a female to her offspring. Such females, also called Fragile X intermediate or pre-mutation carriers, are phenotypically normal although with an increased risk of POF. We report a case of a healthy, 34-year-old woman who had premature ovarian failure (POF), (FSH measurement = 102,00 mIU/ml, LH measurement = 18,00 mIU/ml). Her condition was considered idiopathic because she did not show any of the known POF-related conditions such as ovarian surgery, previous chemotherapy or radiotherapy, or autoimmune disease. Laboratory tests prior to initiating a stimulation cycle were normal including karyotype. GnRH and follicle stimulating hormone treatment was recommended but the first treatment cycle was unsuccessful in inducing ovulation. Before the second cycle, analysis of FMR1 genotype repeat size was performed and result showed a permutated FMR1 allele (90 Cogs repeats). Patient was derived to a Preimplantational Genetic Diagnosis Program. The association between premature ovarian failure (POF) and the *FMR1* repeat number has been widely investigated and must be taken in account in Reproductive and Infertility Programs.

P04.16

Genetic polymorphisms of Glutathione S-transferase M1, T1 and P1 in Tunisian infertile men

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Introduction: Genetic causes are responsible of 60% of cases of idiopathic male infertility. Polymorphisms of genes that encode Glutathione S-transferases (GSTs), a group of phase II enzymes that detoxify endogenous and exogenous electrophiles, can affect the biotransformation of toxic compounds to which the male productive system is exposed. Some reports attested the association of GSTs gene polymorphisms with male infertility. In order to investigate whether there is an impact of genetic variations of GSTs on semen quality and male fertility, we studied three genetic polymorphisms in GSTT1, GSTM1 and GSTP1 in infertile men and controls from Tunisia.
Methods: Participant's were 159 men with idiopathic infertility and 102 fertile men. Basic semen analysis was performed including total sperm count and concentration, motility and morphology. Genotyping of GSTM1 and GSTT1 polymorphisms were performed using the multiplex PCR. The GSTP1 Ile 105 Val polymorphism was determined using PCR-RFLP.
Results: GSTM1 null genotype (GSTM1 0/0) was significantly associated with reduced sperm count in infertile men semen (oligozoospermia) (P=.001) and GSTT1 null genotype (GSTT1 0/0) was significantly associated with low sperm motility (P=.001). However, infertile men had a higher prevalence of the wide type of GSTP1 allele (GSTP1 Ile 105) than the fertile group (80.5% and 72.54%, respectively; P=.034) and the presence of the homozygote mutant genotype (GSTP1 Val/Val) was less common in infertile men than in fertile group.
Conclusion. Our results suggest that both GSTM1 and GSTT1 gene polymorphisms have a negative impact on semen quality and are associated with male infertility in Tunisia.

P04.18

An association between the IGF2 ApaI polymorphism and spontaneous abortion

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The imprinted IGF2 gene encodes insulin-like growth factor II, displays loss of imprinting (LOI) or aberrant imprinting in human diseases is important for human placental development. Accordingly to GWAS, IGF2 is associated with human longevity and an association between the IGF2 ApaI polymorphism and body mass index was confirmed.
The PCR- RFLP analysis has been performed and the distribution of ApaI IGF2 genotypes was determined in 146 patients with muscle weakness and elevated creatine kinase, in 107 miscarriages' chorionic villi samples (MCV) and control samples. RNA from 41 MCV samples was reverse-transcribed and subjected to IGF2 loss of imprinting (LOI) analysis.
The differences of AG and GG ApaI IGF2 genotypes frequencies between experimental and control groups were significant. The AG ApaI IGF2 genotype was detected in 69.2% of MCV versus to 22.5% in control and 40.4% in patients with muscle weakness and significantly increases the risk of spontaneous abortion (OR=7.72; CI: 3.30-18.03). MCV samples with confirmed heterozygous AG genotype undergo to LOI analysis and expression from both alleles was detected in 37 (90.2%) samples. The revealed deviation from Hardy-Weinberg equilibrium in the control group can be explained by the possibility that embryos with the AG ApaI IGF2 genotype are more often eliminated compared with other genotypes that could be realized by the loss of imprinting mechanisms.

P04.19

The role of thrombophilia in implantation failure and recurrent spontaneous abortion

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It`s accepted, that trombophilia is one of the possible etiological factors of *in vitro* fertilization (IVF) failure and recurrent pregnancy loss (RPL). We conducted this study to determine the role of thrombophilic inherited and acquired factors in the etiology of infertility in 150 families. We analysed clinical, laboratory and genealogical data of families in two groups: in the first group we enrolled women who had had at least two IVF failure (n=75), but no deliveries. Second group consisted of women (n=75) who had conceived spontaneously and had uneventful pregnancies (at least two pregnancy loss previously). All the women underwent a complete screening for congenital (factor V Leiden-FVL, factor II c.20210G>A, antithrombin, proteiin C, proteiin S deficiency, MTHFR c.677C>T and c.1298A>C) and acquired (APC resistancy) thrombophilia risk factors. Conventional cytogenetical analysis was performed in all women and their partners. Inherited thrombophilia mutations were revealed in 82.4% of in the first group versus 64.8% in RPL group. APC resistancy was diagnosed in 3% among IVF failure patients and 30% in RPL patients. About half of the cases in both groups it was caused by F V Leiden mutation. No differences was found between groups concerning MTHFR mutations. Combined thrombophilia (the presence of two genetic factors of thrombophilia) was present in 5% of first and 12% among second group of patients. Our data suggest that factor V Leiden mutation alone or combined with acquired factors can have a role in recurrent fetal loss in families without conceiving problems.

P04.20

Application of a Scoring System in PGD for late onset diseases and cancer predispositions.

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Introduction: PGD is widely used for cancer predisposition mutations with variable penetrance and additional late onset diseases. The number of families affected by these conditions that opt for PGD increases continuously as molecular techniques are advancing. Nonetheless, the indication for PGD in these cases keeps raising ethical issues.
Aim: to summarize our experience in PGD for couples affected with late onset diseases following the evaluation of each case by a quantitative Scoring System (SS).
Methods: PGD was performed by embryo biopsy, followed by single cell multiplex nested PCR for the familial predisposition mutation and for 4-8 flanking polymorphic markers. The devised SS considers disease characteristics (onset, severity, penetrance, inheritance pattern) and patient clinical variables (carrier status, infertility, objection to terminate pregnancy, additional genetic syndrome) resulting in an absolute numeric value for every patient.
Results: the evaluation of 31 couples by the SS showed that some conditions such as FAP or Huntington definitely justify PGD while others like BRCA or HNPCC require the contribution of patient variables in order to justify inclusion into PGD program. Seven healthy pregnancies were achieved.
Conclusion: the employment of a SS that takes into account the disease characteristics as well as the patients' clinical variables allows for the objective determination wherein PGD is justified. We envision that the continuous discovery of cancer predisposition and late onset mutations will highlight PGD as the most appropriate reproductive option for preventing the perpetuation of severe inherited predisposition in families with several affected or deceased members.

P04.21

Interleukin 6 polymorphism in recurrent pregnancy loss

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Introduction
About 15 - 20% of clinically recognized pregnancies end in miscarriage among Caucasians. The etiology of recurrent pregnancy loss remains unclear and the possible immunological etiologies of pregnancy failure have been intensively investigated.
The maternal immune system confronts the embryo/ fetus with a host

defense reaction, based on the recognition of paternally derived antigens. Cytokines have been described to play a major role in the pathogenesis of recurrent miscarriage.

Objective

We purpose to investigate the relationships between recurrent pregnancy loss and single nucleotide polymorphism - 174 G / C in the promoter region of the interleukin 6 gene in Romanian population.

Material and methods

The diagnosis of RPL was based on a documented history of at least two spontaneous consecutive miscarriages. Each woman underwent a diagnostic work - up to rule out a verifiable cause for the recurrent miscarriage.

We studied 37 women with recurrent spontaneous abortions (>2) and 40 women experiencing at least one live birth and no abortions referred to Life Memorial Hospital. DNA extraction and PCR were employed to genotype women for the presence of a polymorphism in the promoter region of interleukin 6 gene.

Results

There was a not significant difference in the - 174 C/G genotype frequency (GG vs. GC/CC) between the women with RPL and controls (p value = 0,47). We did not detect any homozygote for C allele in 77 subjects.

Conclusion

The interleukin 6 polymorphism investigated was not associated with recurrent pregnancy loss in Romanian population.

P04.22

Iron deficiency and targeted deletion of iron regulatory protein 2 affect sperm motility and male fertility

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The iron metabolism is regulated both, at the systemic and cellular level, amongst others, by the iron regulatory proteins (IRP) 1 and 2. IRP2 regulates proteins involved in iron transport and storage such as transferrin receptor 1 (TfR1), ferroportin and ferritin. *Irp2* knock out mice exhibit misregulated expression levels of these proteins and develop anemia and a late onset progressive neurodegeneration.

Infertility of *Irp1*$^{+/-}$/*Irp2*$^{-/-}$ breeding pairs and the abundant expression of IRP2 in the testis of wild-type mice indicate an important role of IRP2 in the regulation of testicular iron homeostasis. This project focused on how testicular iron metabolism, spermatogenesis and spermiogenesis are affected by IRP2 deficiency. Spermatogenesis and fertility of *Irp2*$^{-/-}$-mice are not affected. However, sperm motility of IRP2-deficient males is significantly enhanced in comparison to age-matched C57BL/6J wild-type controls.

We kept *Irp2*$^{-/-}$-mice and age-matched controls on a low iron diet in order to analyze if the increased sperm velocity is a result of iron deficiency and if lack of iron could affect spermatogenesis and male fertility in general. While sperm of wild-type mice on a low iron diet mimic the behaviour of *Irp2*-deficient mice under normal conditions, sperm motility and fertility of homozygous *Irp2* knock out mice on a low iron diet are significantly reduced. Whether this effect is mediated by structural abnormalities of the sperm flagellum and/or due to a lower ATP production in IRP2-deficient sperm is currently under investigation.

P04.24

Array-CGH analysis in male infertility

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In order to identify genetic causes of male infertility, many individual genes have been studied for the presence of mutations in infertile patients. However, these studies were rather disappointing. Therefore, we looked for the presence of copy number variations (CNVs) in 9 infertile men with a maturation arrest of spermatogenesis and in 20 control males with normal sperm parameters. After several elimination steps, 7 regions remained. These regions, including four deletions and three duplications, were further investigated by qPCR in patients and in a large number of controls. The 4 deletions were present in heterozygous form and contained the SLC25A24, FAM82A1, C17ORF51 and SIRT4 genes. By sequencing no mutations were detected in the non-deleted copies of these genes. The three duplications involved the genes THRAP3+C1ORF113, SYT6 and PLSCR2. Due to their known function the genes SYT6, PLSCR2 and SIRT4 have a potential role in male infertility but are presumably not involved in maturation arrest at the spermatocyte stage. SLC25A24 has two transcript variants of which only variant 2 is testis-specific. Besides multiple heterozygous deletions of the start codon region, we detected one patient with a maturation arrest of spermatogenesis (not tested by array CGH) who was having a homozygous deletion of this region. More patients and controls are being investigated. In addition, immunohistochemistry is performed to determine the localization of the SLC25A24 proteins in testicular tissues. The role of the genes FAM82A1 and C17ORF51 is under investigation. These studies will clarify whether these genes are linked to male infertility.

P04.25

A methylation-sensitive SNP in PIWIL2 is associated with male infertility

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Epigenetic mechanisms have recently emerged as playing a crucial role in the pathogenesis of various common diseases. We hypothesized that aberrant DNA methylation patterns also contribute to the causes of male infertility. The aim of this study was to identify CpG loci differentially methylated in infertile men as compared to fertile male controls.

Using the HumanMethylation450k BeadChip DNA from peripheral blood cells obtained from 33 infertile men, as well as 10 fertile male controls was analyzed. A total of 596 differentially methylated CpG loci were revealed by this approach (p<0.001). These genes were enriched for PIWIL family members (Enrichtment=39.32). As PIWILs are involved in the regulation of spermatogenesis, we further focused on this group of genes.

Notably, one differently methylated CpG site, which was located in the S-shore region of PIWIL2 showed a significantly lower methylation level in patients as compared to controls (p<0.01). Remarkably, DNA methylation at this site showed a bimodal distribution with very low levels in a subgroup of 8 patients (mean methylation = 37.8% vs. 83.7% in controls). Further evaluation revealed that this site contains a rare single nucleotide polymorphism (SNP) and that lower methylation levels in the affected patient subgroup were reflected by heterozygosity for this SNP. Based on our findings we propose that heterozygosity for a methylation-sensitive SNP in PIWIL2 is associated with male infertility.

P04.27

Y chromosome haplogroups do not confer susceptibility to partial AZFc deletions in Tunisan infertile men

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Introduction: Partial AZFc deletions related to testis-specific gene families are common mutations of the Y chromosome, but their contribution to spermatogenic impairment is still unresolved, and the risk factors related to the onset of microdeletions remain unknown. With this in mind, we investigated the possible association between Y chromosome haplogroups and predisposition to partial AZFc deletions a Tunisian population. Materiel et methodes : The study involved 216 infertile patients (68 azoospermic, 63 oligospermic and 85 normospermic). Identification of haplogroups was made by PCR using a set of binary markers. We also screened partial microdeletions of Y chromosome AZFc region by polymerase chain reaction (PCR) according to established protocols. Redultats : Eleven haplogroups were identified (E3b2, J1J*, E1, E3b*, F, G, K, P/Q, R*, R1and R1a1), with a high frequency of E3b2 (35.18%) and J1J* (30.9%), knowing that E3b2 is the most frequent haplogroup in the north African populations. Only 30 patient carried a partial AZFc microdeletion (13.88%). Gr/gr was found in 80% of patients (24/30) with seven different haplogroups (E1, E3b*, E3b2, F, J1J*, R1a1* and R1). E3b2 was the most frequent (54.16%). However, the frequency of J1J* was only 16.66%. Conclusion : This study suggests lack of significant evidence of increased of AZFc partial microdélétion in a specific haplogroupe of Y chromosome in Tunisian infertile men.

P04.28

Application of mFISH in identification of supernumerary marker chromosome in a female with infertility

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Supernumerary marker chromosome (SMC) belongs to a heterogeneous group of structurally rearranged chromosomes, it is difficult to be identified due to small size. The occurrence of SMCs is 0,1-0,72/1000 in phenotypically normal individuals (Blennow et al., 1994).
After introduction of multiplex fluorescrence in situ hybridization (mFISH) it has become possible to investigate origin of SMCs.
In our case a phenotypically normal 27-year-old female with infertility (3 years) was analysed after two unsuccessful ICSI courses. The cytogenetic analysis identified mosaicism of cells with SMC. The female patient's karyotype was 47,XX,+mar[15]/46,XX[20]. The karyotype of her husband was normal.
The following banding methods for studing SMC were used: GTG, QFQ, CBG,DA/DAPI. The SMC appeared to be of a small size, metacentric, bisatellited,DA/DAPI positive at both centromeres. mFISH was performed using Spectra Vysion Assay (Abbott Inc, USA) and the Applied Imaging Spectra Vysion Imaging System.
SMC was identified as an inverted duplication of chromosome 15.
This is the most widespread group of SMCs - approximately 50% of healthy SMCs carriers have SMC derived from chromosome 15(Liehr et al., 2004). A review of studies involving this type of SMCs suggested that they can be divided into several groups. Our case belongs to the group with those SMCs that are inv dup (15) with breakpoints in 15q11 and contain very little or no euchromatic material and exert no phenotypic effect.
It should be taken into account that when the next ICSI procedure is successful, prenatal diagnosis should be subsequently performed.

P04.29

Non-disjunction of the bivalent is not the predominant mechanism leading to aneu-ploidy in humans

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Results obtained by array comparative genomic hybridization (aCGH) of first polar bodies (PB1) suggested that non-disjunction of the bivalent is not the predominant mechanism leading to aneuploidy in humans (Gabriel et al., 2011) and that a greater number of chromosome segregation errors taking place at second meiotic division (MII) compared with first meiotic division (MI) (Fragouli et al., 2011). The aim of our study was to confirm both observations by analyzing the segregation errors of chromosomes X, 13, 15, 16, 17, 18, 21 and 22 in PB1 and PB2 in a large cohort of patients (3307 cycles) aged 24 to 48 years.
PBs were analyzed by FISH with 5-chromosome probes (15 846 oocytes from 2958 cycles) or with 8-chromosome probes adding chromosomes X, 15 and 17 (1970 oocytes from 394 cycles). The data were allocated to the age groups ≤ 35 years, 36 to 40 years and > 40 years.
Our data confirm the observations by Gabriel et al., 2011, that premature chromatid seperation is the predominant mechanism leading to aneuploidy in humans (Angell's hypothesis). In oocytes of reproductively older women there is an excess of chromosome errors originating from MII compared with MI. The data suggest that the decrease in fertility of older women is due to a significant increase in segregation errors both in MI and to a larger extent in MII. These data were supported by preliminary data from 36 cycles analyzing all chromosomes in PB1 and PB2 from 202 oocytes by aCGH.

P04.30

Functional assessment of mismatch repair

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Introduction: Mismatch repair (MMR) plays an important role in repairing mismatched bases and insertion/deletion of loops. MMR genes were shown to be expressed in the early stages of mammalian development. However, the gene expression profile alone cannot determine the activity/functionality of the repair pathways. Investigating the repair capacity of MMR proteins in preimplantation embryos is important since the rates of cell proliferation and DNA replication are high in the early embryo. This study aims to develop a functional assay to assess the efficiency of MMR in preimplantation embryos.
Methods: Complementary synthetic oligonucleotides were used to form a heteroduplex DNA fragment of 180 base pairs in length. The constructs were exposed to nuclear/whole cell extracts. Semi-quantitative analysis of the mismatched base was performed by minisequencing. Control studies were carried out in the absence of any nuclear/whole cell extracts. MMR efficiency was also studied in four mouse blastocysts. MMR efficiency was assessed as described for the nuclear/whole cell extracts.
Results: Heteroduplexes were repaired in nuclear and whole cell extracts. Control studies in the absence of nuclear and whole cell extracts showed no repair. Repair was observed in the mouse blastocysts though the efficiency was not as high as the nuclear/whole cell extracts.
Conclusion: MMR efficiency in designed mismatched heteroduplexes was successfully analysed in the presence of nuclear/whole cell extracts and in mouse blastocysts. This assay can easily be modified to detect different/multiple mismatches with different lengths in addition to modifying the construct into assessing insertion/deletion loops with different sizes.

P04.31

Cytogenetic analysis of 102 missed and spontaneous abortions in the western regions of Ukraine

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Background: Approximately 15% to 20% of pregnancies result in spontaneous miscarriages and missed abortions and most often occur in the first trimester. In 60-80% of cases they are caused by chromosomal changes in the embryo/fetus. The present study displays frequency and spectrum of chromosomal abnormalities in embryos derived from missed and spontaneous abortions in the western regions of Ukraine.
Methods: The study population consisted of 102 embryo tissues from women with the final diagnosis of missed (99 cases) or spontaneous (3) abortions. Cytogenetic analysis was performed in the uncultured chorionic villi samples, using standard G-banding.
Results: Karyotype results were obtained in 50 of 102 cases (49%), one of them from spontaneous abortion. Among the abortions the gonosomal constitution of XY prevailed (n = 36), followed by XX (n=14). Chromosomal abnormalities were found in 25 cases (50%): autosomal trisomies in 9 cases (36%), gonosomal trisomies 47,XXY in 2 cases (8%) and triploidy in 14 cases (56%) (9 XXY : 5 XXX). Autosomal trisomies involved chromosome 2 (one case), 16 (tree cases), 19 (one case), 20 (one case, spontaneous abortion) and 22 (tree cases). One case of triploid abortion showed mosaicism with chromosome 20 - 70,XXX,+20/69,XXX.
Conclusions: Conventional methods of cytogenetic investigation of material from missed abortions produce results in half of the cases. Presence of Y chromosome in 72% of incidents shows prevalence of male gender in affected pregnancies. Triploidy was predominant followed by autosomal and gonosomal trisomy. Among autosomal trisomies, chromosomes 16 (12%) and 22 (12%) were prevalent.

P04.32

Identification of differentiallymethylated genes in the extraembryonic tissues of human embryos with mosaictrisomy 16

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Chromosomal mosaicism results from mitotic non-disjunction in aneuploid or primarily euploid cell. However, the inducing factors of mitotic instability are poorly investigated. Earlier we showed
the high frequency of epimutations of two cell cycle control genes (*RB1* and *P14ARF*) in the extraembryonic tissues of miscarriages with mosaic trisomies involved different chromosomes. So, we can not exclude that the increase of a certain chromosome dose may lead to abnormal epigenetic status of genes. The present work was aimed to identify a spectrum of differentially methylated genes in the cytotrophoblast (CT) and in the extraembryonic mesoderm (EM) of 13 miscarriages with trisomy 16, and 7 first trimester induced abortions using HumanMethylation27 BeadChip (Illumina). The level of aneuploid cells was 4-93% in the CT and 65-98% in the EM. There were no differentially methylated CpG-sites in the CT with pure trisomy (≥90% of trisomic cells) and in the EM, whereas there were 20 hypermethylated CpG-sites in 20 genes in the CT with mosaic trisomy (<90% of trisomic cells). These 20 differentially methylated genes belong to the following functional groups: transport (*ABCC3, P2RY6, PDZD3, PKDREJ, SEC31B, SLC17A4, SLC22A18, TRPV6*), signal transduction (*ADRB3, P2RY6, PDZD3, PKDREJ*), cell proliferation (*THBS4*), cell death (*NOL3*), response to stress (*ADRB3, NOL3, TIMP3, TP53TG1*), cell adhesion (*TECTA, THBS4*), etc. Thus, hypermethylation of these genes is most likely associated with trisomy rescue events and mosaicism origin but not to the increase of chromosome 16 dose. This study was supported by Federal Program P1161.

P04.33

Polymorphism of folate cycle and integrins genes and pregnancy loss

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The causes of pregnancy loss are diverse. Polymorphism of candidate genes may influence on the early stages of embryogenesis. The role of gene polymorphisms of folate cycle, and coagulation factors in pregnancy loss is discussed. We analyzed the frequency of gene polymorphism MTHFR (C677T), MTRR (A66G), MTR (A2756G), ITGB3 (T1565C), ITGA2 (C807T), FGB (-455G-A), PAI 1 (-675 5G/4G) among 124 women with pregnancy loss in the first trimester. The control group included 114 women with normal pregnancy. The frequency of the investigated genotypes among two groups of women is not different. The exception was the gene methionine synthase. The frequence of 2756GG genotype was three times higher in the group of women with pregnancy loss than control (OR = 3.71). The frequency of allele 2756G MTR gene was 0.26, which exceeds the controls (P = 0.028). The number of homozygotes-455AA FGB was 2.6% in the control group and 6.5% in the comparison group (OR = 2.79).The combination of gene polymorphisms of folate cycle and integrins increase risk of pregnancy loss in the first trimester. The frequence of integrins polymorphisms did not differ in women with pregnancy loss and control group. However the combination of polymorphisms of three genes (MTRR, MTR ITGB3) increases the risk of pregnancy loss is 5 times.

P04.36

Birth of healthy twin after implanting a singleton male embryo, a hemophilia PGD case

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A family with one hemophiliac child who had come for prenatal diagnosis (PND), requested to have a healthy child via preimplantation genetic diagnosis (PGD). The affected boy's mutation had been characterized in the previous PND and the mother was an obligate carrier. For performing PGD we tested several STR markers on the affected boy and his mother's DNA to determine the informative ones. Reprogrammed induction of super-ovulation was performed by HCG injection and 34 to 36 hours later oocyte were picked up using ultrasound guide. Three days after insemination, embryo biopsy was performed using SATURN laser. The biopsy pipette was inserted through the hole, and the selected blastomere was removed gently by aspiration. In total eight cells were removed from eight embryos. Each cell was analyzed by two rounds of multiplex nested PCR for STRs and direct DNA sequencing. Only one healthy male and two none carrier female embryos were identified. The mother insisted on the transfer of only the male embryo. Pregnancy progressed well and sonography in week 8 indicated twin pregnancy. The mother was disappointed in having twin boys and decided to terminate pregnancy. Intensive counseling was provided and she was persuaded to continue the pregnancy. Both fetuses were checked for hemophilia during 11th weeks of gestation using CVS. Chromosomal abnormalities were checked by means of QF PCR and karyotyping. The children were delivered with no complication and later coagulation analysis revealed no complication. The mother is now happy in deciding to continue the pregnancy to term.

P04.37

Translocation and aneuploidy analyses of polar bodies and trophectoderm cells using 24sure: First results in a clinical setting

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Objective: Recent studies have shown that biopsy of polar bodies (PB) from oocytes and trophectoderm cells (TE) from blastocysts followed by array comparative genomic hybridisation (aCGH) might be a good strategy for the detection of genomic imbalances in human embryos. The combined detection of aneuploidies for all chromosomes and of unbalanced aberrations in just one experiment makes aCGH-analyses very helpful, especially in gametes and embryonic cells from translocation carriers. Here, we report our first experiences and data from aCGH analyses in a real clinical setting.

Methods: Polar bodies of more than 20 cycles and trophectoderm cells of more than 10 cycles (including different translocation carriers) were biopsied. The samples were amplified by whole genome amplification (WGA) using the SurePlex Kit [BlueGnome] and their genomes examined by aCGH using 24sure and 24sure+ Cytochips [BlueGnome].

Results: aCGH results of more than 150 PB- and more than 50 TE-samples are presented. Technical problems and methodical limitations are discussed. Different strategies are shown for practicable and cost effective analyses of polar bodies and trophectoderm cells in a clinical setting using 24sure and 24sure+ Cytochips.

Conclusion: Molecular karyotyping by aCGH allows a combined detection of aneuploidies and structural aberrations and therefore, may help to identify euploid embryos with higher implantation potential.

P04.38

Detection of PRDM9 variations in patients with meiotic disorders

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In the last seven years genetics and molecular biology have begun to uncover the role of PRDM9 in mammalian meiosis as a major determinant of meiotic recombination hotspots. PRDM9 contains an N-terminal KRAB domain, a central SET domain and a C-terminal tandem-repeat zinc-finger array. It is known that the zinc-finger array serves for binding to the DNA and marking the hotspots and that the H3K4 trimethylase activity of the SET domain could be responsible for activating these hotspots by chromatin remodeling. The zinc-finger array is coded by a variable microsatellite (differences in nucleotides and number of microsatellites).

Due to its role in homologous recombination, mutations/variations in PRDM9 might be associated with different pathologies of meiotic origin. Mutations in any of its domains could be the cause of some cases of infertility. Moreover, according to several authors, variation within the PRDM9 zinc-finger domain might be involved in the mechanisms responsible for de novo genomic disorders that result from unequal meiotic exchanges at minisatellites.

Therefore, the PRDM9 gene has been sequenced in 3 distinct groups (controls=83, infertile=14, transmitting parents of de novo rearrangements=20) in order to analyze the presence of mutations/variations associated with infertility and the origin of de novo genomic disorders. Preliminary results show the presence of SNPs and uncommon variants of the zinc-finger array in all groups. However, this presence has not been associated with any statistical significance.

Further studies including a bigger number of analyzed samples will contribute to determine the role of PRDM9 in pathologies of meiotic origin.

P04.39

Investigation of Angiotensin II Type 2 Receptor Gene A1675G Polymorphism and Distribution of Genotypes in Preeclampsia and Normal Pregnancies

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Angiotensin II type 2 receptor (AT2R) gene polymorphism in the development of preeclampsia in pregnant woman might be thougt to play an active role. Therefore, we assessed whether AT2R gene A1675G polymorphism increase the risk of preeclampsia.The study cohort was a group of 95 woman with preeclampsia and 78 healthy pregnant woman subjects from the general turkish population. We were studied, by RFLP analysis, to validate the role that the AT2R gene A1675G polymorphism plays in preeclampsia. Among controls, the AT2R Hyp188III genotypes of G/G, A/G, and A/A were observed in 10%, 49%, and 41% , respectively, whereas the G/G, A/G, and A/A genotypes were observed in 24%, 45%, and 31% of case patients, respectively. The G/G genotype of the Hyp188III site in the AT2R gene were associated significantly with the risk of developing preeclampsia.Polymorphisms of AT2R gene in the renin angiotensin system pathway were associated significantly with preeclampsia risk.

P04.40

A genome-wide expression profile of decidual tissue in preeclampsia

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Preeclampsia is a common pregnancy-specific disorder with unknown etiology diagnosed in 5-17% of pregnancies. It is the leading cause of maternal and perinatal morbidity and mortality. Candidate genes associated with preeclampsia have not been fully described. To investigate how the expression of maternal genes contributes to the mechanisms underlying the progression of the disease, we investigate global placental gene expression in preeclampsia using microarray technology. Genome-wide transcriptional profiling was performed on decidua basalis tissue from preeclamptic (n=10) and normal (n=11) pregnancies. Among the 26000 genes that were screened, 79 were found to be differentially expressed between normal and pre-eclamptic tissues. Among these candidates, 59 were up-regulated and 20 were down-regulated. The up-regulated genes included *LEP, BHLHB2, SIGLEC6, RDH13, BCL6, SYDE1,* which are well-known differentially expressed genes for pre-eclampsia, as well as *CORO2A, CEBPA, HK2* which was recently proved to be linked with the etiology of this disease. Gene ontology analysis further revealed several biological processes that could be associated with the development of pre-eclampsia, including response to stress, immune system process, regulation of cell communication, intracellular signaling cascade etc. Furthermore, when our patients were classified as cases of mild or severe pre-eclampsia, the expression of 10 genes could be correlated with the severity of this disorder. This finding may provide insight into the pathophysiology of the disorder and lead to new therapeutic possibilities for this disease. This work was supported by the Russian Foundation for Basic Research.

P04.41

Polymorphisms in the ANXA5 gene promoter in Japanese women with pre-eclampsia

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Annexin A5 (ANXA5) forms an antithrombotic shield on the apical surface of syncytiotrophoblasts in the placental villi. A common haplotype called M2 consisting of minor alleles of four SNPs in the promoter region of the ANXA5 gene is more frequent in women with recurrent pregnancy loss. In this study, association between the M2 and pre-eclampsia (PE) was examined. Placental DNAs from 47 Japanese PE patients and 50 normotensive controls were genotyped. The M2 was recorded in 12 out of 47 PE placentas (25.5%) but 5 out of 50 controls (10%), and the difference was statistically significant (P=0.04). However, when maternal blood samples were genotyped, the M2 was observed only in 7 out of 34 PE patients (17.5%) and 5 out of 22 controls (13.2%) (P=0.66). In case of placentas carrying the M2, mothers were also found to carry the M2 in 2 out of 4 (50%) in the control group, and the frequency was not high in PE group (4 out of 6, 67%). Placental expression of ANXA5 was examined by qRT-PCR, but not different between PE and controls (P=0.71). However, the expression levels were lower in M2 carriers than in non-carriers in each group (P=0.076, P=0.004). Our data might indicate that hypomorphic alleles in the ANXA5 promoter in placenta, not in maternal blood, are essential to the onset of PE, and that the reduced expression of ANXA5 in placental villi carrying the M2 might be associated with local hypercoagulable state possibly leading to the onset of PE.

P04.42

Preimplantation genetic diagnosis (PGD) after trophectoderm biopsy - results from 2010 to 2012

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The German Supreme Court (BGH) stated in July 2010 that preimplantation genetic diagnosis, PGD (or PID) is in accordance with the Embryo Protection Act (ESchG) and can be offered couples at risk for monogenic diseases and chromosomal aberrations. Here we present the results of PGD for monogenic diseases as well as for reciprocal and Robertsonian translocations employing trophectoderm (TE) cells from day 5 blastocysts. Protocols for mutation analysis for a number of genes involved in severe monogenic inherited disorders were established and optimized to fit into the short time span of 24 hours between biopsy and embryo transfer. PCR strategies for mutation detection involved either indirect methods (linkage analysis with polymorphic markers), or direct mutation detection by sequence or fragment length analyses. For couples, who are carrier of a reciprocal or Robertsonian translocation, array CGH (24sure technology) was performed. Here, only a minority of TE samples (approx. 33%) revealed an euploid, balanced result, whereas the majority of samples (approx. 66%) were aneuploid showing an unbalanced karyotype due to the balanced translocation in the parents, or due to aneuploidies of other chromosomes than expected from the parents´ karyotype. In the majority of cases, at least one embryo was unaffected, which led to a transfer of one or two embryos in each cycle. Our results of >100 samples clearly demonstrates the reliability of trophectoderm biopsy for PGD. Even more, the pregnancy rate strikingly increased to a rate above 60% per embryo transfer.

P04.43

First pregnancy after preimplantation genetic screening (PGS) in a balanced translocation carrier with 46,XX,t(4;14)(q25;q32.1) karyotype

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We report on the outcome of assisted reproductive treatment comprising PGS of a couple with a healthy daughter and the desire for a second child. The 33 year old consulter was diagnosed as balanced translocation carrier of a t(4;14)(q25;q32.1), her non-consanguineous partner showed a normal karyotype. They reported a history of seven pregnancy losses. Cytogenetic diagnostic on the most recent product of conception revealed a genetic cause demonstrating in the karyotype of 46,XX,der(14)t(4;14)(q25;q32.1)mat. The couple agreed to array based PGS, which was performed by trophectoderm analyses of five day 5-blastocysts. While embryos were vitrified, laser dissected biopsies of two to six cells each were subjected to whole genome amplification and then hybridized to 24sure+ microarrays utilizing the SurePlex DNA amplification system and the 24sure protocol (BlueGnome, Cambridge, United Kingdom).

Although presenting with normal morphology, four of the blastocysts were assigned a minimal potential for nidation and normal development due to the presence of multiple aneuploidies. As suspected, three of them displayed partial and whole chromosome imbalances of chromosomes 4 and 14 originating from maternal derivatives. Interestingly, two of them harboured a plethora of imbalances affecting additional chromosomes, another one a trisomy 9 as sole abnormality. One biopsy appeared euploid and the respective embryo was transferred. Ongoing pregnancy was confirmed biochemically and by ultrasound. This case illustrates the benefit of PGS for translocation carriers with chromosomal breakpoints leading to a low risk for the birth of a child with imbalanced karyotype but elevated risk for still births or early pregnancy losses.

P04.44

Cytokine gene expression in women with embryo loss

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Recurrent miscarriage is often defined as three or more consecutive miscarriages. While it has a number of causes, the more important ones relate to chromosomal and genetic abnormalities and autoimmunity. An aberrant maternal immune response, whereby the requisite adaptations to support conceptus development fail, is one likely mechanism contributing to unexplained recurrent miscarriage. In this study, we examined expression of IL-1β, IL-6, IL-10, and TNF-α in human chorion and endometrium tissues from women with normal and miscarriage pregnancies using quantitative RT-PCR assays. We found that IL-1β mRNA expression is higher in endometrium than in the chorion both in miscarriage and normal pregnancy. In miscarriage expression level is higher than in normal pregnancy (in the chorion and endometrium). IL-6 mRNA expression lower than half the samples in the endometrium than in the chorion and in miscarriage expression level lower than in normal pregnancy. IL-10 and TNF-α mRNA expression in two compared groups did not differ. Inadequate expression of IL-6 and IL-1β mRNAs in chorion and endometrial tissues may predispose to recurrent miscarriage through a perturbed maternal immune response, effects on decidual tissue remodeling and angiogenesis, or dysregulated trophoblast differentiation and invasion.

P04.45

Gene mutations of EG-VEGF and its receptor genes in patients of recurrent pregnancy loss

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Recurrent pregnancy loss (RPL) is a multi-factorial disorder and up to 50% of cases remain undetermined causes after detailed clinical examination. Angiogenesis plays a critical role in early gestation and endocrine gland-derived VEGF (EG-VEGF) is a newly found angiogenesis-associated gene. The role of EG-VEGF and its two receptor genes (PKR1, PKR2) in human early pregnancy was believed to have a direct effect on both endothelial and trophoblastic cells and are likely to play important roles in placentation. We previously found gene polymorphisms of PKR1, PKR2 were significantly associated with human RPL using tag SNP analysis. We now direct sequenced these genes in 100 RPL patients and 100 normal controls, trying to fine map the variation sites that interfere with early pregnancy and further functional validate in vitro studies. We found allele and genotype frequencies of PKR1(I379V) and PKR2(V331M) were significantly higher in the normal controls and may play protective roles in RPL (p<0.05). Both variants induced nonsynonymous change of amino acids and located in the intracellular C-terminal domains of G protein-coupled receptors. We further demonstrated PKR1(I379V)- and PKR2(V331M)-overexpressed cell had altered intracellular calcium influx and significantly higher ability of cell invasiveness in both HEK293 and JAR (trophoblast) cell lines. We therefore concluded that PKR1(I379V) and PKR2(V331M) may play protective roles in preventing RPL by altering intracellular calcium signaling and enhancing trophoblast cell invasion ability.

P04.46

The distribution of HLA-G 14 bp insertion/deletion polymorphism and IL-10 SNP -1082G/A,- 592C/A, -819C/T in the case of recurrent pregnancy loss (RPL)

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Pregnancy prolongation considerably depends on non-classical HLA-G antigen and is associated with HLA-G 14bp (+)insertion/(-)deletion polymorphism. HLA-G gene transcription in trophoblast cells is under control of cytokines secreted by placenta. The interregulation of HLA-G and IL-10 genes expression is discussed. The aim of our research is to analyze the distribution of HLA-G 14bp insertion/deletion polymorphism and IL-10 SNP-1082G/A, -592C/A, -819C/T among the families with RPL. Methods: DNA extraction from peripheral blood cells and chorionic villi, PCR, agarose gel electrophoresis. Results: 140 women with RPL, 86 spontaneously aborted embryos and 100 reproductively healthy women have been observed. Significantly higher HLA-G gene genotype +14bp/+14bp frequency has been shown in the group of women with RPL (P<0.05) and in the group of spontaneously aborted embryos (P<0.05) in comparison with the control group. The presence of this genotype in women, or embryos is associated with 3-fold increased risk of RPL (OR = 3.41, CI: 0.98-11.85 and OR = 2.75 CI: 1.10-6.90). The analysis of distribution of IL-10 SNP-1082G/A, -592C/A, -819C/T genotype has shown the significantly higher 1082GG-genotype (P<0.01) and 592CC, 819 CC-genotypes (P<0.05) frequency in the group of women with RPL in comparison with the control group. The increasing of risk of RPL up to 4 times with 1082GG-genotype (OR=3.43; CI: 1.72-6.84) and 592CC, 819 CC-genotypes, (OR=3.87; CI: 1.23-12.20) has been established. Conclusions: HLA gene mutations and changes in the genes which interact with HLA-system can cause the reproductive dysfunction among women and lead to early fetal loss.

P04.47

Problems with Robertsonian translocations between chromosomes 13 and 14 may also cause problems for men.

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Robertsonian translocation is the most common type of chromosome rearrangement with a prevalence of 1 in 1000 in the general population. The most common subtype of Robertsonian translocation is seen between chromosomes 13 and 14. According to available literature, the der(13;14) women have a 1% chance of having a baby with trisomy 13, whereas the males with any Robertsonian combination have a slim chance - below 1%, of their children being affected.

We present three cases of unbalanced form of Robertsonian translocations detected by fluorescent in situ hybridisation, quantitative fluorescent polymerase chain reaction and spectral karyotyping.*The first case* is represented by a fetus in whom trisomy 13 syndrome 46,XY,+13,der(13;14)(q10;10) was revealed during prenatal screening. Consequently, oligohydramnion and cheilognathopalatoschis was observed in the fetus and the pregnancy was terminated. Translocation 45,XX,der(13;14) was found in the mother. Her medical history was significant for eight spontaneous abortions. The *second case* features a fetus with trisomy 13 syndrome 46,XX,+13,der(13;14)(q10;q10). The pregnancy was terminated because of multiple malformations. Balanced translocation 45,XY,der(13;14) was found in the father. In most cases couples carrying Robertsonian translocation may request preimplantation genetic diagnosis in order to select embryos with no genetic imbalance and hence increase their chances of a successful pregnancy. But our *third case* describes a couple evaluated for primary sterility - translocation 45,XY,der(13;14) was found in men. The couple had a history of three unsuccessful attempts on in vitro fertilisation with PGD. Their first spontaneous pregnancy ended in miscarriage. It was found karyotype 46,XX,+13,der(13;14)(q10;q10) from the sample.

P04.48

Role of TNF-α, IFN-γ, IL-6, TGF-β1 and IL-10 in Pathogenesis of Recurrent Pregnancy loss

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Aim : According to previous investigations, certain cytokines may play a role in recurrent pregnancy loss (RPL) and also some cytokine gene polymorphisms may affect the level of cytokine production. The aim of our study was to investigate the potential associations between IL-6 (-174), IL-10 (-1082, -819), IFN-γ (+874), TGF-β1 (codon 10/25) and TNF-α (-308) gene polymorphisms and RPL.

Method: A case control study was carried out in 49 RPL patients and 39 healthy control women. Cytokine genotyping was performed by PCR-SSP.

Results: RPL patients had significantly higher frequencies of TNF-α polymorphism in both GA genotype (high expression) (p=0.020) and A allele (p=0.026). No statistically significant differences were observed between groups in genotype and allele frequencies of IL-6 and IFN-γ genes. The haplotypes of TGF-β1 and IL-10 were compared in terms of their expressions and it was shown that the CC/GC, CC/CC, TT/CC, TC/CC haplotypes (low expression) of TGF-β1 had significantly decreased in the patients (p=0.049), whereas there were no statisticaly significant differences in the haplotypes of IL10 (p>0.05).

Conclusion: Our results showed that the high expression of TNF-α gene was associated with susceptibility PRL. The low expression of TGF-β1 gene may be a risk factor for the development of PRL. This would further help in efficient management of immunologically mediated recurrent miscarriages at the sample/individual level. This study which is **the first** to search eight polymorphisms of five cytokine genes at the same time in RFLP patients.

** E.O. and S.P. contributed equally to this work.*

P04.49

DNA fragmentation status in patient's with necrozoospermia

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Introduction : Necrospermia is still a poorly documented cause of male infertility, Several hypothesis have been made forward to explain the relationship between necrospermia and DNA fragmentation. Our aim was to determine if a relationship exists between the levels of sperm DNA fragmentation and necrospermia in infertile men.

Patients and methods: Semen samples obtained from 70 infertile men were analysed according to world health organization guidelines .The eosin-nigrosin viability test was performed and the percentage of viable and non viable sperm were assessed by counting a minimum of 100 spermatozoa. Patients were subdivided in three groups: normozoospermia, moderate necrozoospermia and severe necrozoospermia. DNA fragmentation was detected by TUNEL assay, approximately 500 cells were counted, and DNA fragmented index (DFI) was calculated. Results: The DFI was 9.28 ± 2.98 % in patients with normal level of dead spermatozoa, 20.25 ± 3.21 % in patients with moderate necrozoospermia, and 35.31 ± 5.25 % in patients with severe necrozoospermia. Sperm DNA fragmentation was significantly higher in patients with necrozoospermia. A strong correlation was found between the degree of necrozoospermia and sperm DNA fragmentation; In addition statistically significant correlations were found between the DFI level and sperm motility, abnormal sperm morphology. However no evident correlation was found between the DFI and sperm count or paternal age.

Conclusion: our study showed a high DFI in necrozoospermic men, particularly when necrozoospermia exceeding the proportion of 80 %. So severe necrozoospermia is a predictive factor for an increased risk of sperm DNA damage.

P04.50
Association study of single nucleotide polymorphisms in SLC6A14 gene with male infertility

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The gene SLC6A14, encoding for amino acid transporter related to appetite control has been found to be in association with X-linked obesity. The results of several studies point to an increased likelihood of abnormal semen parameters among overweight men. Possible association of three single nucleotide polymorphisms (SNPs) in the SLC6A14 gene and male infertility was the subject of our study. We have analyzed 123 infertile males of different ethnic origin (83 Macedonian, 30 Albanian, 10 of other origin) which have previously been diagnosed either with idiopathic azoospermia (50) or oligozoospermia (73) in comparison to 127 fertile men (98 Macedonians and 29 Albanians) as controls. The methodology included multiplex PCR followed by single nucleotide extension reaction and capillary electrophoresis on ABI 3130 Genetic Analyzer for detection of SLC6A14 303 A/T (SNP1), 20649 C/T (SNP2) and 22510 C/G (SNP3). The allele frequencies showed a significant difference between the infertile patients and fertile controls (p=0,044) only for the 22510 C/G SNP located in the 3’ untranslated region of the SLC6A14 gene (0,463 in infertile men versus 0,339 in fertile controls for the minor C allele). The distribution of haplotypes including the three SNPs, as well as only SNPs 2 and 3 that lie less than 2kB apart was also analyzed. The ACG and CG haplotypes were more frequent among fertile control men than among infertile patients (0,520 vs. 0,366; p=0, 014 and 0,661 vs. 0,537; p=0, 044 respectively). In conclusion, this is the first report that links the SLC6A14 polymorphisms with male infertility.

P04.51
PRM1 and PRM2 gene polymorphism in Czech and German men with idiopathic oligozoospermia

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The aim of our study was to verify the impact of the most frequent ACC haplotype formed by PRM1 230A>C and PRM2 298G>C/373C>A variants on spermatogenesis in Czech and German males with idiopathic oligozoospermia.

PRM1 and PRM2 sequencing was performed on 3130xl Genetic Analyzer in 52 men with idiopathic oligozoospermia, in 52 normozoospermic and in 75 Czech males with proven fertility. The three SNPs were also analysed by Taqman assays in 108 German males with less and 160 with more than 20 million sperm per millilitre.

In PRM1 we detected the common variant (230A>C) with an overall minor allele frequency (MAF) of 28.5% and three rare variants (c.54G>A, c.102G>T and c.166C>T) with overall frequencies of 0.56%, 0.28% and 0.28%, respectively. In PRM2 we detected the two common polymorphisms (298G>C and 373C>A) with overall MAFs of 49.1% and 29.3%, respectively, and two rare variants (c.201C>T, c.377C>T), both with overall frequencies of 0.28%.

Despite the ethnical difference the allele prevalences of the most frequent PRM1 and PRM2 polymorphisms are identical in Czech normozoospermic and Czech fertile as well as German normozoospermic males, except the four rare PRM1 and PRM2 SNPs were present only in Czech males. The prevalence of all detected variants and the ACC haplotype was not significantly different between men with idiopathic oligozoospermia and controls in Czech and German males. In conclusions, we could not confirm the impact of PRM1 and PRM2 gene variants on sperm counts

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P04.52
Investigation of mutations in the Synaptonemal Complex Protein 3 (SYCP3) gene among azoospermic infertile male patients in the Turkish population

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Objective: To investigate possible mutations and/or single nucleotide polymorphisms in the synaptonemal complex protein 3 (SYCP3) gene among non-obstructive azoospermic infertile males in a Turkish population.

Design: Nine exon deep intronic primers belonging to the SYCP3 gene were designed and amplified by PCR, and the nucleotide sequences were identified by automated DNA sequence analysis.

Patients: Seventy-five non-obstructive azoospermic infertile male patients were included in the study. These patients were unrelated to each other and had 46,XY chromosome structure without Y microdeletion. In addition, 75 individuals whose fertility was proven by reproduction were enrolled in the study as controls.

Main Outcome Measure(s): PCR and automated DNA sequence analysis to detect mutations and/or single nucleotide polymorphisms in the SYCP3 gene.

Results: No mutations were detected in the 9 exons of SYCP3. A total of 11 variations, however, were detected: 7 have been identified in the NCBI SNP database, whereas 4 have not.

Conclusions: Based on the results, we agree with the idea that SYCP3 mutations are not associated with the genetic susceptibility for meiotic arrest in infertile male patients with non-obstructive azoospermia in the Turkish population and that further studies investigating the other components of the synaptonemal complex protein (SYCP1, SYCP2) should be conducted.

P04.53
Factor II G20210A and factor V G1691A mutations and methylenetetrahydrofolate reductase C677T polymorphism in 155 women with repeated pregnancy loss

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Introduction: pregnancy is the process from the fertilized ovum to the fetus with capability of extra uterine survival. Pregnancy loss is the most common complication of pregnancies. About 1 in 300 couples and 0.5-2% of women are involved in repeated pregnancy loss (RPL). Various etiological factors involve in RPL and the main part of them remains unknown. Among them the thrombophilic factors are important.

Material and methods: Genetic counseling program was done for 158 couples suffering from RPL. Three molecular genetic variations were investigated in main thrombophilic agents: G20210A in factor II, G1691A in factor V and C677T in MTHFR gene. The method was PCR-RFLP.

Results: No G20210A mutation was found in Factor II gene. Heterozygote G1691 mutation in factor V gene was found in 3 women (1.94%). But, C677T polymorphism in MTHFR gene was found in 33 women (21.3%). Among them, 4 cases (12.12%) were homozygote and 29 cases (87.88%) were heterozygote.

Discussion: Assessment of variations in thrombophilic related genes can be useful in etiologic evaluation and planning of effective treatment in RPL women. Genetic counseling, clinical aspect of abortions and genotype-phenotype correlation should be considered for request of molecular thrombophilic tests in RPL.

P04.54
DNA fragmentation in chromosomal translocation carriers

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Carriers of a chromosomal structural abnormality, as reciprocal or robertsonian, have a normal phenotype but often have fertility problems. Infertility of men with chromosomal translocations could therefore be partly explained by high DNA fragmentation in sperm. The aim of this study was to analyze the sperm DNA fragmentation in translocation carriers.

One robertosonian translocation carrier 46,XY,der(13;14)(q10;q10), 3 reciprocal translocations carriers: 46,XY,t(6;19)(p22;q12); 46,XY,t(2;3)(q33;q29); 46,XY,t(1;5)(p22;q32),t(6;12)(q15;q21) and 5 fertile donors were recruited. Semen analysis was evaluated according to WHO guidelines (2010). The method used to assess sperm DNA fragmentation was the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay.

DNA fragmentation frequency in sperm from donors was 0,26±0,09%, whereas that from translocation carriers was 0,91±0,53%. Significantly increased rate was seen among oligoasthenoteratoozoospermia patients: 46,XY,der(13;14)(q10;q10) and 46,XY,t(2;3)(q33;q29) (1,4% and 1,35%, respectively). Sperm DNA fragmentation rate in 46,XY,t(6;19)(p22;q12) and 46,XY,t(1;5)(p22;q32),t(6;12)(q15;q21) teratozoospermic patients in both cases was 0,45%. In the present study, the DNA fragmentation rates were significantly different between the carriers of a chromosomal structural abnormality with abnormal and normal spermogram (p=0,0007). Therefore, the present results suggest that the DNA fragmentation rate may depend not only of the presence of a structural abnormality but also on the spermogram parameters.

In conclusion, the infertility of men carrying a chromosomal structural abnormality could be explained by the poor-quality semen, and/or the elevated rate of DNA fragmentation.

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P04.55

TSPY1 copy number variation and male infertility

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Testis-specific protein, Y-linked 1 (TSPY1) gene is located on the short arm of Y chromosome (Yp11.2) and is present as an array of approximately 18-76 gene copies. It encodes a testis-specific protein that is thought to have a role in sperm differentiation and proliferation. It has recently been suggested that TSPY1 copy numbers may influence spermatogenic efficiency. In this study, we compared the relative TSPY1 copy number between men with spermatogenic failure and fertile controls. The study group included 60 azoospermic men, 66 men with oligozoospermia and 119 fertile controls of similar ethnic origin. Relative TSPY1 copy number was determined by quantitative PCR compared to a single copy HPRT1 gene. Y chromosome haplogroups were determined by analysis of 28 single nucleotide polymorphisms (SNPs) by multiplex SNaPshot. Infertile patients showed higher mean dCt values in comparison with the fertile control men with a borderline statistical significance (p= 0,0785). Oligozoospermic men showed statistically higher mean dCt value when compared with the fertile controls (p=0,0170). This difference was even higher when Macedonians with oligozoospermia were compared with the Macedonian fertile controls (p=0,0098). The dCt differed between different Y chromosome haplogroups (p=0,0027), but no difference was observed between infertile and fertile men with the most common Y chromosome haplogroups. In conclusion, the initial results of the study investigating relative TSPY1 copy number in infertile men showed an association of TSPY1 gene copy number with oligozoospermia. Our results also showed that the TSPY1 gene copy number differs between different Y chromosome lineages.

P04.56

Association between ubiquitin-specific protease 26 (USP26) gene variations and male infertility in Iranian men.

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The human X chromosome is enriched with testis-specific genes that may be crucial for male fertility. Recently, mutations in ubiquitin specific protease 26(*USP26*) gene have been proposed to be associated with male infertility. This gene locates on $X_{q26.2}$. Some mutations and haplotypes on this gene have been proposed to be associated with male infertility. In this study, five different mutations on USP26 were investigated: 1737G>A,1090C>T, 370-371ins ACA,494T>C and 1423C>T. The study included 120 infertile men with non-obstructive azoospermia and 60 fertile men. Besides family history of reproduction, hormonal evaluation and semen analysis were performed. DNA was extracted from blood samples. PCR-SSCP, PCR-RFLP and PCR Product Cloning methods were used and resumed by sequencing to insuring about the mutations. Moreover, USP26 gene expression was studied by Real-Time PCR after RNA extraction followed by cDNA synthesis from testis biopsy in obstructive and non obstructive azoospermia patients. Surprisingly the mutation frequency was the same in both groups (6.67%). The results indicate that there is a haplotype between three observed mutations in Iranian population include:370-371insACA, 1423C>T and 494T>C,as reported before in some other populations.This haplotype was seen in the control group as well. Serum testosterone concentrations and testicular volume did not differ in the mutation positive group compared with the non-mutation group. About the USP26 gene expression, there was no significant difference among these two groups. These results indicate that these alterations might not be involved in male infertility in Iranian population and their role in infertility is still controversial.

P04.57

Molecular characterisation and analysis of spermatozoa in an infertile man with oligozoospermia and mosaicism of an unbalanced Y;autosome translocation

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We report on an infertile male patient with an unremarkable general medical history and clinical examination including genital exam. He had severe oligozoospermia in two semen samples with slightly elevated FSH levels of 7.6 U/l and otherwise normal hormone values.

Conventional cytogenetic analysis on peripheral lymphocytes revealed a mosaicism of cells with an unbalanced Y;autosome translocation found in 10 metaphases and in 20 metaphases an apparently normal karyotype summarised as 45,X,der(Y)t(Y;21)(q12;q21),-21[10]/46,XY[20]. FISH analysis demonstrated the presence of the subtelomeric probe DXYS224 on the derivative Y chromosome indicating a breakpoint closely to the telomere region of Yqter. CGH analysis using the 400k array set from Agilent revealed loss of 9.2Mb spanning the chromosomal region 21q11.2-q21.2 with loss of 13 genes. Loss of Y chromosomal material was not observed. Both, FISH and CGH also indicated mosaicism with aberrant and normal cells.

Furthermore, we analysed spermatozoa by FISH using BAC probes for region 21q11.2 (inside the deleted region) and 21q22.3 (as control probe) and centromeric probes for the X and Y chromosomes. The FISH results demonstrated a normal signal pattern with one normal chromosome 21 and one X or one normal Y chromosome in most spermatozoa (73%). On the other hand, 10% of the analysed spermatozoa showed only one normal chromosome 21 without any specific gonosomal signal. These spermatozoa would lead to fertilised eggs with only one X chromosome. Other signal constellations were only found in few spermatozoa each.

P04.58

Screening for Microdeletions in the AZF Region of the Y Chromosome in Patients with Disorders of Sex Development due to 45,X/46,XY Chromosome Abnormalities

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The etiology of the disorders of sex development (DSD) in patients with 45,X/46,XY karyotype is not yet completely understood. Deletions of AZF region (AZFa, AZFb and AZFc sub-regions), which might predispose to Y loss, have been identified in Klinefelter syndrome (KS) and in 45,X/46,XY subjects. **Objective**: to screen Yq microdeletions in Brazilian patients with DSD due to 45,X/46,XY chromosomal abnormalities. Twenty-six 45,X/46,XY or 45,X/46,X,idic(Y) subjects were selected: 16 with mixed gonadal dysgenesis (MGD) and 11 with Turner syndrome (TS). Eight Y *loci* were screened using PCR: DYZ3 (centromere); DYS280, *UTY* (AZFa); DYS216, DYS231, DYS224 (AZFb); *DAZ*, *PPP1R12BP1* (AZFc). **Results**: Yq microdeletions were detected in 6 (22%) patients (3 MGD; 3 TS). Regarding MGD patients, the 3 deletions span at least 6, 4.5 and 3 Mb. In the 3 TS patients the deletion spans at least 3 Mb. **Discussion**: Yq deletions identified in these patients involved the AZFb and AZFc regions. The longest deletions of Yq were identified in 2 MGD patients with male phenotype. The AZFc region was deleted in all 6 patients. Likely, the AZFc deletions are the most common identified in patients with idiopathic infertility due to oligozoospermia or complete absence of germ cells as in KS. In 45,X/46,XY patients, there are a few reports identifying deletions in AZFb/AZFc regions. Extensive studies are needed to establish the exactly association between Yq microdeletions and the various degrees of gonadal dysgenesis in these patients and to confirm the role of this mechanism for the formation of 45,X cell line.

P05. Prenatal and perinatal genetics

P05.02

Changes in age of pregnant women who undergo amniocentesis

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Karyotyping of fetal cells obtained by amniocentesis reveals the number and structure of chromosomes. Amniocentesis is offered to all women of advanced maternal age, in Croatia 35 years of age and older. Serum screening and ultrasonography are noninvasive techniques and can assist all women, regardless of their age, in a decision concerning invasive testing. There has

been an increasing trend among women to delay childbearing, a characteristic in the most countries, including Croatia. The reason is associated with the increasing number of women wanting to receive higher education and to achieve financial independence.
During the last two decades the average age of mothers at birth of their child in Croatia increased by 3.3 years. Our results show that the average age of pregnant women who undergo amniocentesis follows the increasing trend of maternal age by four years, from 33 to 37 years of age. At the same time, the percentage of pregnant women with the single indication for the amniocentesis of advanced maternal age (AMA) is slightly decreased, while those with pathological ultrasound and/or biochemical screening increased. Although the majority of pregnant women who performed amniocentesis were aged 35 years or older, during the 15-year period, the proportion of women under 35 increased from 17.5% to 23%.
These results are evidence of an increased access to prenatal diagnosis of younger women in Croatia at increased risk for fetal chromosomal aberration. Decision about accepting or declining the prenatal testing should be made by pregnant women with their partners after genetic counseling.

P05.03

DNA diagnostics of human chromosomes numerical abnormalities using fluorescent quantitative PCR analysis in Belarus

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Numerical abnormalities of human chromosomes are the most frequent cause of congenital human pathology. The frequency of chromosome 21 trisomy (Down syndrome) constitutes 1:780 in Belarus.
One of the most effective strategies for molecular diagnostics of aneuploidies and triploidies is based on quantitative PCR analysis. We selected and tested the most informative DNA-markers of chromosomes 13, 18, 21 and X. For sex determination and Klinefelter syndrome identification we tested the microsatellite markers of chromosome Y.
On the basis of current molecular genetic technologies there has been developed a method of DNA diagnostics of the most frequent aneuploidies using multiplex PCR and automated capillary electrophoresis based on the simultaneous testing of 15 microsatellite markers of chromosomes 13, 18, 21, X, Y in a single analysis. The method was tested on samples with chromosomal pathologies established by karyotype. According to the results of testing DNA samples from fetuses with Down, Edwards, Patau, Turner and Klinefelter syndromes, changing of quantitative characteristics of the alleles of respective chromosomes was detected in all the samples, indicating a high informative capacity of the developed protocol. Then with this particular test we analyzed 70 DNA samples from fetuses having the risk of chromosomal pathology. DNA was extracted from the amniotic fluid cells. Numerical abnormalities of human chromosomes were detected in 5 cases.
DNA diagnostics of human chromosomes numerical abnormalities using fluorescent quantitative PCR analysis has high throughput and low costs, and DNA analysis results can be obtained in less than 6 hours.

P05.04

Moving from chromosome analysis of cultured amniocytes: implementation of SOGC guidelines for amniotic fluids in a Canadian setting

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Chromosome analysis has been gold standard for prenatal detection of aneuploidies for decades. To supplement this procedure many diagnostic labs have introduced QF-PCR as a rapid aneuploidy screen for chromosomes 13, 18, 21, X or Y. There is also an increasing use of microarray testing for more complex prenatal cases with ultrasound abnormalities. In Sept 2011, the Society of Obstetricians and Gynaecologists of Canada (SOGC) and the Canadian College of Medical Geneticists (CCMG) published a joint clinical practice guideline that supports replacement of conventional karyotyping with QF-PCR whenever prenatal testing is performed solely because of an increased risk of aneuploidy for chromosomes 13, 18, 21, X or Y. Implementing this major change in analysis criteria at the same time as increased availability of prenatal microarray brings its own set of challenges: it is highly desirable to maintain a DNA source for all pregnancies with normal QF-PCR results to enable possible downstream molecular testing. Retention of a DNA source also facilitates testing should anomalies be detected later in the pregnancy, necessitating microarray. We have developed an algorithm for prenatal testing of pregnancies at risk for trisomy vs those at risk for other genetic imbalances which allows for the maintenance of a DNA source without extensive culturing. Our approach reduces chromosome analysis of cultured amniocytes by 75% while maintaining clinically appropriate testing. This approach and its implementation are discussed.

P05.05

Chromosome microarray analysis in routine prenatal diagnosis practice: a prospective study on 2800 clinical cases

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Objectives: Although several studies have demonstrated the usefulness of chromosome microarray analysis (CMA) in clinical prenatal diagnosis practice, only limited conclusions could be drawn due to the small size of the cohorts analysed. To assess the feasibility of offering CMA for prenatal diagnosis as a first-line diagnostic test, a large-scale prospective study was performed on a cohort of 2800 consecutive prenatal samples, with parallel processing for both CMA and conventional cytogenetic analysis.
Methods: Women undergoing amniocentesis or chorionic villus sampling (CVS) for standard karyotype were offered CMA. A total of 2800 prenatal samples were processed in parallel using both CMA and G-banding for standard karyotyping.
Results: Clinically significant copy number variations (CNVs) were identified in 94(3.4%) samples, 70(74.5%) of which were also detected by conventional karyotyping. In 24 cases (0.9%), CMA identified pathogenic CNVs that would have remained undiagnosed if only a conventional karyotype had been performed, 16 of which were concerning well-established syndromes. The selection of an array platform specifically developed for prenatal applications, allowed us to detect a single occurrence of variation of unclear significance. CMA was also able to detect chromosomal mosaicisms as lower as 3.3% level.
Conclusions: The results of this study demonstrate that CMA improves the detection of fetal chromosome aberrations than conventional karyotyping, without missing potential pathogenic chromosomal abnormalities, with no appreciable increase in results of unclear clinical relevance. These findings provide substantial evidence for the feasibility of introducing CMA into routine prenatal diagnosis practice as a first-line diagnostic test.

P05.06

ArrayCGH as diagnostic tool for genetic analysis of spontaneous abortions

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Spontaneous abortions are common, with 10-15% of all clinically recognized pregnancies ending in early pregnancy loss. In 40-50% of these cases, fetal chromosomal abnormalities are responsible. Identification of these abnormalities helps to estimate recurrence risks in future pregnancies.
For the last decades chromosome analysis has been the golden standard to detect genomic imbalances in spontaneous abortions. However, due to culture failure or maternal contamination often no fetal karyotype can be obtained. Since DNA-based technologies do not require dividing cells , array comparative genomic hybridization can overcome some of these limitations.
To evaluate the efficiency of arrayCGH as an alternative method for identification of chromosome anomalies in abortion material, we present the results of a study on more than 50 cases referred to our laboratory for cytogenetic analysis. So far, about 40 percent of our cases show genomic imbalances. We present two cases with an interesting correlation of clinical observation and genomic imbalance.

P05.07

Array-CGH results in fetuses with central nervous system

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The central nervous system (CNS) anomalies are seen in 1:1000 ratios in new born population. 5-50 % is identified to have chromosome anomalies referred during antenatal ultrasonography (USG) due to any CNS abnormalities. CNS malformations are related to specific chromosome anomalies, such as ventriculomegaly with trisomy 13, 18, 21, and microduplication of 16p; holoprosencephaly with trisomy 13, 18, microdeletions of 7q, 2p, 13q, 18p, 21q, and microduplication of 3p; hydrocephaly with trisomy 13, 18, 9, microdeletion of 4p, microduplications of 1q, 3q, 5p, 9p and mosaicism of trisomy 8; corpus callosum agenesis (CCA) with trisomy 8, 18, 13, microdeletions of 2q, 6q, 15q, 13q, 1q, 3p, 3q, microduplicatios of 8p, 11q, and triploidy; choroid plexus cyst (CPC) with trisomy 18, 21, and 13; Dandy Walker malformation with trisomy 13, 18, microdeletions of 3q, 6q, 13q, 11q and microduplication of 5p, 8p, 8q and triploidy.

Array-CGH technique specifically designed for molecular karyotyping, which is the finest detection method of chromosomal abnormalities enable us to detect the imbalances as low as 5 Mb.

We aimed to investigate 35 fetuses, with various CNS anomalies, for possible submicroscopic imbalances, with Array-CGH (CGX-720K, NimbleGen-Roche), previously known to have normal karyotypes. Patient collective includes cases with ventriculomegaly (n:16), holoprosencephaly (n:6), hydrocephaly (n:5), CCA (n:3), CPC (n:3), Dandy-Walker malformation (n:2). Two chromosomal imbalances were revealed in the collection. One was 11.57 Mb deletion in 7q35qter in a case with holoprosencephaly and the other was 137.507 Kb deletion in 3p26.1p25 with CCA.

Final results will be presented.

P05.09

Optimization and validation of RHD and KELL genotyping for non-invasive prenatal diagnostics

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Introduction: There are two reasons for establishing a methodology for non-invasive determination of RHD and KELL genotypes in early pregnancy.

1) To identify fetuses which are at risk of hemolytic disease of fetus and newborn by alloimmunized pregnant women.

2) To prevent alloimmunization during pregnancy.

There is no method validation on a representative number of samples in the Czech Republic, which would allow to introduce methodology into clinical practice. Project is supported by IGA MZ CR: NT12225.

Aim: Evaluate two different cell free fetal (cff) DNA separation procedures based on adsorption on the surface of silica gel and on the separation on magnetic particles. Optimize and evaluate RHD and KELL genotyping.

Material and methods: We tested both isolation procedures in 76 cffDNA samples.

Together 200 control samples were used for genotype assessment. Optimization and calibration of RHD and KELL genotyping was done using Real-Time PCR and by capillary electrophoresiss minisequencing .

Results: There were found significant differences in the yield of cell free fetal DNA between the tested cffDNA isolation methods. Silicagel membrane based method for isolation of cffDNA shorter molecules is more suitable than the magnetic particle one.

To determine the sensitivity threshold there were performed RHD and KELL calibrations by Real time PCR and capillary electrophoresis with a dilution series RHD and KELL genotypes. Both methods are able to clearly recognize the fetal genotype.

The optimization was further examined to detect RHD and KELL genotypes simultaneously and together with multiplex SNP assay as an internal cffDNA control.

P05.10

Clinical application of array-CGH in prenatal diagnosis: case reports of pregnancies with abnormal ultrasound findings and apparently normal karyotype

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Conventional G-banding karyotyping is a standard method used in prenatal diagnosis to detect chromosomal abnormalities larger than 5 Mb. However, many genetic syndromes are often associated with sub-microscopic deletions or duplications. Array-CGH is a modern method, which allows detection of small variations in the genome. Its application is mainly in postnatal diagnosis, where it helps to clarify diagnosis, prevention and prognosis. In prenatal diagnosis it has limited use. This is mainly because a number of aberrations detected by array-CGH have not been described and their clinical significance is unknown. Due to this fact we use a BAC-based array (BlueGnome), which is focused on areas in the genome having demonstrable connection with 110 microdeletion syndromes described in the OMIM database.

We used array-CGH to detect sub-microscopic aberrations in carefully selected 67 prenatal cases, which were primarily indicated for conventional cytogenetics based on serious ultrasound findings. Case reports, in which array-CGH enabled detection of changes that conventional cytogenetics did not reveal and cases, where array-CGH clarified the origin of a genetic extra-material (i.e. marker chromosomes) will be presented. In total, array-CGH detected clinically relevant chromosomal abnormalities in 13,5 % cases (9/67).

From our four-year experience we can say, that array-CGH is an appropriate tool with high resolution to detect small changes in the genome and provides important diagnostic information for prenatal genetic counselling. It can be applied in cases where amniocytes failed to grow for conventional karyotyping, in cases of clonal selection or for detection of sub-microscopic changes caused by inversion/translocation.

P05.11

Limitations of prenatal detection by FISH method - a case report with a complex chromosomal mosaic with 6 cell lines

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In the period 2004-2011 in Prenatal Diagnosis Laboratory of „Cuza-Voda" Maternity Iasi were made 1425 prenatal cytogenetic analysis by FISH method, using Aneuvision probes for chromosomes 13, 18, 21, X and Y. Unfortunately, conventional chromosomal analysis based on amniocytes culture could not be applied and thus the results were formulated only by FISH. We present a case showing one of the limitations of FISH technique in prenatal diagnosis. Pregnant women, aged 26 years, were investigated in our laboratory at 16 weeks of pregnancy because of a risk of 1/200 for trisomy 21 at triple test. Analysis on the UV emission microscope revealed two chromosomal fluorescent signals for chromosomes 13, 18, 21 and X, and one signal for chromosome Y. Because the XXY trisomy is not a reason for therapeutic abortion, pregnancy continued and resulted in the birth of a male child with 2750 g weighth and 47 cm length. Neonatal clinical examination showed scaphocephaly, hypertelorism, epicantus, antevertate nostrils, prominent upper lip, micrognathia, bilateral cryptorchidism and hypotonia. In order to elucidate the causes of facial dysmorphia was made a constitutional chromosomal analysis, which revealed a mosaic with 6 cell lines and chromosomal formula: 48,XY,+mar,+mar[29]/47,XY,+mar[14]/48,XY,r(X),+mar[10]/49,XY,r(X),+mar,+mar[3]/46,XY[3]/50,XY,r(X),r(X),+mar,+mar[3]. By FISH technique with probes for centromere of chromosome X the X ring chromosomes was confirmed, while the origin of marker chromosomes has not been established. Our paper shows the failure of characterization of prenatal chromosomal mosaics by FISH technique and the need for other methods to confirm the diagnostic.

P05.12

The use of chromosome microarray analysis as a first-line test in pregnancies with *a priori* low risk for detection of submicroscopic chromosomal abnormalities

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Objectives: In this study we aimed to explore the usefulness of chromosome microarray analysis (CMA) in groups of pregnancies with *a priori* low risk for detection of submicroscopic chromosome abnormalities, usually not considered an indication for testing, in order the assess if CMA improves the prenatal detection rate of chromosomal aberrations.

Methods: A total of 2800 prenatal samples were processed. The indications included: advanced maternal age (AMA), abnormal results of maternal serum screening tests (MSS), abnormal ultrasound findings (AUS), known abnormal fetal karyotype (AFK), parental anxiety (PA), family history of a genetic condition (FIS), cell culture failure (CCF).

Results: the use of CMA resulted in an increased detection rate regardless of the indication for analysis. This was evident in high-risk groups AUS-AFK (7/114, 6.1%), and also in low-risk groups, such as AMA (7/1033, 0.7%) and PA (10/1569, 0.6%). A total of 24(0.9%) fetal conditions would have otherwise been overlooked if only a standard karyotype had been performed, 17(0.6%) of which if offering CMA to high-risk pregnancies only.

Conclusions: The results of this study demonstrate that more widespread testing by CMA in fetuses would result in a higher detection of chromosome abnormalities prenatally, also in low risk pregnancies. Our findings provide substantial evidence for the utility of using CMA as a first-line diagnostic test to all pregnant women undergoing invasive prenatal testing, regardless of risk factors.

Indication	No. Samples analysed (%)	No. Samples with chr. Abn. (%)	No. Samples w/ chr. abn. not det. by conv. karyotyping	aCGH detection rate	
				Indic.	% abnormal results
AUS	59 (2.1)	14 (23.7)	3		21.4
AUS + AMA	31 (1.1)	12 (38.7)	3	9.7	25.0
AMA	1033 (36.9)	36 (3.5)	7	0.7	19.4
PA	1569 (56.0)	25 (1.6)	10	0.6	40.0
AFK	24 (0.9)	4 (16.7)	1	4.2	25.0
MSS	27 (1.0)	3 (11.1)	0	0.0	0.0
FIS	24 (0.9)	0 (0.0)	0	0	0
CCF	33 (1.2)	0 (0.0)	0	0	0
High-risk pregnancies (AUS+AFK)	114 (4.1)	30 (26.3)	7	6.1	23.3
Low-risk pregnancies (AMA+PA+MSS +FIS+CFF)	2686 (95.9)	64 (2.4)	17	0.6	26.6
Total	2800	94 (3.4)	24	0.9	25.5

P05.13
Optimization of isolation of cell-free fetal DNA from plasma of pregnant women

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Cell-free DNA released from fetal cells (cffDNA) represents important alternative source of material for non-invasive prenatal diagnostics. Because of the low quantity and increased fragmentation of cffDNA in maternal plasma, the DNA extraction method is crucial step for further analyses of cffDNA.

The aim of this study was to directly compare the yield of extracted DNA after using three commercial kits widely used for isolation of nucleic acids.

For cffDNA extraction from plasma of pregnant women carrying male fetuses three commercial kits and corresponding original protocols were used (QIAamp DNA Blood Mini Kit, QIAamp DSP Virus Kit, QIAamp Circulating Nucleic Acid Kit). Extracted DNA was used for amplification by qPCR. Marker - gene DYS14 located on the Y chromosome was used for comparison of circulating fetal DNA recovery. Gene for androgen receptor located on the X chromosome was used as a marker for detection of total circulating DNA. Ct values from qPCR were used for determining the relative quantity. Variability between categories was estimated by Repeated measures ANOVA and Tukey´s test.

Statistically significant difference was proved after Ct values comparison (F=48.43, p<0.0001). The yield of isolated DNA was significantly higher using Virus Kit than Blood Mini Kit (p<0.0001) and CNA Kit than Blood Mini Kit (p<0.0001). Virus Kit and CNA Kit did not significantly differ in the amount of isolated cffDNA.

According to our finding CNA Kit and Virus Kit are equally suitable for extraction of fragmented circulating DNA derived from fetal cells, when yield of isolation is important.

P05.14
Do uniparental isodisomies or microimbalances lead to early losses of pregnancy?

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Aneuploidy is known to be a common cause of spontaneous abortion. We designed a combined platform utilizing array based comparative genomic hybridization (aCGH) and single nucleotide polymorphisms (SNPs) to further elucidate genetic causes underlying pregnancy losses displaying 46,XX- or 46,XY-karyotypes. Simultaneous determination of imbalances at high resolution and genome-wide heterozygosity state was performed on a targeted CGH+SNP 8x60K microarray (BlueGnome, Cambridge, UK). Results were juxtaposed to those of a second panel displaying aneuploidies. Copy number imbalance and loss of heterozygosity (LOH) findings were compared to cases reported in the literature to determine their clinical relevance.

There was no difference in the type or frequency of microimbalances between the group of samples with aneuploidies and the group of samples with normal karyotypes. Most small imbalances could be identified as copy number variations, only few unknown variants remained and were equally distributed to cases with and without aneuploidy. No aberration associated with any common microdeletion or microduplication syndrome was detected in cases with normal karyotype. A complete hydatidiform mole was identified showing genome wide uniparental isodisomy. Segmental stretches of copy number neutral LOH occured at comparable frequency in both groups of samples. They varied from 8,9-14,2 Mb in size and mapped to various chromosomes with very little overlap. A thorough investigation was done to differentiate pathogenic changes from statistically unusual though benign features of the genome.

Overall, even in this relatively small number of cases, aCGH+SNP analysis picked up aberrations of putative pathological relevance, which were missed following standard diagnostic procedure.

P05.15
Prenatal array CGH identifies genomic imbalances associated with congenital diaphragmatic hernia (CDH)

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Congenital Diaphragmatic Hernia (CDH) is caused by a defect in the formation or closure of the diaphragm, with incidence of 1.7 - 5.7 per 10,000 live-births. Genetic factors have long been considered to play an important role in the pathogenesis of both syndromic as well as isolated CDH. We have previously published our findings from a retrospective study using a targeted custom design array. Here, we present our findings from both the retrospective and on-going prospective study into the use of a high-resolution genome-wide oligonucleotide array for prenatal diagnosis. In this study we have now screened over 60 cases of CDH by array CGH and identify 6 genomic imbalances responsible for the diaphragm defect. Interestingly, the array results highlight duplications of EFNB1 as a cause, and redefine the minimal deleted CDH region at 15q26 to a single gene NR2F2. This study provides more insight into the genetic etiology of CDH.

P05.16
Analysis of CYP21A2 gene in Congenital Adrenal Hyperplasia patients from Bashkortostan Republic of Russia

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Congenital adrenal hyperplasia (CAH) describes a group of autosomal recessive disorders caused by complete or partial defects in one of several steroidogenic enzymes. More than 95% of all CAH cases are caused by 21-hydroxylase deficiency.

We analyzed CYP21A2 gene in 101 unrelated CAH patients from Bashkortostan Republic. The large gene deletions or large gene conversions (delA2/LGC) were found in 28.5% of 202 unrelated CAH alleles. The most frequent point mutations were R356W (14.1%), I2splice (13.9%), I172N (6.2%) and Q318X (4%). Other mutations (V281L, P30L and P453S) met rarely with frequency from 2.7% to 0.5%. The clusters of mutations of CYP21A2 gene in one chromosome: Q318X+R356W (5%), I172N+Q318X (1%), delA2orLGC+V281L (1%), were found in 7 CAH patients.

Furthermore, we identified two additional mutations of CYP21A2 gene in CAH patients. In one patient with salt wasting form (SW) of CAH we found previously described missense mutation R426C in exon 10. The other allele carried the I2splice mutation. In another patient with SW we found previously unreported deletion of 3 nucleotides in exon 9 of CYP21A2 gene that led to the deletion of isoleucine in 384 amino acid sequence. The novel mutation delIle384 was identified in heterozygous state with delA2/LGC of CYP21A2 gene.

In conclusion, previously described diagnostically significant CYP21A2 gene mutations were present in about 72.3% of unrelated CAH alleles. The new mutation corresponds to 0.5% of mutant alleles in our cohort of 101 patients. The discovery of the novel mutation increases our knowledge of CAH caused by 21-hydroxylase deficiency.

P05.17
A cytogenetic study from chorionic villus of 110 spontaneous abortions

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A total of 110 cases of first trimester spontaneous abortions were analyzed cytogeneti-cally. A long term culture was used for the chorionic villi. The maternal age ranged from 21-42 years and the gestational age from 6-12 weeks. In 7 cases, it was not possible to perform the karyotype (in vitro culture failed). Abnormal karyotypes were found in 52 cases (47,3%). The sex ratio (male/female) in chromosomally abnormal abortions was 0,42 and in normal abortions, it was 0,20 which is different from 1,2 found in earlier studies. The most frequent chromosome abnormalities found were trisomies (61,5%), followed by polyploidy (17,3%), structural anomalies (9,61%) abnormality of gonosomal chromosome (9,61%), double trisomy (1,9%) and monosomy (1,9%). Among the trisomies, the prevalent ones were of chromosome 21 (8 cases), followed by chromosome 16 (4 cases), chromosome 14,19,20 (3 cases each), the chromosome 1,8,13,10,15,17 (6 cases). In one case, there was a double trisomy (48,XY,+13,+15). Although polyploidy is a rare abnormality we detected tetrapoidy in 5 cases, all with 92,XXYY and triploidy in 5 cases, with 69,XXX (2 cases) and 69,XXY(2 cases). The abnormality of the gonosomal chromosomes were found in 3 cases of X monosomy, followed by 2 cases of XXY. Structural anomalies were represented by Marker chromosomes (2 cases), unbalanced translocations (2 cases) and Robertsonian translocations trob(13;14) with trisomy 13 (1 case). Cytogenetic investigations of spontaneous abortions provide valid information as to the cause of abortion. This information may be helpful for genetic counseling and could contribute to prenatal diagnosis in subsequent pregnancies.

P05.18

Prenatal expression of dehydrated hereditary stomatocytosis

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Dehydrated hereditary stomatocytosis belongs to the group of autosomal dominant haemolytic anemias. No causative gene is known but a linkage was found with the 16q23-q24 chromosomal region. In addition to chronic haemolysis, dehydrated stomatocytosis can be responsible of pseudohyperkalemia, hyperferrinemia and perinatal edema.

From 1996, 5 families have been reported with prenatal / perinatal ascitis or hydrops. The reason of these effusions is not elucidated but the anemia is probably not the unique mechanism.

We present 2 novel families with prenatal ascitis. In the first family, due to the severity of the ascitis, a pregnancy was medically stopped at 28 weeks of amenorrhea. The disease was inherited from the father, presenting with moderate haemolytic anemia, splenomegaly, hyperkaliemia and hyperferritinemia. In the second family, the prenatal and neonatal ascitis of the 2 affected children was spontaneously regressive. The affection was inherited from the asymptomatic mother who only presented macrocythemia and hyperferritinemia.

In case of unexplained prenatal / perinatal ascitis, the diagnosis of dehydrated hereditary stomatocytosis must be considered and the parents investigated.

P05.19

Double trisomy in pregnancy

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Double trisomy is extremely rare in liveborns, but it is a relatively common event in pregnancies ending in early abortions and usually involves at least one nonviable trisomy. Prevalence of double trisomy is higher within first trimester spontaneous abortions (~2%) than within the second trimester pregnancy (~0.01%). Double trisomy is associated with advanced maternal age and mean gestational age has been described to be significantly lower for double trisomy cases than that reported for single trisomy ones.

Our results of only two double trisomy cases from samples of amniocentesis, using routine cytogenetic analysis of cultured amniotic fluid cells with GTG-banding confirm the fact that double trisomy in second trimester of pregnancy, also in liveborns, usually involves the sex chromosomes in combination with potentially viable autosomal trisomies such as 21, 18 and 13. Both of them were in mosaic form (48,XXX,+21/46,XX and 48,XXY,+21/47,XY,+21).

Double trisomies within first trimester of spontaneous abortions, in regular and mosaic form, were present in 1.86% of cases. These cases were lethal in first trimester of pregnancy due to the presence of nonviable autosomes in double trisomies or combination of autosomal and gonosomal trisomies.

Our cases emphasize the importance of genetic counseling to assess the recurrent risk of double trisomy.

P05.20

Investigation of *MTHFR* and *MTHFD* polymorphisms as maternal risk factors for Down syndrome

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Members of the family of B9 vitamins are commonly known as folates. A number of studies have associated polymorphisms found in genes involved in folate metabolism to an elevated maternal risk for Down syndrome (DS). Central role in this process is played by

MTHFR, *MTR*, *RFC1* and *MTHFD* genes. In this study, we evaluated the role of three common polymorphisms in folate

metabolizing genes as possible risk factors for having a child with DS. The prevalence of these variant genotypes in mothers of DS children (case mothers) (n=26) was compared with controls (n=46). Investigated polymorphisms include methylenetetrahydrofolate reductase (*MTHFR*) 677C>T and 1298A>C and methylenetetrahydrofolate dehydrogenase (*MTHFD*) 1958 G>A. Present results indicate that none of the, *MTHFD* 1958G>A, *MTHFR* 677C>T, and *MTHFR* 1298A>C polymorphisms is an independent risk factor for a DS offspring at a young maternal age. The combined *MTHFR* 1298CC/*MTHFD* 1958GG, *MTHFR* 1298(AC or CC)/*MTHFD* 1958GG, *MTHFR* 1298AA/MTHFD 1958GA, *MTHFR* 1298CC/*MTHFD* 1958GA and *MTHFR* 1298CC/*MTHFD* 1958AA genotypes compared with the *MTHFR* 1298AA/*MTHFD* 1958GG genotype was associated with increased DS risk (with related P values: 0.019, 0.038, 0.046, 0.05 and respectively 0.019). These results show that maternal polymorphisms of folate metabolism could be implied in pathogenesis of Down syndrome.

P05.21

Prenataldiagnosis of a *de novo* euchromatic variant 16p11.2

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Introduction: Chromosome euchromatic variants most commonly result from copy number variation (CNV) of gene and pseudogene containing polymorphic genomic segments that became visible at the cytogenetic level. Intrachromosomal rearrangements of the proximal short arm of chromosome 16, leading to 16p+ morphology can raise interpretation problems because both euchromatic variants (16p11.2v) and unbalanced duplications have been described in this region.Case report: An amniocentesis performed at 16 weeks gestation, due to maternal age, revealed a *de novo* 16p+ chromosome by GTL banding. C banding and N/DAPI staining were negative. WCP labeled all 16p+ chromosome. MLPA analysis with eleven probes for 16p11 (kit P343) was normal, while, array CGH pointed to a 1.9 MB duplication in 16p11.2. Fetal ultrasound did not show any morphological defects. Postnatal FISH study with a panel of RP11 BACs confirmed the presence of a duplication involving the CNVs regions of proximal 16p11.2 (euchromatic variant) and no evidence of a duplication in the autism or development delay region 16p11.2. Discussion: The occurrence of *de novo* structural euchromatic chromosome abnormalities presents a genetic counseling dilemma, particularly when ascertained in the prenatal setting, as the prognosis can be more reliably discussed when the chromosome abnormality is transmitted by a phenotypically unaffected parent. Fetal ultrasound, postnatal clinical reports, molecular cytogenetic analyses may all contribute with critical data to the genetic counseling. Professionals involved in prenatal diagnosis and genetic counseling should be aware of the euchromatic variant 16p11.2v in order to establish the differential diagnosis and provide parents with proper information.

P05.22

Indications for Fetal Karyotyping and Ultrasonographic Findings in Common Trisomies; Alterations in over 2 Decades

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Advanced Maternal Age (AMA) was the first screening parameter of trisomies at the beginning of prenatal diagnosis era. The developing of the new screening techniques using biochemical parameters in maternal serum (MS) and ultrasonographic (USG) markers allowed us to get higher detection rates for common trisomies in all pregnancies.

We retrospectively evaluated the results of 25808 prenatal cases obtained in 2 periods (from 1989 to 1999 and 2000 to 2011), under the aspect of the presence of AMA, MS- biochemical screening and USG findings prior to the cytogenetic diagnosis. This series covered 23427 amniotic fluid and 2381 chorionic villi samples in which 462 trisomy 21, 127 trisomy 18 and 51 trisomy 13 cases were diagnosed.

We determined to reveal the alterations of the indications of the fetal karyotyping over time, and further more to identify the most frequent USG findings detected in trisomies.

AMA, which was the most common parameter in the 1st period, decreased, while MS- biochemical and USG screening tests became more effective over time.

The most common observed USG findings was nuchal translucency in all trisomies. Cystic hygroma, ascites in trisomy 21 and 18; cardiac anomalies in trisomy 18 and 13; urogenital anomalies in trisomy 13; omphalocele in trisomy 18 were more frequently identified USG findings.

Widely usage and increased experience in screening tests led to detect more cases with common aneuploidies with reduced number of prenatal invasive procedures in high risk pregnancies. Further more, younger women gained a chance for prenatal diagnosis for chromosome anomalies.

P05.23

Non- invasive fetal sex determination using a converntional nested PCR analysis of fetal DNA inmaternal plasma

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Prenatal diagnosis is usually performed by collecting fetal samples through amniocentesis or chorionic villus sampling. However, these invasive procedures are associated with some degree of risk to the fetus and/or mother. Therefore, in recent years, considerable effort has been made to develop non-invasive prenatal diagnostic procedures. One potential non-invasive approach involves analysis of cell-free fetal DNA in maternal plasma or serum. The objective of our study was to investigate the feasibility of using fetal DNA in maternal plasma for prenatal diagnosis.

For this experimental study, to develop a nested PCR technique for fetal SRY gene identification using cell-free fetal DNA in maternal plasma. Peripheral blood samples were obtained from 32 pregnant women at the gestational period from 8 to 13 weeks and cell-free DNA was extracted by the phenol/chloroform method from plasma. The nested PCR was carried out to amplify the fragment of SRY gene by two sets of PCR primer pairs. Analysis was then performed on the PCR product.

Specifically, the presence of Y-chromosome sequences in maternal blood plasma indicates that the fetus is male, whereas lack of a signal will indicate that the fetus is female.

Among the 32 pregnant women bearing male fetuses, SRY sequences were detected in 16 plasma samples after nested PCR amplification, while the 18 women bearing female fetuses had the positive results. The sensitivity were 87.5% (28/32). The nested PCR amplification of SRY sequence is a convenient and low-cost approach for the noninvasive early prenatal diagnosis of sex-linked inheritant.

P05.24

Qualitative analysis of the results from the first trimester screening

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Introduction

The first trimester screening is based on the following criteria - maternal age; double test (PAPP-A; free ß-HCG), FHR, nuchal translucency, facial angle, tricuspid flow, ductus venosus flow, nasal bone.

Objectives

The aim is to estimate a qualitative analysis of the first trimester screening based on the rate of detection and on the false positive rate - FPR.

Material and method

During the aug.2010-dec.2011 we performed the first trimester screening at 671 patients; 37 patients had multiple pregnancies. The ultrasound screening was performed on GEVoluson730Expert, at a fetal CRL of 44-85 mm. The PAPP-A; free ß-HCG was carried out on BRAHMS or Delphia.The calculation of risk was evaluated with the FMFsoftware and all the ultrasound criteria (NT, FA, NB, TR, DV) were analyzed in 538 cases. Based on the results of the screening we performed 31 invasive procedures: 28 CVS and 3 amniocentesis (the amniocentesis cases refused CVS).

Results

Our results are the detection of a number of 7 chromosomal abnormalities :T21 - 4, T18 - 1, T13 - 1 and 1 case of triploidy. At a cut-off level at 1: 150 we obtained a FPR of 3.5% and if we set the cut-off level at 1: 100 - the FPR is 2.38%. All the cases with chromosomal abnormalities belong to the group of risk > 1: 100.

ConclusionThe setting of the cut-off level at 1: 100 and at 1: 150 doesn't modifies the rate of detection of chromosomal abnormalities, only the FPR increases from 2.38% to 3.5%

P05.28

Investigation of HbQ-Iran in couples referred to Pasteur Institute of Iran

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Introduction :

Alpha thalassemia is the most common inherited disorder of hemoglobin synthesis in the world . Single nucleotide mutation in α1 or α2 genes produce abnormal α-chain hemoglobin . Hb Q disorders are regard as rare Hb variants . Several Hb Q have been reported sofar including Hb Q-iran , Hb Q-thailand , Hb Q-india .

Materials & Methods :

In this study one couple refered from primary health care (PHC) centers to Pasteur institute of iran with MCV>80 , MCH>28 , HbA2=2 and Hb variant=14 . Genomic DNA was extracted by salting out method. DNA sequencing using Big Dye from ABI was used .

Result :

A total of 1000 individuals with microcytic hypocromic anemia were screened for the most common type of α-thalassemia . We investigated molecular basis of Hb variant in the couple using multiplex gap PCR , MLPA &direct DNA sequencing . No deletion was found . DNA sequencing revealed codon 75 G>C , Asp>His in α2 gene mutation in both couple .

Discussion:

Heterozygous individuals for Hb Q -Thailand generally present with moderate red cell microcytosis due to the association of the mutation with deletion -α4.2 Kb , but those carrying Hb Q-Iran or Hb Q-India are hematologically normal and no association with α-thalassemic phenotype has been reported . Since Hb Q-Iran is usually associated with normal CBC , it may not be detected through routine National Screening Program , therefore it's true ferequency can not easily be determined.

P05.29

Chromosomal Imbalances in Holoprosencephaly Sequence; Results of 87 Cases Diagnosed Prenatally

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Holoprosencephaly sequence (HPE, # MIM 2361000), is the most common developmental defect of midline cleavage in human embryonic forebrain, with a variable phenotypic expression. Several mechanisms play a role in the etiopathogenesis; genetic, environmental, multi-factorial, and unknown.

The rate of chromosome abnormalities is ~ 40 % in patients with HPE. The most common chromosomal abnormality involved is trisomy 13, followed by trisomy 18, triploidy and deletion 7q36.3. In approximately 18%-25% of cases monogenic syndromes are diagnosed. To date, 12 genes are known to be associated with HPE. Human Sonic Hedgehog gene (SHH*)* located on chromosome 7q36 is the best known gene related.

This study comprises the results of cytogenetic, molecular cytogenetic and molecular karyotyping studies obtained in tissue samples (51 fetal blood,19 chorionic villus and 17 amniotic fluid) of 87 fetuses diagnosed ultrasonographically as having HPE.

Karyotype analysis was performed in all cases. Molecular cytogenetic technique using subtelomeric probe7q36.3 was applied in 12 cases. Six cases with normal karyotype were investigated by oligonuclotide Array-CGH (Roche-NimbleGen).

Chromosomal abnormalities were detected in 51.7 % of cases (45/87). The predominant chromosomal abnormality was trisomy 13 (n:24), which was followed by trisomy 18 (n:5), and triploidy (n:5). Terminal 7q deletion being the most frequent structural anomaly observed (n:9), was *de novo* in 6 cases and unbalanced product of maternal translocations in 3. Furthermore, deletion of 18p and paternally inherited balanced inversion of chromosome 11 was observed in single cases.

The clinical and laboratory findings will be discussed in view of the literature.

P05.30

Prenatal screening for aneuploidies in Iranian families using QF-PCR

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Quantitative fluorescence polymerase chain reaction (QF-PCR) has been introduced in a number of genetic laboratories as an inexpensive, rapid and reliable method for prenatal recognition of aneuploidy in chromosomes 13, 18, 21, X and Y. The aim of this study was to investigate the efficacy of QF-PCR for the prenatal recognition of common aneuploidies and compared our findings with cytogenetic results in Iran.

A multiplex-PCR involving 15 short tandem repeat (STR) sequences was established for aneuploidy screening and chromosomal study was performed for all samples and the results werecompared.

Total of 654 prenatal samples were analyzed including 616 amniotic fluid and 38 chorionic villous samples (CVS). The following abnormalities were detected in 21 (3.2%) individuals: 11 (1.7%) with Down syndrome, 4 samples (0.6%) with Edward syndrome, 2(0.3%) samples with Patau syndrome, 1 (0.15%)sample with Turner syndrome, and 1 (0.15%) 47,XYY. All of the CVS samples were normal. In addition, 2 cases (0.3%)showed triploidies. All aneuploidies detected by QF-PCR, were confirmed by cytogenetics results. Elevensamples (1.7%) shown maternal cell contamination in whichthree of their results were failed. Additional chromosomal aberrations; inversion 9, t(9;14) and XX/XY mosaicism, were detected in 3 cases by karyotyping. In conclusion,usingQF-PCR with cytogenetic study simultaneously for all prenatal cases provide a rapid andreliable method in families at risk for aneuploidies. We also recommend all families that are seeking prenatal diagnosis of single gene disorders a QF-PCR to be added to their work up.

P05.31

Prenatal diagnosis of a complex de novo translocation without ultrasound abnormalities

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Apparently balanced de novo aberrations without any abnormalities in the ultrasound at prenatal diagnosis are a challenging setting for genetic counselling, especially if multiple breaks have occurred.

Here, we report on an amniocentesis performed because of advanced maternal age that revealed a complex de novo translocation involving chromosome 2, 7 and 18, and showing multiple breakpoints on each of the implicated chromosomes. The risk for mental retardation in balanced de novo aberrations increases by the number of chromosomal breaks. As we could find at least eight breaks by chromosomal analysis, a high risk for mental retardation was assumed even without ultrasound findings. A prenatal microarray analysis was recommended. Four major abnormalities were found, on chromosome 2 two deletions (328kb in 2q22.1 and 2415kb in 2q22.1-q22.3) and one duplication (777kb in 2q14.3), a further deletion in 18q12.2 (1935kb), as well as additional minor changes on chromosome 7. The genetic content of the rather large aberrations on chromosome 2 was not helpful with regard to the postnatal prognosis. Nevertheless, the deletion in 18q12.2 including the gene KIAA1328 was found to be described in association with mental retardation and only mild dysmorphic features. Finally, the parents decided to terminate the pregnancy.

P05.32

Prenatal diagnosis of multiple small supernumerary marker chromosomes (sSMCs) of different centromeric origin

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Small supernumerary marker chromosomes (sSMCs) are structurally abnormal chromosomes that cannot be identified or characterized unambiguously by conventional banding techniques alone (Liehr et al. 2004). Fluorescence in-situ hybridization (FISH) with centromeric and subcentromeric probes enables the rapid identification of sSMCs originating from pericentromeric regions.

We report on a fetus with up to 5 markers of different origin in one cell. Amniocentesis was undertaken for the reason of advanced maternal age. Chromosome analysis revealed the occurrence of one to six sSMCs (mean 2.6 per cell) in both independent cultures. Molecular karyotyping using an SNP-Array (Affymetrix) revealed amplification of 6.7 Mb from 4p12-q12 (arr 4p12q12(46.567.172-53.241.307)x3). FISH using a centromeric probe confirmed the homology to chromosome 4 of one of the sSMCs. The largest marker amongst the other sSMCs, probably a ring chromosome, originates from chromosome 9 and includes mainly the heterochromatic band of the long arm: min(9)(:p12→q12:). The remaining markers are very small and contain centromeric material from chromosomes 6, 14 and 22 only. Due to the fact that phenotypic abnormalities have been described in patients with duplications of the 4p12 region, the couple decided to terminate the pregnancy. The aborted fetus could not be investigated. Chromosome analyses of the parents´ lymphocytes were inconspicuous.

The maternal origin of markers from the amplified 4p segment and the different centromeric origin of the sSMCs, point to the possibility that the degrading second polar body had been incorporated into the zygote. We compare the present case with previously published cases.

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P05.33

Array-CGH identification of de novo mosaic supernumerary marker chromosome 19 in prenatal diagnosis

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Small supernumerary marker chromosomes (sSMC) are relatively rare in the general population, found in approximately in 0,1-1/1000 live births. They are defined as additional structurally abnormal chromosomes which cannot be identified with conventional cytogenetic techniques. The frequency of supernumerary markers found in prenatal diagnosis is about 0,076%. The clinical significance of sSMC varies widely, and the phenotype associated with presence of a de novo sSMC may differ from normal to severely abnormal.

We describe a finding of a „de novo“ mosaic supernumerary marker chromosome 19 , its genomic characterisation and its possible impact on the phenotype.

Amniocentesis was performed on a 33 year old primigravida because of an increased risk finding at maternal serum and nuchal translucency screening.

The cytogenetic analysis detected a supernumerary marker chromosome: 47,XY,+mar(56)/46,XY(47), in three independent cultures, confirmed using FISH as chromosome 19. To define the exact duplicated region array-CGH was performed. The aCGH finding indicated three copies of 19q12q13.11, 5,05 Mb in range. The final cytogenetic result was: 47,XY,mar.ish der(19)(wcp19+).arr 19q12q13.11(32545077-37601048)x3 dn.

According to involved genes in the duplicated region on 19q carrier could have a phenotipic consequences, especially developmental delay. Although the ultrasound scan was normal, the parents decided to terminate the pregnancy.

In order to offer appropriate genetic counselling an accurate identification of marker chromosomes „de novo“ found in prenatal diagnosis is fundamental. We emphasize the importance of array-CGH in prenatally detected „de novo“ sSMC and determination of genotype-phenotype correlation for better risk evaluation.

P05.34

Whole genome microarray in clinical practice: Investigation of 2,024 miscarriage, stillbirth and fetal malformation referrals

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We have used whole genome microarray as a replacement for conventional chromosome analysis to investigate genomic abnormalities in approximately 2,000 miscarriage, stillbirth and fetal malformation referrals. NimbleGen 135K CGH array was employed in the early phase of the program (1,248 referrals) prior to using Illumina Cyto-SNP12 array (776 referrals). Greater than 98% of samples yielded informative results, a substantial improvement over conventional chromosome analysis which typically has a success rate of 60-95% depending on referral type. Male to female sex ratio was 1.2 in keeping with strict tissue selection protocols.

Whole chromosome aneuploidy predominated, accounting for 578 abnormal results. However, 82 (4.1%) CNVs of unknown, uncertain or pathogenic significance were also identified. Clearly pathogenic genomic disorders were significantly enriched in this series. These included 7q11.23 (Williams) deletion (2 cases), 7q11.23 duplication (1 case), 5q35.2q35.3 (Sotos) deletion (1 case), 15q11.2q13 (Angelman) deletion (1 case) and 22q11.21 (Di George) deletion (2 cases), accounting for approximately 1 in 285 (0.35%) referrals. We found little evidence for submicroscopic, unbalanced rearrangements being transmitted by balanced carrier parents. Such rearrangements do not contribute significantly to the burden of miscarriage or stillbirth. Large (>6 Mb) genomic imbalances were identified in 31 cases. Uniparental disomy (UPD) was rare. One case of paternal isodisomy 14 was identified in 762 informative results obtained by SNP array. Chimerism was identified in 4 pregnancies; complete mole in 12 cases. Our data demonstrate that whole genome microarray is a powerful tool for investigating genomic abnormalities that contribute to miscarriage, stillbirth and fetal abnormality.

P05.35

Prenatal diagnosis of microdeletion 17q12 in a fetus with cystic kidney disease

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Genomic rearrangements such as deletions, duplications and insertions result in copy number variation (CNV) that may cause phenotypes by affecting dosage-sensitive genes, disrupting genes, creating fusion genes, and other mechanisms. Until now, numerous microdeletion and microduplication syndromes with characteristic phenotypes have been described.

We report a case of a 32-year-old pregnant woman. Sonographic examination in the 22nd week of gestation showed bilateral cystic kidney disease and oligohydramnios. Amniotic fluid sampling was performed for molecular genetic testing and karyotyping. The cytogenetic karyotype was normal (46, XY). HNF1B analysis by sequencing showed no mutation but MLPA revealed a heterozygous deletion of the complete gene. For precise characterization of the extent of the deletion Array CGH analysis was done showing a deletion of at least 1,3 Mb corresponding to the cytogenetic region 17q12 (arr17q12(34437534x2, 34817422-36168104x1, 36473175x2). This contiguous gene deletion syndrome comprises 11 OMIM genes, including HNF1B.

Involvement of the HNF1B gene causes cystic renal disease and maturity onset diabetes of the young (Renal cysts and diabetes syndrome, RCAD, OMIM 137920). According to data of the literature and DECIPHER database, patients with microdeletion 17q12 may exhibit cognitive impairment, speech delay, seizures, autism and psychiatric disorders, possibly due to haploinsufficiency of the LHX1 gene. Dysmorphic features are reported to be only mild.

The pregnant woman decided to continue pregnancy. Delivery date is in March 2012. The pregnancy outcome will be reported.

This case report underlines the importance of Array CGH analysis in the diagnostic work up of prenatally detected fetal anomalies.

P05.36

The Efficiency Of Multiplex Ligation-Dependent Probe Amplification Technique In The Diagnosis Of Fetal Chromosomal Abnormalities

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With standard karyotyping techniques chromosomal abnormalities are detected in only about 20% of patients with multiple congenital abnormalities/mental retardation (MCA/MR). At the 500-600 band level the resolution for detection is about 5 Mb. Fluorescence in situ hybridization (FISH) technique can unveil submicroscopic aberrations (<5Mb), which increases the detection rate an additional 4-8% in the etiology of MCA/MR. Molecular techniques can identify abnormalities <5 Mb.

The clinical findings of MCA/MR cases can guide the clinician toward syndrome-specific FISH probes. However, this is usually limited for prenatal cases. Multiple Ligation-dependent Probe Amplification (MLPA) extends the boundaries of the diagnosis of chromosomal anomalies that are undetectable by classical methods.

In this study, we aimed to search for subtelomeric aberrations and syndrome-related microdeletions/duplications by using SALSA P070 and P245 probe-sets of MLPA in 66 samples of fetuses with major ultrasonographic abnormalities carrying normal karyotypes with conventional methods.

Two microdeletions (18p11.3->pter and 7q11.23) and one microduplication (18q23) were identified (4.5 % - 3/66) by MLPA. Deletions were confirmed by FISH and duplication was confirmed by microarray.

The advantage of MLPA is the ability of searching multiple loci in one test run, which could be very helpful in prenatal diagnosis of cases with abnormal ultrasound findings where the karyotypes were normal.

P05.37

Possibility of MLPA prenatal detection of the most clinically important chromosomal abnormalities

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The aim of this study is the verification of the possibility to extend of the diagnostic spectrum of MLPA by simultaneous implementation of aneuploidy, centromeric and subtelomeric kits to detect aneuploidies of all chromosomes.

The 65 samples of fetal cell cultures derived for prenatal diagnosis with normal karyotypes (20) and different chromosomal abnormalities (45) were reexamined by MLPA MRC Holland kits. The combination of kits for the examination of aneuploidies of chromosomes 13, 18, 21, X and Y (PO95-A2), centromeres (P181-A2/182-B1) and subtelomeres (P036-E1 and P070-B2) was used.

MLPA results were identical with karyotypes in all euploid samples as in cultures with trisomies 2, 4, 5, 6, 9, 13, 14, 15, 16, 18, 21, double trisomy 15 and 18, syndromes XXX, XYY and XXY, except triploidy 69,XXX. Abnormal clone in mosaics was disclosed in 3/4 cases except 45,X/46,XX. All four balanced Robertsonian and reciprocal as well as unbalanced Robertsonian (13;14, 14;15, 14;21) and 4 reciprocal translocations (3;4, 3;6, 4;21, 9;15) were confirmed. The origin of 4 extra marker chromosomes derived from chromosomes X, Y, 15 and 21 was revealed. The 5 cases with the deletions of chromosomes Y, 9p and 18p and 1 case of duplication 3p were disclosed. Only ins(3;4)(p21;q26) and Y chromosome inversion were missed, because the breaks were not in the detectable regions of used kits.

This pilot study suggests that the MLPA kits combination might allow rapid, reliable prenatal detection of clinically significant abnormalities of all chromosomes, except 69,XXX and inversions/insertions.

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P05.38

Discrepancies between QF-PCR and karyotype results in a rare prenatal case of mosaic trisomy 18 and supernumerary marker chromosome 18

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Chromosomal aneuploidy is a common cause of human genetic disorders and cytogenetic analysis remain the standard method of diagnosis. Quantitative Fluorescence PCR (QF-PCR) is a rapid assay used to identify most common aneuploidies of chromosomes 13, 18, 21, X, Y. This method uses highly DNA polymorphic markers (STRs) specific for each chromosome, to determine the number of alleles of the analyzed chromosomes. In this rare case a sample of amniotic fluid was referred for prenatal diagnosis with a high risk indication 1:2 for Edwards Syndrome after combined first trimester screening, in the absence of any detectable fetal malformation. QF-PCR was followed by cytogenetic analysis of cultured cells. The results of QF-PCR revealed an abnormal pattern of allele ratio suggesting mosaic of three full copies of chromosome 18, whereas cytogenetic analysis revealed a 50% mosaic of a supernumerary centromeric marker 18. Due to the discrepancy of these results, genetic counseling advised to continue the investigation with a second sample either fetal blood or amniotic fluid. Also, array-CGH was recommended to clarify these unusual results. The results of the second amniotic fluid sample confirmed the presence of extra marker 18. After counseling about the associated risk of abnormal outcome, parents decided to continue the pregnancy. Postnatal blood karyotype revealed a third cell line of full trisomy 18 in 5% of peripheral cells, confirming the QF-PCR findings. QF-PCR technique is a trustworthy method for detecting aneuploidies and even mosaics in a low percentage. The results should always be considered in any circumstances.

P05.39

Prenatal diagnosis of a case with mosaic 22q11 microdeletion syndrome

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Mosaic microdeletion syndromes are rarely reported and the prenatal diagnosis of those cases might be difficult because low levels of mosaicism can be easily missdiagnosed. We present a case of a pregnant women who at the 20 weeks of gestation, after a routine ultrasonography screening found polyhidramnios, intrauterin developmental delay, tetralogy of Fallot, timus hypoplasia for the fetus. Amniocentesis was performed and FISH analysis was done. Besides common aneuploidies probes, also LSI TUPLE1 probe (Abbott) for DiGeorge syndrome was used for diagnosis confirmation. A total number of 100 cells were evaluated for each probe. Results were normal for common aneuploidies. In 13 cells a 22q11.2 microdeletion was found and the finding was conclusive for mosaic 22q11.2 microdeletion (13%). Genetic counselling was offered to parents who decided to terminate the pregnancy and requested to be informed about the risk for the following pregnancy. The indemn parents were screened for the same deletion by using FISH analysis which revealed that the father also presents a very low level of mosaic for microdeletion of chromosome 22q11.2 in his peripheral lymphocytes.

This case sustains the importance of widening the FISH studies spectrum when ultrasonography shows cardiac malformations in association with other defects which can be a hallmark of a specific chromosomal deffect allowing an ethiologic diagnosis. The prenatal diagnosis of an affected fetus facilitated in this case the identification in a parent with normal phenotype of a very low level of 22q11.2 microdeletion and allowed an adequate genetic counselling.

P05.41

Next generation sequencing application for a heterogenous disorder: a pilot study on inherited peripheral neuropathies

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Inherited peripheral neuropathies (IPN) are a heterogeneous group of disorders with an overall prevalence of 1 in 2,500 and more than 50 genes implicated. Bristol Genetics Laboratory currently provides a specialist UKGTN Sanger sequencing service for 12 neuropathy genes.

The aim of this project is to evaluate the application of targeted capture NGS technology to the diagnosis of IPN using a pilot cohort of ten recruited/consented patients; three unrelated individuals with a known mutation/SNP profile including quantitative changes and seven with an uncharacterized genetic aetiology. A solution-based oligonucleotide capture array was designed using Agilent eArray (SureSelect) to capture a 450KB target encompassing coding exons and 5‘ and 3‘ UTRs of 65 neuropathy genes. DNA libraries were run on Illumina GAII and MiSeq sequencers for comparison and to aid in evaluating bioinformatics approaches to variant calling and dosage enumeration. Data analysis will be performed using open source tools (Galaxy).

We aim to assess the quality of genetic data, the extent of genetic variation in patients with IPN, the utility of current bioinformatics packages and databases in assigning variant status, the ability to detect quantitative changes, and potential new genotype-phenotype correlations.

The experience gained through validating a new technology in a clinical context and the workup of various approaches to downstream analysis together with clinically relevant variant findings will be presented.

This work will further the knowledge of the genetics of IPN and contribute to improved genetic testing for patients with these diseases.

P05.42

The non-invasive prenatal tests available in Romania

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The detection of cell-free fetal DNA in maternal circulation opened a new, noninvasive approach for prenatal diagnosis (NIPD). The NIPD approach has been studied for more than a decade. It started with the fetal RHD genotyping and gender determination, allowing at the moment the noninvasive detection of specific mutations causing diseases and selected chromosomal aneuploidies.

We performed the noninvasive detection of fetal RHD genotype in Romania since 2010 and we considered this approach an improvement in the management of mother-fetus RhD incompatibility in our country.

This short study aim was to improve the protocol for non-invasive fetal RHD genotyping in our laboratory since we notice a nonspecific amplification band in some RHD negative samples.

A three-step method was used for noninvasive fetal RHD genotyping. For the ccftDNA extraction we proceed from 1ml maternal plasma using the QIAamp® DSP Virus kit. The fetal RHD genotyping was determined using specific primers for two sequences in the exon 5 and exon 7 of the RHD gene; we also included the GAPDH, β-globin, DYS14 and SRY sequences detection as internal controls. To overcome our issue, five different PCR reactions were performed. The PCR products were automated analyzed by high-resolution capillary electrophoresis.

We tested 93 plasma samples using both protocols and the results were confirmed by the invasive method.

We conclude that our protocol for RHD genotyping is rapid and feasible. Also, the fetal gender determination is currently performed as a useful tool for screening the pregnancies at risk for inheriting an X-linked recessive disorder.

P05.43

Clinical application of non invasive RHD genotyping using cell free fetal DNA in maternal plasma

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To assess the accuracy of non invasive fetal RHD genotyping in the second trimester of pregnancy using cell free fetal DNA in maternal plasma and the practicality of avoiding the use of prophylactic antenatal anti-D gammaglobulin in RhD negative fetuses.

Fetal RHD genotyping was offered to RhD negative pregnant women attending the West Barcelona Health district. A total of 284 cases were collected at 24-26 weeks of gestation and tested for fetal RhD status using multiplex rt-PCR amplification of exons 5, 7 and 10 of the RHD gene. Women carrying RhD negative fetuses were counseled about the possibility of avoiding prophylactic anti-D immunoglobulin. Diagnostic accuracy and feasibility of routine application of non invasive RHD genotyping were compared with established postnatal RhD typing in umbilcal cord blood.

A total of 183 fetuses were genotyped as RhD positive (64 %) and 96 RhD negative (34%). Two samples were not informative as RhD sequences were detected in amount compatible with a maternal positive genotype. Three RHD variants were identified (1%), all results were confirmed in the newborns. No false positive or negative results were observed in sigleton pregnancies. One false positive result was observed in a twin pregnancy, follow up allowed determining a paternal RHD variant as its cause. Antenatal anti-D immunoglobuline was only requested in 5 cases of RhD negative fetuses.

Non invasive fetal RHD genotyping in the second trimester of pregnancy proved to be efficient and reliable. This approach allowed avoiding unnecessary use of antenatal immunoglobulin in our population.

P05.44

Preimplantation genetic diagnosis of metabolic diseases in Gennet

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In 2007 we completed the first in vitro fertilization cycle (IVF) followed by preimplantation genetic diagnosis(PGD). Since than , we have performed over 170 cycles with scheduled PGD for 53 different monogenic disorders. One third of those were metabolic diseases with X-linked and autosomal recessive types of indications.

PGD in our centre is based on principle of haplotyping analysis for determination of disease-associated haplotype derived from family members. During the IVF cycle haplotyping technique by multiplex PCR is used on products of multiple displacement amplification (MDA) from one blastomere biopsied from the cleavage-stage embryo on the day 3 (In rare cases of ambiguous result from the blastomere , analysis may be repeated from trophectoderm and embryo is still managed to be transferred on the day 5).

PGD is available for a large number of monogenic disorders. Presented group of metabolic disease forms the most frequent group of examinated disorders in our centre. We have completed 15 IVF cycles - in 11 cases the embryo was transferred. The success rate of PGD procedures of metabolic diseases is approximately 40% (gravidity confirmed by the fetal heart beat).

PGD is a reproductive option for couples at substantial risk of conceiving a pregnancy affected with known genetic metabolic diseases who wish to avoid the emotional burden associated with an affected child or termination of pregnancy.

P05.45

Preimplantation genetic diagnosis and polar body diagnosis for Fragile X syndrome

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Introduction: Fragile X (FRAXA) syndrome is one of the more frequent indications for preimplantation genetic diagnosis (PGD). However, FRAXA premutation carriers are at increased risk for premature ovarian failure and have to cope with significantly lower pregnancy rates per oocyte retrieval (ESHRE data collection X: 17,5%) when compared to PGD for other single gene disorders (ESHRE 22,1%).

Methods: 13 oocyte retrieval cycles (ORC) for polar body diagnosis in 8 families at risk for FRAXA and 26 ORC for 15 families at risk for Cystic Fibrosis (CF) as control group.

Results: For the FRAXA group less metaphase II oocytes could be retrieved (mean 8.31/cycle; CF: 12.15/cycle). With identical embryo transfer rates of 77% per ORC a significantly lower rate of clinical pregnancies (2/10=20%; CF: 9/20=45%) and life births (2; CF: 8 + 1 ongoing pregnancy) was obtained in the FRAXA group per embryo transfer cycle when compared to the CF group.

Discussion: PBD allows the earliest view on oocyte maturation in heterozygous FRAXA carrier females in a diagnostic setting. Our data indicate that PBD results for FRAXA are at least comparable to PGD on day 3 embryos. However, in concordance with the ESHRE PGD data we also observed a reduced number of metaphase II oocytes, lower impregnation and pregnancy rates for Fragile X syndrome when compared to PBD for Cystic Fibrosis, which should be emphasized early during genetic counseling.

P05.47
Virus detection from amniotic fluid and peripheral blood in pregnant women

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Background: Transplacental viral infections was widely investigated in last decades. Embryo-fetal infections have been reported to cause recurrent spontaneous abortions, and fetal malformations. The possible mechanisms include production of toxic metabolic byproducts, fetal or placental infection, chronic endometrial infection, and chorio-amnionitis. The aim of our study was to detect EBV, CMV, HHV-6, HHV-7, HHV-8,HPV and Torque teno virus DNA from amniotic fluid samples.

Materials and methods: Amniotic fluid (during artifitial membrane rupture) and periferal blood samples were collected at delivery, from 106 pregnant women. DNA was isolated with silica adsorption method. Viral DNA was determined with real time PCR method. IgG and IgM was also determined form peripheral blood samples.

Results: Viral DNA was detected in 27 of 106 amniotic flud samples. We detected CMV DNA in nine, HHV-7 DNA in eight, HHV-8 DNA in five, EBV DNA in four amniotic fluid samples. CMV, HHV-7 and EBV positivity was two-twofour fold higher in amniotic flud samples than in peripheral blood samples. 69,81% of maternal blood samples was positive for HHV6 IgG.

Conclusions: Our results suggest that viral infections occure more often in pregnancy, than previously where supposed. In case of abnormal prenatal/ ultrasound findings, and amniotic fluid sampeling rises the possibility of PCR viral detection from aniotic fluid.

P05.48
Deletion 18q21.2q21.31 encompassing *TCF4* diagnosed by CGH-Array in a fetus presenting with hypoplastic corpus callosum

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We report on a fetus with subtotal corpus callosum agenesis and bilateral ventriculomegaly diagnosed by ultrasonography at 25 weeks of gestation. After MRI confirmation, pregnancy was terminated. Conventional cytogenetic analysis on amniotic fluid cell cultures revealed a normal karyotype (46,XY). Fetal autopsy showed hypoplastic corpus callosum with fragmented fibers and bilateral ventriculomegaly. Array-CGH analysis (Agilent®, 4x44K) was performed on DNA extracted from frozen fetal tissue samples and showed a 3 Mb interstitial 18q deletion: arr 18q21.2q21.31(49190680-52188365)x1. FISH on metaphase spreads from amniotic fluid cultures with RP11-344A12 BAC probe confirmed the deletion. This deletion which occurred *de novo*, encompasses 10 genes including *TCF4*.

Haploinsufficiency of *TCF4* was identified as the underlying cause of Pitt-Hopkins syndrome. Pitt-Hopkins syndrome (PTHS, OMIM 610954) is characterized by severe intellectual disability, typical facial gestalt and daily episodes of hyperventilation followed by apnea. Other common findings are epilepsy and brain abnormalities such as hypoplastic corpus callosum. About sixty cases of PTHS confirmed by molecular studies have been reported in the literature. Most of them result from mutations or intragenic deletions or insertions. In 30 % of cases, PTHS results from submicroscopic deletion including *TCF4* and detected by Array-CGH analysis. Clinical reevaluation found facial features suggestive of PTHS.

This is the first report of a submicroscopic deletion including *TCF4* found after prenatal diagnosis of a corpus callosum abnormality. These results allow reassuring genetic counseling for later pregnancies and give the opportunity to discuss the usefulness of Array-CGH in isolated hypoplasia/agenesis of the corpus callosum.

P05.49
Study of the methylenetetrahydrofolate reductase and the reduced-folate carrier-1 gene polymorphism in healthy and severe pre-eclamptic patients

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One-carbon cycle is involved in the synthesis of purins and pyrimidines required for DNA synthesis and repair. These one-carbon groups are served by the tetrahydrofolate and the S-adenosylmethionine. Deficiencies of the folate, and methionine pathway leads to elevated homocysteine levels. These disorders have been implicated in placental diseases. Studies have shown that homocysteine levels are elevated by patients with severe pre-eclampsia than by healthy pregnant normotensive women. Methylenetetrahydrofolate reductase (MTHFR) gene C677T missense mutation is connected with elevated serum homocysteine levels. The mutation G80A of the reduced-folate carrier (RFC-1) gene leads to higher plasma folate levels. We identified polymorphisms of these genes by severe pre-eclamptic patients and healthy controls.

Blood samples were collected from healthy pregnant normotensive women (n=82) and women with pre-eclampsia (n=75). DNA was isolated and quantitative real-time PCR method combined with melting curve analysis was performed for the detection of the two polymorphisms.

The frequency of A allele in the RFC-1 gene was 46,57% by controls and 41% by pre-eclamptic patients. Overall distribution of genotypes was not significantly different between the two groups (p=0.58). In the study groups by the MTHFR gene the frequency of T allele was 32% in pre-eclamptics, 35.92% in controls. The distribution of genotypes was not significantly different between the two study groups (p=0.15).In pre-eclampsia the one-carbon cycle is disturbed. We studied single nucleotid mutations in the genes of two enzymes involved in the cycle and found no significant differences. Examinations of other genetical compounds help understanding elevated homocysteine levels in pre-eclampsia.

P05.50
Preimplantation diagnosis of hearing impairment

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In our centre we have been providing preimplantation genetic diagnosis (PGD) since 2007. Up to now we have accomplished 173 IVF cycles followed by PGD in about 50 genetic diseases. We used genetic haplotyping technique by multiplex PCR on products of MDA (multiple displacement amplification) from 1 blastomere biopsied from embryo. On average 5-6 embryos were biopsied in one IVF cycle. The success rate of the procedure (calculated on the fetal heart beat pregnancy) is approximately 30%.

We present four families in which both parents are heterozygous for 35delG mutation in GJB2 gene (Connexin 26) and have one deaf child, homozygote of the same mutation.

One family with Waardenburg syndrome, autosomal dominant inherided, where the mutation p.Phe45Leu in PAX3 gene was confirmed and the haplotypic analysis for PGD is prepared.

Six IVF cycles was realized in these families undergone PGD, recommended due to the high risk of recurrence of hearing impairment in offspring. One cycle was not followed by embryotransfer. Three pregnancies was confirmed. One of them was finished by missed abortion in 9th week. Three hearing children were born - one girl and twins, boy and girl.

One woman patient conceived spontaneously before repeated IVF cycle and gave birth to one healthy hearing son.

P05.51

A generic, fast and flexible protocol for preimplantation HLA-typing alone or in combination with a monogenic disease

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HLA-typing of *in-vitro* fertilization (IVF) embryos aims to establish a pregnancy that is HLA-compatible with an affected sibling who requires haematopoietic stem-cell transplantation. The procedure can be performed with or without preimplantation genetic diagnosis (PGD) for exclusion of a single-gene disorder (SGD). HLA-PGD is, however, a multistep, technically challenging procedure at every stage. To address the aspect of genetic analysis through simplifying patient work-up and PGD application, we developed a fast, reliable, accurate HLA-PGD protocol, to allow minimal work-up time and high flexibility for combination with any SGD.

Recent HLA-PGD requests included 11 families to treat β-thalassaemia and 1 family each for Diamond-Blackfan anaemia, Chronic Granulomatous disease, Sideroblastic anaemia and preimplantation-HLA-typing only.

For HLA-haplotyping we selected 22 short tandem repeats distributed across the entire HLA-locus (4Mb) following published guidelines. PCR primers were designed with properties allowing multiplex analysis in any combination. The resulting one-step, single-tube, multiplex fluorescent touchdown-PCR, was minimally modified to incorporate multiplex protocols for direct and indirect genotyping of the SGDs, supporting concurrent SGD exclusion and HLA-typing. Amplification efficiency and allele-dropout, from single lymphocyte testing, ranged from 97.6-100% and 0-6.2%, respectively. Five clinical cycles were performed with a diagnosis achieved for 92.8% of amplified biopsied blastomeres. Embryo transfer took place in three cycles and one pregnancy was established.

Our protocol enables HLA-typing in a single-PCR, reducing risk of contamination and cost and providing faster results. For different SGDs, it requires minimum optimization before clinical application, decreasing the waiting time from referral to treatment for all HLA-PGD cases.

P05.52

Prenatal BoBs complements excellently karyotyping in routine prenatal diagnostics

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The new bead-based multiplex assay Prenatal BACs-on-Beads (PN_BoBs™) was validated and utilized for routine use in prenatal diagnostics. PN_BoBs assay detects aneuploidies (13,18,21,X,Y) along with nine of the most common microdeletion syndromes. The validation contained 57 different fetal samples with a success rate of 98.2%. The diagnostic cases included fresh amniotic fluid (AF), chorion villus (CV) samples (group A) and tissue samples (skin or placenta) from cases with unexplained fetal loss (group B). We have now studied 118 samples (group A 55 and group B 63 cases) the success rate being 96.6 %. In group A PN_BoBs revealed two microdeletions (monosomy 22q11), one monosomy X, trisomy 18, and trisomy 21. No false positive results were detected, but one false negative result (69,XXX) was reported, and one sample with maternal cell contamination (MCC) was detected. In group B three cases of trisomy 21 and one trisomy 13 were detected. The overall abnormality detection rate was 1/12 and the additional detection rate of PN_BoBs over karyotyping was 1/59, revealing two microdeletions. The advantage of PN_BoBs is the simultaneous detection of both trisomies and microdeletions in addition to reliability and quickness. Furthermore PN_BoBs is unsensitive for MCC and it detects even 30% mosaicism. 6/118 had minor interpretation difficulties but in case of abnormalities no interpretation difficulties have arised. The reason for failures (3,4%) were due to low level of DNA. In conclusion PN_BoBs has additional informational value over QF-PCR as it also detects common microdeletions and therefore it better complements karyotyping.

P05.53

24 chromosomes in 24 hours: Validation study of a new assay for rapid detection of aneuploidies and terminal imbalances of all chromosomes in chorionic villous samples

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Conventional karyotyping after cell culture still represents the gold standard in prenatal diagnosis. In general, results are available 10-12 days after sample reception. To bridge this gap, rapid testing for common aneuploidies using QF-PCR or FISH is performed. In chorionic villous samples (CVS), direct or short-term incubation preparations are recommended to obtain an early complete chromosome analysis. However, the labour input is substantial and the results are often of poor quality. As the likelihood for chromosomal aberrations other than trisomies 21, 18 and 13 in CVS is higher as compared to amniocytes, we evaluated the KaryoLite™BACs on Beads™ technology (Perkin Elmer, Turku, Finland) as an alternative testing method for aneuploidies and terminal imbalances of all chromosomes within 24 hours. Endpoints of the study were analytic sensitivity and ease of use.

This molecular karyotyping assay consists of 91 BAC coupled beads providing information about the dosage of proximal and terminal regions of each chromosome arm using comparative hybridisation.

In this validation study, we investigated 102 CVSs with known aneuploidies (n=93, autosomal=69, gonosomal=24), polyploidies (n=3) and terminal imbalances (n=6) previously analysed by QF-PCR and karyotyping.

From our data we conclude that Karyolite™ BoBs™ provides correct and rapid results on all aneuploidies and - if covered by the assay - terminal imbalances, based on minute (<50ng) amounts of DNA in a single assay. As expected, due to methodological restrictions, female triploidies could not be diagnosed. The test has an acceptable workload and is easy to use.

P05.54

Undetected sex chromosome aneuploidy by chromosomal microarray - practical implications

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We report on a case of a female fetus found to be mosaic for Turner syndrome (45,X) and trisomy X (47,XXX) by karyotype, with 3:1 ratio between the two cell lines respectively. However, chromosomal microarray analysis (CMA) failed to detect the aneuploidy due to a normal average dosage of the X chromosome in the direct amniotic fluid sample. This case represents an unusual instance in which CMA may not detect chromosomal aberrations. Such a possibility should be taken into consideration in similar cases where CMA is used in a clinical setting, and especially in cases suspected for sex chromosome aberrations, in which mosaic states are common.

P05.55

Chromosomal mosaicism in invasive prenatal investigations in 1998-2010 in a fetal medicine department.

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Counselling after a diagnosis of chromosomal mosaicism in a prenatal invasive sample is difficult, as the impact of the finding on the fetus is difficult to predict. We review all cases of level II and III mosaicism in our prenatal invasive samples between 1998 and 2010 and present the results. 4803 amniocentesis (Amnio) were performed with 26 (0,5%) cases of mosaicism, and 643 CVS with 23 (3,6%) cases of mosaicism. Of 23 ascertained outcomes of Amnio pregnancies 4 were terminated, 1 miscarried, 16 resulted in birth of a normal baby and 2 babies had abnormalities, which were probably unrelated to the cytogenetic finding. Of 22 ascertained outcomes of CVS pregnancies 5 were terminated, 2 miscarried, 14 resulted in a birth of a normal baby and 1 baby had abnormalities, which were unrelated to the cytogenetic finding. Negative outcomes were found in mosiacist variants of chromosomal aneuploidies which are in a pure form connected with known syndromes (trisomy 21, monosomy X and so on).

P05.56

Fetal RHD genotyping in maternal plasma: 2 years of experience

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backround

Fetal RHD genotyping in maternal plasma is carried out for almost 2 years in the laboratory.

Aims: The analysis performed from 10 weeks of pregnancy involves three of the 10 exons of this gene: exons 4, 5 and 10 by real-time PCR. Two genes controls are used: SRY and CCR5 gene.

The criteria for technical and biological validation have been defined. Any negative or abnormal results (absence of amplification of the three exons or amplification of one or two of the three exons) must be controlled on a new sample taken 15 days later.

The reporting of results is standardized with well-defined biological comments. The report is accompanied by a tracking sheet of pregnancy. The feedback at birth in the laboratory by the motherhood, (indicating RH1 phenotype at birth and sex of the child) can be difficult and allows tracking of results, and is an quality indicator, the target being the absence of false negatives.

Results: Since 2010, 64 analyzes were performed: 38 results were positive, 14 negative and 12 undetermined usually corresponding to a maternal RHD gene variant. In several cases, variant RHD genes were found in the newborn.

Conclusion: the experience of almost two years shows that this technique guided by procedures and very precise and rigorous criteria of validation is reliable. Situations of maternal variants are the most difficult situations that should lead to careful interpretation of the results.

The laboratory is also part of a quality initiative, by participating and developing an inter-laboratory quality control.

P05.57

Evaluation of Prenatal BoBs® for the detection of mosaicism in prenatal diagnosis

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Objective: To evaluate the effectiveness of a new prenatal diagnostic platform - prenatal bacterial artificial chromosomes-on-Beads® (Prenatal BoBs®) in detecting mosaicism and comparing it's performance with quantitative fluorescence polymerase chain reaction (QF-PCR)

Methods: A validation study of Prenatal BoBs® was firstly performed using artificially constructing mosaic samples involving various aneuploidies and microdeletion conditions. Furthermore, we compared the accuracy between Prenatal BoBs® and QF-PCR in 16 archived real clinical mosaic cases according to the conventional karyotype results.

Results: In the validation study, Prenatal BoBs® allowed the detection of mosaicism at a level of 20 to 40%. Among 16 real clinical mosaic cases, 4 (25%)cases could be identified by both Prenatal BoBs® and QF-PCR but 8 (50.3%) cases were missed by both tests. Three cases (18.8%) were detected by Prenatal BoBs® but missed by QF-PCR, while QF-PCR detected 1 case which was missed by Prenatal BoBs® due to the mosaic region did not having probe covered. The overall sensitivity of Prenatal BoBs® in detecting mosaicism is 43.8% (7/16) which is slightly higher than 31.3% (5/16) of QF-PCR.

Conclusion: Prenatal BoBs® has a sensitivity of 44% in the detection of real clinical mosaic cases. The threshold mosaic level to be detectable by Prenatal BoBs® is 20% according to the validation study. This assay is likely superior in detecting mosaicism.

P05.58

Clinical use of array-CGH in foetuses with ultrasound anomalies and normal karyotype

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Prospective application of array-CGH in pregnancies with ultrasound anomalies and to a lesser extent in pregnancies referred for other reasons has recently become a common investigation in clinical practice. We summarize our experience on prenatal array-CGH analysis performed on 40 pregnancies with foetuses presenting isolated or multiple malformations, and/or intrauterine growth retardation detected by ultrasound and with a normal karyotype on chorionic villi or amniotic fluid samples. All parents received genetic counselling and gave their informed consent. We used the 44k Agilent-based oligonucleotides array and performed 3 hybridisation experiments per case: the DNA samples of the foetus and both parents were independently co-hybridized with a reference commercialised DNA. Eight out of 40 samples (20%) carried copy number variations (CNVs). The rate of clinically significant de novo alterations was 1/40 (2.5%) as was the rate of de novo findings with uncertain clinical significance. We found a single inherited CNV in two foetuses while 3 other foetuses each carried 2 inherited variants . Our results support the ability of array CGH to identify cryptic chromosomal abnormalities which cannot be detected by standard karyotyping. Thus, the percentage of prenatal genetic diagnosis increases, which has a strong impact on genetic counselling. We encourage the use of CGH-array for clinical practice in foetuses with ultrasound anomalies and normal karyotype.

P05.59

‚I do not want my baby to endure as I did'. Prenatal and Preimplentation Genetic Diagnosis for BRCA1/2 Mutations

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Prenatal and preimplantation genetic diagnosis (PND/PGD) can be employed to prevent transmission of BRCA1/2 mutations, thereby, obviating adverse clinical-psychological associations. It remains debated whether PND/PGD should be offered for low penetrance, late onset syndromes. A case history and issues related to PND/PGD for breast-ovarian cancer syndrome in Israel and across countries is presented.

Sarah, 34 years old, 9-10 weeks spontaneously pregnant seeks PND for BRCA1 mutation. She was diagnosed with bilateral breast cancer at 29 and 32 years of age, respectively, followed by mastectomy, reconstruction, and chemotherapy. Her mother had breast cancer at age 46; no other case of cancer in the family was reported.

This case history stimulates several questions. How personal and family history of cancer should be regarded in the context of PGD/PND; does the gender of the fetus, or the carrier status (whether due to a BRCA1 or BRCA2 mutation) should be considered; is it ethical to allow PGD or PND for late onset diseases with incomplete penetrance.

PGD/PND for hereditary breast-ovarian and bowel cancer was considered in 2006, in UK. Israel and Germany advocate and ban, respectively, PGD/PND for cancer susceptibilities, whilst other Western countries are somewhere in between. The criteria that each country adopts in favor or against PGD/PND, for late onset diseases may reflect the complex social and family ethical values regarding the technology of assistance reproduction. Comprehensively, with genetic diagnosis at hand these days, PND/PGD for BRCA1/2 mutation carriers is at the front door and becomes relevant within the clinical setting.

P05.60

Unexpected findings in prenatal diagnosis of fetal ultrasound abnormalities using SNP array

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Objective: To get insight into the frequency and nature of the so-called "unexpected findings" when using SNP array in prenatal diagnosis. This is important for setting-up a good pre-test counseling.

Methods: We performed HumanCytoSNP-12 array (Illumina) analysis of uncultured amniotic fluid cells and LTC-villi in 344 cases of fetal anomalies after exclusion of the common aneuploidies and triploidy. For correct interpretation, simultaneous analysis of both parents was performed in most cases. According to our policy, only clinically relevant copy number variants (CNVs) were reported to the clinical geneticist who counselled the parents.

Results: On a total of 344 arrays, 36 (10,5 %) CNVs were found that were interpreted as clinically relevant based on current knowledge. 24 (7 %) were most probably causative explaining the ultrasound abnormalities and 12 (3,5 %) were so-called "unexpected findings" that are most likely not directly associated with the fetal anomalies. In the latter cases the involved CNVs were risk factors for mental disability, autism spectrum disorder, schizophrenia etc. CNVs involving genes like DMD or BRCA2 were not found so far.

Conclusions: These findings stress the importance of a pre-test counseling with special attention for this type of risk factors, taking into account that risk figures are based upon postnatal studies and that the prenatal manifestation of such factors is not yet studied. In our opinion, the pregnant woman should get the opportunity to decide whether she wants to be informed about these CNVs, which may potentially complicate decision-making on continuation or termination of the pregnancy.

P05.61

The results of the Pilot Project for prenatal diagnostics of fetal malformations in Tomsk region

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In 2010, the Russian Government has appointed Tomsk region as the participant in the Pilot Project for prenatal diagnostics of child malformation on the basis of integrated (ultrasonic and biochemical) screening in the first trimester of pregnancy. The sonographic marker of chromosomal anomalies (CA) in this Program was the measure of nuchal translucency by 3D ultrasonic apparatus accompanied by the maternal serum markers of free beta-HCG and PAPP-A. On the basis of these parameters we calculate the cumulative risk of CA and form the group of expectant mothers with high risk for birth of a child with defects. These women are recommended to pass the invasive diagnostics.

During the first year of realization of this Project in Tomsk Region we examined 9.575 expectant mothers (93.5% out of women registered in first trimester or 73.4% out of all pregnant women).

As a result of prenatal screening, 124 women were selected to high-risk group of CA (1.4 % of all examined). We carried out 119 invasive manipulations for karyotyping of fetal cells. In 34 cases (28.6%) CA was diagnosed by cytogenetic methods, including FISH. Down syndrome was the most common among the detected fetal CA (17 cases). Edwards syndrome was detected in 5 cases; Patau syndrome was found in 3 cases; Klinefelter syndrome was observed in 4 cases; Turner syndrome was detected in 2 cases. Other chromosomal anomalies were detected in 2 fetus.

Thus the “Prenatal Diagnostics” program allows effectively diagnosing the fetal malformations and preventing the birth of children with CA.

P05.62

XXY by QF-PCR- is it always Klinefelter?

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Quantitative Fluorescent PCR analysis (QF-PCR) is now widely used for prenatal detection of the most common autosome aneuploidies - trisomy 21, trisomy 18, trisomy 13 and some of the sex chromosomes aneuploidies. It provides rapid and accurate results. Aneuploidies involving sex chromosomes detected by QF-PCR could reflect different types of underlying chromosomal rearrangements including mosaics and structural abnormalities.

We report two prenatal cases of XX disomy in male fetuses (SRY positive) detected by QF-PCR on uncultivated amniocytes. Both patients were referred for amniocentesis because of positive maternal serum screening.

DNA was extracted from uncultivated amniocytes, amplified with commercial QF-PCR kit Aneufast (Molgentix SL, Barcelona, Spain) and analyzed on ABI 3110 XL Karyotyping was performed on cultured amniocytes using standard protocol.

QF-PCR showed results consistent with sex chromosomes aneuploidy, SRY was present. Pseudoautosomal markers AMEL, X22, DXYS218 were in diallelic trisomic pattern in case 1. Markers AMEL, DXYS218, DXYS267 were in diallelic trisomic pattern in case 2. Markers on chromosome X were in hemizygous pattern in both cases. Cytogenetic analysis revealed 45, X/46, XY - [75%]/ [25%] karyotype in case 1 and 45, X in case 2.

The wide range of clinical manifestations of 45, X/46, XY mosaics is a genetic counseling challenge during pregnancy.

Pathological QF-PCR results involving the sex chromosomes require additional cytogenetic analysis in order to clarify the underlying chromosomal rearrangement.

P05.63

Study of the reduced folate carrier-1 (RFC-1) *A90G* polymorphism in Down syndrome

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RFC-1 gene encodes the reduced folate carrier 1 protein which plays role in the folic acid adsorption by transporting 5-methylenetetrahydrofolate to cells. Polymorphisms in folate metabolic genes have been associated with the development of Down syndrome. We determined the *RFC-1 A*80*G* polymorphism in Down syndrome.

Materials and methods: DNA was isolated from amniotic fluid of 92 Down syndrome and 76 heathy cases by silica adsorption method (High Pure PCR Template Preparation Kit, Roche, Germany). PCR was performed with LightSnip mixture of *RFC-1* (TibMolbiol, Germany) with LightCycler DNA Master HybProbe kit (Roche). Following melting curve analysis aleles were assigned and statistical analysis was performed to compare the allele and genotype frequencies.

Results: The melting point of the PCR product was 55 ºC for the *G*, and 65 ºC for the *A* allele. We observed significant difference in the frequency of the *G* allele in Down syndrome group (69.1% vs. 30.9%), higher what we expect having three alleles from chromosome 21. Accordingly it was similar with the genotypes. We found 31.8% *GGG*, 40.6% *AGG, 17,6% AAG* and 9.8% *AAA* genotypes in Down group and 28.4% *GG*, 51.4% *GA* and 20.2% *AA* in control group. **Discussion**: Polymorphism in the folate metabolism enzymes could increase the risk of chromosomal segregation and the risk of development Down syndrome. We detected high frequency of G alleles in Down syndrome cases.

P05.64

Lack of association between plasminogen activator inhibitor-1 4G/5G polymorphism and retinopathy of prematurity in premature neonates

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INTRODUCTION: Retinopathy of prematurity (ROP) is a proliferative vascular disorder in premature neonates. Due to differences in individual responses to the treatment, various genetic factors have been investigated in the etiology of ROP. We investigated the gene polymorphism of plasminogen activator inhibitor (PAI-1) 4G/5 as a risk factor of ROP development.

METHODS: 73 neonates with ROP and 101 controls were enrolled to study. The mean gestational ages were 29.4±0.8 weeks and 30±1.4 weeks, respectively. The mean birth weight was 1322±431 g and 1414±313 g, respectively. Genotyping was analyzed using real time polymerase chain reaction methods.

RESULTS: We found no significant differences in allele frequency of the PAI-1 genes between control group and neonates with ROP (*p*=0.540 and p=0.527, respectively). The proportion of 4G/4G, 4G/5G and 5G/5G genotypes did not differ statistically between the ROP and control groups (p>0.05). Having PAI-1 4G/4G genotype polymorphism seems to developed the risk of ROP (OR =0,702; 95% CI: 0.300-1.639) less than PAI-1 4G/5G polymorphisms (OR =1.064; 95% CI: 0.469-2.410). 4G/4G genotype frequency was decreasing as the stages of ROP were increasing though there was no statistically significant difference between proportion of genotypes and ROP stages.

CONCLUSION: This study showed that PAI-1 4G/5G genotype which is known as a risk factor for angiogenesis is not a predisposing factor for ROP development. Our study is the first report to investigate the association of PAI-1 gene polymorphisms on retinal angiogenesis and given clues of decreased risk for ROP development within the 4G homozygous neonates.

P05.65

The reliability of maternal serum triple screening for the prenatal diagnosis of fetal chromosomal abnormalities in Turkish women

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The purpose of this study was to evaluate the reliability of maternal serum triple marker screening of alpha-fetoprotein, human chorionic gonadotropin, and unconjugated estriol for the prenatal diagnosis of fetal chromosomal abnormalities in Turkish pregnant women. Medical records were used to analyze indications of amniocentesis and quantitative fluorescent-polymerase chain reaction. A total of 1725 pregnancies with chromosomal abnormality risk according to triple test screening were accepted for fetal chromosome analysis and quantitative fluorescent-polymerase chain reaction. Weeks of pregnancy of the subjects ranged between 13 and 22. Chromosomal aberrations were observed in 56 (3.2%) cases. About 44.6% of the abnormalities detected were numerical aberrations; however, 55.3% of the abnormalities were structural aberrations. Abnormalities detected were inversion of chromosome 9 in 20 cases, trisomy 21 in 14 cases, 46,XX/47,XX, + 21 in 1 case, trisomy 18 in 2 cases, trisomy 13 in 1 case, 47,XXY, in 1 case, 45,X, in 1 case, structural abnormalities in 12 cases, and mosaic or tetraploidy in 6 cases.

Second trimester triple test is an effective screening tool for detecting fetal Down syndrome in Turkish women.

P05.66

The relationship between haplotype & IVSII-745 mutation in β-thalassemia

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The relationship between haplotype & IVSII-745

The hemoglobin disorders are a group of autosomal recessive disorders causes anemia with different severities.

β-thalassemia is one of the commonest genetic disorders characterized by either absence or reduced β-globin chains synthesis. One of the mutations causing β-thalassemia especially in south and north of Iran is IVSII-745. There are numerous polymorphic base substitutions within the β -glibin-gene cluster, that are in linkage disequilibrium with β -globin-gene mutations.

The aim of this study was to analyze the relationship between β-globin cluster haplotype and IVSII-745 mutation.

Materials and Methods:

After obtaining informal consent, DNA was extracted from 5 ml of peripheral blood of β-thalassemia carriers referred from primary health care centers(PHC). ARMS- PCR was performed for detecting the common mutations. Haplotype analysis was done by using PCR-RFLP in three different sites. Polymorphisms included: GγHindIII, 3ψβHincII, AvaII/β. Polymorphisms were done by digesting PCR products by aproprate restriction enzyme.

Results and Discussion:

In this study, 35 β- thalassemia carriers and their parents with IVSII-745 mutation were studied. Total of 35 cases with IVSII-745(C>G) , 30% had the pattern type I and type V (- - +). In the remaining cases that have informative pattern (70%) had haplotype VII (- - -). Rest of the cases didn't have informative pattern.

Our study showed that based on haplotype analysis, it became apparent that non-random association of polymorphic restriction sites in the β-gene cluster occurs within the mutations in β-globin gene like IVSII-745.

P05.67

Prenatal diagnosis by array CGH of a fetus with Thrombocytopenia-Absent Radius (TAR) syndrome in the first trimester

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Thrombocytopenia-Absent Radius (TAR) syndrome (MIM 274000) is a very rare condition (0.5:100,000) characterized by absence of the radii with the presence of both thumbs and thrombocytopenia. However, other congenital anomalies can occur, and affect the skeletal, cardiac, gastrointestinal, and genitourinary systems. The mode of inheritance of TAR syndrome is unknown. Presence of a minimally deleted 200-kb region at chromosome band 1q21.1 is necessary but not sufficient to cause the phenotype. One or more as-yet-unknown modifiers are thought to be necessary for the expression of the TAR syndrome phenotype. Whereas TAR syndrome has been described in numerous patients, a very limited number of prenatally diagnosed cases have been reported and only five cases have been genetically tested and verified the microdeletion in the 1q21.1 region. We report clinical, molecular and cytogenetic studies of a prenatally diagnosed fetus with TAR syndrome with a small (334 kb) deletion characterized by the array-CGH technique and emphasize that array-CGH remains a useful tool in the precise description of TAR syndrome as well as other haploinsufficiency syndromes.

P05.68

Triploidies identified at prenatal diagnosis

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Triploidy, the presence of an extra haploid set of chromosomes, is one of the most frequent chromosomal abnormalities affecting human gestation. Its prevalence among all pregnancies has been estimated to be approximately 1% to 3%. Triploidy may be the result of either digyny (extra haploid set from mother) or diandry (extra haploid set from father).

Two distinct phenotypes observed in triploid fetuses have been shown to be associated with parental origin of the triploidy (McFadden and Kalousek 1991). The diandric phenotype is characterised by a normally sized or mildly symmetrically growth retarded fetus with normal adrenal glands, and is associated with an abnormally large, cystic placenta with histological features known as partial hydatidiform mole (Type I). The digynic phenotype is characterised by marked asymmetric intrauterine growth restriction (IUGR), marked adrenal hypoplasia, and a very small, non-molar placenta (Type II).

We present chromosomal, fetal ultrasound and pathological findings in two cases of triploidy diagnosed prenatally. The parental origin of the additional haploid chromosome set was determined based on a range of highly polymorphic microsatellite markers. In the first case, the fetal karyotype was 69,XXY and the histological investigation showed bilateral cystic renal dysplasia. There were external and internal fetal anomalies, and marked IUGR in the second case of triploidy (69,XXX). The parental origin of the triploidies was found to be maternal and both triploidies were screen positive for trisomy 18.

It has been published that digynic triploidy predominates in fetuses, and diandry accounts for about 50-60% of early triploid spontaneous abortions.

P05.69

Screening for prenatal diagnosis of chromosomal abnormalities

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Introduction: Invasive procedures for prenatal diagnosis can be performed on different reasons: at demand of the patient, usually advanced maternal age; determined by the results of biochemical test; combined screening, which includes specialized ultrasonography (visualization of fetal anomalies or soft markers).

Objectives: The aim is to establish the qualitative analysis from the indications of invasive testing for prenatal diagnosis (detection rate of chromosomal abnormalities and the false positive rate - FPR).

Material and method: During ian.2010-dec.2011 we examined a number of 947 pregnant patients. We performed a number of 86 invasive procedures in order to determine fetal karyotype: 15 - advanced maternal age; 20 - biochemical risk + maternal age; 51 - ultrasound criteria + maternal age ± biochemistry.

The calculated risk was estimated by introduction of data in the software Astraia. The cut-off level for the 1st trimester screening was set at 1: 150 and for the 2nd trimester - at 1:250.

Results: Prenatal diagnosis revealed 10 chromosomal abnormalities (T21 - 4, T18 - 3, T13 - 2 and 1 case of triploidy). All this cases belong to the group with ultrasound exam. For the same rate of detection, the FPR is 8% for all invasive procedures. If we exclude the invasive diagnosis on demand, the FPR is 6.44% and if we analyze only the cases (51 pregnancies) with ultrasound screening - the FPR is 4.3%.

Conclusion: We observe that if we analyze pregnancies including ultrasound screening, the FPR drops almost to the half value (from 8% to 4.3%).

P05.70

Paternal uniparental diploidy mosaicism in a fetus with multiple abnormalities including findings of Beckwith-Wiedemann Syndrome

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We describe a fetus with multiple abnormalities in prenatal ultrasound, especially hydrops, hepatomegaly and a retroperitoneal tumour. After fetal karyotyping following amniocentesis showed a normal female karyotype, uniparental disomy was suspected and analysis of microsatellites for chromosomes 13, 18 and 21 was performed, comparing fetus and parents. Results were suspicious for a mosaic paternal uniparental diploidy with a normal biparental cell line. The rate of uniparental cells was about 80%. Uniparental diploidy was also found in fetal blood and after induced abortion in fetal tissue with variant frequencies. Autopsy revealed macroglossy, hemihypertrophy and viszeromegaly of liver, pancreas, adrenal glands, ovaries and skelettal musculature. Furthermore the fetus showed multiple tumours concerning heterotopic tissue of the adrenal glands, nodular nephroblastomatosis of the kidneys and haemangioma of the heart. All features resembling Beckwith-Wiedemann Syndrome. There are few reports on fetuses with mosaic uniparental diploidy. Analysis for genome-wide uniparental disomy should be considered in fetuses with an atypical phenotype.

P06. Cancer genetics

P06.001

Frequency of the DPYD * 2A allele in the Czech population

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5-fluorouracil (5-FU) is used for chemotherapy of many tumors. Enzyme DPD (dihydropyrimidine dehydrogenase) has a key function for 5-FU metabolism and is encoded by *DPYD* gene. Mutant allele DPYD * 2A is caused by inherited mutation IVS14 +1 G> A *DPYD* gene and produce non-functional enzyme, so the 5-FU treatment leads to toxic reactions.

212 males and 210 females (age 18-69 years) of the Czech population were tested. DNA was isolated from oral mucosa or blood by using QIAmp DNA Minikit / DNA QuickGene 810. DPYD * 2 allele was tested by using certificated method strip assay (PGX-5FU) and by new created metod High-Resolution Melting. Were compared to results of both methods with each other and assessed allele frequency in the Czech population.

DPYD * 2A allele was demonstrated in a heterozygous state in 3/422 (0.7%), in the homozygous state was not found. Czech frequency of DPYP * 2A allele 0.36% is 2.5 times lower than the reported frequency 0.91% in the European population. The results of both methods were completely identical.

New cheaper method for detection DPYD * 2A was introduced and validated. Low frequency of DPYD * 2A allele in the Czech population assume the presence of other mutations in the *DPYD* gene and in other genes affecting the metabolism of 5-FU (eg . *TYMS, MTHFR*). Genetic test is recommended prior to cancer treatment by 5-FU, because are suitable the lower doses in the heterozygotes and choose other chemotherapy for the homozygotes with risk of toxic reaction.

P06.002

Concordant change of the 5-hydroxymethylcytosine status and mRNA expression at the LZTS1 loci in breast cancer

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Analysis of 5-methylcytosine (5-mC) patterns in DNA for the identification of epigenetic dysregulation in cancer is well established whereas elucidation of the role of 5-hydroxymethylcytosine has yet to be unravelled. TET-proteins convert 5-mC, usually found in CpG context, to 5-hmC, which is assumed to be involved in gene expression regulation and might prove to be intriguing for cancer diagnostics. DNA samples from breast cancer tissue (n=6) and blood samples from healthy patients (n=6) were glucosylated with 5-hydroxymethylcytosineglucosyltransferase and digested with the restriction endonuclease MspI. As control reaction each sample was treated with MspI without prior glucosylation. Thus, the selective glycosylation of 5-hmC to glucosyl-5-hydroxymethylcytosine (glu-5-hmC) enabled us to distinguish between 5-hmC and methylated or unmodified cytosine. After digestion, the samples were analysed for 5-hmC in 325 loci by a targeted microarray. Through this approach the detection of one potential gene loci with a significant difference (P<0.05) was achieved. The marker LZTS1was validated with qPCR on breast cancer samples (n=32) and normal tissue (n=6), showing a higher level of 5-hmC in normal breast tissue. As next step the mRNA expression of LZTS1 was monitored in the validation set, which enabled us to detect a significant decrease of mRNA expression of the tumor suppressor gene LZTS1 in cancerous tissue. Our detection of 5-hmC DNA changes might contribute to understand possible functions of 5-hmC as an epigenetic regulator, because to our best knowledge we are the first that show a direct connection between 5-hmC alterations and a changed mRNA-expression on experimental basis.

P06.003

Analysis of FLT3 mutations in infant acute leukemia

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FLT3-gene mutations cause leukemic cells to proliferate uncontrollabely and leads to a poor prognosis.The aim of this study is to explore appropriate at diagnostic molecular tests and to screen mutations that occur in patients with acute leukemia.

91 infant Acute Myeloid Leukemia and Acute Lymphoid Leukemiaof patients investigate for FLT3- gene mutations in acute leukemia and ITD mutation (Internal Tandem Duplication) that codes juxamembrane region in FLT3 receptor and also the point mutation that is coded by exon 17 in that is FLT3 receptor kinase region .ITD mutation in FLT3 receptor was analyzed by PCR in 11,12 exons and 11 intron , using designed primers. For analysis of point mutation of Exon 17 in FLT3 receptor gene ,the genomic DNA of patient was amplified using the PCR. Resulted PCR products were studied by ECOR V enzyme and RFLP In cases of positive ITD, the Sequencing Method was applied.

ITD mutation was observed in 7 cases of 91 studied acute leukemia patients. Under investigation the sequence of PCR products in the mutation samples showed that different insertions of ‚s' are seen in JM region.

Also, 2 of 91 patients, studied had point mutation of in which their (D835) distributions in were not identical in FAB subtypes.Studying history of these patients, it was cleared that there was not significant relation between chromosome variations and induction of mutation and it can be decided about the treatment by molecular diagnosis of this mutaions independent of FAB classification and before the treatment get started.

P06.004

B-cell activating factor: variability of selected exonic regions and association with acute lymphoblastic leukemia in paediatric patients

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Introduction: There is emerging evidence that B-lineage neoplasms have aberrant expression of B-cell activating factor (BAFF, TNFSF13B) that enables the B cells to escape apoptosis. The aim of our study was to investigate circulating levels of BAFF in paediatric malignancies related to B-cell growth, i.e. B-cell acute lymphoblastic leukaemia (B-ALL) and B-lineage lymphomas.

Materials and methods: This cross-sectional study included the total of 18 children with B-lineage neoplasms (11 children with B-cell lymphoma, mean age at onset ± SD: 11.4 ± 4.8 y) and 7 children with B-cell precursor ALL, mean age at onset ± SD: 6.1 ± 6.1 y) whose serum levels of BAFF before the start of the treatment were examined using the ELISA-based methodology. Exons 1, 4 and 5 of BAFF gene were investigated using the direct sequencing in all patients.

Results: We observed significant differences in circulating levels of BAFF between the B-ALL patients and B-cell lymphoma patients (Bcp-ALL: 7764 ± 6329 pg/ml, B-cell lymphoma: 2675 ± 1544 pg/ml; p = 0.0268), the circulating levels of BAFF being substantially higher in B-ALL cases than in B-cell lymphoma cases. However, no genetic variability was observed in any of examined exonic regions of BAFF gene.

Discussion: This is the first study to report elevated BAFF levels in acute lymphoblastic leukemia in children. Although highly limited in number of cases, our study provides a potential basis for further evaluation of BAFF as a diagnostic and/or prognostic marker in B-ALL.

P06.005

SNP rs4132601 as a possible risk allele of acute lymphoblastic leukaemia development in children

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Background. Acute lymphoblastic leukaemia (ALL) is the most common cancer among children, with an annual incidence rate of approximately 3.9 per 100 000 children. Recent case control genome wide association studies confirmed single nucleotide polymorphism (SNP) rs4132601T>G of *IKZF1* gene, which is located on chromosome 7, to increase significantly risk of developing childhood ALL. There are no available data about rs4132601. G allele is a risk allele from familial studies evaluating allelic transmission distortion.

Aim. To confirm role of *IKZF1* gene rs4132601G allele in relationship to childhood ALL development.

Material and methods. Eighteen pre-B ALL patient' case-parent trios were recruited at Children's Clinical University Hospital. The presence of polymorphism was analyzed using PCR with subsequent restriction enzyme *MboI* digestion and visualized in polyacrylamide gel. Transmission distortion test was performed as implemented in PLINK 1.07.

Results. SNP rs4132601 G allele frequency in patients was 0,472. Four out of

18 patients were homozygous for the risk allele. There was not found significant association between analyzed SNP in ALL familial studies (p=0.449, OR=0.75, CI95% 0.35-1.58).
Conclusions. In present study, we could not confirm any significant evidence about rs4132601 G allele as a risk allele. However HapMap project in European samples observed allele frequency was 0.301, thus justifying further studies in a larger sample of ALL patients' parents' trios.

P06.006
Detection of DNMT3A Mutation in Iranian patients with acute myeloid leukemia

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Acute myeloid leukemia (AML) is a disease with marked heterogeneity in both response to therapy and survival. The advent of molecular diagnostics has heralded an explosion in new prognostic factors, including mutations in DNMT3A gene that encodes DNA methyltransferase.
In this study, mutation in exon 20 of DNMT3A gene of 25 untreated cytogenetic normal AML patients (The mean age of patients was 39 year) were analyzed. For this purpose, genomic DNA was extracted from peripheral blood (referred to Shariati hospital, Tehran, Iran) by standard methods. PCR amplification and DNA Sequencing was performed for exon 20 of the DNMT3A gene.
DNA sequencing at two patients showed 2 different missense mutations in exon 20 of DNMT3A gene. These missense mutations were predicted to affect amino acid R882 (Polyphen 2). A patient had the R882H (CGC to CAC) variant and another one had R882P (CGC to CCC). These two patients had a normal cytogenetic profile and their white-cell counts were significantly higher than other patients.
In any case, DNMT3A mutations are associated with poor overall survival, suggesting that they have an important common effect on the potential of AML cells to cause lethal disease. Recent study showed that older AML patients with R882-DNMT3A mutation has shorter disease free survival (DFS), while in younger patients (<60 years) non R882-DNMT3A mutation have shorter DFS.

P06.007
The simultaneous usage of BAC based high throughput FISH analysis and the Real-Time PCR technology at the diagnosis of the adult myeloid leukemia cases

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Introduction**:** Leukemia occurs as a result of limitless and uncontrollable production of the blood cells. Among Myeloid Leukemias, CML and AML take place. In our study, Real-Time PCR and BAC Based High Throughput FISH Analysis methods are utilized simultaneously at the diagnosis of the adult myeloid leukemia cases and it is aimed to determine the aberrations that occur in the genome level.
Materials and Methods: Without a gender consideration, peripheral blood samples taken from 47 AML or CML diagnosed individuals between ages 18-80 are examined with the Real-Time PCR method in the RNA level and with the BAC Based High Throughput FISH Analysis method in the DNA level and scanned to see where most of the genetic damages take place.
Results: As a result of the Real-Time PCR studies; translocation in 10 patients (21,3%) and as a result of the FISH Analysis; aberrations with several sizes in various of the genome in 13 patients (27,7%) were encountered. In 27 patients (54,4%) no existence of genetic damages were seen with either methods. The most frequent aberration is the trisomy 8 and loss of Y chromosome which was stated in 3 (6,4%) AML cases.
Conclusion: The simultaneous usage of these two methods for the diagnosis of the leukemia cases provides a new approach to hematology in terms of supporting the diagnosis and determine the prognosis.

P06.008
Pharmacogenetic study in Argentinean children with Acute lymphoblastic Leukemia (ALL)

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The aim of this study was to evaluate the influence of the most common genetic variants in the genes methylenetetrahydrofolate reductase (MTHFR), thiopurine methyltransferase (TPMT), and glutathione-S-transferases P1, T1 and M1 (GSTP1, GSTT1, GSTM1) on the outcome of ALL treatment.
Patients and Methods
Samples from 310 ALL patients treated with 2 consecutive BFM-based protocols were analyzed. Genetic variants in TPMT, MTHFR, GSTP1, GSTM1 y GSTT1 were identified by PCR-RFLP or allele-specific PCR. Toxicity was evaluated in 165 patients during consolidation phase according to WHO criteria. Children received 2 or 5 g/m^2/day of methotrexate (MTX) according to risk group, inmunophenotype and protocol, together with 25 mg/m^2/day of 6-Mercaptopurine. Patients allocated in High Risk Group also received the so-called High-risk blocks. The association between toxicity and genotypes was assessed by Fisher-exact Test. The pEFS was estimated by Kaplan-Meier and differences were assessed by log-rank test.
Results
Children who received 2 g/m^2/day of MTX and carried at least one 677T allele variant in MTHFR showed an increased risk of developing severe leukopenia (p=0.004) and neutropenia (p=0.003) during the consolidation phase. MTHFR polymorphisms did not seem to modulate MTX toxicity at 5g/m^2/day. None of the variants evaluated influenced treatment efficacy significantly. Nevertheless, in the intermediate group risk the pEFS for patients with heterozygous genotype for TPMT was higher than that for the wild type group (87% vs. 71%).
Conclusions
Further studies would be necessary to validate these results in order to better define MTX and 6-Mercaptopurine doses in ALL treatment.

P06.009
Anti-mutagenic and Pro-apoptotic Effects of Apigenin on Human Chronic Lymphocytic Leukemia

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Diet can play a vital role in cancer prevention. Nowadays the scientists are looking for food materials which can potentially prevent the cancer occurrence. The purpose of this research is to examine antimutagenic and apoptotic effects of apigenin in human lymphoma cells. In present study human chronic lymphocytic leukemia (Eheb cell line) were cultured in RPMI 1640 (Sigma), supplemented with 10% fetal calf serum, penicillin-streptomycin, L-glutamine and incubated at 37 ºC for 2 days. In addition cancer cell line was treated by and apigenin and cellular vital capacity was determined by MTT assay.
Then effect of apigenin in human lymphoma B cells was examined by flow cytometry techniques. The apigenin was subsequently evaluated in terms of anti-mutagenic properties by a standard reverse mutation assay (Ames test).This was performed with histidine auxotroph strain of Salmonella typhimurium (TA100). Thus, it requires histidine from a foreign supply to ensure its growth. The aforementioned strain gives rise to reverted colonies when expose to sodium azide as a carcinogen substance. During MTT assay, human chronic lymphocytic leukemia revealed to have a meaningful cell death when compared with controls (P<0.01) Apoptosis was induced suitably after 48 hours by flow cytometry assay. In Ames test apigenin prevented the reverted mutations and the hindrance percent of apigenin was 98.17%.These results have revealed apigenin induced apoptosis in human lymphoma B cells in vitro.

P06.010
The APC gene polymorphisms and implication in colorectal cancer susceptibility

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Colorectal cancer (CRC) is a complex genetic disease, which results from interactions between multiple genes and environmental factors without any single factor having strong independent effects. The goal of the analysis was to determine if exist an associations between four polymorphism of APC gene (rs41116, rs465899, rs2229992 and rs2019720) and colorectal cancer patients. Blood samples were obtained, after informed consent, from individuals with CRC (M:F=95:85) and healthy persons (M:F=27:33). Genomic DNA was extracted from peripheral blood leucocytes using commercial kits and the APC gene polymorphisms were assessed by PCR-RFLP. Among the analyzed polymorphisms, we observed significant differences between patients and control group for polymorphism rs2019720 in terms of the distribution of genotypes and alleles. For this polymorphism from the promoter region of the APC gene we obtained an association with disease. Thus, the CC genotype (OR 2.307) and allele C (OR 1.843) are associated with increased risk, while the AA genotype (OR 0.453) and allele (OR 0.543) are associated with decreased risk for CRC. In this study, APC polymorphism rs2019720 may contribute to CRC susceptibility in Romanian patients. Thus, this potential link must be evaluated between in much powerful studies. This study was supported by Grant CNCSIS TD 224/2008.

P06.011
A novel pathogenic germline mutation in the adenomatous polyposis coli gene in a tunisian family with FAP

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Familial adenomatous polyposis (FAP) is an autosomal dominant disorder which typically presents with colorectal cancer in early adult life, secondary to extensive adenomatous polyps of the colon. In addition to the colonic manifestations, the syndrome presents several extracolonic features including, congenital hypertrophy of the retinal pigment, osteoma and desmoid tumors. In this study, we aimed to investigate the clinical and genetic features in a Tunisian family with FAP. Sequence of the APC gene (Adenomatous Polyposis Coli) revealed a novel mutation (c.2016-2017 del TA) in exon 15,present in all affected individuals in an heterozygous state. The frameshift mutation generates a premature stop codon at amino acid 677 of the APC protein (p. H672Qfs X5). The unaffected family members did not harbor this mutation, however, a first degree relative of the patient aged of 32 year-old was phenotypically normal but carries the c.2016-2017 del TA mutation. This discrepancy can be explained by the effect of modifier gene which can affect the expressivity of the disease. Moreover an other first degree relative who is the patient's daughter, aged 8 year-old carries the mutation in an heterozygous state and should benefit of a colonoscopical follow-up knowing the phenotypic variability in her family.

P06.012
Aurora Kinase A (AURKA) and Never in Mitosis GeneA-Related Kinase 6 (NEK6) Up- regulated Gene Using cDNA Microarray and Real-time Reverse Transcription-PCR in Erosive Esophagitis and Esophageal Adenocarcinoma.

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Background and Aim: Gastroesophageal Reflux disease is a risk factor for esophageal adenocarcinoma but the studies that investigate the relationship between erosive esophagitis and esophageal adenocarcinoma usually focus on symptom-related evidence or on polymorphism but there are not any epigenetic gene expression studies on this topic. In this study we aimed to evaluate the relationship of erosive esophagitis and esophageal adenocarcinomas if there is a genetic tendency for EAC.

Methods:*The Human Epigenetic Chromatin Modification Enzymes RT2 Profiler*^{TM} *PCR Array* was used to detect the expression of 84 key genes encoding enzymes. It was used in 60 patients prospectively (20 patients with control group, 20 patients with erosive esophagitis and 20 patients with esophageal adenocarcinoma).

Results: AURKA, AURKB, NEK6 were expressed at significantly higher levels in the esophageal adenocarcinoma than the control group. MBD2, were expressed significantly lower in esophageal adenocarcinoma than the control group. AURKA, AURKC, HDAC9, NEK6, were expressed at significantly higher levels in erosive esophagitis than control group. There was no upregulated gene difference between erosive esophagitis and esophageal adenocarcinoma. MBD2 was significantly downregulated in esophageal adenocarcinoma than erosive esophagitis. The NEK6 and AURKA were significantly upregulated genes in esophageal adenocarcinomas and erosive esophagitis than control groups.

Conclusion: This is the first and pioneering study about the genetic tendency of erosive esophagitis and esophageal adenocarcinoma. AURKA and NEK6 are two promising genetic markers for erosive esophagitis and esophageal adenocarcinoma.

P06.013
ASCL1 activation as a consequence of a t(12;14)(q23.2;q32) in B-cell chronic lymphocytic leukemia

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B-cell chronic lymphocytic leukemia (B-CLL) is frequently accompanied by characteristic cytogenetic abnormalities. Nevertheless, the molecular mechanisms underlying the disease remain largely unknown.

We investigated a translocation between chromosomes 12 and 14 in a patient with B-CLL. FISH confirmed the involvement of the immunoglobulin heavy chain (IgH) locus on chromosome 14 in the translocation. The breakpoint region on derivative chromosome 12 was amplified using long distance inverse PCR and sequenced. Chromosome 12 was disrupted in the region between the *C12orf42* and *ASCL1* (Achaete-scute complex homolog 1) genes. The breakpoint on chromosome 14 was located in the switch region upstream of the IgH Cμ sequence. As a consequence of the rearrangement the *ASCL1* gene was brought into proximity of the enhancer downstream of the IgH joining region and was highly expressed in the bone marrow of the patient in comparison to normal bone marrow and that of other B-CLL patients.

ASCL1 codes for a basic helix-loop-helix transcription factor involved in neural development. The gene is overexpressed in neuroendocrine cancers like small cell lung cancer and medullary thyroid cancer. *ASCL1* plays a role in cell proliferation and differentiation and interacts directly or through its targets with members of the NOTCH, the WNT and the SHH pathways.

Though *ASCL1* activation in B-CLL seems to be a rare event, deregulation of some of its downstream targets or interaction partners, due to different molecular mechanisms, could play a role in the genesis of B-CLL.

P06.014
Gene expression profiling of B-CLL in Ukrainian patients exposed to low doses of ionizing radiation in post-Chernobyl period

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Introduction: After Chernobyl accident, B-cell Chronic Lymphocytic Leukemia (B-CLL) became a predominant form of hematopoietic malignanices in clean-up workers. We have analyzed gene expression patterns in B-CLL patients exposed to low doses of ionizing radiation in post-Chernobyl period with the aims to identify genes associated with disease progression in order to shed light on the biology of progression.

Materials and methods: The samples of the peripheral blood and bone marrow of 44 Ukrainian B-CLL patients were analyzed morphologically and immunocytochemically according to new WHO classification. Total RNA was isolated, gene expression levels were determined by microarray method comparing with 17 healthy donors.

Results: We investigated interactions using the Ingenuity Pathway Analysis (IPA) software and found 1191 network eligible up-regulated genes and 3398 Functions/Pathways eligible up-regulated genes, 1225 network eligible down-regulated genes and 2657 Functions/Pathways eligible down-regulated genes. Gene networks identified around MYC, HNF1A, and HNF4A, YWHAG, NF-κB1 and SP1 as up-regulated; CEBPA, YWHAG, SATB1 and RB1 as down-regulated. G protein coupled receptor signaling, Arachidonic Acid and Linoleic Acid metabolsims, calcium signaling, metabolsim of Xenobiotics by cytochrome P450 are significant up-regulated pathways. Eif2 and Cdc42 signaling, regulation of eIF4 and p70S6k signaling, protein ubiquitination pathway and oxidative phosphorylation are the most significant down-regulated pathways obtained in our study.

Conclusion: Our study represents one of the rare genomic studies concerning relationship between ionizing radiation and B-CLL. NF-κB gene network was conspicuous in terms of being determined also in our previous studies about the gene expression on prostate cancer and acute myeloid leukemia.

P06.015
Frequencies of BCR-ABL1 fusion transcripts among Iranian patients with leukemia

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In this study, we report the frequencies of BCR-ABL fusion transcript variants studied in leukemia patients from Iran. The leukemia patients including 348 chronic myloid leukemia (CML), 72 acute lymphoblastic leukemia (ALL), 34 acute myeloid leukemia (AML), 25 myelodysplastic syndromes (MDS), and 7 chronic lymphoid leukemia (CLL) were enrolled in this study. Peripheral blood samples were analyzed by multiplex RT-PCR from 486 leukemia patients to detect different types of BCR-ABL transcripts.The BCR-ABL transcript frequencies for CML, ALL, AML, CLL and MDS patients were 92.0% , 12.5%, 26.4%, 14.2% and 4% respectively for all transcripts. The majority of CML patients with positive BCR-ABL expressed one of the p210BCR-ABL transcripts (86.6%) while the remaining showed other transcripts (p190BCR-ABL 25 (7.8%)) and p230BCR-ABL 2 (0.6%)). The rate of co-expression of the p190/p210 transcripts were 16(5%). In other types of leukemia patients the rates of expression of those transcripts were different. we didn‘t observe a significant correlation between BCR-ABL1 variants, sex type, age and WBC count of studied leukemia patients.

P06.016

AHR, AHRR, ARNT gene polymorphisms role in bladder cancer development

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To assess c.1661 G>A AHR, c.565C>G AHRR, c.522G>C ARNT gene polymorphisms contribution in the development and severity of bladder cancer we carried out a study 220 DNA bladder cancer patients samples and 216 healthy individuals DNA samples. No association with disease was observed for AHR polymorphism. It has been established that with increasing c.565C>G AHRR G allele dose, increased its protective effect (additive model, OR=0.61, [p=0.02). It has been shown ARNT GC genotype is a resistance factor to disease development (OR=0.56, 95%CI (0.38-0.85), p=0.005). Thus we can assume AHRR and ARNT polymorphisms make a definite contribution to the development and severity of bladder cancer However, the results should be confirmed in replication.

P06.018

Expression profile of bladder cancer cell lines after treatment with potential new drug- Helix lucorum hemocyanin (HlH)

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Urinary bladder cancer is socially significant healthcare problem- negative environmental factor, infections and aging of the world population are responsible for its growing incidence. This requires elaboration of new drug development. Such a potential new drug is Helix lucorum haemocyanin (HlH) - a highly immunogenic glycoprotein with xenogenic nature and a lot of mannose residues.

The anti-tumor effect of HlH was investigated on CAL-29 and T-24 bladder tumor cell line and results suggest 35%-50% inhibition of cancer cell viability in a dose 500ug/ml of HlH after 72h of incubation.

Gene expression profiling of the tumor cells before and after HlH treatment using the panel for 84 genes for Human Inflammatory cytokines and receptors was performed.

Results presume more than 10 times upregulation of genes for inflammatory response activation, cell mediated immunity and proinflommatory cytokines: C4A, CCR4, CARD-18, IL36A, IL37, CXCR1, IL9, LTA, MIF and TNF in CAL-29 cell line treated with HlH (G1). In the same group more than 4 time overexpression of TOLLIP gene was achieved.

In T-24 cell line treated with HlH (G2): IFNA2, IL37, CXCR1, IL36B, IL5 and AIMP1 genes were significantly overexpressed. In the both group CXCR1 gene (for IL8 receptor) had the highest expression.

Gene for CXCL1 was notably downregulated compare to ABCF1, BCL6, IL1A, IL1B in both groups.

Our results suggest that HlH can perform both: growth inhibiting effect (on cell line level) and immune response activation (according gene expression profile) in bladder cancer.

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P06.019

Association of Gene Variants of IL-12 & IL-18 and Serum IL-18 with Bladder Cancer Risk in North Indian Cohort

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Background and Objective: Bladder cancer (BC) is multifactorial disorder and genetic changes may be a crucial etiologic factor. Interleukin-18 and Interleukin-12 plays an important role as immunomodulatory factors in cancer pathogenesis that augment IFN-γ secretion. To test this hypothesis, we investigated association of IL-18 gene promoter polymorphisms at *-137G/C*, *-607C/A* and *IL12(-16974)A/C* with the risk of BC in a North Indian cohort.

Material and Methods: Genetic polymorphisms were analyzed in 200 BC patients and 200 age, ethnicity and sex-matched controls, using Restriction Fragment Length Polymorphism and Amplification refractory mutation specific-polymerase chain reaction. The concentrations of IL-18 in serum were determined by ELISA.

Results: Significant association was observed with *IL18(-137)G/C* heterozygous genotype having 1.96 folds risk of BC as well as C allele carrier and variant allele having 2 fold and 1.6 fold risk for BC respectively. *IL18(-607) C/A*, CA genotype also showed a high risk (OR=1.59) for BC. While *IL12(-16974) A/C* heterozygote genotype and C allele carrier showed reduced risk for BC. Hetero genotype of *IL18(-137)G/C* was associated with risk of recurrence (HR= 2.35) in BC patients receiving BCG treatment showing least survival. Serum IL-18 levels were significantly higher in BC patients than in the healthy subjects (p=0.025).

Conclusion: Our results suggest functional IL-18 polymorphism contributes to the bladder cancer susceptibility. A relation between IL-18 gene polymorphism and serum content with cancer progression has been registered in present study. Further confirmation in large population based studies is needed.

P06.020

Analysis of mitochondrial DNA and p53 gene mutations in bladder tumors

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Introduction: The effect of mitochondrial DNA (mtDNA) mutations and p53 gene mutations have been investigated separately in various tumors; however, both of these factors were not investigated in bladder tumors. Therefore, to understand the significance of mtDNA and p53 gene mutations in bladder cancer development, these mutations were investigated in bladder cancer patients.

Methods: Bladder cancer tissues were obtained by radical cystectomy or transurethral resection from 30 patients and 27 controls. After the isolation of DNA from peripheral blood, exon 5,6,7 and 8 of p53 gene as well as ATPase6, Cytb, ND1, and D310 regions of mtDNA were amplified by PCR. Mutations were detected with direct sequencing. Results were evaluated statistically.

Results: In patient group, 33 mtDNA gene mutations were found in which six of them are novel and 14 of them cause amino acid changes. Also G8697A, G14905A, C15452A and A15607G mutations were found statistically significant in patients when compared to controls. Additionally, in patient group three, p53 gene mutations were detected in which two of them are novel. In novel mutations, adenine insertion at 12570 was found statistically significant in patients when compared to controls (66%).

Conclusion: Especially high incidence of the novel mutation at position 12570 in p53 gene may be an important marker for the detection of bladder tumors. Therefore it should be investigated with further studies. Also high incidence of other mtDNA mutations and p53 gene mutations in patients suggests that mitochondria and p53 gene could play an important role in carcinogenesis.

P06.021

Distribution of mutations in breast/ovarian cancer susceptibility genes in the north-east of Spain.

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Mutations in susceptibility breast/ovarian cancer (BOC) genes show different spectra according to geographic origin. To date, few *BRCA1* and *BRCA2* mutations are described as founder in Spanish population, intriguingly linked to geographic distribution. Other susceptibility genes that share key roles in Fanconi Anemia pathway are now being scanned for mutations.

A total of 1275 unrelated families have been tested for BRCA mutations in the IBGM. Samples and written informed consent of BOC cases and relatives were collected at the Genetic Counselling Units of East CyL. DNA was scrutinized for BRCA mutations by HA-CAE and subsequently sequence analysis of abnormal patterns. Additionally, MLPA was performed in high-risk patients with familial history of BOC without point mutations. Moreover, other genes as *PALB2* or *RAD51C* were tested in families with specific cancer features.

Seventy-four different deleterious DNA changes in BRCA genes have been identified in our laboratory in 186 unrelated families (69 BRCA1+ and 117 BRCA2+ families). Spanish founder mutations were predominant in our samples: 330A>G, 5242C>A and 5272-1G>A in *BRCA1* and 3036_3039delACAA, 5374_5377delTATG and 9254_9258delATCAT in *BRCA2*. Remarkably, 187_188delAG-*BRCA1* mutation was absent in our region. Five different large rearrangements in seven ovarian unrelated patients have been detected during MLPA analysis. No pathological mutations were identified in either *RAD51C* or *PALB2*.

Our target population shows a particular *BRCA1* and *BRCA2* mutation spectra where Spanish founder mutations are leading. Although mutations in other susceptibility BOC genes do not yield results so far, comprehensive studies with larger number of samples would be performed.

P06.022

Identification of mutations in patients with bone marrow failure syndromes associated with the development and progression of MDS and acute leukemia

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Most bone marrow failure syndromes (bmfs) are associated with a marked propensity to transform into myelodysplastic syndrome (MDS) or acute leukemia, with a cumulative rate of transformation that may exceed 20% (e.g., in the case of severe congenital neutropenia). The genetic (and epigenetic) changes that contribute to malignant transformation in bmfs patients are largely unknown.

To elucidate the underlying molecular mechanisms of cancer susceptibility and progression in secondary MDS or acute leukemia in bmfs patients we conducted a comprehensive genome-wide characterization of genetic aberrations in the malignant cells at high-resolution level. We used high density DNA microarray (Agilent 400k/180 k) and direct sequencing of putative cancer genes to analyze a series of 30 CN patients at different time points during the progression into MDS and AML (50 samples in total). Large genomic alterations, namely monosomy 7/-7q, +21q or +3q were associated with leukemic progression. Beside common copy number variants like *UGT2B*, *GSTT1*, *HEATR4*, no microdeletions or microduplications were detected in the primary or secondary diseases. However, we found recurrent somatic missense and frameshift mutations in the transcription factor *RUNX1/AML1* in 10% of the patients.

Notably, *RUNX1/AML1* mutations have recently been described in Fanconi anemia during leukemic progression. *RUNX1/AML1* may have a more general role in the malignant transformation of patients with bone marrow failure syndromes like congenital neutropenia.

P06.023

Detection by HRM and COLD-PCR-HRM BRAF mutational status in paraffin blocks of melanoma patients

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Over 50% of melanoma tumors have BRAF oncogene mutation. In 2011 we have obtained the first results of clinical trials of targeted therapy with molecules that act on BRAF signaling pathway (Ras / Raf / MEK / ERK). Vemurafenib and GSK2118436 showed an increase in overall survival and disease-free in p.V600E BRAF carriers patients. In the coming months, these molecules will be approved for clinical use in Europe.

The application of analytical methods of high sensitivity in determining the mutational status of BRAF will be crucial to the imminent clinical application of new drugs, as the results will select patients eligible for or untreated.

We have studied the 11 and 15 exons of BRAF by HRM-sequencing, and COLD-PCR-HRM-sequencing in 20 paraffin blocks of melanoma, previously studied by Sanger sequencing.

Mutation was detected p.V600E of exon 15 in 9 of them for HRM, and 11 by COLD-HRM, compared to 8 previously detected by sequencing.

The sensitivity of such methods as HRM and COLD-PCR-HRM is greater than sequencing. These results should be compared with those obtained by the proposed clinical diagnostic tests (eg cobas 4800 BRAF V600 Mutation Test Roche©).

Our previous results show the necessary standardization of the methods of detection of somatic mutations, especially in cases where the determined result to apply the therapy to the patient.

P06.024

Comprehensive BRCA1 and BRCA2 mutational profile in Lithuania

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The germline mutations in BRCA1/2 genes are the most significant and well characterized genetic risk factors for breast and/or ovarian cancer. Detection of mutations in these genes is an effective method of cancer prevention and early detection. Different ethnic and geographical regions may have different BRCA1 and BRCA2 mutation spectrum and prevalence due to founder effect. The population of Lithuania has over several centuries undergone limited mixing with surrounding populations and is mostly of indigenous Baltic origin, which is different from Slavs. The aim of our study was to asses full BRCA1/2 mutational profile in Lithuanian population.

We performed comprehensive mutation analysis of BRCA1/2 genes in 567 unrelated breast and/or ovarian cancer patients (with/without family history) and predictive unaffected patients using high resolution melting (HRM) screening followed by direct sequencing and MLPA for large genomic rearrangements (LGRs). RESULTS. Overall, we have identified 23 different mutations (14 in BRCA1 and 9 in BRCA2 genes). Seven frequent pathogenic mutations in BRCA1 gene comprised 51%, 27%, 9%, 3%, 2%, 1,5% and 1,5% respectively of all BRCA1 mutations; a single BRCA2 mutation (c.658delGT) comprised 42% of all mutations in this gene. Four novel BRCA1 and 4 novel BRCA2 genes mutations; 2 different LGRs were found in BRCA1. The most common c.4035delA (47% of all BRCA1/2 mutations) appears to be true Lithuanian (Baltic) founder mutation. Characterization of BRCA1/2 mutational profile in Lithuania enabled to develop screening protocol using HRM for 7 common BRCA1/2 point mutations, which comprise 89% of all mutations detected in our country.

P06.025

Analysis of breast cancer predisposition genes by direct sequencing and multiplex ligation-dependent probe amplification technique

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Objective: In hereditary breast and ovarian cancer (HBOC) families, a large percentage of cases are attributable to hereditary factors compatibles with autosomal transmission of a major tumor suppressor gene with incomplete penetrance. Screening for BRCA1 mutations is now standard practice for HBOC cases in world, and permits medical follow-up. Estimates in different countries range from 5 to 15% the BRCA1 related cases of hereditary breast cancer due to copy number changes of one or more exons of this gene. Exon deletions and amplifications will usually not be detected by sequence analysis of the complete BRCA1 gene, therefore MLPA screening is needed.

Materials and methods: Hundred probands were fully screened for small mutations, and cases for which no causative abnormality were found (n=34) were screened by MLPA.

Results: A total of 5 pathogenic rearrangements in the BRCA1/2 gene were found, accounting for 7% of all mutations and the families with the disease-causing mutations were 16 percent of all families to review allocated. In addition, more than 80 percent of reassortments are related to the BRCA1 gene and less than 20 percent due to mutations in the BRCA2 gene.

Conclusion: These data demonstrate that dosage analysis is an essential component of genetic screening for cancer predisposition genes.

BRCA,MLPA, HBOC

P06.026

BRCA diagnostics on formalin-fixed, paraffin-embedded (FFPE) tissue

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Introduction:

In about 25% cases, hereditary breast and ovarian cancer (HBOC) is caused by a mutation in the *BRCA1* or *BRCA2* gene. Therefore, analysis of carrier status in a family should begin with an individual that has been diagnosed with HBOC to maximize the chances of identifying the familial mutation. In many HBOC families, all affected individuals are deceased and the only material available for genetic analysis is archived pathological specimens. Sanger sequencing of DNA extracted from FFPE tissue is time-consuming and expensive due to the comparatively small amounts and high degree of DNA fragmentation.

Methods:

Genomic DNA from tumor-free and tumor FFPE tissues from different individuals was isolated using the QIAamp DNA FFPE Tissue Kit (Qiagen) according to the manufacturer´s instructions. All coding regions of *BRCA1* and *BRCA2* were amplified with the multiplex PCR Kit BRCA-MASTR v2.1 (Multiplicom). Next generation sequencing was performed using the 454 sequencing kit on a GS Junior (Roche). Data were analysed by NEXTGENe software (Softgenetics).

Results:

In all samples, data analysis showed an even coverage over both genes of at least 15-fold. In the tumor-free sample, no pathogenic mutation was detected. Analysis of the tumor sample revealed a "homozygous" variation in *BRCA2* that could be verified as heterozygous germline variation in DNA isolated from the individual's lymphocytes.

Conclusions:

NGS is a powerful tool for the determination of germline variations in fragmented DNA from FFPE tissue.

P06.027

Meiotic drive at the BRCAs loci in Spanish families with breast/ovarian hereditary cancer.

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Objective: To analyse the sex and *BRCA* segregation ratios in families with breast and/or ovarian cancer in whom pathogenic mutations were identified.

Methods: From the breast and/or ovarian cancer families referred to our hospital, we selected couples that were proven carriers of a *BRCA* mutation. We compared the sex and *BRCA* allele transmission ratios from 305 descendents assuming an equal (1:1) distribution.

Results: We found a 2.2 fold excess of female births (101 females *vs* 49 males for *BRCA1* and 110 females vs 45 males for *BRCA2*) in our pedigrees. We observed that the mutated *BRCA1* allele segregated more often than the wild type allele (ratio 3:1) in the female offspring and that the number of carriers was higher than expected (74% carriers *vs* 24% non-carriers). Interestingly, in the male offspring the wild type *BRCA1* allele segregated more often than the mutated allele (37% carriers *vs* 63% non-carriers). For the *BRCA2* gene, the mutated allele segregated more often than the wild type allele in the female offspring (carriers 68% vs non-carriers 32%) whereas in the male offspring we did not observe TRD.

Conclusions: The results observed in the female offspring of BRCA1/2 carriers suggest a clear tendency to transmit the mutated allele (ratio 3:1). The finding of a high proportion of female carriers may have important implications for the genetic counselling of these families. Prezygote events, meiotic drive, survival of gametes or preferential fertilisation are possible explanations for the observed TRD.

P06.028

Genomic capture and massively parallel sequencing identifies accurately inherited mutations in several genes in BRCA1 & 2 negative families with strong breast/ovarian cancer history

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Inherited germline mutations in known and yet to be discovered genes predispose for breast and/or ovarian cancer. Mutations within BRCA1 and BRCA2 are the most common, but within the last years more than twenty different genes have been linked to breast and/or ovarian cancer susceptibility. Using BROCA to capture and sequence 21 known breast cancer genes in one test, we screened genomic DNA from 42 Greek patients with breast cancer diagnosed before the age of 40 and with a family history of breast or ovarian cancer. Patients had been previously screened for BRCA1 and BRCA2 by Sanger sequencing. Truncating mutations or missenses previously established as damaging were identified in 8 patients, in BRCA1, BRCA2, CHEK2, PALB2, and MSH2. All mutations were different. All were confirmed by Sanger sequencing with diagnostic primers from patients' genomic DNA. Of the 8 mutations, 3 were genomic deletions detected by read depth from BROCA data. In addition to the 8 patients with confirmed damaging mutations, 5 other patients harbored mutations at splice sites, in ATM, BRCA2, and RAD51C. These splice variants have been confirmed in genomic DNA; their effects on transcripts are being evaluated. We conclude that among Greek patients with familial breast cancer, the mutational spectrum is highly heterogeneous with respect to both loci and alleles. These patients are well served by an approach that detects all classes of mutations in all known breast cancer genes.

P06.029

BRCA1 mutation screening in brazilian hereditary breast cancer and ovary syndrome using High Resolution Melting

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About 10% of cases of breast and/or ovary cancer are characterized as hereditary, where the presence of germline mutations in BRCA1 gene increases the risk of developing these cancers during woman's lifetime. The present study aims to characterize BRCA1 gene mutations associated with Hereditary Breast/Ovary Cancer Syndrome (HBOC). The twenty two coding exons of BRCA1 were analyzed using High Resolution Melting method, followed by DNA sequencing. MLPA technique was also used to detect gross deletions. We investigated 41 patients from the Cancer Genetic Counseling Service of the HCFMRP-USP that fulfilled the criteria for genetic testing according to NCCN Clinical Practice Guidelines in Oncology v.1.2010. A total of 21 mutations were identified, two of which are pathogenic: deletion of exons 17-18 and deletion of exon 19. Both of them are located in the BRCT domain of BRCA1 gene, impairing the binding of essential phosphoproteins critical for the activation of DNA repair complex. Because four missense mutations (Pro871Leu, Glu1038Gly, Lis1183Arg, Ser1613Gly) occurred simultaneously in half of patients, we analyzed the presence of the possible haplotypes also in 82 healthy controls and verified that the haplotype Leu-Gly-Arg-Gly, composed of all the mutated residues, showed significant difference ($p < 0.05$) between the groups suggesting a possible association with increased risk for HBOC. This study suggests that haplotypes consisting of non-synonymous mutations can confer increased risk for HBOC due to the cumulative effect of these mutations on the BRCA1 protein structure. Financial Support: Capes, FUNDHERP, INCTC/CNPq.

P06.030

Germline BRCA1 and BRCA2 mutations in Croatian families with breast/ovarian cancer predisposition: Identification of three novel mutations

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Breast cancer is the most common cancer in women after non-melanoma skin cancer, and it is the leading cause of cancer deaths in Croatia. Ovarian cancer is in the fifth place, both in incidence and mortality. About 5-10% of all breast and/or ovarian cancer cases are hereditary, and germline mutations in BRCA1 and BRCA2 account for the majority of hereditary breast and ovarian cancers. The contribution of BRCA1 and BRCA2 mutations to hereditary breast and ovarian cancer in Croatia is unknown. The purpose of this study was to estimate the incidence and spectrum of pathogenic mutations in BRCA1/2 genes in high risk women in Croatia. BRCA1/2 genes from 167 candidates (145 families) were scanned for mutations using High-resolution melting analysis (HRMA), direct sequencing and Quantitative multiplex PCR of short fluorescent fragments (QMPSF). We identified 14 pathogenic point mutations in 17 candidates, 9 in BRCA1 and 5 in BRCA2. Of those, 11 have been previously described and three were novel (c.5335C>T in BRCA1, and c.4139_4140dupTT and c.8175G>A in BRCA2). No large deletions or duplications involving BRCA1 and BRCA2 genes were identified. Two common BRCA1 sequence variants: c.2077G>A and c.4956G>A, were found more frequently in mutation carriers compared to healthy controls. In silico analyses identified one BRCA1 sequence variant (c.4039A>G) and two BRCA2 variants (c.5986G>A and c.6884G>C) as harmful with high probability and inconclusive results were obtained for one novel BRCA2 variant: c.3864_3866delTAA. Combination of QMPSF and HRMA methods provides high detection rate and complete coverage of BRCA1/2 genes.

P06.031

Identification of *BRCA1/2* mutations in an unselected breast cancer population and referral to the Clinical Genetic Center

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BRCA1/2-screening guidelines are aiming to select women who have a high prior probability to be a mutation carrier. We screened *BRCA1/2* mutations in research setting and evaluated Dutch diagnostic *BRCA1/2*-screening criteria in a consecutive, hospital-based series of breast cancer patients <50 years.

We collected clinico-pathological data of 5433 invasive breast cancer patients, diagnosed <50 years in ten hospitals between 1970 and 2003. Germline DNA isolated from FFPE-tissue was tested for the most prevalent pathogenic *BRCA1/2* mutations. For 1539 cases from 5 hospitals complete family history and hormone receptor status (ER, PR, HER2) data was available. For patients from one hospital (NKI-AVL; n=1588) we identified those referred to the CGC for diagnostic *BRCA1/2*-screening.

Results shown are preliminary, final results will be available at ESHG 2012. We identified 4.9% *BRCA1/2* carriers in this unselected breast cancer cohort. Following current Dutch screening criteria, 60% of these carriers could have been identified (sensitivity 58.7%; specificity 78%). Selection of not only patients diagnosed with a triple-negative tumor at age <40 years, but also those with a triple-negative tumor diagnosed at age 40-50 years increased sensitivity to 73.3% (specificity 71.4%). Of 83 *BRCA1/2* mutation carriers from the NKI-AVL, 46% had been referred to the CGC (data linkage ongoing); of those not referred, the 8 carriers diagnosed after 1994 (discovery of the *BRCA* genes) were less likely to meet the criteria for referral.

A more optimal sensitivity and specificity for *BRCA1/2*-screening of breast cancer patients may be achieved based on age and tumor criteria.

P06.032

Validation of three BRCA1/2 mutation-carrier probability models Myriad, BRCAPRO and BOADICEA in a population-based series of 183 German families

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Many studies have evaluated the performance of risk assessment models for BRCA1/2 mutation carrier probabilities in different populations, but to our knowledge very few studies have been conducted in the German population so far. In the recent study, we validated the performance of three risk calculation models by names BRCAPRO, Myriad and BOADICEA in 183 German families who had undergone molecular testing of mutations in BRCA1 and BRCA2 with an indication based on clinical criteria regarding their family history of cancer. The sensitivity and specificity at the conventional threshold of 10% as well as for a threshold of 20% were evaluated. The ability to discriminate between carriers and non-carriers was judged by the area under the receiver operating characteristics curve. We further focused on the performance characteristic of these models in patients carrying large genomic rearrangements as a subtype of mutations which is currently gaining increasing importance. BRCAPRO and BOADICEA performed almost equally well in our patient population, but we found a lack of agreement to Myriad. The results obtained from this study were consistent with previously published results from other population and racial/ethnic groups. We suggest using model specific decision thresholds instead of the recommended universal value of 10%. We further suggest integrating the CaGene5 software package, which includes BRCAPRO and Myriad, in the genetic counselling of German families with suspected inherited breast and ovarian cancer because of the good performance of BRCAPRO and the substantial ease of use of this software.

P06.033

MASTR assays on the Ion PGM Sequencer, streamlining the diagnostic workflow

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Multiplicom's MASTR assays allow multiplexed PCR amplification of numerous target sequences and therefore substantially reduce processing cost and front-end workload in combination with massive parallel sequencing (MPS) platforms. This is demonstrated by the fast uptake of the BRCA MASTR assay in European clinical diagnostic laboratories resulting from the facts that (i) only 5 robust PCR reactions are required to amplify all coding exons of BRCA1/2 and (ii) multiple DNA samples can be processed simultaneously. Additionally, processing cost is reduced by a factor 2-5, compared to Sanger based sequencing resulting from the combination of highly specific amplification (> 96% on target specificity) and a narrow spread factor (i.e. average number of all reads divided by the read number of the lowermost amplicon) of 2,5.

To increase the accessibility of MPS in clinical diagnostics, we worked out and performed a protocol to sequence the BRCA MASTR assay amplicons on an Ion PGM Sequencer. Hereto, amplicons from each of 14 DNA samples were enzymatically sheared followed by ligation of individual PGM adapters. After mixing the adapter ligated samples, an OneTouch based 100 bp ePCR was performed. The resulting ePCR library was sequenced using the 100 bp sequencing kit in combination with a single "Ion316 chip". The obtained sequencing data showed excellent characteristics and in depth analysis was performed for coverage uniformity on the one hand and base calling accuracy on the other. We will present detailed conclusions related to the performance characteristics and points for future improvements.

P06.034

Study of several hormone receptor gene polymorphisms in breast cancer patients

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Androgen receptor gene polymorphisms and PROGINS have been related to a lower risk of breast cancer. The aim of the present study was identifying polymorphisms in the structure of the genes that code for progesterone and androgen receptors, variations that with or without those of estrogen receptors, could be correlated with breast cancer. 60 tumor samples from breast cancer cases were analyzed in order to find out several polymorphisms in the structure of the genes coding for progesterone and androgen receptors and compared to results obtained from the blood analysis of healthy subjects. The number of CAG repeats in the androgen receptor gene ranged from 8 to 27 repeats. The majority of cases had a range between 17 and 24, the size of the PCR products being around 230 bp. 15 alleles (12.5%) had less than 16 CAG repeats and 9 alleles (7.5%) had 25 or 27 CAG repeats. Considering the lower number of CAG tandem repeats, all cases with 11/8 repeats were in stage III and from the 2 cases with 11/9 repeats, 1 was in stage II and the other one in stage IV. The size of the PCR products of PROGINS receptor gene was 175 bp for the majority of the cases, only 6 (5%) longer alleles with 481 bp were detected. One of the detected cases was in stage IV of malignancy, the others were in stage III and all had ductal carcinoma. The obtained data are important for developing early detection and treatment strategies.

P06.035

Bcl-2 gene expression level in tumor and non tumor breast tissues

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Breast cancer is the most common non-skin cancer and the second most frequent cause of death due to cancer among women. There has been much interest in the development and use of molecular-based research aimed at identifying biologic markers for the diagnosis of the disease, whilst the treatment of breast cancer has progressively improved. This development is underpinned by the knowledge of the genetic molecular alterations in the patient tissues. Several genes responsible for expression have been identified by cDNA microarray study in breast cancer, with the Bcl-2 gene indicated as a likely candidate. In this study, we studied bcl-2 gene expression level in parallel tumor and non tumor breast tissues. Forty samples including 19 tumor, 18 non tumor (marginal) and 3 benign breast tissues which were all pathologically diagnosed, were subjected to RNA extraction and polyA RT-PCR with the expression level of bcl-2 quantified using real-time PCR. By comparing tumor with non tumor tissues we found bcl-2 over-expression in all of the timorous tissues. Our data suggests that dysregulated bcl-2 gene expression is potentially involved in the pathogenesis of breast cancer. In conclusion, using gene expression analysis may significantly improve our ability for screening cancer patients and will prove a powerful tool in diagnosis, prognostic and cooperative group trials in the bcl-2based therapy project.

P06.036
Search for novel hereditary breast cancer (BC) genes suggests a causative role of heterozygous BLM mutation

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Sequencing analysis of candidate genes involved in the maintenance of genome integrity (CHEK1, PARP1, PARP2, BRIP1, BLM, DDB, RNF8, FANCG, BARD1, RAD51C) in 95 hereditary BRCA1/BRCA2/NBS1/PALB2/TP53 mutation-negative BC cases has revealed 2 women with clearly inactivating genetic event. Both cases were heterozygous for c.1642 C>T (Q548X) mutation in the Bloom Syndrome gene, BLM. The subsequent extended study has confirmed frequent occurrence and BC-predisposing role of the above allele (17/1498 (1.1%) BC patients vs. 2/1093 (0.2%) healthy females, p = 0.004). As expected for hereditary BC gene, the BLM heterozygotes tended to be overrepresented in patients with family history of the disease, younger age at onset, or BC bilaterality. Unlike BRCA1-related hereditary tumors, BLM-associated BC were frequently hormone receptor positive (13/18, 72%). While virtually all BC arising in BRCA1/2 carriers contain somatic deletion of the remaining wild-type allele, none of BLM-driven cancers demonstrated loss of heterozygosity at BLM locus. 5 patients carrying BLM mutation were treated by neoadjuvant therapy and therefore were available for immediate evaluation of tumor chemosensitivity: 3 showed nearly complete pathological response, and 2 experienced partial clinical response. Elevated frequency of BLM Q548X mutations in Russian (Slavic) subjects allows to expect noticeable occurrence of Bloom Syndrome in Russia and neighboring countries; absence of appropriate clinical records in major Russian genetic centers suggests that at least some of these patients remain undiagnosed and/or have relatively mild presentation of this disease.

P06.037
Whole genome expression, canonical pathway and gene network analysis in breast cancer

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Introduction: Breast cancer is the most common cancer in women and constitutes 23% of all female cancers. Despite advances in treatment options, such as surgery and chemotherapy, breast cancer still remains being the most deadly second malignancy in women. Therefore, requirement of new prognostic markers is increasing. For this purpose, we studied gene expression profiles of breast cancer.
Materials and Methods: RNA samples were obtained from healthy and cancerous tissues taken from twenty patients diagnosed as breast cancer. These RNA samples were hybridized with microarray chips (Agilent Human 4 X 44K Oligo Microarrays). Gene expression, canonical pathway and network analysis were performed using GeneSpring GX 11.0 software.
Results: In our study, we found 585 downregulated and 413 upregulated genes. The canonical pathways significantly regulated were process of the immune system, T cell differentiation, T cell activation, lymphocyte activation, leukocyte activation, lymphocyte differentiation, leukocyte differentiation, immune system development, cell activation, hematopoietic or lymphoid organ development, hematopoiesis, signal transduction, immune response, signal transmission, the signal process, part of the plasma membrane, T cell receptor complex.
Conclusion: In this study, FOXM1, IFNG and MMP9 pathways has been identified among the data sets. These candidate pathways are important for the development of new biomarker panels to use for breast cancer prognosis in clinical practice.

P06.038
Contribution of truncating *PALB2* mutations to breast and ovarian cancer

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Familial breast and ovarian cancer is associated with mutations in *BRCA1* and *BRCA2* but these genes explain only a minority of cases. Germ-line mutations in *PALB2*, encoding the partner and localizer of BRCA2, have also been identified as breast cancer susceptibility alleles, and the geographical spread and risks associated with *PALB2* mutations are subject of intense investigation. We have scanned the whole coding region of *PALB2* using high-resolution melting analysis and direct sequencing of genomic DNA samples to investigate the prevalence of *PALB2* mutations in a series of 158 German patients with bilateral breast cancer and in a second series of 253 unselected patients with epithelial ovarian cancer from Bashkortostan. 17 sequence alterations were identified. Truncating *PALB2* mutations were identified in 2/158 (1.3%) bilateral breast cancer patients and in 1/253 (0.4%) ovarian cancer patients. One nonsense mutation, p.E545X was new, whereas the two other mutations, c.509_510delGA and c.172_175delTTGT had been previously described. The c.172_175delTTGT deletion, found in one Russian ovarian cancer/ melanoma patient, was subsequently screened in 365 breast cancer cases from Bashkortostan, and two further carriers were detected among patients of Russian descent (0.5%). Our results indicate that *PALB2* germ-line mutations account for a small but not negligible fraction of breast cancer, though they make a minor contribution to ovarian cancer. The observation that two of the three identified truncating mutations have been described in different studies, may provide a rationale for mutation-specific screening approaches in extended series of Eastern and Central European cancer patients.

P06.040
Associations Between HER2/neu ,TOP2A ,Chromosome 17 Copy Numbers and RASSF1A , APC Gene Promotor Hypermethylations of Patients with Breast Cancer

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Associations Between HER2/neu ,TOP2A ,Chromosome 17 Copy Numbers and RASSF1A , APC Gene Promotor Hypermethylations of Patients with Breast Cancer
Backround: Breast cancer is an important public health problem worldwide. The HER2 /neu protooncogene is amplified and overexpressed in approximately 25-30% of invasive breast carcinomas. DNA topoisomerase 2-alpha enzyme controls and alters the topologic states of DNA during transcription. RASSF1A and APC gene are putative tumor-suppressor genes that are frequently inactivated epigenetically in breast cancer.
Method: In this study we analysed retrospective HER2, TOP2A gene and CEP17 copy number alterations by fluorescence in situ hybridization (FISH) in primary tumor core biopsies from 60 high-risk primary breast cancer patients (tumors ≥2 cm and/or lenfatic metastase and/or distant metastases and/or under 40 years) . The methylation levels of the RASSF1A, APC gene promoters were assessed Methylation Sensitive High Resolution Melting Analysis (MS-HRM).
Results: In our study, HER2/neu amplifications were identified in 25% and TOP2A coamplications in 53.4% and deletions in 13.3% of patients. HER2/neu gen amplification is found to be associated with high grade, ER negativity and PR negativity. Polysomy17 was present in 23.3% of patients. RASSF1A and APC methylation frequencies were 96.6% and 43.3%. HER2/neu gene amplification was found to be high RASSF1A promotor methylation levels.
Conclusions:. Our study is important as being the first study that analyzes association between HER2/neu, TOP2A gene copy numbers and RASSF1A, APC gene promotor methylation status in Turkish population.
Key words: Breast cancer, HER2/neu gene, TOP2A gene, FISH, RASSF1A gene, APC gene, Methylation, MS-HRM Analysis

P06.041
Necessity of HPV genome screening in women due to its role in breast cancer

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It seems, there is an increment document that high-risk human papilloma virus (HPV) is involved in tumors in over just cervical cancer. For instances, it is already almost accepted that some HPVs have a major role in a significant proportion of head and neck tumors.It also has long been hypothesized that some tumorigenic viruses, such as HPV, may have etiological or even helping role in some human breast cancers. A number of reports have indicated HPV DNA in breast cancer tissue specimens and some normal or pre-cancerous breast tissues. Many of them rely on standard polymerase chain reaction (PCR), which is criticized for its tendency for contamination. We examined the presence, genotype, viral load, and physical status of HPV in a number of Iranian patients with breast carcinoma utilizing Real-time PCR setting (with sequencing), and immunohistochemistry based on HER2/neu overexpression. In this presentation, we attempt to summaries our achievements in using mentioned technologies to explore how HPVs may help to cause or progress malignancies especially in those that are affected by some other tumorigenic factors. Finally, the exigency of women screening for HPV and its potential importance in developing breast cancer, will be discussed.

P06.042

Multiplex single-nucleotide primer extension assay for detection of low-penetrance breast cancer susceptibility polymorphisms

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Breast cancer is one of the most common cancers among women in developed countries. Approximately 10% of sufferers have a genetic predisposition and 25% of patients with familial breast cancer carry mutations in BRCA1 or BRCA2 high-penetrance genes. Recently, genome-wide association studies have identified several SNPs as low-penetrance breast cancer susceptibility polymorphisms. The aim of this study was to develop an easy, rapid and cost-effective method for genotyping of low-penetrance breast cancer susceptibility polymorphisms within six genes and gene-free genomic regions. We have designed a multiplex single-nucleotide primer extension assay to genotype rs2981582 (FGFR2), rs3817198 (LSP1), rs889312 (MAP3K1), rs3803662 (TNRC9/LOC643714), rs1328615 (8q) and rs1045485 (CASP8) in a single reaction. Using this method, we analyzed 92 breast cancer patients, of which 26 with familial breast cancer and 86 controls from the general population. Our results showed that SNP rs1045485 in CASP8 is significantly associated with decreased breast cancer risk [p=0.0081; OR (95%CI)=2.37 (1.24-4.56)], while rs889312 in MAP3K1 showed a significant association with increased breast cancer risk [p=0.0151; OR (95%CI)=2.31 (1.16-4.61)] only among patients with positive family history of breast cancer. In conclusion, we present an effective new genotyping method that can be applied in medium-scale association studies.

P06.043

Evaluation of cisplatin and cisplatin nanoparticles on the MCF-7 cell line

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Introduction: Breast cancer is one of leading causes of mortality worldwide. Cisplatin is a traditional cancer drug commonly used in chemotherapy for killing breast cancer cells. Modulation at the mRNA levels of apoptotic related genes often correlate with the sensitivity of various types of cancer cells to chemotherapeutic agents. Nanoparticulate drug delivery systems are being developed to deliver smaller doses of chemotherapeutic agents in an effective from and control drug distribution with in the body.
Material and methods: In the present study, we synthesized the magnetic nanoparticles. Then nanoparticles were loaded by cisplatin. Then, cell toxicity was evaluated by the MTT assay. Finally, we compared the effect of cisplatin and cisplatin nanoparticles on the mRNA expression levels *BCL2*-gene in MCF-7 breast cancer cell line.
Result: Our initial results indicate that nanoparticles are effective anticancer agents. We also found that cisplatin nanoparticles induce apoptosis in human breast cancer cell line.
Discussion: In the present study, we have shown that the strongly increased *in vitro* cytotoxicity of cisplatin nanoparticles compared with the free drug in MCF-7 cell line. In summary, our results indicate that cisplatin nanoparticles are effective anticancer agents and justify study of these agents *in vivo*.

P06.044

Association of polymorphisms in low-penetrance genes with breast cancer risk in Bulgarian cohort of familial cases and controls

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Background: Although the germ-line mutations in *BRCA1/2* and other breast cancer susceptible genes account for up to 10% of the familial cases, the majority of patients with this diagnosis do not harbour mutations in the main disease associated loci. This observation has led to the proposal that the susceptibility to breast cancer is determined by a large number of loci, each with a small effect on the breast cancer risk.
Materials and methods: We have performed a case-control study of eight SNPs, previously associated with breast cancer in extensive GWASs. The studied group consisted of 191 Bulgarian patients with family history of breast cancer and 151 healthy controls. The genotyping was performed by TaqMan technology and the results analysed using Plink and VassarStat Statistical Calculator.
Results and discussion: Three of the studied polymorphisms: rs2981579 and rs2981582 in *FGFR2* gene and rs3757318 in C6orf9, demonstrated significant association with breast cancer risk. The genotype A/A of both rs2981579 (OR=1.668; p=0.007) and rs2981582 (OR=1.782; p=0.005) appeared to increase the risk of breast cancer. The homozygosity for the G allele in rs3757318 (OR=1.678; p=0.03) was also associated with increased risk, while the genotype A/G appeared to have protective effect (OR=0.472; p=0.003). The other studied SNPs did not show any statistically significant association with breast cancer in the investigated sample.
Conclusions: Our results demonstrated significant association of rs2981579 and rs2981582 in *FGFR2*, and rs3757318 in C6orf9 with increased breast cancer risk which is in concordance with previous GWASs.

P06.045

Oestrogen Receptor-α Gene Polymorphism (T392C) in Iranian Women with Breast Cancer

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Background: Receptor-mediated oestrogen activation plays a part in the development and progression of breast cancer. Evidence suggests that alterations in oestrogen signalling pathways, including oestrogen receptor-α (ESR1) occur during breast cancer development.
progression of breast cancer.
Methods: The ESR1 gene was analysed in 150 Iranian patients who were newly diagnosed with invasive breast tumours and in 147 healthy individuals. Single-strand conformation polymorphism PCR and direct sequencing were done.
Findings: Silent single nucleotide polymorphisms (SNPs), as reported in previous studies, were found but at significantly different frequencies. The frequency of allele C/C in codon 10 rs2077647 (T/C, S392S) of exon 1 was significantly higher in patients with breast cancer (45.7%) than in the controls (39.8%; p=0.148). We found that allele C/C in codon 10 was significantly more common in patients with breast cancer who had a family history of breast cancer (78.9%) than in those without such a history (40.8%; p=0.001). The allele 1 in codon 10 showed an association with the occurrence of lymph node metastasis (58.7% and 43.3% with and without lymph node metastases, respectively). Therefore, this SNP marker further increased predictive accuracy in the Iranian population.
Interpretation: Our data suggest that ESR1 polymorphisms correlated with various aspects of breast cancer in Iranian women, as determined during pre-surgical assessment, might represent a surrogate marker for predicting breast cancer.

P06.046

Prognostic significance of the urokinase-type plasminogen activator (uPA) and its inhibitors (PAI-1 and PAI-2) mRNA expression in Iranian breast cancer

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One of the most thoroughly studied systems in relation to prognostic relevance in breast cancer is the plasminogen activation system. The system comprises of the urokinase Plasminogen Activator (uPA) and its inhibitors, the Plasminogen Activator Inhibitor-1 (PAI-1) and Plasminogen Activator

Inhibitor-2 (PAI-2). In this study, we are investigating the prognostic value of the expression level of uPA and PAI-1 and PAI-2 in 30 sporadic breast cancer patients.

The mRNA expression level of uPA, PAI-1 and PAI-2 was analyzed in tumor tissues and its adjacent normal tissues from 30 patients by quantitative PCR. Gene fragments were amplified in a ABI 7300 real-time PCR system using gene-specific primers and Taq Man probe. The results were normalized to TFRC and ACTB mRNA. The data of real-time RT-PCR will be compared with clinical course of the disease (three years follow up).

Quantitative real-time RT-PCR is a highly sensitive, reproducible, and fast method for measuring gene expression of uPA, PAI-1 and PAI-2 in breast cancer. Some studies have shown the relationship between high expression of UPA system and aggressive course of breast cancer. In addition *in vitro* studies of high expression of UPA have been shown the modulation of angiogenesis.

P06.047

Real-Time Analysis of the expression of isoforms angionegnicaanti- and pro-angiogenic in breast cancer.

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Breast cancer is the most frenquently diagnosed cancer and the leading cause of cancer death in females. About half breast cancer cases and 60% of the deaths are estimated to occur in economically developing countries. Angiogenesis plays a critical role in local growth of solid tumors and subsequently in the process of distant spread. Numerous studies have demonstrated the importance of angiogenesis in cancer. Nevertheless, 3' alternative splice site selection in exon 8 of VEGF gene results in a sister family of isoforms, VEGFxxxb, which are anti-angiogenic and downregulated in tumor tissues. We quantitatively analyzed the expression of pro-angiogenic and anti-angiogenic VEGF isoforms in breast carcinoma and adjacent normal tissue samples. For that purpose, total RNA from 16 tumor samples and their respective margins were obtained and synthesized cDNA from. We designed and synthesized primers and specific probes for each isoform, which were used for the analyses of expression by real time PCR. So far, were observed different expression between the anti- and pro- angiogenic isoforms in the tumor samples compared to normal tissue. These results evidence that the selection of different splicing sites may actually interfere in tumor angiogenesis and consequent tumor progression and metastasis. A bigger sample size might help in more advanced studies and collaborate to better development of researches on tumor angiogenesis involving VEGF gene. Studies approaching control of VEGF splicing in order to promote the selection of the distal splicing site (anti-angiogenic) instead of proximal site (pro-angiogenic) might promote an efficient therapy for breast cancer.

P06.048

A novel missense mutation in IDH2 gene of a glioma patient

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IDH2 gene encodes mitochondrial NADP-dependent isocitrate dehydrogenase - an enzyme that catalyzes the oxidative decarboxilation of isocitrate to α-ketoglutarate. Mutations in IDH2 and its cytosolic isoform - IDH1, were found in brain tumours and acute myeloid leukemia (AML). While the data about survival benefit of AML patients with mutations are controversial, IDH1 and IDH2 aberrations in gliomas are associated with better prognosis.

A recent analysis by direct sequencing of exon 4 of IDH2 gene in brain tumour tissues, including astrocytomas and oligodendrogliomas, revealed a new mutation in IDH2 gene that was not described to date. It was a homozygous one-nucleotide A-to-G substitution at position 386 which caused amino acid change lysine to arginine (c.386A>G, K129R). The aberration was in a highly conserved region of the protein which suggested that it might be associated with the development of the brain tumour. The mutation was detected in one patient with glioblastoma (grade IV) who had overall survival of 30.3 months. This was similar to median survival time in our group of patients with already known IDH2 or IDH1 mutations - about 32 months vs. 5 months in non-mutated cases. The effect of the newly found aberration on protein structure and function should be studied further.

P06.049

Investigation of the telomerase expression level in colon cancer patients and its relation with multi drug resistance.

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One of the most common causes of mortality in the world is cancer. In spite of recent advances in the treatments of cancer, the clinical outcome is far away from expectation yet. Drug resistant remains a major obstacle to the effective cure of almost all cancers. We aimed to investigate the possible association between telomerase expression level and multidrug resistance in colon cancer patients.

In this regards tumor and adjacent normal tissues of 80 colon cancer patients were assessed for the expression level of telomerase by Real Time RT-PCR.

Here we are presenting our data regarding to the correlation between telomerase level and failure to chemotherapy in this group of Iranian colon cancer patients. To our best knowledge, this is the first data derived from an in vivo study. Our data will be compared with other published reports which had the focus on only in vitro (cell lines) system.

P06.050

Impact of cancer-testis antigens crosstalk with self-renewal cell signaling pathways in esophageal squamous cell carcinoma

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Aberrant expression of cancer-testis antigens (CTAs) is reported in variety of tumor cell types, but their functions and related pathways in tumorigenesis are poorly understood. Several CTAs are expressed in human cancers as well as human mesenchymal stem cells. CTAs play a role at earlier stages of embryonic development, stem cell self-renewal and tumorigenicity. Role of CTAs was examined in tumorigenesis of esophageal squamous cell carcinoma (ESCC) with respect to cell signaling pathways. Gene expression analysis of MAGE-A4, LAGE1 and NY-ESO1, EGFR, TWIST1, PYGO2 and MAML1 were performed using comparative real-time RT-PCR in 44 ESCC patients. Overexpression of all genes was detected in 90.2%, 39%, 41.5%, 43.9%, 41.5%, 36.6% and 43.9% of samples, respectively. There are significant correlations among gene transcripts expression as it was shown in the table below. MAGE-A4, TWIST1 and MAML-1 expression were significantly correlated with lymph node metastasis, MAGE-A4 was significantly correlated with tumor staging and TWIST1 expression was significantly correlated with tumor invasion ($p<0.05$). Hierarchical gene clustering data revealed that TWIST1 is related to CTA genes. Correlated overexpression of CTAs and key factors of cell signaling pathways imply a strong cross talk between them through tumorigenesis in ESCC. Interactions of CTAs with Twist1 could trigger epithelial-to-mesenchymal transition and favor metastasis of tumor cells. This provides a support for role of CTAs in the self-renewal and differentiation of tumor cells.

Gene expression correlations between CTAs and Self-Renewal Genes

	LAGE1	MAGEA4	NY-ESO1	EGFR	MAML1	PYGO2	TWIST1
LAGE1		0.008		0.011			
MAGEA4	0.008		0.018				0.016
NY-ESO1		0.018					0.043
EGFR	0.011					0.001	
MAML1						0.006	0.0041
PYGO2				0.001	0.006		
TWIST1		0.016	0.043		0.041		

P06.051

SDHB germline mutation in a patient with Carney-Stratakis syndrome

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Carney-Stratakis syndrome is a recently described, very rare familial syndrome characterized by gastrointestinal stromal tumors (GIST) and paragangliomas. The majority of cases are caused by germline mutations of the succinate dehydrogenase (SDH) subunit genes *SDHB, SDHC* and *SDHD*. Tumor predisposition is inherited in an autosomal dominant manner with incomplete penetrance.

Here, we present a 47-year old patient with a gastrointestinal stromal tumor (GIST) diagnosed at the age of 17 years. Because of severe anemia, total gastrectomy was performed at the age of 45 years, exhaustive clinical screening revealed a non-functioning, abdominal, extra-adrenal paraganglioma.

As a consequence, the patient was referred to our genetic service and molecular genetic diagnosis was initiated. Family history did not reveal the presence of any paragangliomas, gastric stromal sarcomas or further tumor entities. Molecular genetic analysis by direct DNA sequencing showed a putative splice mutation c.287-2A>G in the *SDHB*-gene. This mutation has been reported previously in patients with inherited paraganglioma but never in the context of Carney-Stratakis syndrome. The damaging effect of the mutation in mRNA splicing could be confirmed on the basis of cDNA analysis. By array-CGH we identified the most frequently reported genetic alteration in paraganglioma, i.e. loss of chromosome 1q. In addition, we observed a loss of chromosome 11.

As a consequence, especially in young patients with GIST and/or paragangliomas molecular diagnostics should be offered to identify germline mutations in SDH genes. This is of high relevance as mutated tumours in most cases do not respond to treatment with tyrosine kinase inhibitors.

P06.052
Evaluation of Caspase-8 -652 6N ins/del polymorphism in breast cancer

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In the present study, we examined the impact of CASP8 -652 6N ins/del (rs3834129) and the risk of breast cancer in a sample of Iranian population. This case control study was done on 236 breast cancer patients and 203 cancer free healthy female. Bi-directional PCR allele-specific amplification (Bi-PASA) was used for detection of CASP8 -652 6N ins/del polymorphism. Our findings indicated that the CASP8 -652 6N del/dl genotype was inversely associated with breast cancer risk (OR=0.33, 95% CI=0.17-0.65, p=0.001). The frequency of del allele in cases and controls were 29.1% and 38.6%, respectively. An inverse association between CASP8 6N del variant and the risk of breast cancer (OR=0.66, 95% CI=0.66-0.87, p=0.002) was found.

In conclusion, the finding suggests that the CASP8 -652 6N del polymorphism plays a protective role in susceptibility to breast cancer in our population.

P06.053
Evaluating of CBX8 Gene Expression in Gastric cancer

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Cbx8 is a member of polycomb group proteins which their functions in development and progression of cancer has been investigated a lot. CBX8 regulates proliferation of diploid human and mouse fibroblasts and its ectopic expression leads to repression of the Ink4a-Arf locus and cellular immortalization. Regarding to the function of this gene in regulation of Senescence we studied the expression of CBX8 in gastric tumors one of the most cause of cancer death worldwide. Using real-time quantitative reverse transcription-PCR assay based on SYBER green we evaluated the expression of CBX8 gene in thirty gastric cancer specimens in comparison with their normal marginal tissues . Despite the differences in the expression of this genes found on each pair samples between tumor and marginal tissues, statistical analysis didn't show significant differences in the mean expression of CBX8 between tumor and marginal in total . But it was possible to divide the data into two group; group1 in which the gene was overexpressed in tumor and group 2 in which the gene was downregulated in tumor samples. On each group the difference in CBX8 gene expression was significant between tumor and marginal tissues. Our data suggest that this variation in the gene expression pattern may be due to different origin of gastric cancer.

P06.054
Detection of genomic imbalances in cervical lesions positive for HPV by microarray comparative genomic hybridization

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Widely known as a sexually transmitted agent, genital human papillomavirus (HPV) is considered to be major factor in the development of cervical intraepithelial neoplasia and cervical carcinoma. HPV can be associated with benign warts (HPV types 6 and 11) and related to anogenital tumors (HPV types 16, 18, 31, 33, 35, 45). Practically 100% of cervical cancer - the second most common tumor in women worldwide, contain DNA sequences of high-risk oncogenic genital papillomavirus. Effective diagnosis of these infections today is almost entirely related to the use of molecular techniques for detection of viral DNA and identification of viral genotypes.

The objective of our study was to investigate the incidence and type of genomic imbalances in cervical lesions with different HPV status. We have applied array Comparative Genomic Hybridization (array CGH) in selected groups. In HPV16 positive cervical cancer samples we discovered pathologic deletions in 11q22.1, 1p36.23 and 3q deletion, as well as some benign polymorphisms. In the group of cervical cancer, positive for low risk HPV, we found only polymorphic copy number variations. In samples with cervical dysplasia, positive for HPV16, pathologic imbalances were detected such as deletions in 4p16.2, 17p13.3 and 22q13.33.

This report summarizes and complements the current information on the prevalence of human papillomaviruses in the Bulgarian population and presents the results of our research on benign, precancerous and cancerous changes using array CGH analysis.

P06.055
Importance of SULT1E1 in the cervical carcinogenesis

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Background:

Persistent infection with a high-risk human papillomavirus type is a prerequisite for the development of cervical cancer (CxCa). However, additional genetic alterations, including the reactivation of telomerase activity, are required. Our previous studies (microcell-mediated chromosome transfer, microarray-analysis, loss of heterozygosity analysis) revealed a role of the chromosomal region 4q13 in the regulation of telomerase.

Objectives:

The aim of the present study was to validate the differential expression of one of our candidate genes, SULT1E1, located within 4q13 in biopsy material. Furthermore the ability of SULT1E1 to suppress telomerase activity and to induce senescence in different functional assays is investigated.

Methods:

Gene expression of SULT1E1 and hTERT was validated by real-time PCR in normal cervical tissues, high-grade lesions (CIN2/3) and CxCa. SULT1E1 was expressed stably in various cell lines in order to analyze its effects on telomerase activity, telomere length and senescence using telomerase PCR ELISA kit, telomere length kit and ß-galactosidase staining, respectively.

Results and Conclusions:

The mRNA expression level of SULT1E1 was significantly (p<0,05) lower in CxCa as compared to CIN2/3 or normal tissue. Only low levels of SULT1E1 were detected in the cervical carcinoma cell lines SiHa, ME180 and SW756. Reconstitution of SULT1E1 via lentiviral-mediated gene transfer in these cell lines resulted in a slightly decreased telomerase activity in SiHa and ME180 cells. Telomere length assays and senescence tests are still ongoing.

Our first results thus far suggest that SULT1E1 maybe a putative telomerase suppressor gene and loss of its function may contribute to the transformation process.

P06.056
A new combined approach at the diagnosis of childhood Acute Lymphoblastic Leukemia patients: Real Time PCR and BAC based high throughput FISH analysis

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Introduction: Acute lymphoblastic leukemia (ALL) is a disease characterized by the accumulation and leukemic transformation of immature lymphoid cells in the blood and bone marrow. Aim of this study was to determine genomic level aberrations for the diagnosis of childhood ALL patients using quantitative real time PCR (Q-RT-PCR) and high throughput BAC based molecular FISH analysis simultaneously.

Materials and Methods: In this study, Q-RT-PCR was performed on RNA samples and high throughput BAC based molecular FISH analysis was performed on DNA samples obtained from peripherial blood and bone marrow of 29 pre-diagnosed ALL patients between the ages of 0-18 regardless of gender.

Results: As a result of high throughput BAC based molecular FISH analysis, aberrations with various sizes were detected in various regions of the genome in 17 (58,6%) patients. The presence of MLL-AF4 fusion gene was identified in a patient (3,4%) among 17 patients using Q-RT-PCR method in addition to the found aberrations. The presence of MLL-AF4 and BCR-ABL fusion genes were found using Q-RT-PCR as an only result in 2 (6,9%) patients. Using both methods no aberrations were found in 10 (34,5%) patients. Gain of chromosomes X and 21 in a frequency of 20,7%, loss of chromosomes 9p and 6q in a frequency of 10,3% were the most common chromosomal aberrations detected in our study.

Conclusion: We think that rapid diagnosis and increased sensitivity is the significance of the simultaneous use of these two methods in hematological malignancies.

P06.057

Dysregulation of FAS and FASL in Chordoma: Study of their Role in Zebrafish Notochord Development and Regression

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Chordoma is a rare malignant bone tumor arising from notochord remnants, characterized by local invasiveness and variable tendency for recurrence. As the apoptotic pathway mediated by FAS-FASL was found to be involved in notochordal cells regression, we studied their expression in 34 chordomas and observed that most of them express FAS, but not FASL. To verify a possible implication of FAS/FASL pathway dysfunction during development leading to tumorigenesis, we started in vivo studies on zebrafish, firstly investigating fas-fasl horthologue genes expression by RT-PCR in whole embryos and larvae. While fas was maternally and zigotycally expressed, fasl showed a stage-specific expression. Following microinjection of a GFP-construct activated by the twhh promoter, we specifically sorted by FACS the GFP-positive notochordal cells at different developmental stages in which we studied fas-fasl expression by means of RT-PCR. We observed that fas was expressed at all the analyzed stages, while fasl is modulated during development. Immunohistochemical experiments showed notochord fasl expression at 48 hpf, 3 and 5 days post fertilization (dpf) and at 9 mm. fasl expression seems to decrease when notochordal cells disappear during chord regression, suggesting the activity of apoptotic mechanisms fas-fasl-mediated. Studies on fas and fasl silencing, by means of morpholino oligos injections, allowed us to observe notochord anomalies such as packed notochordal cells, curved body and bent tail. Further analysis will be performed to verify whether the reactivation of fas-fasl pathway might rescue the normal phenotype, allowing to unravel the role of this mechanism in chordoma development.

P06.058

The role of microRNAs in resistance to imatinib in chronic myeloid leukemia patients

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Chronic myeloid leukemia (CML) is a myeloproliferative disorder characterized by the expression of the BCR-ABL oncoprotein, which is essential for the pathogenesis of the disease. Imatinib, an ATP-competitive selective inhibitor of BCR-ABL, has unprecedented efficacy for the treatment of CML. Nevertheless resistance to imatinib is observed in over 30% cases of CML. Several cellular and genetic mechanisms of imatinib resistance have been proposed, including overexpression of the BCR-ABL gene, the tyrosine kinase domain mutations, pharmacokinetic and pharmacodynamic factors.

The purpose of this study was to investigate the mechanisms of resistance to imatinib in CML patients. We have analyzed mRNA level of BCR-ABL gene by quantitative real time RT-PCR in 114 patients and have studied BCR-ABL mutations in patients with high level of expression of fusion gene. Mutation status was studied by direct sequencing of BCR-ABL cDNA samples. BCR-ABL mutations were founded in 16 (32%) patients with resistance to imatinib treatment. The mutation spectrum included four missense mutation: M351T (7 cases), T315I (4), T315I+M351T (2), M244V (1) and H396R (1). Next we conducted a search for microRNAs specifically targeting 3'UTR of BCR-ABL, using the miRBASE program to scan human genome (http://microrna.sanger.ac.uk/) and analyzed microRNAs expression profile. The down-regulation of miR-203-b (25-fold), mir-323 (4-fold) and miR-196-b (2-fold) in the group of patients with resistance was discovered. In conclusion, our data showed that mutations in the BCR-ABL kinase domain may cause, or contribute to, resistance to tyrosine kinase inhibitors in CML patients, but resistance mechanisms might also be regulated by microRNAs.

P06.059

Chronic myeloid leukemia: molecular monitoring of residual disease by genomic DNA compared to conventional cytogenetic and mRNA analysis in follow-ups up to 8 years.

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Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder resulting from a balanced reciprocal translocation, producing the BCR-ABL1 fusion gene in the Philadelphia (Ph) chromosome. The first line therapy of CML is Imatinib mesylate, which targets Bcr-Abl protein. The gold standard for diagnosing CML is cytogenetic analysis, a direct not-sensitive method to detect Ph-positive cells. The real-time reverse transcriptase PCR (qRT-PCR) is a sensitive quantitative manner to monitor residual leukemia, evaluating levels of BCR-ABL1 transcripts. Undetectable levels of mRNA, however, can reflect either an effective elimination of leukemia cells, or the presence of quiescent leukemia stem cells transcriptionally silent.

We developed a novel highly sensitive method to identify quiescent leukemic cells through quantitative real-time PCR (Q-PCR) based on the DNA.

We monitored eight CML patients in the early chronic phase and in follow-ups up to 8 years under Imatinib treatment. We carried out patient specific Q-PCR assays to monitor minimal residual disease, testing the same samples in parallel by cytogenetic analysis and by standard qRT-PCR. In all positive samples for chimeric transcript we measured positive levels of corresponding genomic DNA. In 13% of samples, with undetectable levels of mRNA, we showed the persistence of quiescent leukemic cells with Q-PCR. Finally we showed a patient with undetectable levels of both mRNA and correspondent DNA in three several consecutive follow-ups during the last year.

In conclusion the DNA genomic Q-PCR is a sensitive and direct technique to identify quiescent leukemic cells and possible candidates to stop imatinib therapy.

P06.060

Measurement of concentration of differentially fragmented DNA with commonly used methods

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Introduction: Circulating nucleic acids are curently studied as potential diagnostic markers of oncological diseases as well as in relation to preeclampsia pathogenesis. Fragmentation and concentration are limiting characteristic features of circulating nucleic acids. Aim of our study was to determine possible impact of DNA fragmentation on measurement of DNA concentration.

Methods: DNA originated from blood of 10 volunteers was isolated using QIAamp® DNA Blood Mini Kit according to the manufacturers protocol. The DNA samples were fragmented by ultrasound with use of Covaris S220 to obtain DNA fragments with various length (150 - 1500 bp). DNA concentration was quantified with three methods: absorbance measurement at 260 nm, fluorescence measurement with PicoGreen® and qPCR with Alu based assay. DNA fragmentation was checked on agarose gel.

Results: DNA quantification with PicoGreen® was affected by degree of fragmentation. DNA concentration significantly decreased as the level of DNA degradation increased (p< 0,0001; F= 65,34). The A_{260}-based quantification was not influenced by the fragment length (p= ns). Regarding the qPCR measurement, DNA concentration of fragments with length above 500 bp was not affected by the fragmentation degree. However, concentration in samples with 150 bp fragments was significantly decreased (p< 0,0001; F= 55,61).

Conclusion: Our study showed, that accuracy of DNA quantification by fluorescence and qPCR depends on the level of DNA fragmentation, while the spectrophotometric measurement of DNA concentration is not affected by DNA fragmentation. Therefore, non-fragmented DNA should be quantified, especially when short fragments are crucial for downstream applications (for example next generation sequencing).

P06.061

Identification of Epstein-Barr virus mixtures associated with Notch1 expression in pediatric patients with Hodgkin's Lymphoma

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Classic Hodgkin lymphoma (cHL) is a lymphoid malignancy characterized by the presence of Reed-Sternberg cells. Approximately 40-60% of cHL have been associated with Epstein-Barr virus (EBV) infection. Transcription factors of the B lineage are not expressed in RS cells but these may express other markers of hematopoietic cell lines. In an effort to identify the participation of Pax5, NOTCH1 and the latent state genes of EBV involved in the cHL oncogenic process, 58 lymph node samples of Mexican pediatric patients were analyzed. Detection and genotyping of EBV was performed by PCR and in situ hybridation. Expression of NOTCH1, Pax5 and LMP1B was identified by immunohistochemistry and immunofluorescence. NOTCH1 expression was detected in 85.3% of cHL cases. 80% of cases of cHL were positive for EBV, B subtype had the highest presentation. With these results we concluded that RS cells have an aberrant immunophenotype in comparison to their cells of origin (B lymphocytes), due to molecular mechanisms involved in the loss of expression of transcription factors of B lineage (Pax5). The expression of LMP1 and LMP2 that constitutively activate Notch1 signaling pathway, induces changes in the identity of B cells, a mechanism suggested in animal models and cell lines. The presence of B subtype in the majority of the cases may suggest a poor prognosis, as it enhances proliferation and immortalization of RS cells. We identified 4 cases with A and B subtypes in the same sample, which is very interesting since this has only been reported in HIV patients.

P06.062

Role of VHL gene mutations and loss of heterozygosity in human renal cell carcinoma development in patients from Bashkortostan Republic of Russia

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Von Hippel-Lindau (VHL) tumour suppressor gene inactivation is associated with clear cell renal cell carcinoma (ccRCC)development. The aim of the study was to provide the analysis of *VHL* inactivation in ccRCC tumors and to evaluate relationships between *VHL* inactivation and tumor hystopathological characteristics. *VHL* genetic inactivation was examined among 92 sporadic ccRCC cases from Bashkortostan Republic using SSCP-analysis followed by Sanger sequencing of *VHL* gene. Besides, loss of heterozygosity (LOH) studies were performed using microsatellite markers (*VHL - D3S1038* and *D3S1317*, *RASSF1 - D3S966* and *D3S1568*, *FHIT - D3S1234* and *D3S1300*) of region 3p12-p26 on paired normal-tumor tissues. Analysis of microsatellite markers of *VHL* gene revealed deletions in 32,5% of ccRCC, *RASSF1* - 30,4%, *FHIT* - 22,6%. *VHL* and *RASSF1* gene deletions frequency was almost equal in groups with stages I, II and III,IV. *FHIT* gene deletions frequency was higher in patients with stages I and II (P=0,008). *VHL* mutations were revealed in 20 tumor tissues. Not a single normal tissue had *VHL* mutations. The majority of mutations were deletions reported previously and touching HIF binding β-domain. We have also detected *VHL* deletions in the 1st and 2nd exons haven't been described previously: c.185_195del(p.Val62_Ser65del), c.216_228del (p.Gln73_Phe76del), c.402_407del (p.Leu135_Phe136del). It is known that exon 2-encoded residues are involved in two functions: substrate protein recognition and transcription-dependent nuclear/cytoplasmic trafficking. We also found 6 undescribed deletions in the third *VHL* exon; their roles are to be determined. It is supposed that different domain mutations inactivate *VHL* function differently that may reflect clinical outcome.

P06.063

Identification of germline alterations in the cancer predisposing gene *CDH1* in patients with orofacial clefts

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CDH1 mutation carriers have a lifetime risk of hereditary diffuse gastric cancer (HDGC) up to 80% and in addition females have a lifetime risk of lobular breast cancer up to 60%. Furthermore, an association between *CDH1* mutations and orofacial clefts (OFC) has been observed, suggesting that E-cadherin, the protein encoded by *CDH1*, is involved in lip and palate development. *CDH1* is a reasonable candidate gene for OFC because E-cadherin plays an important role in cell adhesion and the protein is expressed in the lip and palate region during the critical stages of embryonic development.

To determine whether *CDH1* is a susceptibility gene for OFC and to assess whether *CDH1* mutation screening in OFC patients allows identification of families at risk for HDGC, direct sequencing of the full coding sequence of *CDH1* was performed in a cohort of 56 children with different types of non-syndromic OFC. Two putative pathogenic variants, c.1108G>T (p.(Asp370Tyr)) and c.2413G>A (p.(Asp805Asn)) were detected in two patients. Functional assays on these variants are currently ongoing.

In conclusion, two putative pathogenic germline variants of the *CDH1* gene have been found in a cohort of 56 OFC patients. *CDH1* mutation screening in patients with OFC might increase the chance to identify individuals at risk for hereditary gastric and breast cancer whom could benefit from intensive clinical surveillance.

P06.064

TNIP1 is a novel fusion partner gene to PDGFRB in chronic myelomonocytic leukemia associated with eosinophilia

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Gene fusions involving the catalytic domain of Tyrosine Kinases (TKs) are found in a variety of haematological malignancies. A significant minority of patients with Clonal Eosinophilia carry abnormal gene fusions linking *PDGFRA*, *PDGFRβ*, and *FGFR1*. Rearrangements of Platelet Derived Growth Factor Receptor beta (*PDGFRβ*), a Transmembrane Tyrosine Kinase receptor, are rare but recurrent in patients with Eosinophilia-associated Atypical Myeloproliferative Neoplasie (Eos-MPNs). Using fluorescence in situ hybridization (FISH) we found a cryptic deletion of *PDGFR*β (5q33-34) in a MPN patient with abnormal Eosinophilia. Array CGH in bone marrow tissue confirmed the deletion and led to identification of *tumour necrosis factor α-induced protein 3-interacting protein 1 (TNIP1)* as fusion partner. In a next step, we characterized the genomic breakpoints by gap-PCR and sequencing, revealing that the fusion gene comprises *TNIP1* exons 1 to 12 and *PDGFR*β exons 12 to 23. If accurately spliced, this fusion gene does not contain premature stop codons, and the catalytic domain of *PDGFR*β is fully maintained. Reverse transcription PCR and quantitative PCR finally revealed that the fusion gene is expressed both in the bone marrow and in the blood sample of the patient.

To our knowledge, this is the first report of a *TNIP1-PDGFR*β fusion gene. Patients with *PDGFR*β fusions genes are excellent candidates for treatment with Tyrosin Kinases inhibitors (TKI). The patient is currently treated with Imatinib (400 mg/day).

P06.065

Frequency of germ line MUTYH mutations in patients diagnosed with colorectal cancer in Castilla y León (Spain).

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MUTYH-associated polyposis (MAP) is an autosomal recessive cancer predisposition syndrome associated with the development of colorectal tumors and colonic polyps at an early age. MAP syndrome is associated to germline biallelic mutations in the MUTYH gene which lead to deficient DNA repair through the base-excision repair system and accumulation of G:C→T:A transversions. Occurrence of such mutations in oncogenes and tumor suppressor genes drives colorectal carcinogenesis and is associated with the development of colonic polyps. The aim of this study was to assess the frequency of germline MUTYH mutations in patients with MAP and other hereditary colorectal cancer (CRC) phenotypes. A total of 30 patients were included. Samples were screened for the MUTYH germline mutations by capillary array electrophoresis (HA_CAE) method. In all mutation-positive cases, results were confirmed by sequencing. Biallelic germline MUTYH mutations were identified in 2 of 30 (6.6%) patients with a phenotype of hereditary colorectal cancer. Besides, four monoallelic variants were detected in different samples that are described in LOVD database (MUTYH_00078, MUTYH_00005, MUTYH_00063, MUTYH_00086). A genotype-phenotype correlation has found in these patientns. Germline MUTYH mutation screening should be considered in the differential diagnosis of hereditary colorectal syndromes, we are considering the validity and applicability of MUTYH mutation screening in our population.

P06.066

Pharmacogenetic Markers Associated with Adverse Events in Colorectal Cancer patients treated with 5-FU prodrugs

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Intravenous fluorouracil (5-FU) has been used for the treatment of colorectal cancer over 40 years. Lately, oral 5-FU prodrugs have been additionally developed. However, only a fraction of the administred capecitabine reaches its target cells and is transformed into active metabolites which cause permanent inhibition of the enzyme thymidylate synthase (TYMS) and further DNA synthesis. Polymorphisms in TYMS and MTHFR genes presumably affect the clinical response of 5-FU.

Retrospective pharmacogenetic study was performed to investigate germline polymorphisms of the two genes involved in fluorouracil pathway and their potential association with toxicities in patients treated with capecitabine.

The germline DNA of 36 patients treated with capecitabine was genotyped for two variants (677C>T; 1298A>C) in MTHFR gene and one variant in TYMS (28-bp tandem-repeat) gene. The 28-bp tandem-repeat was genotyped by PCR amplification to discriminate between 2R and 3R alleles, PCR fragments were separated by eletrophoresis on 2.5% agarose gels. MTHFR polymorphisms were genotyped using APEX based microarray.

Adverse events were more frequent in patients heterozygoys for TYMS 2R/3R. Patients carrying the 1298C/C genotype had a low risk of developing side effects compared to patients with the A/C or A/A genotype when treated with capecitabine. Similarly, diplotype (CA-CA) showed a negative effect on the incidence of adverse events. In contrast, MTHFR haplotype (677C-1298C) showed a protective effect.

In conclusion, in colorectal cancer patients, the incidence of side-effects induced by capecitabine might be genetically predicted. However, these preliminary findings must be confirmed in larger and prospective clinical studies.

P06.067

Genetic Screening of Colorectal Cancer Patients to Detect Hereditary Colorectal Cancer Syndromes in Estonian Population

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About 700 new colorectal cancer cases are diagnosed in Estonia annually, which places the incidence of colorectal cancer to the third place of all cancer cases.

Aim of the study was to detect hereditary syndromes in patients diagnosed with colorectal cancer to improve screening strategy in Estonia and provide genetic testing and counceling to family members of the patients.

The study involved a systematic analysis of 180 patients diagnosed with colorectal cancer. MSI testing, BRAF V600E mutation detection, immunohistochemistry and mutation analysis of MLH1, MSH2, MSH6, PMS2, APC and MUTYH genes were performed. MSI and BRAF analyses were performed on tumors from all the patients. Based on revised Bethesda guidelines and the results of MSI and BRAF analyses, samples were chosen for IHC analysis and DNA sequencing of MLH1, MSH2, MSH6 and PMS2. Patients subjected to the sequencing of APC and MUTYH genes were chosen based on family history and histologically described polyposis. The analysis of family history and previous diseases revealed six patients with an indication for hereditary breast and ovarian cancer testing.

MSI-H and BRAF mutation were observed in 30 and 28 out of all cases, respectively. Several polymorphisms in MLH1 (13); MSH2 (11); MSH6 (10) and PMS2 (15) genes, and a few previously not described variants of unknown significance were found. Deleterious germline mutation was found in APC gene. One polymorphism was found in BRCA1, BRCA2, RAD51 and also in CHEK2 gene using Hereditary Breast and Ovarian Cancer Assay. Significance of these findings will be further evaluated.

P06.068

Association between 18q LOH and metastatic potential of colorectal cancer

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Background: Identification of genetic markers to complement clinicopathological evaluation in cancer diagnosis and selection of appropriate treatment is a major challenge for molecular oncology today. Loss of heterozigosity (LOH) at several chromosomal regions harboring tumor suppressor genes or oncogenes, is one of the most promising molecular markers with prognostic significance for colorectal cancer (CRC).

Aim: To evaluate the involvement of 18q LOH in colorectal carcinogenesis and its value as a prognostic molecular marker.

Materials and Methods: A total of 314 randomly selected patients undergoing colon and/or rectum resection for pathohistologically confirmed CRC were included in the study. LOH analysis was performed using fluorescence multiplex PCR with 4 microsatelite markers: D18S46, D18S58, D18S61 and D18S535, and subsequent capillary gel electrophoresis on AbiPrism310.

Results: LOH at 18q defined as loss at any of the evaluated markers is detected in 57% of tumor samples. Most efficient molecular marker is D18S46, conferring LOH status to 65% of cancers with this type of chromosomal instability. Cancers with 18q LOH are prone to development of distal metastases (OR=6.35, 95%CI=1.183-34.042, p=0.02), which is considered a pathohystological criterion for poor outcome. LOH at this chromosomal region is also associated with tumor size, though without statistical significance (p=0.25).

Conclusion: Our results identifying association between 18qLOH and development of metastases indirectly support the hypothesis that 18qLOH is a molecular marker of poor outcome in CRC. Further studies investigating the 5-year survival in our patients will be performed to confirm these findings and provide additional details for the observed association.

P06.069

Study of expression of cancer/testis Antigens PAGE4, SPANX, and SCP-1 as putative biomarkers to predict liver metastasis in colorectal cancer (CRC) in Iranian CRC patients

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Cancer testis antigens (CTAs) are a large family of tumor-associated antigens that mainly express in testis and placenta and in some human cancers with different histological origin. CTAs are considered promising target molecules for early diagnosis, prognostic and immunotherapy.

In this study we aimed to employ conventional RT-PCR and Quantitative real-time RT-PCR to examined whether or not PAGE4, SPANX, SCP-1 antigens are express in colorectal tumors. In this regards, 70 tumor samples and adjacent normal tissues of patients with colorectal cancer were evaluated.

In this presentation we will discuss our data in regards to use of these CTAs as putative prognostic marker for liver metastasis in CRC patients.

P06.070

Identification and confirmation of DNA methylation markers for colorectal cancer testing

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Early diagnosis of colorectal cancer (CRC) is of high importance because prognosis for patients with CRC depends on stage at time of diagnosis. Thus there is great need to identify novel biomarkers for diagnostic improvements of CRC. Patients at risk for CRC are identified by screening programs using the FOBT (fecal occult blood test) which provides 61-96% sensitivity and 91-98% specificity. However recall rate due to many false positives are leading to unnecessary colonoscopy and anxiety. This causes also a high workload for confirmatory initial diagnosis by colonoscopy. There remains a need for a minimally invasive, cost-effective procedure that could be used alongside FOBT screening and eventually prior colonoscopy to improve screening sensitivity. To improve CRC initial diagnosis we have elucidated a panel of candidate DNA methylation biomarkers from tissue DNA testing (tumors: n=18; normal tissue: n=18) using a targeted DNA methylation microarray. The top 24 candidates were then subjected to qPCR based confirmation of microarray data. DNA from blood (n=8) was also included in qPCR analyses; based on these data the minimal set of markers for perfect classification could be reduced to a single gene enabling 100% correct classification (AUC=1). Further we designed qMSP for bisulfite based confirmation of findings. By qMSP 96-100% of all different samples were classified correctly. Thus we have elucidated candidate markers which should be validated on different sample cohorts and might be good targets for minimal invasive CRC testing using cell free DNA from serum. Collaborations for validation of markers are envisioned.

P06.071
Intermediate- and low-methylation epigenotypes do not correspond to CpG island methylator phenotype (low and -zero) in colorectal cancer

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Most recent genome-wide studies on the CpG island methylation in colorectal cancer (CRC) have led to the discovery of at least three distinct methylation clusters. However, there remains an uncertainty whether the CRC clusters identified in these studies represent compatible phenotypes.

We performed comprehensive genome-scale DNA methylation profiling by Illumina Infinium HumanMethylation27 of 21 DNA pools that represent 84 CRC samples divided according to their high-, intermediate-, and low-methylation epigenotypes (HME, IME, and LME, respectively) and 70 normal-adjacent colonic tissues. We have also examined the relationship between three epigenotypes and chromosomal gains and deletions (assessed by Comparative Genomic Hybridization) in a group of 100 CRC samples.

HME subgroup showed features associated with CpG island methylator phenotype - high (CIMP-high) including methylation of specific CpG sites (CpGs) as well as significantly lower mean number of chromosomal imbalances when compared to other epigenotypes. IME subgroup displayed lowest number of methylated CpGs (717 versus 2399 and 2679 in HME and LME, respectively) and highest mean number of chromosomal imbalances when compared to HME ($p = 0.001$) and LME ($p = 0.004$). A comparison between the methylation profiles of three epigenotypes revealed more similarities between the HME and LME (1669 methylated CpGs overlapped) than HME and IME (673 methylated CpGs overlapped).

Our results provide evidence that IME and LME CRCs show opposite features to those that have been previously attributed to CIMP-low and CIMP-0 CRCs. These discrepancies should be considered when interpreting the data from a particular epigenotyping method.

P06.072
Tumor-specific age-dependent DNA methylation of RUNX3, CDKN2A and CACNA1G genes in colorectal cancer

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Promoter methylation of tumor suppressor genes has a crucial role in tumorigenesis. It is widely assumed that epigenetic alterations that accumulate during aging might be involved in aging-related pathologies. However, little is known about the cross-talk between aging and cancer in terms of DNA methylation.

We aimed to explore the association between the methylation status of marker genes in normal and tumor tissues with age in colorectal cancer (CRC) patients.

A cohort of 197 unselected CRC patients was studied (median age=71; range 30-94 years). Tumor (n=197) and normal (n=20) colon tissues were analyzed for the panel of eight markers used to assess the CpG Island Methylator Phenotype (CIMP): *RUNX3, CACNA1G, IGF2, MLH1, NEUROG1, CRABP1, SOCS1,* and *CDKN2A.* This study was approached by Methylation Sensitive MLPA. Microsatellite instability (MSI) and *BRAF* V600E mutation were also analyzed.

We found 22.84% (45/197) of tumors with CIMP+ (cutoff: five positive markers); 5.08% (10/197) with *BRAF* mutation and 9.64% (19/197) with MSI. None of the 20 matched normal tissues analyzed from patients with CIMP+ tumors showed significant methylation (10 patients older and 10 younger than the median age). CIMP+ tumors were associated to older patients (OR=2.23; p=0.022); proximal location (p=0.002); *BRAF* mutation (p<0.001) and MSI (p<0.001). Tumor methylation age-dependent was given by *RUNX3* (OR=3.53; p<0.001); *CDKN2A* (OR=2.26; p=0.007) and *CACNA1G* (OR=2.46; p=0.018).

Our results suggest that methylation at *RUNX3*, *CDKN2A* and *CACNA1G* genes is tumor specific and age dependent in CRC patients. The epigenetic landscape of CRC might be different depending on patient age.

P06.073
A DNA methylation survey of 117 tumor samples and corresponding normal controls revealed more than 640 genes aberrantly methylated in colorectal cancer

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Colorectal carcinoma (CRC) is still a leading cause for cancer related death in the western world [1]. At molecular level colorectal carcinoma is characterized by both, genetic aberrations and extensive alterations in the gene expression pattern.

Epigenetic mechanisms and particularly DNA methylation are well known to contribute to the stability of the genome and to the establishment of tissue specific gene expression patterns [2]. Consequently, alterations in the methylome are a typical hallmark of cancer, contributing the malignant phenotype, e.g. in CRC [3].

In this study we included genomic DNA isolated from 117 CRC tissue samples and the corresponding control tissue from the same patients. Detailed clinical, histological as well as molecular data (e.g. age at diagnosis, treatment regimen, MSI status) were available from all patients.

Within the BMBF funded NGFN-Network „Integrated genomic investigation of colorectal carcinoma" we used Illumina's HumanMethylation450k BeadChip to identify genes aberrantly methylated in CRC as compared to normal colon tissue. This array is designed to assay the methylation status of 485,577 CpG loci in parallel.

By this approach we have identified more than 1,380 CpG loci corresponding to more than 640 genes aberrantly methylated in CRC. Furthermore, hierarchical cluster analysis based on these loci separated the samples into two different CRC subentities and the controls. The putative use for diagnostic, prognostic or therapeutic purposes is currently under investigation.

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P06.074
Dual blockade of IGF-IR and EGFR sensitizes colorectal cancer cells to 5-FU-based radiochemotherapy

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Receptor tyrosine kinases (RTKs) are known to be key players in the development of colorectal cancer (CRC). Recently, we could show that inhibition of the RTKs insulin-like growth factor-I receptor (IGF-IR) and epidermal growth factor receptor (EGFR) in CRC cells by siRNA approach or by small molecule inhibitors NVP-AEW541 and erlotinib, respectively, results in decreased proliferation and induction of apoptosis, with having additive effects when both receptors were blocked simultaneously. In the present study we analysed the effect of RTK inhibition in the context of 5-Fluorouracil-based radiochemotherapy (RCT). Inhibition of IGF-IR and EGFR using NVP-AEW541 and erlotinib results in a sensitization of CRC cells (DLD-1, SW837 and Caco-2) to RCT and a poor survival in therapy treatments. In addition, dual blockade of both receptors in CRC cells enhanced the therapy success. To identify the molecular mechanisms underlying this sensitization of CRC cells we analysed the DNA repair mechanisms. Phospho-H2AX staining as marker for double strand breaks revealed impairment of DNA repair in single inhibitor treatments 24h after RCT and even more pronounced effects were observed when both receptors were inhibited simultaneously. Furthermore, we could show using proximity ligation and co-immuoprecipitation assays that IGF-IR and EGFR form heterodimers in CRC cells in a ligand-dependent manner. Taken together, these results indicate that the RTKs EGFR and IGF-IR play important roles in the resistance of CRC patients to neoadjuvant RCT and that simultaneous treatment of RCT resistant patients with a combination of RTK inhibitors can circumvent these problems.

P06.075
KRAS mutation genotypes and prometer methylation of tumor suppressor genes in cancer tissue and peripheral blood in colorectal cancer patients

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Objective: The aim was to evaluate the value of testing KRAS mutation genotypes and methylation of tumor suppressor genes(TSG) in peripheral blood samples besides colorectal cancer tumor tissue.

Materials and Methods: The promoter methylation status of TSG in tumor tissue and peripheral blood samples in 89 colorectal cancer patients (32F/57M) were evaluated by sodium bisulfite conversion and DNA amplification with methylation specific multiplex PCR technique.Also, KRAS in codons 12 and 13 were analyzed for possible genotypic mutations.

Results:TSG was inactivated in 6 samples in 32 females and 11 of 57 males in peripheral blood ($p>0.05$).Testing of tumor tissue revealed a positivity of TSG inactivation in 17 females and 29 males ($p>0.05$).KRAS mutation was present in 7 females and 22 males in blood samples ($p>0.05$) and 20 vs 36 for females and males,respectively in biopsy specimen. Overall KRAS mutation or TSG inactivation was 8/32 vs 27/57 for females and males ($p<0.02$). Total alterations were 82% vs 41.5% for tissue and blood,respectively ($p<0.001$). Total mutant KRAS ratio in tissue was 63%.

Conclusion: Genes exhibit tumor suppressor activities in tissue and peripheral blood samples. The treatment choice depending on these results should be further evaluated.Screening KRAS and inactivated TSG from tumoral and blood samples may give clinical clues. Mutation or inactivation over 80% and significant gender difference need to be verified by large series.

P06.076

Interlaboratory comparison of the *K-ras* mutation testing techniques

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Treatment of metastatic colon cancer with the anti-EGFR antibodies, cetuximab and panitumumab, is reported to be ineffective in carcinomas with *K-ras* point mutations in codons 12 or 13. We compared four the most frequently used methods for *K-ras* testing: ACRS, SSCP, allele-specific RT PCR and Sanger sequencing in clinical practice. Samples from 62 colorectal cancer patients (frozen tissues after manual dissection) were tested in different laboratories.

The results, received with ACRS, SSCP and allele-specific RT PCR have coincided for 59 carcinomas (95.2 %). To investigate the apparent discrepant results between these methods for three samples we used commercial TheraScreen KRAS Kit (DxS Ltd, Manchester, England). Specificity and sensitivity of allele-specific RT PCR were 100 % (34/34) and 96.4 % (27/28) respectively and with the use of ACRS or SSCP- 94.1 % (32/34) and 100 % (28/28) respectively. Falsely positive findings were absent with allele-specific RT PCR, but were discovered in two cases with ACRS or SSCP analysis (5.9 %). The single falsely negative result was received with allele-specific RT PCR (3.6 %). Sensitivity of direct sequencing was significantly lower than with the use of ACRS, SSCP or allele-specific RT PCR: 78.6 % (22/28).

Allele-specific RT PCR, ACRS and SSCP showed high and similar diagnostic sensitivity and specificity (from 94 to100 %) for the studied series of samples. These techniques are able to detect somatic *K-ras* mutations in DNA samples when the mutant DNA represents at least 5 % of total DNA.

P06.077

miR-21 expression in tumor tissue progressively increases during the development of colorectal cancer

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MicroRNAs (miRNAs) are endogenously expressed noncoding RNAs with important posttranscriptional regulatory functions. Recent data suggest that miRNAs may play significant roles in carcinogenesis.The aim of this study was to identify the differential miRNA expression patterns associated with colorectal cancer (CRC) in Republic of Macedonia and to evaluate their diagnostic and/or prognostic potential. We assessed the expression levels of eleven miRNAs (miR-17-3p,miR-18a,miR-20a,miR-21,miR-92a,miR-106a,miR-125b,miR-137,miR-143,miR-145 and miR-200c) in 89 CRC and 40 normal colon tissues. We observed statistically significant up-regulation of miR-18a, miR-20a, miR-21 and miR-200c and down-regulation of miR-145 in CRC compared to normal mucosa ($p<0.001$). Multivariate analysis showed that miR-20, miR-21 and miR-145 are independent variables for discrimination between normal and tumor tissues ($p<0.001$). Combination of the later three miRNAs as biomarkers for diagnosis increases the power of the test and enables higher accuracy for overall correct classification (95.35%) compared with their separate use (86.05%, 89.92% and 67.44% respectively). Furthermore, we found higher miR-21 expression levels in microsatellite and chromosomal stable tumors ($p=0.039$) which suggests the possibility for distinct mechanism in CRC etiopathogenesis mediated by dysregulation of proto-oncogene miRNAs that predominantly occurs in MSI-/CIN-/CIMP-tumors. In addition, our results indicate correlation between the miR-21 expression levels and CRC clinical stage ($p<0.001$). The expression of miR-21 progressively increases with average fold-change of 1.79, 5.65, 11.59 and 33.85 (stage I,II,II and IV respectively) compared to the control group. This illustrates the potential use of miR-21 expression levels in providing higher accuracy of staging on initial biopsies and identifying candidates for more aggressive initial therapies.

P06.078

MiRNA genes constitute new targets for microsatellite instability in colorectal cancer

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Mismatch repair-deficient colorectal cancers (CRC) display widespread instability at DNA microsatellite sequences (MSI). Although MSI has been reported to commonly occur at coding repeats, leading to alterations in the function of a number of genes encoding cancer-related proteins, nothing is known about the impact such a process could have on microRNAs. In miRbase V15, we identified few human microRNA genes with mono- or di-nucleotide repeats (n=27). Out of the 24 microRNA genes successfully studied, 15 were found unstable in a large series of MSI CRC cell lines and primary tumors. Frequencies of instability ranged from 2.5% to 100%. Following a maximum likelihood statistical method, microRNA genes were separated into two groups that differed significantly in their mutation frequencies and in their tendency to represent mutations that may be or not under selective pressures during MSI tumoral progression. The first group included 21 genes that displayed no or few mutations in CRC. The second group contained 3 genes with frequent (≥80%) and sometimes bi-allelic mutations in MSI tumors. For *hsa-mir-1303*, the only miR expressed in colonic tissues, no direct link was found between the presence or not of mono or bi-allelic alterations and the levels of mature miR expression in MSI cell lines. Any impact MSI might have on the reliable processing of *hsa-miR-1303*, and potentially the production of mutant miRs is still to be determined. Overall, our results provide evidence that DNA repeats contained in human miRNA genes are relatively rare and preserved from MSI mutations in MMR-deficient cancer cells.

P06.079

Molecular characterization of Solid Tumors by 454 Sequencing

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Introduction

The success of a treatment with monoclonal antibodies (mAB) or „small molecules" (e.g. tyrosine kinase inhibitor, TKI) depends on the mutational status of tumor-relevant genes in solid tumors (tab.1).

Materials and Methods

NGS is applied for mutational screening (tab.1) in 40 CRC, 34 NSCLC, 2 Melanoma, 2 GIST. DNA was isolated from FFPE-tumor tissue, amplicons (300bp) were prepared and multiplex identifiers (MID) were added manually. After purification, the pooled library was sequenced using the GS junior (454 Life Sciences, Branford, CT, USA) with an aimed coverage of 1000-fold.

Results

17/40 CRC showed a *KRAS*-mutation, 5/40 CRC a *BRAF*-mutation. Both GISTs had a *c-KIT*-mutation. In 9/34 NSCLC an *EGFR*-mutation could be detected and another three had a *KRAS*-mutation. One melanoma showed a *KRAS*-mutation. Some of these mutations only could be detected due to the high coverage.

Conclusion

Our data show that NGS is a feasible method in routine molecular pathology to examine mutational status of tumor-relevant genes in a cost effective and comprehensive manner. In contrast, conventional capillary sequencing techniques often lack the sensitivity and cost effectiveness to detect tumor mutations occurring at less than 20% frequency. With 454-sequencing, mutations can be detected against a wildtype-background of 95% (Coverage 1000x), which is very important in solid tumors. With NGS, we were able to improve our mutation detection rate, an important step towards personalized medicine.

tab.1:Therapy-relevant biomarker in solid tumors
*also mutations leading to resistance

Tumor Entity	***Related Gene***	***Affected Exon***	***Allel***
Colorectal Carcinoma (CRC)	*KRAS*	2,3	Wildtype
	BRAF	15	Wildtype
Non-Small Cell Lung Cancer (NSCLC)	*EGFR*	18, 19, 20, 21	mutated*
	KRAS	2, 3	Wildtype
Gastrointestinal Tumors (GIST)	*c-KIT*	9, 11, 13, 17	mutated*
	PDGFRa	12, 14, 18	mutated*
Malignant Melanoma	*BRAF*	15	mutated*

P06.080

Exome sequencing reveals genetic predisposition in pediatric colorectal cancer.

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Childhood colorectal cancer (CCRC) is a rare condition, mainly characterized by non-polyposis, microsatellite-stable tumors with a higher frequency of signet ring cell histology than in adults, suggesting a different biology. In many patients the genetic cause remains unknown.

We performed whole exome sequencing on germline DNA of four children with CRC, diagnosed between age 13 and 15. Per patient, we validated an average of 45 candidate pathogenic variants by Sanger sequencing , and tested parental DNA to reveal the modes of inheritance.

In one child a paternal *BRCA2* mutation and a maternal change in *PARP1* were identified. The co-occurrence of these two mutations appears relevant, since both proteins are involved in DNA repair. The second patient, a child of distantly related parents, carried a homozygous *SEC31B* variant. In yeast, Sec31p is involved in cell cycle progression and therefore might play a role in cancer development. *De novo* mutations were identified in two patients, affecting highly conserved protein-coding positions in *CCDC13*, a gene with unknown function and in the RAS pathway gene *SOS2,* respectively. In the latter patient, *in vitro* expression of this mutant in HEK293 cells revealed an increase of RAS activity, suggesting a gain-of-function effect.

Our data indicate that exome sequencing is a powerful tool for unraveling genetic predisposition in CCRC, which turns out to be a heterogeneous condition with different models of inheritance. Elucidating the genetic cause of CCRC will facilitate decisions on surveillance of patients and relatives and might reveal new treatment targets.

P06.082

TGFBR1 Intralocus Epistatic Interaction as a Risk Factor for Colorectal Cancer

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In colorectal cancer (CRC), an inherited susceptibility risk affects about 35% of patients, whereas high-penetrance germline mutations account for < 6% of cases. A considerable proportion of sporadic tumors could be explained by the coinheritance of multiple common low-penetrance variants. We assessed the susceptibility to CRC conferred by 14 polymorphisms and the allele-specific expression (ASE) of *TGFBR1* in a case-control designed study (504 controls and 521 patients with sporadic CRC). Polymorphisms were genotyped with the iPLEX Gold (MassARRAY-Sequenom) technology. Descriptive analyses of the polymorphisms and association studies were performed with the SNPator workpackage. No relevant associations were detected between individual polymorphisms or haplotypes and the risk of CRC. The *TGFBR1**9A/6A polymorphism was used for the ASE analysis by fragment analysis using cDNA from normal tissue. The relative level of allelic expression was extrapolated from a standard curve. ASE was found in 25.4% of patients and 16.4% of controls. No significant differences between the ASE values of patients and controls were identified. Interestingly, a combined analysis of the polymorphisms and ASE for the association with CRC occurrence revealed that ASE-positive individuals carrying one of the most common haplotypes (H2: 20.7%) showed remarkable susceptibility to CRC (RR: 5.25; 95% CI: 2.547-5.250; p < 0.001) with a synergy factor of 3.7. In our study, 54.1% of sporadic CRC cases were attributable to the coinheritance of the H2 haplotype and *TGFBR1* ASE. These results support the hypothesis that the allelic architecture of cancer genes, rather than individual polymorphisms, more accurately defines the CRC risk.

P06.083

Constitutional mismatch repair-deficiency syndrome and high-grade brain tumors in siblings with biallelic MSH6 mutations

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Constitutional mismatch repair-deficiency syndrome (CMMR-D) is a rare autosomal recessive disease, first reported in 1999. It is characterized by hematological malignancies, brain tumors and tumors of the large intestine with an early formation. Skin maculae with diffuse margins and irregular pigmentation are similar to *café au lait* present in neurofibromatosis 1, and they may occur together with hypopigmented areas. Development of this syndrome is caused by biallelic mutations in genes that are associated with Lynch syndrome (hereditary non-polyposis colorectal cancer - HNPCC). *MLH1, MSH2, MSH6* and *PMS2* genes belong to the mismatch repair system and play a basic role in the genome integrity. In the heterozygous state, the mutation of one of these genes causes HNPCC. Biallelic mutations of the aforementioned genes are characterized by occurrence of the first malignancies (brain tumors, leukemias) at the age of 2 and occurrence of other types of tumors with increasing age. *Wimmer* and *Kratz* summarized data from 52 reports covering 92 CMMR-D patients and performed genotype-phenotype correlation output from the last 12 years. We present a case with CMMR-D caused by novel homozygous *MSH6* mutations leading to gliomatosis cerebri and T-ALL in an 11-year-old female and glioblastoma multiforme in her 10-year-old brother, both with rapid progression of the diseases. A literature review on brain tumors in CMMR-D families shows that they are treatment-resistant and lead to early death. Identification of patients with CMMR-D is critical, and specific cancer screening programs with early surgery are recommended.

P06.084

Lack of association between ERCC2 K751Q polymorphism and thyroid cancer risk

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Thyroid carcinomas belong to tumors with well prognosis, slow progress and low benignity but with tendency to recurrences and regional or remote metastasis. Papillary and follicular thyroid cancer are the most frequent in endocrine system with unidentified genetic background.

In this focus searching for molecular markers of disease course, good or poor prognosis and response on medical treatment is fundamental. It is expected that SNP polymorphisms research in genes demonstrating association with neoplastic diseases will be helpful in understanding of molecular mechanisms of thyroid gland tumors development and allow to better diagnosing.

The published data on the association ERCC2 K751Q polymorphism with cancer remained controversial.

We analyzed polymorphism K751Q in ERCC2 gene (c.2251A>C, rs13181) in group of 451 Polish patients with differentiated thyroid cancer and 560 individuals from Polish population. Sequence variants were determined by pyrosequencing.

We didn't observed significant differences in allele frequencies in patient with thyroid cancer and population group (p=0,64). In DTC patient group allele A was present with frequency 0,61 and allele C with frequency 0,39. In population frequency for allele A was 0,59 and for allele C 0,41.

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P06.085

Spontaneous and dioxidine-induced DNA damage in cultured human multipotent mesenchymal stromal cells and peripheral blood lymphocytes

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Numerous studies have shown the possibility of genetic transformation of multipotent mesenchymal stromal cells (MSC). The DNA damage can lead to chromosomal abnormalities and gene mutations associated with risk of malignization. To determine the genetic stability and sensitivity of MSCs to the ROS-mediated DNA-damage, we have studied spontaneous and dioxidine-induced DNA damage in cultured human MSCs from adipose tissue and compared it with cultured peripheral blood lymphocytes.

Dioxidine is a mutagen with prooxidative type of genotoxic action. The peripheral blood lymphocytes from 5 healthy donors and MSCs from adipose tissue of 8 healthy donors were exposed during 24 h to dioxidine in concentrations 0.01mg/ml and 0.1mg/ml. The levels of DNA damage have determined as %tail DNA in alkaline comet assay.

The mean value of spontaneous DNA damage in cultured lymphocytes was higher than that in cultured MSCs (7.7±0.5% vs. 4.8±0.5 %DNA in tail, p≤0.05). The mean values of dioxidine-induced DNA damage in concentration of 0.01 mg/ml were 10.9±0.70 % DNA in tail in the lymphocytes and 13.0±0.9 %DNA in tail in MSCs. After the exposure to dioxidine in concentration of 0.1 mg/ml, 17.1±1.9 %DNA in tail in the lymphocytes and 16.7±2.0 %DNA in tail in MSCs were observed. So, although end-point values of genotoxic effect of dioxidine was similar for both type of cells (p>0.05), MSCs showed greater sensitivity, particularly when agent in lower concentration was used.

In conclusion, spontaneous level of DNA damage in the MSCs was lower compared with lymphocytes, but MSCs indicated higher response to ROS-mediated DNA-damage.

P06.086

Dioxidine influence on whole-genome methylation status in peripheral blood lymphocytes in vitro

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Introduction. Chemical mutagens can lead to genes mutations and chromosomal aberrations as well as to epigenetic changes. Toxins and other exogenous agents can cause nonspecific (whole-genome DNA hypomethylation and histone deacetylation) and specific (hypo- and hypermethylation of several genes) modifications of genome. Dioxidine is well-studied agent with proved mutagenic activity. Frequency of chromosomal aberrations in peripheral blood lymphocytes increases from spontaneous level of 2.25±0.17% to 8.06±0.37% and 17.00±0.86% after dioxidine exposure in concentrations of 0.01 and 0.1 mg/ml respectively. The aim of this study was to estimate dioxidine influence on whole-genome methylation status in human peripheral blood lymphocytes. Materials and Methods. Samples of peripheral blood were obtained from 14 healthy donors. DNA methylation was analyzed using methyl-sensitive Comet assay with additional step of restriction by *Hpa*II and *Msp*I enzymes. DNA methylation in lymphocytes was estimated before cultivation, after 25-hours cultivation without dioxidine and with dioxidine addition in final concentrations of 0.01 and 0.1 mg/ml after 24-hours cultivation of whole blood. DNA methylation level was defined as the ratio of mean of DNA value in comet's tails after *Hpa*II restriction to that after *Msp*I restriction. Results. DNA methylation levels in lymphocytes before and after cultivation didn't differ (45.28% and 44.80% respectively p>0.4). Lymphocytes after the cultivation with low dioxidine concentration have shown the increased DNA methylation level up to 46.14% (p<0.001) however high dioxidine concentration causes DNA hypomethylation down to 42.31% (p<0.001). Conclusion. Nonspecific hypomethylation caused by dioxidine exposure can result in genome instability and diseases including cancer.

P06.087

Altered DNA methylation in early-stage non-small-cell lung cancer

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Background: There is a lack of suitable markers for the early and reliable detection of malignant tumors, including non-small cell lung cancer (NSCLC). We've performed a genome-wide analysis in early NSCLC to find DNA methylation-based biomarker candidates.

Methods: We've analysed samples from 48 patients with stage I NSCLC and 18 matching cancer-free lung samples using microarrays that cover the promoter regions of more than 14,500 genes. DNA methylation changes were correlated with gene expression levels. In addition, survival analysis was performed. The gene expression and DNA methylation changes were validated with real-time qPCR analysis and bisulfite sequencing with HiSeq 2000 instrument (Illumina).

Results: We observed hypermethylation of 496 CpGs in 379 genes and hypomethylation of 373 CpGs in 335 genes in NSCLC. 378 of 869 (43.5%) CpGs discriminating the NSCLC and control samples showed the expected inverse correlation between CpG methylation and gene expression levels. As a result of a survival analysis, we found 10 CpGs in 10 genes, in which the methylation level differs in different survival groups.

Conclusions: We have identified a set of genes with altered methylation in NSCLC. We also found a set of genes that associated with the survival of the patients. These marker candidates of NSCLC will need a further analysis in order to determine their clinical utility.

P06.088

XPD Lys751Gln polymorphism in Romanian patients with myelodisplastic syndrome

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Polymorphisms in DNA repair genes have been associated to repair DNA damages, and might contribute to the individual susceptibility to develop different types of malignant hemopathias. One of the most studied DNA repair system in humans is nucleotide excision repair pathway. The *XPD* gene (xeroderma pigmentosum group D) is involved in nucleotide excision repair system. An *XPD* variant in exon 23 leading to a Lys751G1n aminoacid substitution has been associated with reduced DNA repair capacity.

To investigate the possible association between the *XPD*
Lys751G1n and the risk of myelodisplastic syndrome in a Romanian population, we analyzed DNA samples from a case-control study of 26 MDS cases and 50 healthy subjects as a control group.

The *XPD* codon 751 genotypes were determined using a PCR-RFLP technique. No statistically significant difference was found for the genotypic and allelic distributions of the polymorphisms in the *XPD* gene between the patients and the control subjects. In conclusion polymorphism in *XPD* codon 751 is not associated with the development of MDS.

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P06.089

DNA repair *XRCC3* polymorphism and acute myeloid leukemia

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The X-ray repair cross-complementing group 3 (*XRCC3*), has an important role in homologous recombination (HR), one of the main pathways for the repair of DNA double strand breaks and maintenance of genomic integrity. Acute myeloid leukemia (AML) is characterized by genetic instability. We hypothesized that polymorphisms in the *XRCC3* repair gene may lead to genetic instability and subsequent AML. The aim of this study was to determine the frequency of the *XRCC3* Thr241Met polymorphisms among AML patients. Therefore we performed a case-control study including 50 de novo AML patients and 50 healthy subjects as a control group.

Genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), with the digestion of restriction endonuclease NlaIII. The restricted products were analyzed on 2% agarose gel stained with ethidium bromide and analyzed under UV light. We found a significantly higher frequency of the polymorphic variants *XRCC3* Thr241-Met genes in AML cases, when compared with controls (P = 0.04; odds ratio =2.54). *XRCC3* 241Met allele is associated with an increased risk for AML. In conclusion, we consider that polymorphism *XRCC3* Thr241Met polymorphism may be a risk factor for the development of AML.

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P06.090

DNMT3B C46359T and *SHMT1* C1420T polymorphisms in carcinogenesis of head and neck

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Introduction: Folate is an essential nutrient for the synthesis, repair, and methylation of DNA. Polymorphisms in genes involved in folate metabolism may alter these processes and, consequently, modulate the cancer development. **Objectives:** Investigate *DNMT3B* C46359T (rs2424913) and *SHMT1* C1420T (rs1979277) polymorphisms related to folate pathway in head and neck cancer risk and the association between these polymorphisms with gender, risk factors and clinical histopathological parameters. **Methods:** A case-control study was conducted in 725 individuals in a Brazilian population (237 patients with head and neck cancer and 488 control individuals without cancer history). The Real-Time PCR technique was performed for genotyping the polymorphisms. Chi-square test and multiple logistic regression test were used in the statistical analysis. **Results:** No significant difference in genotypes distribution was observed between groups in both polymorphisms evaluated. Male gender and tobacco consumption were associated with increased risk for head and neck cancer ($P<0.05$). There were no significant associations between the polymorphisms and risk of disease, however, the tobacco and alcohol habits analyzed together showed association with *SHMT1* C1420T polymorphism ($P<0.05$). For clinical histopathological parameters, *SHMT1* C1420T polymorphism was less frequent in patients that had larynx as primary site ($P<0.05$). **Conclusion:** The male gender and tobacco habit may be predictors of the head and neck cancer and the polymorphisms investigated have no association with head and neck cancer risk. However, further studies involving genes related to folate metabolism may contribute to the understanding of this cancer type development.
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P06.091

Developmental pluripotency associated-2 (DPPA2) gene may be a metastatic marker for colorectal cancer

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Colorectal cancer (CRC) is the third most frequent malignancy in the world. Cancer cells have countless behaviors of pluripotent embryonic and germ line cells, such as unlimited proliferation and the capacity of self-renewal as well as migration. Active embryonic genes in tumor cells may be associated with invasiveness and indefinite growth of such cells. Developmental pluripotency associated-2 (DPPA2) is implicated in regulatory pathways maintaining the pluripotency of embryonic stem cells. DPPA2 expression in human germ line and early stage embryo is also being extended to a significant subset of malignant tumors. However, its expression in CRC remains to be clarified. In this study, the expression level of DPPA2 in 38 CRC samples was compared with related normal tissues by real-time PCR assay. Expressional analysis represented the overexpression of DPPA2 in 31.5% of tumor specimens. In the advanced stages (III/IV) of tumor development, the overexpression of DPPA2 was significantly correlated with the lymph node metastasis of tumor cells ($p<0.05$). These results not only emphasize on the probable role of DPPA2 gene overexpression in the development of CRC tumors through advanced stages but also draw attention to the possible function of this gene in epithelial mesenchymal transition (EMT) of colorectal tumor cells. In summary, having revealed the clinical relevance of DPPA2 expression in CRC, we extrapolate the potential of this gene as a promising marker to evaluate the risk of lymph node metastasis and a possible therapeutic target to prevent functional metastasis.

P06.092

Expression of Selected Drug Resistance Genes in Breast and Colon Cancer Patients

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The ABC transporters have been studied extensively for over two decades. Their structure and function is well described as well as their involvement in the resistance of cancers to therapy. Despite being one of the best studied protein families with important effect on the cancer therapy, the ABC transporters are not routinely diagnosed in clinical practice. This has many reasons, one of them being lack of relevant clinical data or their inconclusiveness. The main aim of this study was to collect more information about the relevance of the relationship between the drug-resistance genes and therapy response. We have analyzed expression of selected ABC transporter genes in the tissues of breast and colon cancer patients. Analysis was performed in over 200 patient samples, with diagnoses ranging from benign lesions to invasive cancers. Our real-time PCR-based analysis revealed frequent overexpression of certain ABC genes in all types of cancers. Most frequently overexpressed genes included ABCC1, ABCC2, ABCC5 and ABCC11. The expression status of ABC genes was subsequently correlated to the clinical data of the patient. The effects of the ABC genes expression on the therapy outcome will be discussed.

P06.093

Comprehensive genomic study in ductal breast cancer

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Despite of the large number of molecular studies in breast cancer, the data are still insufficient for understanding its molecular pathogenesis. In this study we have performed whole genome analysis by DNA microarrays for determining the type, incidence and fine mapping of unbalanced genomic aberrations in ten ductal breast cancers. Trisomies of whole chromosomes or chromosome arms with the highest frequency were observed for chromosomes 20 (80%) and 7 (40%), the most common monosomies were discovered for chromosomes 8 and 15 (60%), followed by 4, 18 and 21 (50%). Significant micro-aberrations were determined by selection of aberrant clones with more than 40% frequency and detection of alterations of high amplitude in more than 30% of tumors. Doing this, we detected significant aberrations for known tumor-driving genes such as MUC1 in 1q21, LASP1 (in 17q11-q21.3) and HER-2 (17q21.1), ZNF217 and AURKA (STK6) in 20q13.2-q13.3. In addition, we suggest as new potential oncogenes TNS1 (2q35), SH3BP5 (3p25), HSPB1 (7q11), and ZNF503 (10q22). The genes, located in the most significant deleted small overlapping regions, were: EPS15 (1p32), ARL15 (5q11), CD2AP (6p12), IKBKB (8p11), KIF11 (10q23), ATM (11q22), CYP27B1 (12q13), EFTUD1 (15q26), and CHAF1B (21q22). Our results showed at a high resolution the unbalanced genomic aberrations for whole genome along all chromosomal regions and complement with additional data the genomic characteristics of ductal breast cancer.

P06.094

Absence of epidermal growth factor receptor gene mutations in patients with first diagnosis of prostate cancer

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BACKGROUND: Mutations in the tyrosine kinase domain of the epidermal growth factor receptor (EGFR) gene are known for a variety of human cancers. In prostate cancer they represent a rare event although the increased tyrosine phosphorylation is an important feature of advanced prostate cancer. In this study we investigated the presence of most common somatic mutations in the EGFR gene in patients with first diagnosis of prostate cancer. The aim was to evaluate the utility of EGFR mutation analysis for early identification of prostate cancers with aggressive growth potential.
METHODS: First voided urine was collected from patients after digital rectal examination. RNA was extracted from urine sediment and used for cDNA synthesis. Exon 19 deletions were detected with polymerase chain reaction and capillary electrophoresis. The L858R mutation was detected with PCR followed by MscI digestion and agarose electrophoresis.
RESULTS: Study sample included 65 patients. All had confirmed diagnosis of prostate cancer with positive biopsy and samples were collected before planed surgery. Gleason score 6 was determined for all cases. No frame shift mutations in exon 19 of the EGFR gene were detected and the L858R mutation was also absent,
CONCLUSIONS: Our results show that EGFR mutations did not occur in these

patients suggesting these mutations may be very rare events in early phase of prostate cancer. Consequently the analysis of EGFR mutations appears not to be informative for evaluation of growth potential of prostate cancer and for disease prognosis.

P06.095

Prolonged HPV infection can produce chronic inflammation and link to ESCC in Iran

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Prolonged inflammation, or chronic inflammation, can lead to cancer. This process can be occurred in present of prolonged infections or irritants. Now, it is clear that tumor microenvironment is accompanied by inflammatory cells which their production including cytokines can promote tumor progression.

In previous study, we showed that chronic inflammation is involved in developing of esophageal cancer, esophageal squamous cell carcinoma (ESCC), in Iran. The incidence of ESCC is high in northern Iran. Environmental exposure to polycyclic aromatic hydrocarbons (PAHs) has been suggested to provoke inflammation. They are easily distributed throughout the human body and produce PAH-DNA adducts which are risk factors for ESCC. Also, it is shown that human papillomavirus (HPV) genome integrate into the host cell chromosome and lead to malignant cell transformation. This infection can be contributed to carcinogenesis by inactivating the p53 protein in oral cancers through *p53* gene mutation.

We assessed the HPV infections in ESCC tissues. The samples (tumor and control tissues) were collected from Tehran and the HPV infections were determined by in situ hybridization analysis.

Our data showed that the presence of type of 16 or 18 HPV in esophageal cancerous tissues. Based on above results, it can be concluded that prolonged HPV infection accompanied by presence of PAHs may produce chronic inflammation and link to ESCC in Iran.

P06.096

Mutation screening in Adenomatous Polyposis Coli (APC) gene in patients clinically diagnosed as Familial Adenomatous Polyposis (FAP)

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Colorectal cancer is an important malignant neoplasm. Its development involves storage of mutations in oncogenes and in tumoral supressor genes, as well as epigenetic changes. One of these abnormal genes is the Adenomatous Polyposis Coli (APC), a tumor suppressor that presents mutations which may be associated to colorectal adenomas that develop into adenocarcinomas. Defects in this gene cause Familial Adenomatous Polyposis (FAP), an autosomal dominant pre-malignant disease that usually progress to malignancy. Its main characteristic is the large development of pre-cancerous colonic polyps in the intestines, which invariably evolve for the installment of cancer. The goals of this project are the screening for mutations in the APC gene (GenBank M74088) using High Resolution Melting technique and DNA sequencing, and the search for deletions using MLPA. The research includes 16 patients with clinical suspicion of FAP which were attended at the University Hospital and have signed for the consented forms. Of the 15 exons, 14 were screened so far and a nonsense mutation was found (Arg302Stop), producing an inactive protein of reduced size. This mutation was transmitted from mother to both children. We also found a deletion detected by MLPA in another family; the proband showed deletions of the entire exon 1 and 12 of the APC gene, besides a deletion of the exon 1 of MUTYH gene. All exons of APC and MUTYH will be screened and genetic counseling will be offered to the families carrying mutations. Financial support: FAPESP (2011/11456-0), INCTC.

P06.097

Does FIP1L1-PDGFRA fusion play role in pathogenesis of nasal polyposis?

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Top of Form

Background: Nasal polyposis (NP) is a common chronic inflammatory disease of the nasal mucosa. Although NP occurs 4% of population, the exact mechanisms leading its development are still unknown. On histopatological examination, tissue eosinophilia is a hallmark of NP. Recent studies examining eosinophyl biology have focused on delineating the molecular basis of FIP1L1-PDGFRA fusion gene Considering the eosinophils are the most important cells in NP and the role of FIP1L1-PDGFRA fusion gene in the ethiology of hyper eosinophilic syndrome, it was aimed to investigate FIP1L1-PDGFRA fusion gene at the NP.

Methods: We enrolled the NP patients to this translational study approved by the institutional ethical comittee of Ankara Atatürk Hospital. All individuals provided informed consent, The nasal polyp tissue and normal nasal mucosa biopsy materials were obtained from 20 patients who had undergone a NP operation in the Hospital ENT Clinic. Fluorescent in-situ hybridisation (FISH) was performed using LSI 4q12 Tri-Color Rearrangement probe (Vysis, 05N52-020) on touch-samples slides prepared from fresh biopsies FISH was also performed on nasal mucosa biopsy samples of 20 patients who were operated for nasal septal deviation as control group. Laboratory studies were carried out as blinded.

Results: The FIP1L1-PDGFRA fusion was not found .The nasal polyp tissue and normal nasal mucosa biopsy materials were obtained from 20 patients. The control specimens were all normal.

Conclusions: Further prospective, longitudinal studies are required to establish whether FIP1L1-PDGFRA fusion and the other eosinophilia related biomarkers play role in the pathogenesis of NP.

P06.098

Longitudinal study of t(14;18) positive Follicular Lymphomas identifies different patterns of genetic and epigentic evolution

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Follicular Lymphomas (FLs) are germinal center-derived B-cell lymphomas which in the vast majority of cases carry a t(14;18)(q32;q21). This translocation juxtaposes the enhancers of the immunoglobulin heavy chain (IGH) locus at 14q32 to the BCL2 gene at 18q21. Although crucial in the initiation of the malignant process, the t(14;18) translocation alone is not sufficient to drive lymphomagenesis. As a prerequisite to model the pathogenesis of FL from initiation to disease progression we here investigated the clonal evolution in t(14;18) positive FLs. To this end, we performed morphologic, immunohistochemic, interphase cytogenetic, mutational and epigenetic studies in pairs of initial and relapsed FL tumor biopsies within the framework of the BMBF-funded HaematoSys Systems Biology Network. We studied genomic copy number changes using SNP 6.0-Chips, chromosomal translocations with fluorescent in situ hybridization (FISH), mutations of immunoglobulin (IG)- and non-IG genes via cloning and sequencing and additionally next generation sequencing as well as DNA-methylation by 27K BeadArrays. Integrated analysis of the first 16 tumor pairs obtained after an interval of between 12 and 101 months provide evidence for different modes of lymphoma progression. In particular, phylogenetic trees derived from mutational and cytogenetic studies showed different ways of branching which correlated with patterns of differential DNA methylation. Our findings suggest that genetic and epigenetic changes might interact in the pathogenesis and evolution of t(14;18)-positive FLs.

P06.099

Abnormal methylation of tumor-related genes in gastric carcinomas and their adjacent nontumor areas.

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Gastric cancer (GC) is the second leading cause of cancer death and the fourth most common malignant tumor in the world. GC develops through the accumulation of genetic and epigenetic alterations. Epigenetic silencing of tumor-related genes, due to hypermethylation of the CpG sites in the promoter regions, has emerged as one of the main genetic alterations in cancer development.

We examined 106 frozen gastric carcinoma tissues and their adjacent nontumor areas for CpG-island hypermethylation in 5 tumor-related genes (*CDH1, RASSF1A, MLH1, N33, DAPK*) by methyl-sensitive PCR. Samples for detecting hypermethylation in the nontumor tissues were located no farther than 4 cm from tumor.

Hypermethylation of *E-cadherin*, *N33* and *DAPK* (61%, 66% and 48%) was detected more frequently than that of *RASSF1A* and *hMLH1* (25% and 20%) in carcinoma tissues. Genes *CDH1, N33, DAPK* were methylated both in tumor tissues and in the adjacent nontumor areas. Hypermethylation of *RASSF1A, MLH1* were detected in gastric carcinomas only.

The current study shows that hypermethylation of multiple tumor-related genes is detected frequently in gastric carcinoma as well as in adjacent normal tissues. Our findings suggest that a mechanism leading to CpG-island methylation is likely to be involved in the early gastric carcinogenesis process, by establishing field cancerization in gastric mucosa.

P06.100

CpG-island hypermethylation in 5 tumor-related genes (CDH1, RASSF1A, MLH1, N33, DAPK) as diagnostic and prognostic biomarkers for gastric cancer.

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Promoter hypermethylation of tumor suppression genes is a central mechanism for epigenetic inactivation and silencing of these genes in cancer cells. Analyses of the promoter hypermethylation patterns in different genes have identified specific alterations that may serve as useful diagnostic and prognostic biomarkers.We investigated the clinical and prognostic importance of CpG-island hypermethylation in 5 tumor-related genes (CDH1, RASSF1A, MLH1, N33, DAPK) for gastric cancer patients. We examined abnormal methylation and methylation index (MI) in 106 gastric cancer patients. Methylation index (MI) was defined as the ratio between the numbers of methylated genes to total number of examined genes in each sample. Statistical significance was evaluated using the Mann Whitney U-test.

There was a significant difference in the frequency of hypermethylation of these genes among different clinical groups of patients. Abnormal methylation of *CDH1* and *N33* genes is related to the progress of gastric cancer and metastasis to lymph nodes ($p < 0.05$). Abnormal methylation of *CDH1* is specifically associated with diffuse-type GC ($p< 0,05$). Abnormal methylation of *DAPK* gene, in contrast, is a marker of favorable prognosis, being more frequently methylated in non-metastatic gastric carcinomas ($p< 0,05$). There was no significant difference between groups of patients with IM> 0,5 and IM< 0,5.

The use of molecular genetic markers, such as promoter hypermethylation of tumor suppression genes, may be an additional factor of the diagnosis and prognosis for gastric cancer.

P06.101

Genomic rearrangements in Slovak BRCA1/2 families: rare deletion of complete BRCA1 gene represents a potential founder mutation

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Germline mutations in BRCA1 and BRCA2 genes account for a major proportion of hereditary breast and ovarian cancer cases. Most of these mutations consist of point mutations: deletions, insertions, nonsense or missense mutations, and splice variants, however an increasing number of large genomic rearrangements have been identified. In Slovak HBOC families large genomic rearrangements are responsible for approximately 10% of BRCA1/2 positive families.

Genomic BRCA1, BRCA2 and CHEK2 rearrangements were analysed by MLPA (Multiplex Ligation-Dependent Probe Amplification) and in some cases approved by Plexamp (DNA Quantitative Multiplex Amplification System by Prestagen). The complete deletion of BRCA1 gene was closer characterized by special BRCA1 region MLPA kit and oligonucleotide array based comparative genomic hybridization (aCGH).

Large genomic rearrangements were identified altogether in 5 families, while no LGR was indentified in BRCA2 gene, one deletion of exons 9 and 10 was identified in CHEK2 gene and two different deletions were detected in BRCA1 gene. Deletion of exons 21 and 22 of BRCA1 was previously described in Czech HBOC population and was detected in one family. Complete deletion of BRCA1 gene was previously reported in two Spanish and one German HBOC family and was identified in 3 Slovak families. This deletion was further characterized; however exact breakpoints have not been detected yet.

We report the spectrum and frequency of detected genomic rearrangements of BRCA1, BRCA2 and CHEK2 gene and also the potential founder origin of rare complete BRCA1 deletion in Slovak HBOC families.

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P06.102

Mutation analysis of cKit and PDGFRα genes in GIST patients from Slovakia

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Gastrointestinal stromal tumors (GIST) represent most common mesenchymal neoplasms. In 1998 activating mutations in gene coding cKit tyrosine kinase (KIT) were identified to be present in most of GIST tumors. Later on mutations in other tyrosine kinase - PDGFRα (PDGFRA), were identified as causative in GISTs. Mutation analysis of KIT and PDGFRA could be used in GIST therapy decision and in clinical prognosis of GISTs, therefore KIT and PDGFRA mutation analysis is currently a predictivelly important step in diagnostic protocol with its both prognostic and predictive consequences.

In our study KIT and PDGFRA mutation status was analyzed during differential diagnosis protocol applied to GIST bioptic material in Slovakia. We have performed mutation analysis of most commonly mutated exons in KIT (9, 11, 13, 17) and PDGFRA (12, 14, 18) genes in patients and combined the data with most important clinical parameters.

Totally 278 GIST suspect patients have been screened for mutations in analyzed exons of KIT and PDGFRA. According to information from other publications as well as in silico analysis with PolyPhen-2 predictor 233 patients have been identified with GIST causal mutation. Of those most prevalent mutations were deletions present in KIT exon 11 with proportion of 41.20%. The most frequent single mutation was KIT exon 9 p. 503-504_dup2 with proportion 9.44%. Genotype-phenotype correlation analysis reveal the statistically significant association between intestinal localization of tumors and presence of KIT exon 9 p. 503-504_dup2 mutation ($p<0.001$) and gastric localization of tumors and presence of PDGFRA exon 18 p. D842V mutation ($p=0,021$).

P06.103

Expression analysis of KIT gene splice variants Kit+ and KitA+ in gastrointestinal stromal tumors.

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Gastrointestinal stromal tumors are the most common mesenchymal tumors of the gastrointestinal tract. 5% of GISTs are caused by oncogenic mutations in PDGFRA gene, 60-89% by mutations in KIT gene. Exon 10 KIT pre-mRNA contains splice site. Alternative splicing results in the presence of two mRNAs KIT isoforms: KitA+ and Kit+, containing an in-frame GNNK deletion.

We investigated mutational status of KIT and PDGFRA genes and Kit+/KitA+ mRNA isoforms expression from 25 GIST patients. Analysis of mutations in KIT exons 9, 11 and PDGFRA exon 18 was conducted by PCR following by sequencing. cDNA from normal testicle tissue was used as control. Isoform expression analysis was perfomed by RT-PCR.

We observed mutations in 21/25 (84%) tumors (KIT exon 9 - 8%, KIT exon 11 - 52%, PDGFRA exon 18 - 20 %). Kit+ expression is dominate over KitA+ expression in 21 samples, 2 samples demonstrate equal expression of both isoforms, only Kit+ expression was detected in 2 samples. It’s interesting that equal expression of both isoforms or only Kit+ expression are detected in specimens with KIT exon 9 and PDGFRA exon 18 mutations only. It is known that these mutations are connected with unfavourable prognosis and low response to Gleevec. No correlation was observed between expression level of isoforms and intensity of IHC staining of c-kit.

Our data suggests the possible correlation between the isoforms expression and the mutational status of GISTs. Further investigation may be helpful for the isoform role in GIST development and tumor response to target therapy.

P06.104

Cisplatin induces apoptosis in U87MG and A172 glioblastoma cell lines via up regulation of bax gene and down regulation of bcl-2 gene

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Cancer cell apoptosis can be induced by Cis-diamminedichloroplatinum II (cisplatin), an efficient anticancer agent. bcl-2 and bax are members of the bcl-2 family that play key roles in the regulation of apoptosis. The ratio between bax and bcl2 often determines whether a cell will live or die. In this study, U87MG and A172 cells were treated with various concentrations of cisplatin for different times (24, 48 and 72 h). Then, cell viability was assessed using MTT assay and IC50 was determined. The two glioma cell lines were treated with IC50 dose of cisplatin at 48 h for different times(24, 48 and 72 h), RNA was extracted and cDNA was synthesized. Quantity of bcl-2 and bax genes expression compare to tbp gene were analyzed using very sensitive quantitative Real-time PCR. bcl-2 gene expression was decreased and bax gene was increased in a time-dependent manner by cisplatin, that was statistically significant ($P<0.05$). The results showed that cisplatin exerted a time-dependent inhibitory effect on the viability, via up regulation of bax and down regulation of bcl-2 gene, in U87MG and A172 cells.

P06.105

Molecular alterations of EGFR and PTEN genes in primary glioblastoma: associations with prognostic

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EGFR is frequently amplified, overexpressed or mutated in glioblastoma, the most common and devastating malignant brain tumor. Commonly lost in these tumors is also PTEN, an inhibitor of the PI3K signaling pathway activated by EGFR.

In this study, we aimed to characterize the molecular alterations of EGFR and PTEN in 60 patients diagnosed with primary glioblastoma. We analyzed mutational status of PTEN and EGFR tyrosine kinase domain; we evaluated EGFR amplification and PTEN deletion by FISH and we determined EGFRvIII expression by RT-PCR.

20.8% patients showed pathogenic mutations in PTEN and 68.2% tumors presented PTEN locus deletion. 20% patients with PTEN deletion showed additionally PTEN mutation. Regards to EFGR, no mutations were detected in the tyrosine kinase domain, whereas 32.7% glioblastomas showed EGFR amplification and 31.7% expressed EGFRvIII truncated variant. 46.1% patients with EGFRvIII expression showed EGFR amplification, resulting in the overexpression of constitutively active EGFR lacking I and II extracellular domains. Furthermore, we have found 5 novel EGFR non canonical splice variants in the extracellular domain. Four were truncated proteins and only one generated an in-frame protein lacking aminoacids 20-251 in a tumor with EGFR amplification. In summary, 73.3% of glioblastomas analyzed presented any alteration in PTEN and/or EGFR.

Finally, we investigated the prognostic impact of these alterations in patient outcome. EGFRvIII expression was a significant prognostic factor ($HR = 2.56$, 95% C.I. 1.17-5.57, $p = 0.018$). Patients with EGFRvIII variant expression had a higher median survival than those without it (16.7 months vs. 10.1 months)(Supported by FIS PI10/00219).

P06.106

MGMT methylation and serum albumin in survival for glioblastoma patients

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Glioblastoma is the most malignant astrocytic brain tumor (astrocytoma WHO grade IV) with very poor prognosis of a median survival of 14-15 months after diagnosis, despite applied treatment (surgery, chemotherapy, radiotherapy). Till now, only MGMT (O(6)-methylguanine-DNA methyltransferase) promoter methylation is confirmed molecular prognostic factor and helpful marker for chemotherapy of glioblastoma. Serum albumin level were investigated only in some glioblastoma patients studies.

Where, hypoalbuminaemia was associated with significantly reduced median survival. The aim of this study was to investigate MGMT promoter methylation status in histologically confirmed glioblastoma samples and pre-operative serum albumin level of 70 glioblastoma patients.

MGMT promoter methylation status were determined by MS PCR. Survival was determinated by using the Kaplan-Meier method and a multivariate Cox regression model (SPSS).

This study shows that MGMT promoter methylation and serum albumin are significant predictors of survival in glioblastoma patients.

P06.107

New insights into glioblastoma development by integrative genetic analysis of multifocal glioblastoma multiforme

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Glioblastoma multiforme (GBM) is the most common and malignant type of brain tumor in adults with an average survival time of less than 12 months and targeted therapies are not yet available. Therefore, we investigated different foci in multifocal GBM by an integrative approach in order to identify markers of tumor initiation and progression as well as potential therapeutic targets. We combined high resolution array CGH and expression arrays on fresh frozen tissue from 11 tumor foci from 6 patients with spectral karyotyping (SKY) on corresponding primary cell cultures. Moreover, *PTEN* Sanger sequencing was performed. Array CGH-analysis detected multiple aberrations in the tumors investigated. Additionally, SKY-analysis revealed multiple translocations including complex rearrangements. We found that different tumor foci derived from the same patients shared the majority of aberrations detected, indicating that these aberrations had probably emerged early in tumorigenesis. Interestingly however, some of the aberrations differed between the tumor foci from the same patient and thus must have occurred later in tumor development. These observations support the hypothesis that different tumor foci in multifocal GBM are of monoclonal origin and then develop independently of each other by clonal evolution. In accordance with this hypothesis, some of the tumor foci from the same patient shared the same *PTEN* mutation whereas others displayed different *PTEN* mutations. We are currently analyzing the expression data which will also be presented. Taken together, multifocal GBM provide an excellent model for investigating tumor progression and might help distinguishing between driver and passenger alterations in GBM.

P06.108

High Frequency of Mutations in the PIK3CA Gene Helical and Kinase Coding Regions in a Group of Iranian Patients with High Grade Glioblastomas: Five Novel Mutations

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Glioblastoma multiform (GBM; WHO grade IV) and Anaplastic Astrocytomas (AA; WHO grade III) are highly aggressive and lethal astrocytic brain tumors. To detect cancer specific somatic mutations in two hot-spot regions of PIK3CA gene, the helical and kinase domains (encoded by exons 9 and 20, respectively) in GBM and AA, we examined the respective sequences 31 paraffin-embedded samples (23 GBM and 8 AA). The samples were obtained from a group of Iranian patients affected with high grade glioblastoma (HGG). The overall prevalence of PIK3CA mutations was 23% (7/31) for both tumor types (22% in GBM, and 25% in AA). Five mutations were detected in exon 20, p.Arg992Gln (c.2976G>A), p.Met1005Val (c.3014A>G), p.Ile1019>Val (c.3056A>G), p.Ser1008Cys (c.3024C>G), p. Asn1044Asp (c.3130A>G), and one mutation in exon 9, p.Glu545Ala (c.1634A>C). Additionally exons 4-8 of *P53* gene in four unrelated young patients, who showed no mutations in *PIK3CA* exons 9 and 20, were analyzed. Three mutations were identified: p.Pro72Ala (c.214C>G), g.11608G>T (homozygote splice mutation), and p.Thr170Thr (c.510G>A silent mutation). In conclusion mutation detection in *PIK3CA* in patients with a high degree of malignancy and early age at diagnosis should be included in molecular diagnostic protocols, also with regard to possible upcoming therapies.

P06.109

Genetic Analysis of Long-Term Survivors with High-Grade Gliomas

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Background: High-grade gliomas have poor prognosis. Only 3-5% survive more than five years being referred as long-term survivors. Factors determining this outcome are largely unknown.
Aim: Identify specific genetic parameters that might be associated to long-term survival.
Methods: Evaluation of genomic imbalances by chromosomal CGH and of MGMT and MMR genes methylation levels (%) by MLPA analysis in 3 groups of patients. Group 1: 13 long-term survivors; Group 2: 17 patients with an overall survival (OS)< 5 years and $\text{MGMT}_{av} \geq 25\%$; Group 3: 29 patients with OS<5years and $\text{MGMT}_{av}<25\%$. Statistical analysis was performed using Mann-Whitney and Fisher's Exact tests with p-value adjustment for multiple tests (Bonferroni).
Results: Multiple chromosomal imbalances were observed in all groups being gains at 7p/q regions the most frequent alteration. None of the long-term survivors had 7p12 (EGFR) amplifications and loss of 10q23 (PTEN) region were significantly less frequent: 23,1% in group 1 *v.s.* 88,7% and 86,2% in groups 2 and 3. MS-MLPA analysis revealed that MGMT and 4 of the MMR genes were significantly more methylated in the long-term survivor group than in the others. Accordingly for groups 1, 2 and 3 mean methylation levels were respectively for MGMT (70,6%; 54,1%; 8,6%), MSH2 (6,8%; 0,8%; 0,8%), MSH6 (25,3%; 22,4%; 4,7%); PMS2 (24,7%; 5,5%; 2,2%) and MLH3 (56,5%; 11,2%; 3,1%)
Conclusions: Our data underlies that long-term survival is associated to an absence of 7p12 (EGFR) amplification, infrequent 10q23 (PTEN) loss and higher levels of MGMT; MSH2, MSH6, PMS2 and MLH3 methylation.

P06.110

Association of different ovarian tumor types in a patient with Gorlin Syndrome

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A 29 years old woman came to our attention with the suspicion of Gorlin Syndrome (GS). At age 15 she had undergone unilateral oophorectomy for a large mass of the right ovary and excision of several masses of the left ovary, diagnosed as fibromas; at age 18 she had multiple fibromas of the left ovary removed and two years later she underwent surgery for an uterine septum. Recently, she has undergone left oophorectomy for a large cystic mass: pathological examination revealed a granulosa cell tumor with theca-cell areas.
Maternal family history was unremarkable, whereas paternal family history was largely unknown, except for hysterectomy in her grandmother. The proband has two healthy younger brothers (27 and 18 years old); her mother had three spontaneous miscarriages. Physical examination showed coarse face with hypertelorism and three skin lesions, of recent onset, which were removed and diagnosed as basal cell carcinomas. To confirm the suspicion of GS, the search for germline mutations in PTCH1 gene was carried out, which detected the heterozygous mutation c.1067_1067+3>TT, already reported in GS patients. Although ovarian cysts and ovarian fibromas, often bilateral, are reported in 25-50% of GS female patients, to our knowledge, this is the first case of a granulosa cell tumor associated to multiple ovarian fibromas in a GS patient, whose clinical manifestations were mainly and heavily affecting ovaries.
Other investigations (X-rays of the skull and ortopantogram) are ongoing, as well as molecular analysis in parents and sibs.

P06.111

A conditional mouse model for granulosa cell tumour driven by WT1

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The Wilms tumour protein 1 (WT1) is essential for male sex determination. Not much is known about it's expression and function in the embryonic and adult female genital tract and if WT1 can trigger ovarian tumorgenesis.
To this end, we generated two different conditional mouse models, both based on an inducible Wt1 tTA knockin effector line carrying a tetracycline-dependant activator (tTA) in place of the first exon of the endogenous murine Wt1 locus. In the first model, tripel transgenic animals bearing a tetracycline-responsive transgene for Cre-recombinase, and the Cre reporter line ROSA26 lacZ, the fate of WT1-positive cells was followed in vivo by tetracycline administration using beta-galactosidase to identify cells and tissues derived from WT1-positive precursors. In tripel transgenic animals of the second model, the Wt1 tTA knockin effector line and the tetracycline-responsive Cre-recombinase should excise the third exon of Ctnnb1 (βCat-Flox-Ex3), which may in that case trigger ovarian tumorgenesis.
Histochemical staining of embryos demonstrated earliest Wt1 expression in the urogenital ridge at day 10.5 post coitum (d.p.c.). When the fate of Wt1-positive cells was analysed in the ovaries of adult mice, beta-galactosidase activity could be detected in oocytes, stromal and granulosa cells. Tripel transgenic mice of the second model developed huge ovarian carcinoma. Molecular and histological analysis suggested that this is due to Ctnnb1 overexpression and that the carcinoma were granulosa cell tumours.
Our results show that WT1 has an essential function in the female genital tract and present a new mouse model for granulosa cell tumours.

P06.112

Evaluation of methylation status in glutation S-transferase P1(GSTP1) gene promoter in human breast cancer and it's relation to tumor grade and stage

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Glutation S-transferase P1 (GSTP1) gene methylation in promoter CpG islands has been described as a specific biomarker for many types of cancer including breast cancer as a tumor suppressor gene . At presents study we found the GSTP1 gene promoter to be methylated in breast cancer tissues. To study the difference of sequence in hypermethylated GSTP1 promoter in cancer tissues and non methylated status in normal tissues, we analyzed the cytosine methylation status as epigenetic changes in 50 tumors from patient's with breast cancer and 50 normal breast tissues that obtained from the same breast tumor in adjacent region. In order to study the promoter methylation status for GSTP1 gene in breast cancer, 40 CpG sites [nucleotide(nt) 197,190,187,185,183,182,176,162,155,152,148,145,141,132,127,124,112,109,101,99,81,77,74,71,54,53,48,47,43,42,40,38,23,22,15,14,13,11,8,4] were screened. The GSTP1 methylation was detected in %.41.3 of breast tumors which was associated with higher tumor grade (p=0.467) and tumor stage(p=0.048) .

P06.113

Histone deacetylases activate the HGF/c-Met pathway via repression of microRNA-449 in human hepatocellular carcinoma

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BACKGROUND & AIMS: Histone deacetylation represents an important epigenetic modification in cancer development and is responsible for chromatin remodeling. In this study, we aimed to identify microRNAs affected by histone deacetylation and to understand functional consequences. METHODS: HCC cell lines and immortalized liver cell lines were treated with the histone deacetylase inhibitor TSA. Differentially expressed mRNAs and miRNAs were identified using mRNA and miRNA expression profiling. Findings were validated by siRNA mediated silencing of HDAC1-3, transfection of miR-449 into a HCC cell line, Western blotting and by luciferase reporter assays. RESULTS: Here we show that HDAC1-3 are consistently up-regulated in primary HCC. MiRNA profiling identified hsa-miR-449a to be significantly up-regulated after HDAC inhibition. c-MET encoding the receptor tyrosine kinase for hepatocyte growth factor (HGF) is a putative target gene of miR-449. Indeed, HDAC inhibition and miR-449 transfection resulted in reduced expression of c-MET. Increased apoptosis and decreased proliferation after histone deacetylation and miR-449 upregulation confirmed the miR-449 tumor suppressive role. MiR-449 was found to bind directly to c-MET. Importantly, primary human HCC showed reduced expression of miR-449 and increased expression of c-MET. CONCLUSIONS: This work opens up new avenues for targeted therapies of HCC, either via treatment with histone deacetylase inhibitors or, possibly by direct transfer of specific miRNA-449.

P06.114

Detection of EGFR expression levels in tissues of head and neck cancer patients and cell lines

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Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide and includes epithelial malignancies of the oral cavity, pharynx and larynx. Epidermal growth factor receptor (EGFR) is involved in tumor development and is highly expressed in head and neck squamous cell carcinomas. EGFR is a 170-kDa transmembrane glycoprotein with an extracellular ligand-binding and a cytoplasmic domain with intrinsic tyrosine kinase activity. EGFR overexpression has been observed in both premalignant lesions and malignant head and neck tumors. Eventhough there are several studies which are analysing the expression of EGFR in malignant tumors, we have very limited information about their expression and activation in adjacent normal tissue from head and neck cancer patients. Therefore, we have investigated the differential expression of EGFR a) in tumor tissue b) in adjacent normal tissue c) blood tissue in head and neck cancer patients and d) SCCL-MT1 and USC-HN2 cell lines by using RT-PCR. At tumor and adjacent normal tissues, the amount of EGFR mRNA wasn't different than eachother. Also, there weren't any EGFR mRNA levels in blood tissues. This findings are shown that expressions of the other variations of EGFR also can be effective on head and neck cancers.

P06.115

Polymorphism of GSTT1, GSM1 and GSTP1 genes investigated in patients with head and neck squamous cell carcinoma

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Variations of activation and detoxification of chemical compounds in the xenobiotics metabolism are involved in head and neck tumorigenesis, even as polymorphisms in genes of the glutathione S-transferase superfamily, which act in phase II of this metabolic pathway. Aim: To investigate the A313G and C341T GSTP1 polymorphisms, GSTT1 and GSTM1 null genotype in patients with head and neck cancer and compare with subjects with no history of cancer to evaluate the association between these polymorphisms and the risk factors (smoking and drinking) and the histopathologic characters of the tumor. Methods: Were included 775 individuals, 261 patients and 514 controls. Molecular analysis was performed by PCR-RFLP. For statistical analysis we used the chi-square and multiple logistic regression. Results: The significant results with $p< 0.05$ showed that age ≥48 years, smoking and drinking and the presence of A313G GSTP1 polymorphisms were predictors for the development of cancer of the head and neck. In individuals with the GSTM1 null genotype and age <48 years; the A313G GSTP1 polymorphism and age ≥48 years, smoking and drinking; the GSTT1 null genotype and primary anatomical sites pharynx and larynx present increased chances for the development of head and neck cancer. Conclusion: In conclusion, the presence of GSTP1 A313G variant is associated with decreased risk for head and neck cancer and age ≥ 48 years, male gender and smoking and drinking; GSTM1 null genotype and age <48 years; GSTT1 null genotype and primary anatomical sites pharynx and larynx are associated with increased chances for developing this disease.

P06.116

Deep Sequencing of Hepatitis B Virus in Hepatocellular Carcinoma Patients

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Chronic infection by Hepatitis B Virus (HBV) is one of the most aetiologically associated risk factor for the development of hepatocellular carcinoma (HCC). However, the role of HBV in HCC remains unclear due to technological limitation. Here, we employ novel enrichment and pooling strategy coupled with the next generation state-of-the-art FLX deep sequencing to comprehensively characterize HBV in 48 HCC patients. Our data suggest preferential integration of HBV into genic regions and many are predicted to alter gene regulation. Notably, integration of HBV into tumor tissues is less random than integration into the adjacent non-tumorous tissues. In tumor tissues, there is preferential integration of HBV into selected chromosomes. Within the HBV genome, the preferred region involved in integration is at the 3'end of the HBX gene and the 5' of the precore/core protein. The 3'end of the HBx is often deleted upon integration. The most common type of chimeric transcript observed is the HBx-human transcripts which can be expressed. This study represents a comprehensive characterization of HBV in HCC patients at the genetic level which may facilitate our understanding of the potential role of HBV in HCC development.

P06.117

Functional analysis of 30 putative *BRCA1* splicing mutations in German families with hereditary breast and ovarian cancer

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Since 1997, more than 4500 families fulfilling the criteria for hereditary breast and/or ovarian cancer were screened for *BRCA1* and *BRCA2* mutations at the German consortium of hereditary breast and ovarian cancer (GC-HBOC) centres Cologne, Munich and Kiel. More than 1200 different *BRCA1* mutations have been described as disease-causing. Variants disrupting invariant splice sites are generally considered as clinically significant. However, numerous intronic and exonic variants outside invariant splice sites with uncertain affect on *BRCA1* pre-mRNA processing have been detected. We characterized 30 distinct *BRCA1* variants, which have not been sufficiently described before on transcript level, by quantitative PCR and sequencing using mRNA extracted from blood lymphocytes. 13 variants disrupt invariant splice sites, resulting in exon skipping and/or activation of cryptic splice sites. Experimental analysis of more distant intronic or exonic variants is mandatory and can be supported by *in silico* analyses. Ten intronic mutations were tested using the *Human Splicing Finder* prediction algorithm and analyzed for aberrant splicing. Four mutations caused splicing defects while the remaining ones were neutral, which was in-line with *in silico* predictions. Interestingly, we identified 5 out of 7 exonic variants analyzed affecting *BRCA1* pre-mRNA processing (silent: c.710C>T; missense: c.787A>G, c.4794G>A, c.5193G>C, c.5527G>C). Those variants were located close (≤ 3bp) to the respective intron/exon borders, highlighting the importance of splicing analysis even for silent mutations. In conclusion, our results contribute to the recent knowledge of deleterious *BRCA1* splicing mutations. The clinical consequence of mutations with only minor effects on alternative splicing remains to be explored.

P06.118

Mutation screening of *RAD51D* in non-*BRCA* breast/ovarian cancer families from Spain.

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Introduction: Mutations in the *BRCA1* and *BRCA2* genes are involved in approximately 25% of hereditary breast/ovarian cancer families and predisposition to the syndrome may be attributed to mutations in genes of moderate risk. Recently, germ line mutations in *RAD51D* were identified in families with breast and ovarian cancer cases.

Objectives: We aimed to determine the prevalence of germ line *RAD51* mutations in Spanish breast/ovarian cancer families previously found to be negative for *BRCA1BRCA/2* mutations.

Methods: We performed mutational analysis in 289 index patients: 144 from breast cancer families and 123 from families with breast and ovarían cancer cases. Mutation detection was performed with High resolution melting curve analysis (HRM) or Sanger sequencing, and MLPA for large rear-

rangements.
Results: We found one nonsense mutation (c.674C>T; p.Arg232X) in a patient with ovarian cancer diagnosed at 44 years of age and no family history. We also detected some common polymorphisms and missense variants. The clinical significance of all detected variants is currently under evaluation.
Conclusions: Our preliminary data suggest a very low frequency of deleterious mutations in *RAD51D* in our study population. The results support the association of mutations in this gene with the presence of ovarian cancer.

P06.119

HIWI as a prognostic marker for early stages of colorectal cancer

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Dysregulation of self-renewal pathways is likely a requirement for cancer development. HIWI proteins and their interaction with piRNAs have indicated their key function in stem cell development. Although Hiwi overexpression has been observed in several cancers, the relationship between Hiwi and colorectal cancer (CRC) is unclear. In this study, we assessed the Hiwi mRNA expression in 38 CRC patients (20Male/18Female, Mean age 56) by comparative real-time PCR. Overexpression of Hiwi mRNA transcripts observed in 35% (13/38) of patients. There was not any significant correlation between mRNA expression and patients' survival, tumor size and location. However, it was observed that the stage of tumor has a significant correlation with hiwi expression, in which the hiwi overexpression prohibits the tumor extension toward the higher stages (P=0.034). Furthermore, significant correlations were observed between overexpression of either Hiwi and self-renewal marker SALL4 (p=0.002) or HIWI and cancer testis antigen DPPA2 (p=0.019). Few target molecules have been identified that enable the prognosis of colorectal cancer with a high sensitivity and specificity, especially in the early clinical stages of cancer. This report emphasizes the importance of HIWI as a proper candidate for use in the proggnosis of colorectal cancer in the early stages, and represents a complex network between Hiwi and other self renewal markers such as Dppa2 and Sall4 in colorectal cancer.

P06.120

Alterations of the human β defensin-1 gene in Acute Myeloid Leukemia: association with FLT3 gene

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INTRODUCTION: Defensins are a family of antimicrobial peptides produced by WBCs and epithelial cells that functions in the host innate defense. Both HBD1 and HBD2 have been shown to induce the migration of immature dendrite cells (DC) and memory T cells. *Fms*-like tyrosine kinase-3 (FLT3) provides an important stimulus for resident DC production in vivo and indicating its important in migratory DC generation. We then analyzed the frequency distribution of promoter polymorphisms and determined the effect of these base changes on transcriptional activity of the HBD1 promoter and the prevalence of FLT3 mutation in patients with various AML.
METHODS: Genomic DNA was isolated from a BM slide or BM aspiration with various 47 AML patients and 43 controls using QIAamp DNA kit (Qiagen, Hilden, Germany). The PCR products of hBD-1 gene were then digested by the restriction enzymes *Nla*IV, *Hga*I, and *ScrF*I, respectively.
RESULTS: AML patients had significantly different mutation frequency of at position -44 and -20 in the 5'-UTR (, p=0.000, p=0.05, respectively). Of the 47 patients with AML, alterations in FLT3 gene were detected in 13 (27.6%) patients. These mutations included FLT3/ITD in 9 (19.1%) and FLT3/TKD mutation in 4 (8.5%) patients. Statistically significant differences for -44 allele frequencies were found between patients with AML with the mutation of FLT3/ITD and the wild types of FLT3/ITD.
CONCLUSIONS: These data suggest that HBD-1 is a potential tumor suppressor gene for AML. Promoter point mutations may be responsible for AML-specific loss of HBD-1 expression.

P06.121

Human Papilloma Virus infection and KRAS mutations in lung squamous cell carcinoma patients from Iran

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Lung cancer is the leading cause of cancer death for both women and men. Non-small cell lung cancer (NSCLC) represents more than 80% of lung cancers and is sub grouped in squamous cell carcinoma (SCC), adenocarcinomas (ADC) and large cell carcinoma(LCC). SCC accounts for about 30% of all cases of patients with NSCLC. Mutations in ras oncogens appear to play a significant role in the development of NSCLC. The role of HPV infection in the development of carcinomas was also demonstrated. The aim of this study was to determine the association between HPV infection and K-ras mutations in patients with SCC. DNA was isolated from Fifty patients with histologically confirmed SCC. HPV typing was done by Nested-PCR and direct sequencing. High risk HPV was detected in 9 (18 %) patients. HPV-18 was the most frequent type in the cases. To investigate gene- virus interactions, DNA from the 9 HPV positive and 9 HPV negative patients, as controls, were subjected to mutation analysis in exons 2 and 3 of the K-ras gene using direct sequencing. Among the two K-ras exons tested, no mutation was found in two groups. Our overall findings demonstrate that the HPV infection has a significant impact on NSCLC. However, due to the small number of patients, our finding could not demonstrate a positive association between HPV-infection and K-ras mutation in patients with lung SCC. Our results are preliminary and larger cohorts are needed for better understanding the contribution of genetic alternations and HPV infection in lung SCC.

P06.122

Comparison of Methylation-Specific qPCR (MS-qPCR) and pyrosequencing for the analysis of *p16/INK4a* in lymph node samples

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Introduction: Promoter hypermethylation of tumor suppressor gene p16/INK4a has been found in a wide variety of neoplasia, suggesting in some cases its utility as diagnostic or prognostic tool. The aim of this study was to compare two methods (MS-qPCR and pyrosequencing) for the quantitative methylation analysis of this gene.
Methods: 62 patients with confirmed diagnosis of non-small cell lung cancer were included (56.9% with ganglionar metastasis). DNA from lymph nodes was extracted and sodium bisulfite modification, required for both techniques, was accomplished. The same portion of CpG island containing 11 CpG sites was independently analyzed using both techniques. For the MS-qPCR, pre-amplification with universal primers followed by TaqMan-based qPCR with methylation-specific primers and probe was used. The relative methylation percent was estimated based on a standard curve build with variable proportions of fully-methylated/unmethylated control and using MYOD1 to normalize the DNA input. On the other hand, templates for pyrosequencing were obtained amplifying bisulfite-treated DNA with biotinylated primers. Methylation percent was calculated by averaging across all CpG sites interrogated.
Results: Differences in the methylation percent were found between the two techniques, resulting in different cut-offs for MS-qPCR and pyrosequencing (2.25 and 9.95 methylation percent, respectively). According to MS-qPCR, p16/INK4a promoter was considered hypermethylated in 33.33% of the cases with metastasis, and in 24.24% for pyrosequencing.
Conclusion: Pyrosequencing and MS-qPCR show good linearity for known methylation percents. In this cohort positive predictive value was 100% for MS-qPCR and pyrosequencing.
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P06.123

Inflammatory Response Evaluation in EGFR Mutation Negative Lung Adenocarcinomas

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Activation of the epidermal growth factor receptor (EGFR) constitutes a major molecular pathway involved in the carcinogenesis of nonsmall cell lung carcinomas. In EGFR mutation negative cases, K-ras activation represents an alternative molecular pathway responsible for primary resistance to tyrosine kinase inhibitors. We investigated a number of 34 randomly selected cases of advanced lung adenocarcinomas from patients with a long history of active smoking. The DNA samples extracted from formalin fixed, paraffin embedded tumoral material were initially tested for EGFR mutations by PCR and sequencing of exons 18, 19 and 21. Since the studied cases showed no mutations on any of the EGFR exons, they were subsequently tested for K-ras mutations in codons 12 and 13 using the PCR-RFLP method. K-ras codon 12 mutation was identified in 12 lung adenocarcinomas (35.29%). Considering the worse prognosis in K-ras positive cases, we further assessed the tumoral associated inflammatory response compared to EGFR-negative, K-ras negative patients. The inflammatory infiltrate was significantly increased in K-ras negative tumors, opposed to K-ras positive cases where inflammatory cells showed reduced counts. Our findings indicate a medium negative correlation (r = -0.35) between K-ras mutational status and the inflammatory response in EGFR-negative advanced lung adenocarcinomas, which was statistically relevant (p = 0.001). These findings indicate a possible impact of K-ras mutation on tumoral biology in diminishing the level of inflammation elicited by the tumor, favouring cancer progression and lowering prognosis.

P06.124

Identification of candidate genes involved in maintaining Interstitial Cajal Cells and Interstitial Cajal-like Cells in mutant mice

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Interstitial Cajal Cells (ICC) are involved in gastro-digestive tract motility and neurotransmission, but also in gastro-intestinal tumors (GIST) pathogenesis. Other extra-digestive organs (gallbladder, heart, uterus, etc) have been shown to contain Interstitial Cajal-like Cells (ICLC), whose function is yet unknown. Both cell types express the Kit protein, a tyrosine-kinase receptor with essential role in maintaining these phenotypes.

Our study was focused on comparative investigation of gene expression in normal and mutant mice by DNA microarray, in order to contribute in understanding the physiology of ICC and ICLC.

Total RNA was extracted by RNeasy Mini Kit (Qiagen) from heart and gallbladder tissues sampled from control and Kit mutant mice (WBB6F1/J-KitW/KitW-v/J strain) and analyzed by Bioanalyzer (Agilent Technologies). DNA microarrays from Whole Mouse Genome Microarray Kit (Agilent Technologies) hybridized and scanned by Agilent DNA Microarray Scanner were analyzed by Feature Extraction5.1.1. and GeneSpring GX10 Software (Agilent Technologies).

In the myocardium, three genes demonstrated differential expression by >2 fold (p<0,05) in mutant versus control mice and may be involved in maintaining ICC and ICLC phenotypes: the Timp4 and Dnaja1 genes down-regulated and the Serpinb2 gene up-regulated.

Of the 22 genes different expressed in the gallbladder, only one was found down-regulated in mutant mice (the PYCARD gene, involved in apoptosis); some of the 21 over-expressed genes are involved in different metabolic processes: signal transduction, cell adhesion, proteolysis (NOS1AP, ITGB3, Ctsj genes), etc.

Some of these genes may become candidate biomarkers for studying ICC, ICLC and associated pathology in humans.

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P06.125

Detection of the JAK2V617F mutation in polycythemia vera and other myeloproliferative disorders in Iranian patients

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A somatic mutation in the JH2 autoinhibitory domain of the Janus kinase 2 (JAK2) tyrosine kinase have been implicated in polycythemia vera (PV), essential thrombocythemia (ET), myelofibrosis as well as other myeloproliferative disorders. This mutation, (V617F) a change of valine to phenylalanine at the 617 position, appears to render hematopoietic cells more sensitive to growth factors such as erythropoietin and thrombopoietin.

The aim of this study was identification of JAK2 mutations among patients with PV , ET and idiopathic myelofibrosis who were refered to our center since 2008. We have analyzed leukemic patients by ARMS-PCR method for screening of patients for a single point mutation.

The JAK2 (V617F) mutation was studied in 236 patients, of whom 33 (34%) out of 97 patients with PV were identified, 64 (49.2%) out of 130 patients with ET and 1 (11.1%) of 9 patients with idiopathic myelofibrosis were detected. In PV patients 14.5% were homozygous and 19.5% were heterozygous, these values were 9.2% and 40% among ET patients and all of the mutant alleles detected for myelofibrosis patients were in heterozygous form. In previous studies, the JAK2 mutation was identified in 60-70% patients with PV, 30-40% of ET patients and 11-14% of patients with idiopathic myelofibrosis and the range of heterozygosity and homozygosity in patients with PV were 48% and 25%, respectively. The difference between our results and the other studies could be due to the misdiagnosis of the patients by the physicians who referred the patients for molecular diagnosis in Iran.

P06.126

Ion AmpliSeq™ Cancer Panel - Accurate and Sensitive Detection of Over 700 Somatic Mutations in a Single Day

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Selective enrichment of the coding regions from genomic DNA has proven beneficial in the detection of genetic disorders without the cost, labor, and time associated with sequencing the entire genome. Widespread diagnostic utilization of whole exome sequencing has been has been limited by the extensive time required for typical hybridization-based exon capture strategies, which can take several days. We have developed a quick, easy-to-use, multiplex PCR based, enrichment method for next-generation sequencing. The process combines the speed and specificity of PCR reactions with the efficiency of unprecedented levels of plexy. The Ion AmpliSeq™ Cancer Panel consists of 190 amplicons and surveys 739 known cosmic mutations in approximately 20 kb of sequence from 46 genes including EGFR, KRAS and BRAF. This kit is ideally suited for both fresh and archival (FFPE) samples, requiring only 10ng of DNA per reaction. When the libraries are processed and sequenced with the Ion OneTouch™ and PGM™ Sequencer, mutations at only 5% frequency can be quantified from DNA to results in less than 12 hours.

The Ion AmpliSeq™ Cancer panel was tested using FFPE preserved, KRAS mutation containing cells lines mixed together at various concentrations. Several known mutations were verified and quantified down to 5% frequency. The results were further confirmed by CAST PCR.

Ion AmpliSeq™ Primer Panels are available in custom assay sets and scalable to over 1,000 amplicons per tube. With barcode compatibility, and multiple chip capacities, this technology enables rapid and efficient focused sequencing in a variety of settings.

P06.127

Combined point mutations in codon 12 and 13 of KRAS oncogene in prostate carcinomas with high gleason score

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The G to T transversions in codon 12 and C to T transitions in codon 13 of KRAS proto-oncogene are predominant point mutations that occur in about 20% of different cancers in human. It was aimed to investigate the prevalence and predictive significance of KRAS mutations in patients with prostate carcinomas. In a total of 30 fresh tumoural tissue specimens were investigated in patinet with prostate carcinomas of different scores(GS). All tumoural specimens were histo-pathologically diagnosed and genotyped for codon 12, 13 KRAS point mutations by reverse hybridisation and direct sequencing methods. KRAS mutations were found in 12 (40%) samples with 29 samples deriving from adenocarcinomas and 1 sample was small cell prostate carcinoma. In 1 (3.44%) sample codon 12 was found to be mutated and in 2 (6.8%) samples codon 13 and in 9 (31%) samples combined codon 12 and 13 were found to be mutated particularly in higher grade of tumoural tissues. Current preliminary results indicate that combined point mutations in codons 12 and 13 KRAS gene play crucial role in prostate carcinomas with high GS.

P06.128

Study of HER-1 497K polymorphism in EGFR gene in patients with laryngeal cancer

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Introduction and aim

Head and neck squamous-cell carcinoma is the sixth most frequent neoplasia worldwide and the most malignant tumour in the superior aero-digestive tract. The highest rates in the whole world of laryngeal cancer are encountered in Spain, especially in men, although the number of women is rapidly increasing. 95% of laryngeal cancer is developed in men between 45 and 70 years old, with a maximum of incidence in the sixth decade. It has been described that EGFR is over expressed in the epidermoid carcinoma of the larynx, and the HER-1 497K polymorphism has been associated with a higher risk of mortality in these tumours.

In this study we have analysed the possible association between this polymorphism and the developing of laryngeal cancer.

Methods

Genomic DNA was extracted from peripheral blood leukocytes by standard techniques. We selected 65 patients from Salamanca (Spain) with cancer of the larynx and 385 healthy subjects as controls. Clinical characteristics have also been studied. The HER-1 497K polymorphism in the EGFR gene was realised by DHPLC (Denaturing High Performance Liquid Chromatography).

Statistical analysis was performed comparing the different distribution of the polymorphism in the EGFR gene in subgroups of patients and controls.

Results and conclusion

No statistical differences have been shown in the distribution of the HER-1 497K polymorphism comparing cases and controls (p<0.05).

Our results do not support the hypothesis that the HER-1 497K polymorphism is associated with increased susceptibility to suffer laryngeal cancer.

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P06.129

In vitro sensitivity profile of Laryngeal cell carcinoma

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Introduction: The research of chemosensitivity is important to screen new therapeutic agents, identify patterns of chemosensitivity for different tumor types and to identify chemotherapy regimens to patients because antineoplastic agents increase acute toxicity and side effects and tumors may exhibit resistance to chemotherapeutic. Objectives: To evaluate the sensitivity of *Hep-2* cells line (Laryngeal carcinoma) to MTX chemotherapeutic *in vitro* in three different concentrations. Methods: Cells were plated in six-well culture plates at a density of 1 × 10^5/well and incubated with three different concentrations 0.25 μM, 25 μM, and 75 μM of the Metrotexate for 24 hours at 37 °C. Cell Apoptosis was evaluated by double staining with fluorescein isothiocyanate (FITC) label Bcl-2 (100: sc-509) by Flow Cytometry Technique. Statistical analysis was performed by Chi square test (X2) to compare the cell viability. Correlation coefficient of Pearson (R2) between the concentrations also was measured. Results: For the treatment with 0.25 uM, 25.0 uM and 75.0 uM MTX concentration, the viable cells were 85,43%, 22.46% and 8.42%, respectively. There was positive association between the 0.25 mM (X2=55.001; p<0.0001), 25 mM (X2=2991.3; p<0.0001) and 75 mM (X2=5091.7; p<0.0001) MTX concentrations. There was a good correlation between cell viability and the different doses of the chemotherapy (R2 = 0.9276). Conclusions: According to the dose was increasing, the cells became more sensitive and lower resistant to the MTX chemotherapeutic. Moreover, there are a positive correlation between cell viability and the three different doses of the MTX chemotherapeutic. Financial support: Fapesp, CNPq, Capes FAMERP/FUNFARME.

P06.130

The Expression of RLIP76 in Leukemia Cell Lines

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RLIP76 is a membrane-located protein mediating the transport of multiple molecules, including both glutathione-conjugated or lipid-oxidized metabolites and drugs.

Besides this transport function, RLIP76 acts as an anti-apoptotic protein, interacting with Ras/Ral and EGF-R signaling. Studies have shown that there is an increased expression of RLIP76 in several types of cancer cells and that it plays a prominent role in drug resistance. The expression of RLIP76 in leukemia cells has also been reported, but the cell type and differentiation-related alterations are not yet known.

The aim of this study was to investigate RLIP76 expression in leukemia cells. The analysis of RLIP76 expression was performed at the mRNA level on several leukemia cell lines by using quantitative PCR.

Our results suggest that the expression of RLIP76 may exhibit a differential pattern in several types of leukemia cells.

P06.131

Prognostic impact of del(17p) and del(22q) as assessed by interphase FISH in sporadic colorectal carcinomas

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Purpose: Most sporadic colorectal cancer (sCRC) deaths are caused by metastatic dissemination of the primary tumor. New advances in genetic profiling of sCRC suggest that the primary tumor may comprehend a cell population with metastatic potential. Here we compare the cytogenetic profile of primary tumors from metastatic versus non-metastatic sCRC.

Patients and Methods: We prospectively analyzed the frequency of numerical/structural abnormalities of chromosomes 1,7, 8, 13, 14, 17, 18, 20, and 22 in 58 sCRC patients (31 non-metastatic vs. 27 metastatic) through FISH analysis.

Results: From a total of 18 probes, significant differences emerged only for 17p11.2 and 22q11.2. Patients with metastatic sCRC showed an increased frequency of del(17p11.2) (10% vs. 67%;p<.001) and del(22q11.2) (0% vs. 22%;p=.02) as compared to non-metastatic cases. Multivariate analysis of prognostic factors for overall survival (OS) showed that the only clinical and cytogenetic alterations that had an independent impact on patient outcome were the presence vs. absence of del(17p) with a 17p11.2 breakpoint and del(22q11.2). Based on these two cytogenetic variables patients were classified into three groups: low- (no adverse features), intermediate- (one adverse features) and high-risk (two adverse features)- with significantly different OS rates at 5-years (p<.001): 92%, 53% and 0%, respectively.

Conclusion: Our results unravel the potential implication of del(17p11.2) and del(22q11.2) chromosomal abnormalities in the sCRC pathogenesis, as these are intrinsically related to an increased metastatic potential and extremely poor outcome. Additional prospective studies in larger series of patients are necessary to confirm the clinical utility of the new prognostic markers identified.

P06.132

The Relationship of Metalloproteinase Gene Polymorphisms with Disease and Prognosis in Non-Small Cell Lung Cancer Patients Undergoing Resection

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In this study, we searched the relationship of matrix metalloproteinase (MMP) 2, 7, 13 gene expressions and polymorphisms with susceptibility and prognosis in patients operated for NSCLCs. The study group consisted of 132 patients who had undergone radical surgery in our clinic from 1997 to 2008. In the pathology arm of the study, sections were obtained from paraffin embedded blocks and then were analyzed immunohistochemically after treatment with MMP 2, 7 and 13 antibodies by using streptavidin-biotin method. In the genetic arm of the study, DNA samples were isolated from pathology blocks for NSCLC and from blood samples for the control group. MMP gene polymorphisms were analysed by PCR-RFLP method. The obtained results were compared with those of the control group to evaluate disease susceptibility, correlation with other clinical parameters and with survival and prognosis by using appropriate statistical methods. When MMP polymorphisms are compared in healthy and NSCLC DNA, a decrease was seen in MMP2(-735) GG genotype and increases were seen in MMP13(A77G) AG and GG genotypes (p=0.008, p=0.047, p=0.047 respectively). Median overall survival time was found as 29.5 months in MMP13 AA/AG genotypes and 9.3 months in GG genotype. MMP2, 7, and 13 expressions were not found to have a statistically significant influence on the prognosis of the patients. Decreases in MMP2(-735) GG genotype and increases in MMP13(A77G) polymorphism AG and GG genotypes increase the risk for NSCLC. Furthermore, the presence of MMP13(A77G)GG genotype is an unfavorable prognostic factor.

P06.133

Human Papilloma Virus in Lung Cancer Patients From Iran

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Lung cancer is the leading cause of cancer-related deaths across the world. Prevalent inconsistencies in the association of the high-risk Human Papilloma Virus (HPV) infection with lung cancer were found amongst recent studies.We evaluated the frequency of HPV positivity in NSCLC in an open case control study.50 recently diagnosed patients with squamous cell carcinoma of the lung were selected for HPV DNA extraction from paraffin-embedded blocks HPV DNA was the detected by nested-PCR. DNA amplification was initially performed using MY09/MY11 as the outer and GP5+/GP6+ as the inner primers. DNA was then sequenced for the determination of high-risk HPV types.Saliva samples of 94 control cancer-free subjects were collected for DNA analysis. High risk HPV was detected in 9 (18 %) patients and 6 (5.3%) control subjects, which was proven to be statistically significant. HPV-18 was the most frequent type both in the cases and controls. HPV-6 DNA was detected in one patient from each of the case group and the controls. In conclusion, HPV infection has a significant impact on NSCLC. Despite HPV-16 having a stronger impact, HPV-18 is more likely to cause malignant degeneration in such cancers amongst some communities. It is vital to introduce and conduct immunization schedules in health care systems to protect communities to some extent.

P06.134

Human papillomavirus DNA and abnormal p53 Tumor Suppressor Gene in lung carcinoma

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A powerful relationship has been established between high-risk human papillomaviruses (HPV) and lung cancer. Inactivation of P53 is the most common genetic abnormality in lung cancer. We evaluate the frequency of HPV types and TP53 mutations in squamous cell carcinoma (SCC) of lung, among patients from the north-west of Iran. 50 Paraffin embedded blocks of lung SCC were selected for detection of HPV DNA by Nested PCR with the MY09/11 and GP5+/6+ primer sets, Then DNA sequenced for HPV typing. Equal numbers of positive and negative samples for the HPV DNA were examined for the presence of mutations in exons 5-7 of the TP53 gene by PCR and direct sequencing. 9 (18%) out of 50 samples presented the HPV DNA: eight were HPV-18 and one was HPV-6. TP53 mutations were found in 5 samples (27.7%). Of these, 4 cases showed mutations in exon 5 and one case contained a mutation in exon 7. One of HPV negative samples had a mutation in exon 5. Three of the HPV positive cases demonstrated a mutation in exon 5 and one in exon 7. The frequent mutation in exon 5 was the C to G transversion (c.409C>G), and the T to A tansversion (c.770T>A) in exon 7. Both anomalies were missense mutations, L137V and L257Q respectively. Our study showed that HPV-18 is more likely to result in the development of lung cancer in some communities. Genetic alterations, alongside environmental factors, thus play a significant role in the pathogenesis of lung SCC.

P06.135

Cervical cancer as a part of Lynch Syndrome?

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Background: Pedigrees from Lynch families often include cases of cervical cancer. Cervical cancer is however not defined as an extra colonic cancer in Lynch syndrome. Therefore we set out to investigate if cervical cancer could be a part of the Lynch syndrome.
Materials and method: We used data from the Danish HNPCC register on women from Lynch families with in situ carcinoma in cervical tissue (CIN III) or cervical cancers. We obtained tissue samples from 20 out of 29 patients. We conducted immunohistochemical staining for MMR genes (MLH1, PMS2, MSH2 and MSH6). We used a HPV (*human papilloma virus*) genotyping analysis to identify infection.
Results: Among the cervical cancers 5 were adenocarcinoma and 2 were squamous cell carcinoma. There were full correlation between the mutation in the family and the loss of either MLH1 or MSH2 expression. Loss of expression of MMR genes was found in 5 of 7 (71%) cases of cervical carcinoma but in only one of the carcinoma in situ (p=0.007). All samples positive of HPV were detected in squamous cell carcinoma in situ. Conclusions: The results indicate a relation between the MMR mutation found in families and loss of MMR genes in cervical tumors. Our data also suggest that women with Lynch syndrome are at higher risk of developing adenocarcinomas than the background population. Loss of MMR gene expression seems to be a late event in the carcinogenesis of cervical cancer. Further research is needed to determine the precise relationship between Lynch syndrome and cervical cancer.

P06.136

ALC1 as a candidate oncogene in human malignant glioma

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Copy number gain of chromosomal arm 1q is quite a frequent aberration in malignant glioma. Therefore, we aimed to identify novel glioma-relevant oncogenes located on 1q. We screened glioblastoma cell lines by array-based comparative genomic hybridization (array-CGH) and detected an amplification in 1q21.1 encompassing the *ALC1* (amplified in liver cancer 1) gene in LN308 cells. *ALC1* (syn.: *CHD1L*) encodes the chromodomain helicase DNA-binding protein 1-like protein, a chromatin-remodeling enzyme implicated as an oncogene with a major impact in hepatocellular carcinoma development. To explore whether *ALC1* is of relevance for the tumorigenesis of malignant glioma, we analyzed *ALC1* copy number, mRNA and protein expression in 28 glioblastomas and 11 glioblastoma cell lines, and functional effects of *ALC1* overexpression. Array-CGH detected a copy number gain of 1q21.1 including *ALC1* and increased *ALC1* mRNA levels compared to astrocytes in around 20% of glioblastomas, and protein expression was significantly increased in glioblastoma cell lines compared to astrocytes. While *ALC1* overexpression showed a significant increase in viability of glioblastoma cells, which was reversible by RNAi, no significant influence on proliferation was detected by determination of growth curve and cell cycle analysis, and colony formation and migration capacity were decreased in some glioblastoma cell lines. No regulatory effects of *ALC1* overexpression were observed on epithelial (E-cadherin, α-E-catenin and β-catenin) or mesenchymal (N-cadherin, vimentin, fibronektin, α-SMA, snail and slug) marker expression. While *ALC1* showed copy number gain and increased mRNA and protein expression in a fraction of glioblastomas, no important functional effects of *ALC1* overexpression were detected.

P06.137

Increased gene copy number of *MET* in the bronchial mucosa of patients with malignant pleural mesothelioma

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Background: c-Met has been shown to be overexpressed in malignant pleural mesothelioma (MPM) tumor tissues as compared with normal pleura. However, the amplification of this proto-oncogene in the airways of patients with MPM has not been reported.
Objectives: To investigate the amplification of c-Met in bronchial mucosa of patients with MPM.
Method: Bronchial mucosa biopsy specimens obtained bronchoscopically from three patients with histologically proven MPM, were subjected to interphase fluorescence in situ hybridization (FISH) analysis. The cell touch preparations were obtained by lightly pressing fresh tissue onto slides. FISH was performed according to standard procedures using a c-Met probe in combination with a chromosome 7-specific centromere probe (CEP-7). Specimens were considered to be amplified if the gene copy number was ≥3 times the pooled mean for corresponding normal tissue.
Results: Increased c-Met copy number was identified in all patients.
Conclusion: Our findings demonstrate that amplification of c-Met is not restricted to the MPM tumor tissue, but is also amplified in the bronchial mucosa.

P06.138

MC1R gene variants and Malignant Melanoma susceptibility in the Canary Islands population

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Several *MC1R* variants are associated with increased risk of malignant melanoma (MM) in a variety of populations. The high diversity within the *MC1R* gene observed in populations living in higher latitudes is explained by either relaxation of selective pressure or by selection for lighter pigmentation in countries with reduced ultraviolet radiation. We aim to examine the influence of the *MC1R* variants (red-hair colour, RHC: D84E, R151C, R160W and Non-RHC: V60L, R163Q, T314T) on the MM risk in Caucasians and then restrict the analysis to a well defined population (Canary Islands), adjusting for the main phenotypic pigmentation features.

Methods: 938 Caucasian individuals were genotyped for *MC1R* variants by SNaPshot® and direct sequencing. 447 were MM patients (350 with three generation of Canarian ancestors) and 491 were healthy control subjects (296 Canarians) from general population. The analysis was adjusted for age, sex, hair colour, eye colour, skin phototype and ancestry.

Results: Carriers of the R151C and R163Q variants were at an increased risk of melanoma (OR=2.78 (1.58-4.90) and OR=5.45 (2.44-12.18), respectively) in the overall cohort. We observed similar results In the Canarian sample for carriers of the R151C variant but the R163Q variant showed even a higher risk for MM (OR=7.15; 2.38-21.52). The risk of carrying RHC variants was 3.12 (1.92-5.05) in the Caucasian population and 2.27 (1.31-3.91) in the Canarian individuals.

Conclusion: R151C and R163Q variants confer an increased risk of MM in the analysed population. Our results highlight the importance of the sample population selection in this kind of studies.

P06.139

Heading for a more effective and individualized patient care in multiple endocrine neoplasia type 1 (MEN 1)

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MEN1 is an autosomal-dominant inherited disorder characterized by the combined occurrence of primary hyperparathyroidism (pHPT), gastroenteropancreatic neuroendocrine tumors (GEP), adenomas of the pituitary (APA), adrenal cortical tumors (ADR) and other tumors. As the tumors appear in an unpredictable schedule, uncertainty about screening programs and genotype-phenotype correlation is persisting. In the German database a total of 683 tumors occurred consisting of 273 pHPT, 138 APA, 166 GEP, 57 ADR, 24 thymic - and bronchial-carcinoids as well as 25 neoplasms of other tissues.

The age-related penetrance was determined as 10 % , 35%, 67 % , 81 % and 100 % at 20, 30, 40, 50 and 65 years respectively. Although pHPT being the most frequent first manifestation (41 %), also GEP (22 %) or APA (21 %) were found to be the first presentation.To initiate a supranational data base, statutes have been elaborated to ensure a consistent data collection to define the influences of epigenetic factors in manifestation and progression of the syndrome.As a first step a summary file of 10 major items was developed. Every interested center is invited to an active collaboration. The aims are 1.) a multidisciplinary approach comprising clinicians of different specialities as well as basic scientists involved in the field 2.) providing diagnostic certificates and folders to all known European MEN 1 patients and 3.) the implementation of a supranational MEN1 patient association and a data base.

P06.140

Identification of a new frameshift mutation in the MEN I gene

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Multiple endocrine neoplasia type 1 (MEN1) is an uncommon autosomal dominant cancer syndrome. The classical form of MEN1 is characterized by tumors of the parathyroid, pituitary, and pancreas. MEN1 is a tumor suppressor gene whose germline mutations have been reported in MEN1 syndrome. Although hyperparathyroidism in MEN1 syndrome is the most common manifestation, parathyroid carcinoma is rare. This study aimed to identify mutations in an Iranian pedigree with MEN1. In this report, we presented a male patient who was diagnosed at 44-year-old with a primary hyperparathyroidism (PHPT), parathyroid tumor, recurrent renal stones, maxilla giant cell granuloma, thymic tumor, diabetes mellitus, and developed Cushing syndrome secondary to hypersecretion from neuroendocrine tumor. Genetic analysis revealed a novel germline frame shift mutation in exon 10 of the MEN1 gene, c.1642-1648dup. This heterozygote mutation is a duplication of seven nucleotides (GGTCCAG) which results in a premature termination codon at 558 (p.Val550fs). Three generation of his family members were also evaluated for *MEN1* gene, and the same mutation was detected in one of his sons. Menin truncated protein due to the premature stop codon in MEN1 gene seems to prevent interaction with various cellular proteins, inhibiting tumor suppress activity of menin protein and increasing the potential for tumorgenesis. Finding the same mutation in a younger member of this patient's family allows for prophylactic thymectomy and hopefully the avoidance of the malignant course seen in this patient. We suggest that in patients with ACTH-producing thymic NETS, the possibility of MEN1 should be considered.

P06.141

New epigenetic markers of breast cancer identified by unbiased screening of differential methylation of the genomes

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We have performed screening of 100 breast cancer (BC) samples and adjacent tissues by use of a method for unbiased screening of differential methylation that integrates amplification of intermethylated sites, capillary electrophoresis and computational biology and is designated AFLOAT (Amplified Fragment Length Oriented Analysis Technique). This has allowed us to identify abnormal methylation of CpG islands belonging to different regions of the *LAMB1, RAI1, KCNH8, DOCK6, GPC2, SH3KBP1, PPP2R5C, PHF1, ATMIN, C2CD2, TAF4, KIAA1324L, IQSEC2, AX746725/AK127124, TMEM176A/TMEM176B, FOXM1/HKMT1188* genes and intergenic CpG islands located on 1p33, 5p15.33, 12q13.13, 13q32.1.

SH3KBP1 and *PHF15* genes demonstrate significantly more frequent methylation in BC neoplastic grade 2 (G2) compared to G3. At the same time, *GPC2* reveals differences within G2, which is in agreement with the anxiety of other researchers regarding the subjectivity and insufficient reproducibility of the grading. Possibly, molecular markers might supplement morphological characteristics in defining neoplastic grade.

Methylation markers panel incorporating all of the 20 genes demonstrates 83,7% sensitivity and 93,9% specificity in discriminating BC against adjacent tissues. Exclusion of the least informative markers has led to the development of two systems that are optimized by the number of markers. The first includes 13 markers and is characterized by a sensitivity of 93.9% and a specificity of 93.9%, the second consists of seven markers, with a sensitivity of 89.8% and a specificity of 100%.

Methylation frequencies of loci under study;
n / s - the differences not statistically significant

Loci	Methylation in breast cancer, %	Methylation in adj. tissue, %	p	Methylation in nor. mammary gland, %	*13 markers system*	*7 markers system*
LAMB1 (17q31.1)	16	9	n / s	0(0/4)	-	-
TAF4 (20q13.33)	100	88	<0,01	100 (4/4)	+	+
RAI1 (17p11.2)	72	0	<0,01	0(0/4)	+	+
KCNH8 (3p24.3)	81	12	<0,05	0(0/4)	+	+
DOCK6 (19p13.2)	35	3	<0,01	0(0/4)	+	+
GPC2 (7q22.1)	56	9	<0,01	0(0/4)	+	+
SH3KBP1 (Xp22.12)	64	55	n / s	0 (0/4)	-	-
1p33	79	17	<0,01	0(0/4)	+	+
5p15.33	87	39	<0,01	0(0/4)	+	+
PPP2R5C (14q32.31)	15	0	<0,01	0 (0/4)	+	-
PHF15 (5q31.1)	19	0	<0,01	0 (0/4)	+	-
ATMIN (16q23.2)	18	0	<0,01	0 (0/4)	+	-
C2CD2 (21q22.3)	7	3	n / s	0 (0/4)	-	-
KIAA1324L (7q21.12)	60	50	n / s	0 (0/4)	-	-
IQSEC2 (Xp11.12)	27	3	<0,01	0 (4/4)	+	-
12q13.13	23	6	<0,01	0 (0/4)	+	-
13q32.1	15	6	n / s	0 (0/4)	-	-
AX746725/AK127124 (2q21.1)	8	6	n / s	0 (0/4)	-	-
TMEM 176A/176B (7q36.1)	27	9	<0,05	0 (0/4)	+	-
FOXM1/HKMT1188 (12p13.33)	8	6	n / s	0 (0/4)	-	-

P06.142

Evaluation of methylation pattern in promoter region of E-cadherin in Iranian Patients with Squamous Cell Carcinoma of Esophagus (SCCE)

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It has proven that E-cadherin to be widely down-regulated and tightly associated with tumor invasion and metastasis in multiple human cancer types. Recent researches have shown that aberrant methylation around gene promoter region attributes to E-cadherin silencing. However, the detailed information about this epigenetic inactivation in squamous cell carcinoma of esophagus (SCCE) is rare. For this reason, we studied for methylation at the E-cadherin gene promoters on 44 fresh tumor tissues and 19 non-tumor adjacent normal tissues, obtained from 44 patients affected by squamous cell carcinoma of esophagus in Iran. Up to now, we have done DNA extraction with phenol- chloroform method on tissue samples and the bisulfate treatment on DNAs for carrying up methylation-specific polymerase chain reaction assay (MS-PCR). MS-PCR has done with two set of specific primers for methylated and unmethylated status of E-cadherin gene promoter. Moreover, we have examined the expression of this gene by RT-PCR with two set of specific primers for and β- actin. For this step, we tried to synthesis the cDNA on RNAs Extracted from above tissues. Though, we have finished these experiments on most of samples, the results showed the 40%Methylation at the E-cadherin gene promoter in the tumor samples, while none of the non-tumor tissues exhibited the aberrant methylation. Also, RT-PCR experiments confirmed the expression of E-cadherin in all of non-tumor samples and unmethylated tissues. These data suggest that epigenetic silencing via aberrant methylation of the E-cadherin promoter is a common cause of inactivation of this gene in SCCE.

P06.143

Hypermethylation of TWIST1 and NID2 in tumor tissues and voided urine in urinary bladder cancer patients

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Purpose: We aimed to investigate the methylation patterns of TWIST1 and NID2 genes in bladder cancer and assess the use of these epigenetic changes in urine for sensitive/spesific detection of bladder cancer.

Method: The methylation status of 2 genes (TWIST1 and NID2) was analyzed by methylation-spesific PCR (MSP) in 56 cases of urinary bladder cell carcinoma samples. 24 cases had paired voided urine samples for analysis. In addition, 15 normal voided urine samples from sex- and age-matched (±5) controls were included.

Results: Methylation of TWIST1 was detected in %98.2 (55/56) of the cases and methylation of NID2 was detected in %96.4 (54/56) of the cases in urinary bladder tumor tissues. Furthermore, methylation of TWIST1 and NID2 could be detected in %87.5 (21/24) and %95.8 (23/24) of the voided urine samples of bladder cancer patients, respectively. In 15 normal urine controls, no aberrant methylation was detected except for TWIST1 methylation in only 1 normal urine sample (%6.6). The comparison of methylation analysis with urine cytology for cancer detection is one of the issues that will be explained at the presentation of the study.

Conclusion: This two-gene biomarker panel seems as a promising candidate for non-invasive detection of bladder cancer.

P06.144

MGMT promoter hypermethylation is a frequent event in glioma patients but has no prognostic value

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O-6-methylguanine-DNA methyltransferase (MGMT) gene encodes a DNA-repair protein that removes alkyl groups from the O6 position of guanine. Epigenetic modifications, such as hypermethylation along the promoter region, are a cause of gene inactivation and they are associated with better prognosis and benefit from alkylating agents. Assessing promoter hypermethylation of MGMT is therefore of great interest because it might have prognostic value, or be a predictive marker for therapy responses.

We examined the promoter methylation status of 103 glioma patients by Methylation-Sensitive High Resolution Melting (MS-HRM). After DNA extraction from paraffin-embedded tumour tissues, genomic DNA was bisulfite modified. Initially a PCR was run using primers specific for modified DNA but with no effective CpG nucleotides, then the HRM was performed. Finally, the data were statistically analyzed.

MS-HRM demonstrated positive methylation of MGMT in 80 patients (77.7 %) and no methylation in 23 patients (22.3%). In the group of long-term survivors MGMT methylation frequency was even higher (14 out of 15 patients, 93.3%). However, no statistical significance was reached comparing the methylated and unmethylated groups according to the overall survival - the median survival was 10.5 and 8.2 months for the methylated and the unmethylated group, respectively (p>0.05). Also, no significant survival benefit was found in the methylated group of patients treated with chemotherapy or radiotherapy.

Our results agree with the high MGMT methylation frequency shown in analogue reports but contrary to them the methylated group in our study did not show any survival benefit.

P06.145

Deletions of the MGMT gene region on chromosome 10q26.3 in gliomas

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Sensitivity of gliomas to alkylating agents is determined to a great extent by the activity of the DNA repair protein O^6-methylguanine-DNA-methyltransferase (MGMT) in the tumor cells: high activity of this protein forms the resistant phenotype of the tumor. The *MGMT* gene located on chromosome 10q26.3 is a classic tumor suppressor gene. It is known that methylation of the *MGMT* promoter leads to both inactivation of the gene and increase of the tumor's sensitivity towards alkylating drugs. Methylation of the *MGMT* gene promoter is currently considered to be the main marker of the tumour's sensitivity to the alkylating agent temozolomide. Deletion of 10q26.3 locus containing the *MGMT* gene can be an alternative mechanism of its inactivation. We have been the first to conduct a targeted analysis of loss of heterozygosity (LOH) at the *MGMT* gene region on chromosome 10q26.3 in gliomas. A panel of microsatellite markers to detect LOH in the area under study, which includes four intragenic and five flanking microsatellite polymorphisms, has been developed and characterized. Frequency of LOH evaluated by this assay in gliomas equals 51% (18/35). In all cases the deletion size exceeded that of the *MGMT* gene. Thus, the deletion of the *MGMT* gene happens more frequently than the methylation of the gene's promotor. Substantial frequency of the *MGMT* gene's deletions calls for more detailed research as a potential marker of the gliomas sensitivity to alkylating drugs.

P06.146

miRNA expression in two colon cancer cell lines treated with an histone deacetylase inhibitor

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Introduction: the presence of HDAC is necessary for the normal regulation of gene expression. Multiple studies have already revealed that inhibition of these proteins may lead to deregulation of gene expression and increase in cell proliferation. We have analyzed the effect of a histone deacetylase inhibitor (HDACI) in colon cancer cells, measuring miRNA expression in cell cultures after treatment.

Experimental design: we performed an array expression analysis of miRNA. Previously, we quantified the amount of miRNA using a comercial Small RNA kit (Agilent Technologies) allowing miRNA quantified in total RNA.

Results: array expression analysis using bioinformatic programmes showed that after treatment with the histone deacetylase inhibitor, the miRNA were grouped in 7 different expression clusters. These clusters group the miRNA with a similar expression throughout the treatment.

Conclusion: Analysis of expression of these clusters will allow to check if important pathways are modified for cell viability after treatment with these inhibitors.

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P06.148

Matrix Metalloproteinase-9 as Prognostic Marker of Cancer

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MMPs are being studied in a variety of tumor systems to ascertain their role in tumor progression. MMPs contribute in multiple ways to all stages of malignant progression, including tumor invasion, metastases and angiogenesis. The aim of this study was to investigate MMP-9 gene expression and to identify MMP-9 (-1562 C/T) gene promoter variations in breast, non-small cell lung cancer (NSCLC) patients' blood and tumor samples, and in prostate cancer patients blood. **Materials and methods:** A total of 188 patients with histopathologically diagnosed breast, NSCLC or prostate cancer tumors were enrolled to the study. MMP-9 gene expression was assessed by reverse transcription-PCR method. The MMP-9 (-1562 C/T) polymorphism variants were determined by the polymerase chain reaction-based restriction fragment length polymorphism method. **Results:** MMP-9 expression in breast cancer patients' blood correlated with disease stage (p=0.041) and tumor differentiation grade (p=0.037). Also a significant association (p=0.018) between clinical stage and MMP-9 polymorphism was found. MMP-9 expression and all polymorphism variants (CC, CT, TT) were detected in all NSCLC patients' samples, but without statistical correlations. For prostate cancer patients with identified CC or CT MMP-9 polymorphism variant survival time was longer compared with those patients with TT variant (p<0,001). There were no statistical correlations with Gleason score or PSA. **Conclusions:** MMP-9 expression and identification of polymorphisms variants could serve as prognostic marker for breast and prostate cancer. Additional studies with larger population are warranted for NSCLC.

P06.149

Expression of MRP1 and LRP in breast cancer patients: Correlation with response to chemotherapy treatment

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Background: Drug resistance is still a great obstacle to the success treatment of breast cancer. In this study we attempted to investigate the possible correlation between MRP1 and LRP and clinical response in women with breast cancer.

Materials and Methods: Tumor and adjacent normal tissues from 54 breast cancer patients were assessed for the expression level of MRP1 and LRP by Real Time RT-PCR.

Results: A statistically significant increase in MRP1 and LRP expression level was observed when tumor tissues were compared with normal breast tissues. Furthermore, MRP1 and LRP expression levels were significantly different in patients responding to chemotherapy compared to noresponding patients.

Conclusion: Our results suggest that MRP1 and LRP in human breast cancer cells may affect the clinical response to treatment and determination of MRP1 and LRP (either alone or in combination) may be valuable for the prediction of the chemotherapy outcome in breast cancer patients which remains to be cleared.

P06.150

The Frequency of mtDNA Alterations of G13397A, 12308G and G10398A in Involvement of Metastasis on IRANIAN Breast Cancer Patients

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Background and aim: The role of mtDNA alteration is recognized as being in carcinogenesis. mtDNA alteration G13397A and alteration of 12308 in tRNA Leu gene have been reported in involvement in metastasis but to date there was not shown on clinical breast cancer samples as a prognosis factors. It has been also shown that mtDNA alteration G10398A in breast cancer patients correlated with increased breast cancer risks and metastasis. The aim of this study was to address the question if the mtDNA alterations of G13397A, 12308G&G10398A can play a role in promoting of tumor and leading to metastasis.**Patients and tissue specimens**; 69 paired Fresh tumor and adjacent normal samples were obtained from patients with BC (31of 69 metastasis and 38 of 69 non metastasis) who underwent surgery for mama mastroctomy between October 2007 and Nov 2009. No patients had received any preoperative chemotherapy and or radiotherapy. Follow-up was continued until Jan 2012.We searched for mtDNA alterations of G13397A, 12308G and G10398A were analyzed by means of PCR sequencing.**Result and conclusion:** The G13397A mutation was not seen for all patient including metastasis and non-metastasis.It has not been found any role for G13397A mutation in involvement in metastasis on IRANIAN breast cancer patients. The frequency of 12308 G alteration was 28 %(8 0f 31) for metastasis patients. The rate of mtDNA alteration G10398A was 50 %(17 of 34) for BC patients including 9 of 15 (60.3%) for metastasis subjects and 8 of 19 (42.1%) for non-metastasis subjects.

P06.151

MTHFR gene C677T polymorphism is associated with the increased risk of cervical cancer in Russians

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Methylenetetrahydrofolate reductase (MTHFR) catalyzes the synthesis of 5-methyltetrahydrofolate, which is involved in the methylation of homocysteine to methionine. A common variant of this enzyme, resulting from a 677C>T (Ala>Val) substitution in the gene, has been shown to have reduced activity and is associated with hyperhomocysteinemia. Altered homocysteine levels, a functional marker of folate inadequacy, might contribute to the carcinogenic process. There is a growing body of epidemiological evidence suggesting that the MTHFR 677T allele and reduced dietary folate may increase the risk of cervical cancer. The aim of the study was to examine the association of the MTHFR genotype with the odds ratio (OR) for cervical cancer among women. MTHFR gene variants were determined in 127 women with cervical cancer and 175 healthy controls (all Caucasians and citizens of Russia). The MTHFR 677T allele frequency was significantly higher in women with the cervical cancer compared to controls (36.2 vs 22.0%; P = 0.0002). Those women with TT genotype were at six times the risk for cervical cancer [OR, 5.948; 95% CI, 2.406-14.704] compared to women with the homozygous MTHFR CC genotype. In conclusion, the MTHFR 677T allele and TT genotype are associated with the increased risk of cervical cancer in Russian women.

P06.152

What about the relatives who have MTHFR C677T polymorphism?

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Methylenetetrahydrofolate reductase (MTHFR) which affects both DNA synthesis/repair and methylation, plays a crucial role in regulating folate metabolism. MTHR C677T polymorphism has a role about cardiovasculer diasease, stroke, early pregnacy loss and in puplished datas show that MTHFR C677T polymorphism can be responsable some of malignancies such as lung and breast cancer.

We examined if the patients whose MTHFR C677T polymorphism had been studied have any malignancy stories in their family. We called 18 female and 7 male who have homozygote mutant genotype, 31 female and 25 male who have heterozygote genotype and 32 female and 24 male homozygote wild genotype for MTHR C677T polymorphism.

In homozygote mutant group(n:25) ; there are 14 relatives have malignancy stories, 7 relatives had lung cancer and 3 relatives have breast cancer. 56%, OR 3,8, p:0,007 (Compared to wild group)

In heterozygote group(n: 56); there are 17 relatives have malignancy stories, 3 relatives had lung cancer, 3 relatives had meningioma, 2 relatives had prostate cancer and 2 relatives had leukemia. 30 %, OR 1,308 p: 0,526 (Compared to wild group)

In wild group(n:56); there are 14 relatives have malignancy stories, 5 relatives had lung cancer and 5 relatives had breast cancer. 25 %

Published data on the association between the MTHFR C677T gene polymorphism and malignancy stories in their family who have MTHFR gene polymorphisms are inconclusive. We need further studies lead us understanding of the role of the MTHFR polymorphism and the mechanism of cancer development.

P06.154

Lynch-Syndrome or not? MUTYH associated polyposis in patients with MSI-H tumors and immunohistochemical loss of MMR proteins

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Lynch-syndrome is caused by germline mutations in DNA mismatch repair (MMR) genes predisposing for early-onset colorectal cancer/associated tumors with microsatellite instability, negative immunohistochemical staining and dominant inheritance, but in ~10-15% without germline mutation detection.

The recessively inherited MUTYH associated polyposis (MAP) shows a variable phenotype which can overlap with Lynch-syndrome. We analysed the two common MUTYH mutations p.Tyr197Cys and p.Gly396Asp in 83 MMR-gene mutation-negative patients with MSI-H tumours showing loss of MMR protein staining.

We detected the pathogenic MUTYH mutation p.Tyr197Cys homozygously in one patient with clinical presentation of two adenocarcinomas and rectal adenomas (56 years), urinary bladder carcinoma, sebaceous gland carcinoma (revealing loss of MSH2 and MSH6 proteins and MSI-H) (66 years) and positive family history. Tumor sequencing of the sebaceous gland carcinoma showed two somatic pathogenic transversion mutations in MSH2, other tumors had transversions in KRAS.

In three patients with MLH1-deficient tumors a heterozygous, monoallelic mutation in MUTYH was identified, but no second mutation or deletion in MUTYH, OGG1 and MTH1.

The incidence of 1,2% for biallelic MUTYH mutations in unsolved patients shows that two somatic mutations in MMR genes due to a base excision repair deficiency is rare, but can mimick Lynch-Syndrome.

P06.155

Promoter hypermethylation of tumor suppressor genes in serum as potential biomarker for the early diagnosis of nasopharyngeal carcinoma

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Nasopharyngeal carcinoma (NPC) is a common head and neck cancer in Southern China. Studies have shown that promoter hypermethylation of tumor suppressor genes may serve as a promising epigenetic biomarker for early diagnosis of NPC, which is of great significance in improving patient's survival rate. Resulting from DNA leakage due to tumor necrosis or apoptosis, cell-free circulating DNA in blood has been proven sharing a similar hypermethylation status as the primary tumor. Therefore, cancer-derived DNA in serum may be used for promoter hypermethylation status screening of tumor suppressor genes.

In this study, cell-free circulating DNA is extracted from 40 NPC patients before treatment and 40 age- and sex-matched healthy subjects. Promoter hypermethylation status of five tumor suppressor genes (*RASSF1A*, *CDKN2A*, *DLEC1*, *DAPK* and *UCHL1*) was assessed by methylation-specific polymerase chain reaction assay (MSP) after sodium bisulfite conversion. Differences of methylation status of five tumour suppressor genes and clinicopathological parameters (staging, age) between NPC patients and healthy subject would be compared.

To date, promoter hypermethylation status of four genes has been analyzed in 19 NPC samples. *RASSF1A*, *CDKN2A*, *DLEC1* and *DAPK* were found to be methylated in 5.3%, 0%, 5.3% and 5.3% patients, respectively. Hypermethylation of at least one gene was observed in 15% of the patients. Preliminary data suggest that the sensitivity of promoter hypermethylation detection of these fours genes in serum samples was low. A more definite conclusion has yet to be testified by future study.

P06.156

Differential DNA damage response in breast cancer cells with genetic deficiencies in *BRCA1* or *NBN*

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Genetic predisposition towards breast cancer includes *BRCA1* and *BRCA2* mutations but also mutations in genes encoding the MRE11-RAD50-NBN (MRN) complex which plays a major role in radiation-induced DNA damage response (DDR). Double-strand breaks initiate localization of DDR proteins such as gammaH2AX, 53BP1 and MDC1 forming nuclear foci which provide a platform for subsequent assembly of signalling proteins and disappear after successful double-strand break repair. Here we comparatively analysed the DNA damage response in two different breast cancer epithelial cell lines from patients with germ-line mutations in *BRCA1* or *NBN*, respectively.

We performed immuncytochemical assays to monitor the cellular response after irradiation with different doses (1.5Gy, 6Gy) and after different time-points (0.5hrs, 24 hrs, 48hrs). There was a dramatically reduced level of gammaH2AX and MDC1 foci formation in *NBN*-deficient breast cancer cells at 30 min after irradiation in comparison to wild-type, while 53BP1 foci were two-fold reduced but clearly detected. *BRCA1*-deficient breast cancer cells, in comparison with wild-type and *NBN*-deficient cells, showed a heightened response to irradiation with an up to two-fold increase of gammaH2AX, 53BP1 and MDC1 foci formation 0.5 hrs after treatment (1.5Gy, 6Gy). In addition, these cells showed a prominent delay in repair kinetics after irradiation.

In summary, the present results indicate a defective DNA damage response in two breast cancer cell lines with genetic mutations in double-strand break repair pathway genes. However, these deficiencies manifest at differential stages and time-points, suggesting that breast cancer can be functionally dissected according to the underlying germ-line mutations.

P06.157

Analysis of the nucleotide sequence diversity within the SUZ12 gene and its pseudogene SUZ12P to investigate the signature of nonallelic homologous gene conversion

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Nonallelic homologous recombination (NAHR) and nonallelic homologous gene conversion (NAHGC) are alternative processes depending upon whether the respective recombination intermediates are resolved with or without crossover. Since NAHR events give rise to genomic rearrangements, it is likely that recombination intermediates are more frequently resolved by the non-crossover pathways associated with NAHGC. Low-copy repeats exhibiting meiotic NAHR activity have been shown to be involved in frequent NAHGC-mediated sequence exchange. In addition, the LCRs located within the NF1 gene region (termed NF1-REPs), which mediate meiotic NAHR causing type-1 NF1 microdeletions, manifest an increased SNP frequency suggestive of frequent NAHGC. In this study, we have investigated whether NAHGC might also operate between the SUZ12 gene and its pseudogene (SUZ12P), duplicated sequences that flank the NF1-REPs and undergo mitotic NAHR giving rise to type-2 NF1 microdeletions. To this end, we determined the pattern of variation (SNP density and occurrence of shared SNPs between SUZ12 and SUZ12P) within the NAHR breakpoint cluster regions observed in patients with type-2 NF1 microdeletions. We did not however identify a significant increase in SNP frequency within the analysed NAHR breakpoint clusters of 20 healthy individuals of European descent above the genomic average SNP frequency of 1 SNP/kb. Furthermore, no evidence was noted of greater homology between SUZ12 and SUZ12P within the NAHR breakpoint clusters that would have been indicative of concerted evolution. We conclude that, in contrast to the flanking NF1-REPs, the SUZ12 gene and its pseudogene SUZ12P are not involved in frequent sequence transfer by NAHGC.

P06.158

Investigation of the gene conversion patterns associated with mitotic NAHR in the neurofibromatosis type-1 (NF1) gene region causing NF1 microdeletions

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Nonallelic homologous recombination (NAHR) is an important mutational mechanism underlying polymorphic and pathogenic copy number variation in the human genome. It is generally assumed that NAHR is mechanistically similar to allelic homologous recombination (AHR) between homologous chromosomes during meiosis. AHR-associated crossovers are processed by Holliday junction intermediates. Where interacting homologous DNA sequences exhibit nucleotide differences, mismatches in the recombining heteroduplex DNAs are to be expected. Recombination-associated mismatch repair is typically biased, with the unbroken DNA-strand being used as a mismatch repair template. This results in regions of marker loss in the broken DNA-strand, which are termed conversion tracts. Since mismatch repair frequently proceeds via an excision-based mechanism, AHR-associated conversion tracts are usually continuous. However, the breakpoint regions of several meiotic NAHR-mediated rearrangements have been shown to exhibit a pattern of discontinuous or 'patchy' conversions. In order to investigate whether patchy gene conversion is also a feature of mitotic NAHR events, we investigated the sequence characteristics of the breakpoint regions of 25 type-2 NF1 microdeletions mediated by postzygotic (mitotic) NAHR between the SUZ12 gene and its pseudogene, SUZ12P. However, instead of patchy gene conversion, we observed precise transitions between sequences derived from SUZ12P and SUZ12, respectively, as evidenced by the analysis of paralogous sequence variants and SNPs. Further, we did not observe distinct types of breakpoint-spanning fragments which would have been indicative of uncorrected mismatches resulting from the recombination. We conclude that recombination-associated gene conversion events resulting from heteroduplex-associated mismatch-repair are likely to be differently processed during meiotic versus mitotic NAHR.

P06.159

Mechanisms underlying non-recurrent microdeletions causing neurofibromatosis type-1 (NF1)

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NF1 microdeletions encompassing the NF1 gene region at 17q11.2 are present in 5-10% of patients with NF1. In particular recurrent NF1 microdeletions have been investigated in detail. However, the mechanism underlying non-recurrent (atypical) NF1 microdeletions are not well delineated. NF1 microdeletions with non-recurrent breakpoints are heterogeneous in terms of their size, breakpoint position and number of deleted genes. Furthermore, extended sequence homology is not observed in the respective breakpoint regions. In this study, we have analysed 12 atypical NF1 deletions using high resolution custom made array CGH. We could assign the breakpoints to regions of 1.2-6 kb. In six of these 12 atypical NF1 deletions, we identified the breakpoints at basepair level. Four of these six deletions were mediated by non-homologous end joining (NHEJ) as concluded by the absence of or only minor (1-2bp) homology at the breakpoints. Two of these six NF1 deletions exhibited microhomologies of 24 and 33 bp at the breakpoint sites indicative of microhomology-mediated end joining (MMEJ) as underlying mechanism. We conclude that NHEJ or MMEJ are the prevailing mechanisms underlying non-recurrent NF1 deletions that lack any complexity at the deletion breakpoint sites. However, three of the 12 NF1 deletions investigated by us represent complex rearrangements most likely caused by replication associated erroneous template switches. Our study indicates for the first time, that also non-recurrent NF1 microdeletions are mediated by a variety of mutagenic processes including mechanisms of double strand repair such as NHEJ and replication based mechanisms such as Fork stalling and template switches (FoSTeS).

P06.160

Improved detection of type-2 NF1 microdeletions and identification of breakpoint clusters

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Neurofibromatosis type-1 (NF1) is an autosomal dominant inherited disease that occurs with a frequency of 1:3000. Whereas 95% of all NF1 patients harbour mutations within the NF1-gene, 5% of NF1 patients exhibit large deletions of the NF1-gene and its flanking regions (termed NF1 microdeletions). Four types of NF1 microdeletions have been identified (type-1, type-2, type-3 and atypical) that differ with respect to breakpoint localization and the underlying causative mechanism. Multiplex-ligation-dependent probe-amplification (MLPA) has frequently been employed to identify NF1 microdeletions. However, the unambiguous typing of NF1 deletions is impossible in many instances due to the spacing of the probes included in the currently available MLPA-kit (P122-C1, MRC-Holland). Indeed, distinguishing between type-2 and certain atypical NF1 deletions is impossible using this MLPA-kit. In this study, we have developed an improved set of MLPA-probes that allows the unambiguous identification of type-2 NF1 deletions and potentiates breakpoint-mapping by PCR. Using a combination of this improved MLPA-technique and breakpoint-spanning PCR, we analysed 29 NF1 microdeletions initially considered to be type-2 deletions according to results obtained with the MLPA-kit P122-C1. We determined that 23 of the 29 deletions were indeed classical type-2 microdeletions, with breakpoints located in the SUZ12 gene and its pseudogene SUZ12P. However, 6 deletions turned out to be atypical exhibiting only one of both breakpoints within the SUZ12 sequences. Taken together with 16 previously identified type-2 deletions whose breakpoints have been localized, the analysis of a total of 37 type-2 NF1 deletions revealed a significant clustering of breakpoints within the SUZ12 sequences.

P06.161

BRCA1 and BRCA2 diagnosis by next-generation sequencing: A highly efficient methodology

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BRCA1 and BRCA2 are the most important breast cancer susceptibility genes. The conventional BRCA1 and BRCA2 mutation screening, performed by heteroduplex analysis and/or Sanger sequencing, is time consuming and has relatively high costs due to the absence of hot spots and to the high number of exons per gene. Usually, several months are necessary to complete the diagnosis. To overcome these limitations and to reduce the time needed for diagnosis, we designed a next generation sequencing protocol enabling simultaneous detection of variants in the two genes.

For resequencing BRCA1 and BRCA2, we used Multiplex Amplification (MASTR by Multiplicom) and the 454 GS Junior DNA sequencing instrument (Roche Diagnostics), which is based on pyrosequencing. Bioinformatic analysis was performed by two complementary approaches: Our pipeline (which comprises mapping and comparing reads against the reference sequence, aligning reads, classifying and identifying of SNPs and mutations), and by the Amplicon Variant Analyzer (AVA) software from Roche. We successfully validated this technology with a set of 6 cases carrying known mutations previously detected by Sanger Sequencing, and a reference HapMap sample (NA12144).

The application of this technology in 51 cases with suspected hereditary breast cancer, allowed the identification of 11 pathogenic mutations (3 missense, 1 novel nonsense, 6 frameshift and 1 splice variant), 6 unclassified variants and a high number of polymorphisms.Massive parallel resequencing of the BRCA1/BRCA2 genes is effective as a high throughput, rapid and cost-effective methodology for routine diagnosis of hereditary breast and ovarian cancer.

P06.162

Participation of miRNAs in conditioning of the colon polyposis

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Two types of miRNA: miR135a and miR-135b, encoded by three genes, are involved in the regulation of APC gene expression. The miRNA genes are located respectively, on chromosomes 3, 12 and 1. The miR135a and miR-135b interact with the 3' UTR sequences of the APC mRNA on the basis of partial complementarity and lead to its degradation. It was assumed that changes in the sequences encoding miR135a and miR-135b can affect their affinity to the 3 ,UTR of APC gene mRNA and, by increasing its degradation, cause a decrease in amount of functional APC protein, which can cause similar to haploinsufficiency effects. In addition, changes in the 3 ,UTR sequences may influence the affinity to the sites recognized by miRNA or induce the formation of new sites complementary to the miRNA sequences leading to faster degradation of mRNA. The study group consisted of 300 patients with FAP without detected mutation in APC gene and 300 healthy controls. The sequence of genes encoding miR135a and miR-135b, and 3 ,end sequence of the APC gene were tested. We analyzed occurrence of mutations in the studied fragments and compared frequencies of polymorphisms in the studied groups. The research was funded by Polish Ministry of Science and Higher Education, project number N401 331936.

P06.163
Effect of the PARP-inhibitor PJ34 on *NIS* expression and epigenetics modifications in human thyroid tumour cells

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Sodium iodide symporter (NIS) expression is crucial for the management of thyroid patologies, cancer in particular. Unfortunately NIS expression is often reduced in thyroid cancer; in many cases also his functionality is damaged. Since PARP-1 is supposed to be part of a multimeric repressor involved in cancer NIS uderexpression, in this study various human thyroid tumour cell lines (TPC1, BCPAP, FRO, WRO) were treated with the PARP-inhibitor PJ34, and the effects on the expression of NIS and several thyroid-specific transcription factors, together with the activity of NIS promoter, were evaluated. PJ34 treatment didn't affect thyroid-specific transcription factors expression, whereas we observed a strong increase in NIS mRNA levels in all the cell lines. Accordingly, in transfection experiments performed in TPC1 cells, treatment with PJ34 increased NIS promoter activity. It is well known that post-translational histone modifications are involved in the control of gene transcription. Thus, we have also investigated the epigenetic status of NIS promoter after PJ34 treatment in TPC1 cell line. In addition to an increase of activatory histone modifications (H3K9K14ac, H3K4me3) surprisingly we observed also an increase of H3K27me3, a classical repressive mark.

We concluded that PJ34 action, that implies mechanisms acting on epigenetic marks, is specific on NIS expression, suggesting it as a potential strategy to induce radioiodine sensitivity in human thyroid tumours.

P06.164
Whole genome microarray analysis in non-small cell lung cancer

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Lung cancer is a serious health problem, since it is the leading cause for death world widely. Molecular-cytogenetic studies could provide with reliable data about genetic alterations which could be related to disease pathogenesis and used for better prognosis and treatment strategies. We have performed whole genome oligonucleotide microarrays-based comparative genomic hybridization in ten samples of non-small cell lung cancer. Trisomies were discovered for chromosomes 1, 13, 18 and 20. Affected by genetic gain were chromosome arms 5p, 7p, 11q, 20q and Xq**,** and by genetic losses - 1p, 5q, 10q and 15q. Microstructural (<5 Mbp) genomic aberrations were revealed in regions 7p (containing EGFR) and 12p (containing KRAS) for gains and in 3p26 and 4q34 for losses. Based on high amplitude of alterations and small overlapping regions, new potential oncogenes have been suggested - *NBPF4* (1p13.3); *ETV1*, *AGR3* and *TSPAN13* (7p21.3 -7p21.1); *SOX5* and *FGFR1OP2* (12p12.1-12p11.22); *GPC6* (13q32.1). Significant genetic losses were assumed as containing potential tumor-suppressor genes: *DPYD* (1p21.3); *CLDN22*, *CLDN24*, *ING2*, *CASP3*, *SORBS2* (4q34.2-q35.1); *DEFB* (8p23.1)**.** Our results complement the picture of genomic characterization of non-small cell lung cancer.

P06.165
Molecular genetic testing in patients with Non-Small Cell Lung Carcinoma

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Introduction: Non-Small Cell Lung Carcinoma (NSCLC) is one of the most serious cancers. For targeted biological treatment, sensitive and specific identification of genetic changes is necessary. Identification of genetic changes (mutations) within EGFR, KRAS and ALK oncogenes, associated with NSCLC, allows choosing the patients, which benefit from biological therapy.

Material and Methods: DNA is isolated from biopsy and cytology specimens with verified histological diagnosis. Mutation detection is performed by real-time PCR, fragment analysis, primer-extension analysis and mutant-enriched PCR. Wt-EGFR patients (e.g. with no mutation detected) are tested for ALK gene rearrangement, causing the EML4-ALK fusion gene formation. Analyses are performed on histological slides using the FISH method.

Results: Since 10/2010 till 12/2011, 199 DNA samples were analyzed. Out of these, 12 patients (6 %) were found to be positive for activating mutations within EGFR gene. Since 07/2011 till 12/2011, 54 patients were analyzed for ALK gene rearrangement. This change has been proven in 2 cases.

Discussion: Activating mutations of the EGFR gene correlate with therapeutic response to tyrosin kinase inhibitors. Frequency of mutations within the EGFR gene is 5 - 20 %. ALK gene rearrangement can predict therapeutic response for ALK inhibitors. These rearrangements are found approximately in 5 % of NSCLC patients. Our results correlate with abovementioned findings**.** Occurrence of certain mutations, e.g. T790M, as well as KRAS gene mutations and ALK gene rearrangements predicts resistance to TKIs therapy.

Conclusions: Determination of tumor mutational status can provide powerful tool for setting up strategy and therapeutic protocols in NSCLC patients.

P06.166
Extending the spectrum of PTPN11 germline mutations associated with juvenile myelomonocytic leukemia in children with Noonan syndrome

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Introduction Noonan syndrome (NS) is a genetic disorder caused by a germline mutation in the PTPN11 gene in about 40% of cases. These patients are predisposed to develop a myeloproliferative-myelodysplastic syndrome (MPD), the juvenile myelo-monocytic leukemia (JMML). To date, few data concerning its incidence and prognosis are available.

Methods 562 patients carrying a germline mutation of PTPN11 have been studied. JMML was diagnosed in 21 of them (NS-JMML). In 11 other patients, hematologic anomalies suggestive of a MPD have been noted. Cytologic, genetic and clinical features of these 2 groups of patients have been compared with 24 patients presenting a PTPN11-associated sporadic JMML.

Results Hematological anomalies are found in 32/562 (5.7%) of NS patients and encompass a broad phenotypic spectrum ranging from transient MPD to JMML. Hematologic presentation of NS-MPD and NS-JMML is not different from sporadic JMML but the overall survival is considerably altered in NS-JMML patients and boys. The mutation D61H of PTPN11, never reported in NS, is found in 2 patients with a particularly severe neonatal course. More generally, the spectrum of PTPN11 mutations is narrower in NS-MPD and NS-JMML patients than in the whole cohort of NS patients, with an increased incidence of mutations of the 61, 139 and 506 codons. We were not able to identify additional recurrent genetic alteration with the SNP array analysis of 12 NS-JMML patients.

Conclusion Some PTPN11 mutations are associated with an increased risk of JMML. Additional cooperating factors may explain the genotype-phenotype correlation in the other cases.

P06.167
Platform comparison of expression data and identification of novel mutations in *CIC* in oligodendrogliomas

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Various studies indicate that the success of treatment and thus the overall survival time of patients suffering from gliomas depend on molecular features of the tumor.

We analyzed 17 oligodendroglial tumors by high resolution array CGH (Agilent 400K) and for the first time by systematic comparison of platforms for gene expression analysis. We performed transcriptome next generation sequencing (RNA-Seq) using a 2x100nt paired-end approach on the Illumina-HiSeq2000-platform, miRNA array, 8x60k expression array and Exon array analyses (Agilent). Additionally, Sanger sequencing of candidate genes *(IDH1*, *IDH2*, *CIC*, *FUBP1)* was carried out.

Array CGH revealed structural changes including the previously described 1p/19q-codeletion in 11/17 tumors (69%). We identified seven novel mutations in the *CIC* gene on 19q (altogether 9/17), including one exon-spanning deletion. In the latter, RNA-Seq revealed a notably decreased gene expression. The *IDH1* c.395A>G mutation was the most frequent alteration found in 14/17 tumors (82%). Sequencing of *FUBP1* on 1p uncovered mutations in three cases.

This let us observe a strong association between the presence of *CIC* and *IDH1* mutation and the 1p/19q-codeletion, since all tumors showing mutations in *CIC* also contained the *IDH1* mutation as well as the 1p/19q-codeletion except for one case (*CIC* and *IDH1* mutation only). These results emphasize the critical role of *CIC* for the development of oligodendroglioma.

RNA-Seq data will help to identify aberrant mRNAs and new fusion-genes. In the long run, these studies aim to contribute to the understanding of the complex tumorigenesis of oligodendrogliomas and the finding of molecular targets for directed therapy.

P06.168

Genotype-fenotype correlation in optic nerve gliomas in Slovak NF1 patients

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Introduction: Neurofibromatosis type 1 (NF1; OMIM 162200) is in 15% of cases complicated by optic nerve gliomas (ONG). Genotype-fenotype correlations in patients with NF1 and ONG help to determine the risk group for developing a severe form of NF1.

Materials and methods: We evaluated 51 Slovak patients with NF1 and divided then into two groups 1) with ONG (21 patients), 2) without ONG (30 patients). All of them underwent a clinical examination and molecular diagnostics of *NF1 gene* using protocol based on RNA.

Results: In the group with ONG patients, there was a significantly higher incidence of freckling (95%), brain hamartomas (71%) and neurofibromas (70%), compared to group without ONG. A half of mutations in the ONG group were located in the first 5'tertile (first 16 exons) of the *NF1 gene*. There were 15 novel mutations identified.

Discussion: Our results confirm the clustering of mutations in the 5' tertile of *NF1 gene* in patients with optic nerve glioma and sugguest the incidence of a more severe form of NF1. This may contribute to prognosis prediction.

P06.169

The contribution of MLPA and aCGH to establish the genetic profile of Oral cancer

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Oral squamous cell carcinoma (OSCC) is one of the most common malignant lesions of head and neck. Besides technologic advances, the genetic mechanisms involved in the pathogenesis and progression of this disease are still not clear; thus the improvement in the diagnosis and treatment is limited, which means scarce benefit to the patients. The main goal of this study was to characterize the genetic profile of the OSCC by Multiplex Ligation-dependent Probe Amplification (MLPA). We also applied array-CGH, not only to corroborate the MLPA results but also to identify putative key regions associated to OSCC. To achieve these purposes, biopsies of tumor and biopsies from resection margin of the same patient, were acquired from 23 patients with diagnosis of OSCC. Tissue from healthy donors was used as control. The array-CGH from tumor biopsies was performed using a 4x180K oligonucleotide microarray. With the MLPA we detected frequently losses in chromosomes regions of 3p, 4q, 5q, 8p, 9p, 11q and gains in chromosomes regions of 3q, 6p, 8q, 11q, 16p, 16q, 19q and 20q. The analysis of the tissue from resection margin identified alterations spread over several chromosomes, namely in 6p, 9p, 16p, 17p, and 19q. With array-CGH we detected imbalances in all chromosomes. In conclusion, this kind of studies improves the understanding of these devastating tumors through identification of key chromosomal regions involved in tumor biology, which may be very useful to the follow-up of these patients and also allows the development of novel therapeutic targets.

P06.170

Vitamin D receptor gene polymorphism as prognostic indicator for oral cancer

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It has been reported that genetic polymorphisms in the vitamin D receptor gene (VDR) could influence the risk of oral cancer. Among numerous identified polymorphisms in the VDR gene, FokI (rs2228570) is considered as functional polymorphism and results in synthesis of 427 amino acids long protein, while mutated form results in synthesis of protein shorter for three amino acids. It could be assumed that this functional polymorphism has influence on survival and may be used as oral cancer prognostic indicator. The goal of this study was to investigate the association of VDR FokI polymorphism with oral cancer survival. The study was performed in 110 patients with diagnosed oral cancer. Genotypes were determined by the PCR-RFLP method. All data were statistically analyzed using the SPSS software. The VDR FokI polymorphism was associated with a decreased overall survival (p=0.042, log-rank test). Patients with *wild type* ff genotype had significantly worse survival (p=0.012, log-rank test) compared to the heterozygous and mutated variants together. Stratified analysis by the lymph node involvement and tumor stage revealed that *wild type* ff genotype was associated with the poor survival in groups with and without lymph node involvement (p=0.025, p=0.040, respectively) and in stage III tumors (p=0.026). Multivariate Cox's regression analysis revealed that VDR FokI could be considered as independent prognostic factor (HR=0.600, 95% confidence interval= 0.377-0.954, p=0.031).

Our data suggest that VDR FokI polymorphism is associated with the survival and could be considered as independent prognostic indicator for oral cancer.

P06.171

Ovarian carcinoma - profiling of the gene expression and candidates for targeted therapy

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Ovarian cancer is the second most common gynecologic malignancy and the fifth leading cause of cancer deaths in women. The genome-wide microarray consisting of approximately 38,500 transcripts enabled us to obtain comprehensive gene expression profiles related to phenotypic and biological information in cancer cells. We have identified multiple targets that may be applicable for development of novel anti-cancer drugs and/or diagnostic biomarkers. Through gene-expression profile analysis of 22 epithelial ovarian cancers coupled with purification of cancer cell population by laser microbeam microdissection (LMM) on a microarray, we identified a number of transcripts that were over-expressed in ovarian cancers. Altogether, we identified 273 transcripts that were commonly up-regulated and 387 transcripts that were down-regulated in ovarian carcinomas. Among these 273 transcripts identified, only 87 (31.9%) transcripts were reported as up-regulated genes in previous microarray studies, in which bulk cancer tissues and normal ovarian tissues were used for the analysis. We further propose a number of genes probable to be good candidates for a target therapy of ovarian cancer. Among them we focus on *CHMP4C* (chromatin-modifying protein 4C) that was over-expressed very commonly in ovarian carcinoma, but were not expressed in the normal human tissues examined. Our data should be helpful for a better understanding of the tumorigenesis of ovarian cancer and should contribute to the development of diagnostic tumor markers and molecular-targeting therapy for patients with ovarian cancer.

P06.172

Clinical trials for p53 marker validation

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The moderate efficacy of cancer therapy is still the major challenge in surgical oncology.

The use of genetic markers has been suggested to improve treatment efficacy. However, a clear algorithm for the clinical evaluation of a potential marker is currently lacking.

Based on the well-established phase I-III clinical trials we present an algorithm, which we propose for reliable clinical evaluation of genetic markers like p53.

Phase I marker studies aim to demonstrate the robustness, specificity and prevalence of the potential new marker and the formulation of a marker hypothesis. Phase II marker studies focus on the marker test; concerning reproducibility, sensitivity and specificity of the test, and the result interpretation. Phase I and phase II marker studies may be performed retrospectively using adequately collected samples. Phase III marker trials aim to confirm the clinical relevance of the marker providing a high level of evidence (level I). The latter trials have to be prospective, randomised, controlled investigations taking the qualified trial design into particular consideration. The clinical utility of a marker depends on its ability to guide three therapeutic decisions: "Who to treat", "How to treat" and "How much to treat". These questions have to be answered by different marker types -prognostic, predictive and pharmacodynamic- implicating that the projected phase III trial endpoints have to be different. As a marker, p53 has passed phase I and II. Currently phase III trials evaluating p53 are missing probably because it is not clear whether p53 should be evaluated as prognostic or predictive marker.

P06.173

Phase II clinical trial for optimising radiotherapy for rectal cancer using genetic markers

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In rectal cancer preoperative radiation plus surgery has proven superiority to surgery alone and is widely accepted as standard therapy. However, a significant reduction in local recurrence has been achieved by a questionable risk/benefit ratio.

Radiation therapy is suggested to depend on sufficient tumour oxygenation and to act via induction of apoptosis. TP53 gene mutations represent a crucial defect in the apoptosis pathway, are involved in regulation of tumour vascularisation and are present in 60% of rectal cancers.

The study aims to correlate pathological tumour-response to radiation therapy with three parameters: the genetic status of the marker TP53, differences in perfusion MRI results eight weeks after radiation treatment and differences in the concentration of circulating angiogenesis factors in blood.

Eligible patients in our study receive standard preoperative short-term radiation for 5 days followed by surgery after a delay of eight weeks. The sample size calculation is based on an estimated 40% difference in response rates comparing patients with TP53 normal and mutant tumours. The required patient number to reach the study endpoint is 60.

The TP53 status is analysed in tumour tissue from diagnostic biopsies. Tumour stage is assessed by MRI at time of diagnosis and immediately before surgery. MRI staging will be compared to pathohistological staging. Concentrations of circulating angiogenesis factors are assessed at several time points.

The trial prepares the way for interventional trials and offers potential restriction of preoperative radiation for rectal cancer to those patients who will benefit, saving patients from negative side effects.

P06.174

Novel p53 gene mutations at codons 65 and 100 among Iranian esophageal cancer patients: it may modify MDM2-p53 interaction

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The incidence pattern of esophageal cancer is different among Iranian population and up to 171/100000 in Northern Iran. In previous study, we showed that p53 gene mutations occurred in esophageal squamous cell carcinoma (ESCC) from Iran frequently. The p53 protein has an important role in tumorgenesis in various types of cancer. This protein is a transcription factor and it can induce apoptosis to prevent the development of cancer or tumor growth. Also, Several studies were described the p53 genetic polymorphism in exon 4, at codon 72, and susceptibility to several types of cancer and diseases. We studied association of the p53 genotypes at codon 72 with esophageal cancer risk in Iran.

To investigate the p53 Pro72Arg genotype among healthy controls and ESCC patients, we collected samples from blood and tumor tissues. The p53 genotypes were determined by direct DNA sequencing and PCR-RFLP analysis. During this study, we found novel p53 gene mutations at codon 65 (AGA→AAA) and codon 100 (CAG→ CAA) among patients.

Additionally, we assessed protein expression of p53 and MDM2 in esophageal tumor tissues by immunohistochemistry method. We found that abnormal accumulation of the p53 protein was not associated with MDM2 protein expression.

The p53 and MDM2 proteins are related to cancer. The p53 protein prevents tumor growth, but, MDM2 promotes proliferation of tumor cells. It is described that MDM2 protein can bind to prolin-rich region in p53 protein (residues 61-94). So, p53 point mutation at codon 65 may modify MDM2-p53 interaction.

P06.175

Whole genome expression, canonical pathway and gene network analysis in the cases of papillary thyroid cancer

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Introduction: Papillary carcinoma is the most frequent thyroid cancer and constitutes % 75-80 of thyroid cancers. Finding of scientific markers for papillary thyroid cancer and its variants can make easier to confirm the results taken from Fine Needle Aspiration cytology. The objective of this study consists in elucidating the role of genetic factors in the mechanisms of the development of papillary thyroid cancer and to screen patterns of whole genome expressions in patients with papillary thyroid cancer.

Materials and Methods: RNA samples were obtained from healthy and cancerous tissues taken from cancer detected nodule from eight patients diagnosed as papillary thyroid cancer. These RNA samples were hybridized with microarray chips (Agilent Human 4 X 44K Oligo Microarrays). Gene expression, canonical pathway and network analyisis were performed using GeneSpring GX 11.0 software.

Results: 40 downregulated and 124 upregulated genes were detected in our study. The canonical pathways significantly regulated were extracellular region, collagen, multicellular organismal process, cell adhesion, biological adhesion and multicellular organismal development.

Conclusion: Upregulation of HMGA2 gene which was reported before as a novel molecular marker in development of thyroid carcinoma is noteworthy in our study. This gene is upregulated in malignant forms of thyroid cancers has been reported. It's suggested that HMGA2 might be used as a molecular marker for classification of thyroid tumors in terms of being malignant or benign forms.

P06.176

Characterization of PMS2 rearrangements in Lynch syndrome patients uncovers the first deleterious PMS2-PMS2CL hybrid allele

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Heterozygous *PMS2* germline mutations are associated with Lynch syndrome. Up to one third of *PMS2* mutations are genomic deletions. Their detection is complicated by a pseudogene (*PMS2CL*), which-owing to extensive interparalog sequence exchange-closely resembles *PMS2* downstream to exon 12. A recently re-designed multiplex ligation-dependent probe amplification (MLPA) assay identifies *PMS2* copy number alterations with improved reliability when used with appropriate reference DNAs. We used this assay to study 13 patients with PMS2-defective colorectal tumors. Three presented deleterious alterations: (i) An *Alu*-mediated deletion strengthening the view that the high frequency of *PMS2* deletions is related to a high density of *Alu* elements within the genomic sequence of the gene. (ii) A 125-kb deletion encompassing at least two further genes with tumor suppressing functions in a young colorectal cancer patient. This raises the possibility that *PMS2* mutations of this type may confer a higher penetrance of tumor susceptibility. (iii) The first *deleterious* hybrid *PMS2* allele produced by recombination with crossover between *PMS2* and *PMS2CL*, with the breakpoint in intron 10 (the most 5‘ breakpoint of its kind reported thus far). We discuss mechanisms that might generate this allele in different chromosomal configurations (and their diagnostic implications) and describe an allele-specific PCR assay that facilitates its detection. Our data indicate that, for gDNA-based *PMS2* mutation analysis, the re-designed *PMS2* MLPA assay is a valid first-line option that can identify roughly a quarter of all *PMS2* mutations.

P06.177

Mutation of the PPP2R1A gene as part of the sporadic endometrial serous carcinoma genetic profile

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Introduction and Aim: Endometrial serous carcinoma (ESC) is the most aggressive subtype of endometrial carcinoma. Some of its mainly mutated genes are p53, p16, E-Cadherin, accompanied sometimes by mutations in PTEN, PIK3CA, KRAS, BRAF. Recent studies show the involvement of PPP2R1A in the pathogenesis of ESC. PPP2R1A encodes a constant regulatory subunit of Ser/Thr phosphatase 2 implicated in the negative control of cell growth and division. Although its role in carcinogenesis remains still unveiled, molecular studies indicate that its mutation may be a factor to take into consideration when doing a genetic mapping, thus influencing the treatment of the disease.

Our aim was the study of the PPP2R1A gene in order to determine the frequency of mutations in the sporadic ESC in Spanish patients.

Patients and Methods: A set of 12 patients with sporadic endometrial carcinoma with serous component was studied through analysis of the tumoural DNA by PCR, CSGE, cloning and automatic sequencing of the full coding region and the exon-intron boundaries.

Results: The results are annexed in the table. We found 4 previously reported PPP2R1A pathogenic mutations (33.33%), two of the patients carry additional gene mutations.

Conclusions: The study indicates that PPP2R1A gene mutations play an important role in the carcinogenesis of sporadic ESC in Spanish patients and as such should be included in its molecular profile.

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Table: Pathogenic (*) and unknown significance mutations found in the studied genes. (H) Homozygous

	PPP2R1A	PTEN	P16	TP53	CDH1	PIK3CA	BRAF	KRAS
Pat. 1	wt	wt	wt	wt	wt	wt	wt	wt
Pat. 2	wt	wt	wt	c.G818A; p.R273H (H)*	wt	wt	wt	wt
Pat. 3	wt	c.C388G; p.R130G*	wt	wt	wt	wt	wt	c.G38A; p.G13D*
Pat. 4	wt	wt	wt	wt	wt	wt	wt	wt
Pat. 5	wt	c.G513C; p.Q171H (H)*	wt	c.458_459ins G; p.153fsX179* c.C832T; p.P278S*	wt	wt	wt	wt
Pat. 6	c.C767T; p.S256F*	wt	wt	c.G744A; p.R248Q*	c.G271A: p.R90Q	wt	wt	wt
Pat. 7	wt	wt	wt	c.T821C; p.V274A*	wt	wt	wt	wt
Pat. 8	c.C536G; p.P179R*	wt	wt	wt	wt	wt	wt	wt
Pat. 9	wt	wt	wt	c.A715G; p.N239D	wt	wt	wt	wt
Pat. 10	c.C536G; p.P179R*	wt	wt	IVS6+2T>C*	wt	wt	wt	wt
Pat. 11	wt	wt	wt	wt	wt	wt	wt	wt
Pat. 12	c.C536G; p.P179R*	wt	wt	c.G796A; p.G266R c.G524A; p.R175H*	wt	wt	wt	wt

P06.178

First steps towards an individualized immunotherapy for primary liver cancers.

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Primary liver cancer is within the top ten most common cancers worldwide. Over 500‘000 patients are diagnosed with hepatocellular carcinoma each year. The prevalence in Europe and the USA has been rising constantly over the last decades. Yet, therapeutic options are limited. The BMBF supported project ‘IndividuaLIVER’ aims at establishing an individualized immunotherapy for primary liver cancers.

This approach is based on the detection of somatic mutations in tumor tissue. Hence, we sequenced a pair of tumor tissue and blood sample in one patient suitable for vaccination. Both samples were conditioned and sequenced using a targeted whole-exome resequencing approach. The on-target ratio was 0.79 / 0.77 (tumor / blood), overall coverage at a depth of 10x was 91.6 % / 90.44 %. With stringent filter criteria, we were able to detect a total of 23 somatic sequence changes suitable for vaccination. The laboratory turnaround time is estimated to be 6-7 weeks. Validation was carried out using a second bioinformatic analysis method and a deep-sequencing approach with customized targets (av. depth: 728x). We were able to confirm 12 somatic variants. All sequence changes were further prioritized by an HLA-allele-specific SYFPEITHI-score. Taken together a total of 3 sequence changes were rated suitable for HLA and mutation specific vaccination.

Using this approach, we are able to detect somatic sequence changes within tumor tissue that might lead to successful individualized immunotherapy within a reasonable time frame. The next steps will be design and administration of an individualized multi-peptide anti-cancer vaccine.

P06.179

S-adenosylmethionine alters the transcription profile in prostate cancer cells

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Epigenetic alterations are critical steps in cancerogenesis. DNA hypomethylation in cancer cells is probably as frequent as DNA hypermethylation and might activate oncogene transcription. Recent studies point to a role for S-adenosylmethionine (SAMe), a major methyl donor in biological transmethylation events, as a demethylation inhibitor. In prostate cancer cells, treatment with SAMe leads to increased methylation levels of promoters from several genes like the urokinase plasminogen activator and thus causes the downregulation of the respective genes. To gain a more general overview concerning the effects of SAMe treatment on cancer cells, we performed whole transcriptome shotgun sequencing on prostate cancer cells treated with SAMe. We found altered transcription levels of 160 genes, 90 of which were downregulated. Most of these genes are associated with biological processes critical in cancerogenesis e.g. epithelial-mesenchymal-transition, invasion, migration and proliferation of cells. The expression levels of some genes (e.g. KLF8, BDNF, S100P etc.) were confirmed by realtime PCR and methylation specific PCR was carried out. Treatment of prostate cancer cells with SAMe was found to result in an increased global methylation status of the DNA suggesting that reversing DNA hypomethylation might be one major mechanism of SAMe action. Next, we performed functional studies with SAMe-treated cancer cells as well as human fibroblasts and discovered a decreased potential for proliferation, migration and invasion of cancer cells but not of fibroblasts. Taken together, we provide a more comprehensive overview of effects caused by SAMe and present novel target genes for therapeutic options in prostate cancer.

P06.180

Can mycoplasma-mediated oncogenesis be responsible for formation of prostate cancer?

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Aim : The origin of chronic inflammation preceding the development of prostate cancer (PCa) remains unknown and chronic inflammation associated with infections has been defined as an important cancer-promoting condition. In our study, to investigate relationship between mycoplasma sp. infection and PCa were aimed.

Method: Benign prostate hyperplasia and tumor tissue samples from 31 patients with PCa and healthy control groups (benign prostate hyperplasia) were studied. Moleculer DNA analyses was done after nested-PCR performed in two steps with seven primers (four auter and three iner) that can recognize at least 15 different Mycoplasma using two different PCR methods.

Results: Mycoplasma sp. DNA was detected in benign prostate hyperplasia, prostate tumor tissue samples at ratio 12.9% (4/31) and 35.9% (11/31), respectively. No mycoplasma DNA was determined in tissues of 31 healthy control group.

Conclusion: The relationship between mycoplasma sp. infection and Prostate cancer has been investigated for the second time in literature, and a significantly high existence of Mycoplasma sp. In conclusion, our data suggest that mycoplasma infections could be play a role in the etiology (mycoplasma-mediated multistage carcinogenesis) of prostate cancer. Further experimental and clinical studies are needed for development of a tool for early diagnosis and treatment of prostate cancer.

P06.181

Search for prognostic biomarkers in prostate cancer patients after radical prostatectomy

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New biomarkers are needed to better predict the clinical outcome of patients with clinically localized prostate cancer. The available clinical and histopathological variables currently used lack accuracy to predict the course of the disease often leading to over- or under-treatment. Molecular scores have the potential to improve risk-stratification with respect to timing and intensity of treatment. We have developed an mRNA-based expression signature in a cohort of more than 150 patients who underwent radical prostatectomy between 1993 and 2006.

We measured the expression of approx. 200 genes in RNA derived of formalin-fixed, paraffin-embedded (FFPE) tumour samples. We used the Nanostring nCounter gene expression system, a digital, enzyme-free technology which features excellent reproducibility and high sensitivity on partially degraded RNA extracted from archival FFPE tumour samples. We identified several genes that significantly predicted biochemical relapse in univariate analysis. A multi-gene molecular score was developed which predicts biochemical relapse in univariate and multivariate analyses when controlling for clinical and pathologic variables. The prognostic value of the score is currently being retrospectively assessed in an independent cohort.

P06.182

Expression of the SHB gene in prostate cancer

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SHB (Src homology 2 domain-containing adapter protein B) is involved in receptor tyrosine kinase signaling, angiogenesis, apoptosis and cell regulation regulation. A detailed analysis of expression of the SHB gene has not been conducted in prostate cancer so far. We aimed to compare the SHB expression in prostate cancer and benign prostatic hyperplasia with clinicopathological data and evaluate its diagnostic and prognostic potential.

Isolation of mRNA from prostate cancer in 56 patients has been performed in period 2008 - 2011. As a control group, 26 patients with benign prostate hyperplasia were used.

Statistically significant lower relative expression of SHB in prostate cancer tissue was detected (p< 0.001). In comparison of patients distributed to localized (T2) and locally advanced (T3, T4) groups, decreased expression in locally advanced disease with statistical significance (p< 0.0236). In comparison of groups divided by Gleason score (GS <7 and GS ≥ 7), age and PSA, no differences have been detected. According to our results the level of SHB expression analysed by using RT-PCR in patients with prostate cancer can give additional prognostic information.

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P06.183

Analysis of V89L polymorphism of the testosterone 5-alpha-reductase II gene in prostate cancer patients from Bashkortostan Republic of Russia

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Prostate cancer (PC) is one of the most common visceral malignancies in human. In recent years, its incidence has increased in Russia. The study was performed to investigate the role of various genotypes involved in steroid metabolism and synthesis in the causation of prostate cancer. We investigated the *SRD5A2* gene Ala49Thr (rs9282858), Val89Leu (rs523349) polymorphisms and variation of TA)n repeats in a cohort of 91 PC patients and 100 controls, matched for sex, age and area of residence. The enzyme 5α-reductase, which converts testosterone to dihydrotestosterone (DHT), performs key functions in the androgen receptor signaling pathway. There were no significant correlations of A49T polymorphism and (TA)n repeats with the disease. The analysis of the V89L polymorphism showed significant differences in the distribution of allele and genotype frequencies between PC patients and healthy individuals. The frequency of genotype *SRD5A2*V/V* in patients was significantly higher (54.1%) than in controls (36.5%, χ2=4,27, p=0,04, OR=2,05, CI95% =1,03-4,09). The frequency of genotype *SRD5A2* L/ L* in control group was higher (18.9%) compared with patients (8.23%), but differences were not statistically significant (P>0.05).

The V89L variant was not associated with the grade or stage of prostate cancer, or with patient age.

Thus, the genotype of *SRD5A2*V/V* and allele and *SRD5A2*V* are showed to be markers of the increased risk for prostate cancer development in Bashkortostan Republic. Further large-sample studies will be required to confirm the association and to assess any interactions with environmental factors.

P06.184

The TMPRSS2/ERG fusion gene expression in tumor epithelium and tumor-associated stromal cells in prostate cancer.

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Due to intensive investigations for the last ten years it has become increasingly clear that tumor microenvironment plays a critical role in prostate carcinogenesis. Tumor microenvironment undergoes significant modification such as protein expression alterations and various genetic changes of stromal cells. Accumulation of multiplicity genetic alterations is typical not for only cancer epithelial cells but tumor-associated fibroblasts and endothelial cells as well. Further more these stromal cell alterations are not tumor epithelium specific.

We investigated *TMPRSS2/ERG* fusion gene expression in prostate cancer and tumor microenvironment. Tumor epithelia and tumor-associated stroma 34 prostatectomy specimens from patients with pT1-T4 stage prostate cancer were isolated using laser capture microdissection. mRNA expression of *TMPRSS2* and *TMPRSS2/ERG* significant in prostate carcinogenesis were investigated using RT-PCR following by sequencing.

We found *TMPRSS2/ERG* expression only in 65% (22/34) tumor epithelium samples and neither of adjacent tumor stroma. Also we detected *TMPRSS2* expression in all tumor epithelium samples and 5/34 stroma specimens. The possible explanation of *TMPRSS2* expression in the microenvironment is the presence of single tumor cells, which we observed by cytokeratine IHC staining. Nevertheless the fusion gene expression is not characteristic for the tumor-associated stroma.

The finding of frequent genetic alterations in tumor-associated stroma suggests a more important role for stromal fibroblasts in prostate cancerogenesis than was previously appreciated. Some molecular alterations are common for prostate cancer and tumor microenvironment but *TMPRSS2/ERG* fusion gene is the tumor feature only.

P06.185

Genome-wide increase in differential DNA methylations in TMPRSS:ERG fusiongene negative prostate tumours

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Prostate cancer is the second most common cancer among men worldwide. Despite intensive scientific efforts basic molecular reasons mostly remained obscure. Lately, it became evident that alterations in the DNA methylation pattern can be one of the leading causes for tumour formation. Therefore, we initiated the first high throughput sequencing study investigating genome-wide DNA methylation patterns in a large cohort of 104 human prostate tissues including normal controls using MeDIP-Seq. Comparative analyses identified more than 147,000 cancer-associated epigenetic alterations, affecting more than 75% of homeobox genes and 50% of the known cancer associated genes in their promoter region.

We could show that the increased expression of EZH2, originating in about 50% of prostate tumours in an ERG involving fusiongene (TMPRSS2:ERG), might explain the remarkable differences in DNA methylation between tumour and normal tissues. The methylation patterns, however, are strikingly more dissimilar in TMPRSS2:ERG fusiongene negative samples to normal prostate tissues than those in fusiongene positive tissues. We identified a mechanism of hypermethylation of miRNA-26a as an alternative pathway of ERG independent EZH2 activation which in turn can explain the observed increase in differential methylation in fusiongene negative tumours.

P06.186

Interaction of the RAD51 paralogs in the mammalian 2/3 hybrid system

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The five RAD51 paralogs, RAD51B, RAD51C, RAD51D, XRCC2 and XRCC3, play an important role in homologous recombination, a process crucial for the error-free removal of DNA double-strand lesions. These proteins share 20-30% homology and interact with each other and the RAD51 recombinase, an ortholog of E. coli RecA. Previous investigations in the yeast two-hybrid system, co-immunoprecipitations from human cell extracts and co-expression in the baculovirus system have shown that the paralogs form two complexes, RAD51C/XRCC3 and RAD51B/RAD51C/RAD51D/XRCC2. RAD51B has been reported to stabilize the interaction of RAD51D and XRCC2. Here we aimed at extending these findings in a human cellular environment. For that purpose we used the mammalian two- and three-hybrid system (M2/3H). We cloned full-length cDNA of each of the five RAD51 paralogs into the pM and pVP16 vectors. In M3H studies, the additional expression vector pCMV-Tag3b was used. Firefly luciferase (pGl4.31) served as reporter gene for the assay. As control for transfection efficiency we co-transfected renilla luciferase (pRL-null). As internal standard and positive control we employed the reported interaction of FANCA and FANCG. In M2H studies the previously reported interactions were confirmed, except that of RAD51C and RAD51D. This weak interaction required the presence of RAD51B for activation or stabilization, whereas XRCC2 did not boost it. Likewise, some of the other interactions were be influenced by a third paralog in M3H studies. Additional RAD51 protein seemed not to effect interactions of RAD51C/RAD51D and RAD51D/XRCC2 but those of RAD51C/XRCC3 and RAD51C/RAD51B. Bidirectional testing showed slight differences in interaction strength.

P06.187

Detection of micro-metastases of renal cell carcinoma by CA9 marker using Real-time PCR

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The global prognosis of renal cell carcinoma (RCC (remains poor, about 40% of patients will develop metastasis after nephrectomy. There is a strong need to identify the early metastasis with conventional and molecular risk factor. The analysis of molecular markers provides a new tool for prediction of prognosis for early metastasis. The present study aimed to test if analysis of the CA9 gene in peripheral blood can provide useful information to predict Micro metastasis.

In this experimental study, patients (n=30) with a renal cell carcinoma were evaluated for peripheral blood CA9 expression none randomly. Data of tumor grade were received from pathologists. Total RNA extraction and cDNA synthesis was performed and CA9 gene expression level were compared between patients and normal group (n=16) by Real-time PCR.

Six of patients show high CA9 expression (3 in grade I, 2 in grade II and 1 in grade III) but no significant difference was found between CA9 expression level and tumor grade. After one year follow up 4 patients were found to have a metastasis, but no significant difference was found between CA9 expression level and metastatic patients. (p>0/05)

Ca9 is a tumor-specific marker for RCC with a high degree of expression in the conventional renal cell carcinoma. On the basis of the results of this study, the detection of Ca9 gene expression in the peripheral blood of patients with RCC may prediction factor for increasing risk of micro metastasis.

P06.188

Identification of new diagnostic markers for renal cell carcinoma

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The renal cell carcinoma (RCC) is one of the most frequent malignant tumours and is often associated with the loss of function of the von-Hippel-Lindau gene (VHL). The ubiquitin ligase VHL triggers the degradation of the hypoxia-inducible factor α (HIFα) under normoxic conditions. HIF is the major transcription factor reacting to hypoxia. As in the majority of cases this tumour is resistant to chemotherapy. Therefore, the aim of the study was to identify possible new diagnostic and therapeutic target genes, like VHL, in the HIF pathway in 24 RCC cell lines derived from patient tumour tissue. The cell lines were characterized with respect to mutations in VHL, the expression profile of HIF associated genes (HAF, FIH, VEGF, CMET, MITF, EGFR, TGFβR) at RNA and protein level, proliferation, anchorage independent growth and activation of signalling pathways. The cell lines show a high variability concerning generation time in general and with respect to serum reduction and anchorage independent growing. The identified expression profile of HIF associated genes reveals possible target candidates for diagnostics. VHL sequencing identified both known and unknown mutations. Most of the VHL mutations were found in exon 1. 11 of 18 analysed cell lines had no VHL mutations in exon 2 and 3. First results gained by linking immune blots and expression profiling suggest a correlation of FIH protein expression with HIF target gene vascular endothelial growth factor (VEGF) expression. New possible targets associated with HIF for example FIH were identified to diagnose RCC and could be therapeutically relevant.

P06.189

Vitamin D receptor polymorphisms and renal cancer risk in Bashkortostan Republic of Russia

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Renal cell carcinoma (RCC) is the most common neoplasm affecting the adult kidney. One of the most important events in RCC is metabolism of vitamin D, which exerts its activity through binding to the nuclear vitamin D receptor (VDR).

The aim of investigation was to analyze risk of RCC in patients from Bashkortostan Republic depending on *VDR* polymorphisms. A case-control association study included 176 RCC patients and 165 controls, matched for age, sex and area of residence. We used PCR-RFLP genotyping of *VDR* gene polymorphisms at tree localizations rs731236 (TagI), rs7975232 (ApaI), rs1544410 (BsmI), rs2228570 (FokI).

Statistically significant differences were observed in the TagI genotype t/t between RCC patients and controls (p=0.024, OR=2.93 (95%CI 1.13-7.87)). Analysis of other gene polymorphisms didn‘t show significant differences between patients and controls (p>0.05) in general cohort and taking into consideration sex, pathological stage and histological grade of RCC.

The polymorphisms *BsmI, ApaI, TaqI* of *VDR* gene demonstrated strong linkage disequilibrium (D‘>30%). The FokI wasn't in linkage disequilibrium with any of the other examined *VDR* polymorphisms (D‘<30%).

Haplotype analysis showed that haplotypes tAB (p=0.0030), tAb (p=0.0125), TAB (p=0.0393), TaB (p=$1.9606e^{-5}$) were significantly prevalent in RCC patients. The most patients with lower stage of RCC had haplotype tAb (p=0.002), whereas patients with higher stage had haplotype tAB (p=0.02). We revealed haplotype tAb (p=0.002) of *VDR* gene to be a risk factor for RCC development in males.

The analysis of genetic variation in *VDR* gene may provide insight into the role of vitamin D in RCC development.

P06.190

Amplicon-Based Ultra-Deep Next-Generation Sequencing and its application to characterize mutated transcripts of RB1 gene in peripheral blood cells of childern patients with germinal retinoblastoma

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Introduction: RB1 (gene controlling cell division) mutations constitute a disease-defining molecular aberration in Retinoblastoma, the most common primary ocular malignancy (retina cancer) of childhood. In germinal retinoblastoma (40% of all retinoblastomas) a germline RB1 cancer-predisposing mutation is present in all of the body‘s cells. Therefore molecular diagnostics of germinal retinoblastoma is performed by PCR assays targeting the RB1 gene with genomic DNA, obtained from peripheral blood. Mutational analysis of RB1 transcripts obtained from peripheral blood using current sequencing standard - Sanger capillary sequencing (which sensitivity is about 15%) is complicated by the nonsense-mediated mRNA decay (NMD), an mRNA surveillance pathway that ensures the rapid degradation of mRNAs containing premature translation termination codons. The Next-Generation Sequencing (NGS) - technology Roche 454 is based on pyrosequencing. It means that each sequenced fragment is clonally copied. This allows increasing of sensitivity under 1%

Method: In amplicon covering transcript of RB1 gene we applied the 454 Titanium chemistry assay (454 Life Sciences) to perform ultra-deep sequencing of specific cDNA PCR products using the GS Junior System sequencer from Roche's 454 Life Sciences. In median, 5 446 reads per amplicon were generated, thereby allowing a highly sensitive assessment of mutational burden in RB1transcripts of retinoblastoma patients. To assess sequencing error rates, we included a control amplicon from the normal transcript.

Conclusion: We here demonstrate that amplicon-based ultra-deep NGS is a suitable method to accurately detect and quantify the variety of transcript aberrations with high sensitivity and enables an individualized monitoring of disease.

P06.191

Renal tumour after maternal transmission in a SDHD-linked pedigree

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The succinate dehydrogenase complex-subunit D (SDHD) gene, located on chromosome 11q23, encodes an anchoring subunit of the mitochondrial respiratory chain complex II. It functions as a tumour suppressor gene and causes hereditary paraganglioma type 1 when mutated. Renal tumours have also been associated to SDHD mutations.

Mutations in SDHD exhibit a clear parent-of-origin effect, with autosomal dominant paraganglioma occurring only with paternal transmission, though maternal transmission has been rarely described. Since the maternal allele of SDHD is not methylated, a number of hypothetical mechanisms have been suggested to explain the observed transmission pattern: 1) an unidentified tumour suppressor gene is inactivated in the imprinted region on chromosome 11p15; 2) the maternally derived SDHD is partially inactivated; 3) tissue-specific quantitative imprinting of SDHD confers subtle allele-specific expression differences.

Our aim was to characterise the inheritance pattern of SDHD-Trp5Stop mutation in a 4-generation family, mainly affected with paraganglioma.

Twelve microsatellite markers on 11p15 and 11q13-q23 were genotyped in germline DNA from 15 mutation carriers, as well as in tumoral DNA from the 6 patients (4 paraganglioma, 1 pheocromocytoma, 1 renal tumour). All the tumours, except the renal one had paternal inheritance of the mutation and showed LOH of the twelve microsatellites. The mutation was inherited from the mother in the patient with a renal tumour, who also had prostate cancer. In the latter, LOH of the twelve microsatellites was found in the renal but not in the prostate tumour, which confirms that maternal inheritance of SDHD mutations can cause this disorder.

P06.192

shRNA Mediated RHOXF1 Knock Down in Breast Cancer Cell Lines

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Background: RHOXF1 has been shown to be expressed in embryonic stem cells, adult germline stem cells and some cancer lines. It has been proposed as a candidate gene to encode transcription factors regulating downstream genes in the human testis with an antiapoptotic effect. Its expression in cancer cell lines has implied a similar role for it in the process of tumorigenesis.

Method: The human breast cancer cell lines MDA-MB-231 and MCF-7 were cultured in DMEM medium supplemented with 10% fetal bovine serum,100 U/ml penicillin, and 100 μg/ml streptomycin. Cells were transfected with pGFP-V-RS plasmid having RHOX1 specific shRNA cassette and plasmid with scrambled sequence cassette as the negative control. Transfection efficiency was monitored by the expression of green fluorescent protein (GFP) 24 hours post transfection. RNA was extracted from cells 48 hours after transfection and cDNA was synthesized. Quantitative real-time RT-PCR was performed for RHOXF1, CASP8, BCL2 and HPRT genes.

Results: Decreased RHOXF1 expression was confirmed in cells after transfection. shRNA mediated knock down of RHOXF1 resulted in significant decrease in BCL2 expression in both cell lines but no change in CASP8 expression.

Discussion: RHOXF1 can mediate transcriptional activation of the BCL2 in cancers, so render tumor cells resistant to apoptotic cell death induced by anticancer therapies. shRNA targeting RHOXF1was shown to specifically mediate the RHOXF1 gene silencing. shRNA mediated knock down of RHOXF1 can be effective in induction of apoptotic pathway in cancer cells via BCL2 downregulation, so it can have potential therapeutic usefulness in human breast cancer.

P06.193

Evaluation of silibinin on CD82 gene in prostate cancer PC-3 cells using quantitative Real-time PCR

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Prostate cancer is one of the most common cancer in developed countries. Most of cancer deaths are due to the development of metastasis. Hence, the prevention of metastasis process is critical. Silibinin is a flavonoid component that inhibits cell proliferation and causes cell death of human prostate cancer. In this study, the expression of CD82 gene in PC-3 cells treated with escalating concentrations of silibinin was evaluated that can result in new view for prostate cancer therapy.

In this study, PC-3 cells were treated with different concentrations of silibinin at 24h. The IC50 was determined. RNA was extracted by trizol. Then cDNA was synthesized. Precise primers were designed for CD82 and GAPDH genes by specific software. Quantity of CD82 gene expression compare to GAPDH gene in different concentrations of silibilin was analyzed using very sensitive quantitative Realtime PCR. CD82 gene expression in PC-3 cells treated by 100, 150 and 200μg/ml of silibinin at 24h were increased as 1.97±0.26 (P<0.05), 3.00±0.26 and 3.43±0.43 (P<0.01), respectively.

The results of quantitative Real-time PCR indicated that silibinin can probably decrease metastasis, by up-expression of CD82 metastasis suppressor gene in PC-3 cells.

P06.195

The ancestral haplotype 8.1 (AH8.1) in the major histocompatibility complex (MHC) region is a strong and selective risk factor for small cell lung cancer

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AH8.1 is a haplotype which extends through the whole MHC region in the short arm of chromosome 6. It is the most frequent, very conservative haplotype in the Caucasian population. Previously we have reported on a strong association of AH8.1 and colorectal cancer with an odds ratio of 2.51 which was higher in subjects of <65 years (4.07) or among women (3.77). It is far higher than any risk reported for SNPs in the GWAS or candidate gene studies. Literature data indicate that AH8.1 is a strong risk factor for ovarian cancer and for non-Hodgkin lymphoma as well. Here we have determined the carrier state of AH8.1 in 105 patients with small cell lung cancer (SCLC) (61.6±7.8 years), 91 patients with non- SCLC (58.7±9.0 years) and 252 age-matched control subjects (66.7±7.3 years). Subjects carrying all the four marker alleles of AH8.1 (C allele of AGER 429T>C, G allele of HSP70-2 1267A>G, A allele of TNFalpha -308G>A as well as G allele of LTA 252A>G polymorphisms) were considered as AH8.1 carriers. 23/105 (22%) SCLC patients, 13/91 (14%) non-SCLC patients and 32/252 (13%) healthy controls carried the AH8.1 haplotype. Odds ratio of the AH8.1 carriers for SCLC was 1.93 (1.07-3.49), p=0.036) (for men: 3.15 (1.23-8.07) p=0.025), while for non-SCLC it was not significant. These findings indicate that carriers of the AH8.1 haplotype are at increased risk for SCLC similarly to colorectal and other types of cancer. This high risk is most probably due to the altered immune system of the AH8.1 carriers.

P06.196

Splicing functional assays of a single minigene with eight exons of the BRCA2 gene

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Splicing disruptions is one key pathogenic mechanism in inherited diseases. We are currently investigating the contribution of aberrant splicing of BRCA1/2 genes to hereditary breast/ovarian cancer. A powerful approach to study the splicing outcomes of DNA variants is a splicing reporter minigene especially when patient RNA is not available. We constructed a single minigene of 8 BRCA2 exons (19 to 26) in a pSPL3-derived plasmid in 5 cloning steps, which is, as far as we know, the largest BRCA2 minigene ever reported. The genomic fragment from exons 19 to 26 is more than 27 kb in length that was reduced to a final insert of 4.7 kb with internal deletions of introns 20, 21, 24 and 25.This construction was transfected in HeLa cells and we observed a main RNA isoform of the expected size of 1.5 Kb that contained the vector constitutive exons and BRCA2 exons 19 to 26. Several splicing variants of each exon were generated in the wild type minigene by PCR mutagenesis and assayed to demonstrate the usefulness and reliability of this large construction. Splicing reporter minigenes are straightforward and robust tools to distinguish between pathogenic mutations and innocuous variants. The use of minigenes with several exons facilitates the analysis of putative splicing variants in a single minigene and emulates the physiological genomic context where the splicing reactions take place.

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P06.197

Application of High Resolution Melting Technique for detection of Germ Line single Nucleotide Polymorphisms in STK11 Gene among Patients with Various Gastrointestinal Cancers

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High Resolution Melting is a method that analyzes genetic variations such as Single Nucleotide Polymorphisms in PCR amplicons. Since HRM characterizes nucleic acid samples based on their disassociation (melting) behavior, the nucleotide sequence of amplicon is an important factors affecting the melting curve.The STK11 gene encodes a member of the serine/threonine kinase and regulates cell polarity and function as a tumor suppressor gene. The germ-line mutations in this gene are associated with Peutz-Jeghers syndrome. The patients with this syndrome are prone to some types of neoplasm.

Genomic DNA was extracted from the whole blood samples of 56 patients with various gastrointestinal cancers. The nucleotide changes in the entire STK11 gene were analyzed by Real-time PCR and HRM technique.

The nucleotide screening by HRM technique showed two types of SNPs in introns 6 and 7 of STK11 gene in 10 patients. Four patients had C / T substitution [cluster id/dsSNP/rs9282860] with homozygous genotype in intron 6, and six patients showed a C/G substitution [cluster id/dsSNP/rs2075607] with heterozygous genotype in intron 7. The direct sequencing of the fragments confirmed that the results obtained by HRM were 100% reliable.

In this study we found no SNP in exons of STK11 gene. However, two SNPs were found in the introns of this gene. Our results show that the primary screening of the STK11gene by the HRM technique is easily applicable to detect the unknown germ line and somatic mutations in patients with neoplasia at a relatively low cost.

P06.198

Recurrent and novel SS18-SSX fusion transcripts in synovial sarcoma: description of three new cases

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Synovial sarcoma (SS) is an aggressive type of tumor, comprising approximately 10% of soft tissue sarcomas. Over 90% of SS cases are characterized by the t(X;18)(p11.2;q11.2) translocation, which results mainly in the formation of oncogenic SS18-SSX1 or SS18-SSX2 fusions. In a typical SS18-SSX fusion transcript, exon 10 of SS18 is fused to exon 6 of SSX1/2. However, several variant fusion transcripts have been already described. In the present study, we examined the fusion transcript type in a series of 40 primary untreated SS tumor specimens using reverse transcription polymerase chain reaction (RT-PCR). We detected SS18-SSX1 transcript in 22 (55%) patients and SS18-SSX2 transcript in 17 (42,5%) patients, while in one patient none of SS18-SSX1/2 fusion transcripts were identified. Among the cases under study, two tumors carried novel SS18-SSX1 and SS18-SSX2 variant translocations that were allegedly created by an alternative splicing and in additional case an unusual translocation variant previously described by other group was found. Our data suggest that alternative splicing may play an important role in novel fusion transcript formation and additionally we show that it may be a recurrent event in SS. Furthermore, we describe the first case of a complex rearrangement possibly linking SS to REPS2 gene.

P06.199

Genomic and epigenomic characterization of T-cell prolymphocytic leukemia (T-PLL)

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T-PLL is an aggressive postthymic T-cell malignancy with distinctive clinical, morphologic, cytogenetic and molecular features (*Dürig et al., Leukemia, 2007*). We have initiated a comprehensive genetic and epigenetic profiling of T-PLL. First, we investigated the presence of the hallmark changes inv(14)(q11q32)/t(14;14)(q11;q32) and t(X;14)(q28;q11) by interphase FISH using probes for the TCRAD locus in 14q11 and its both partners involved in the named changes, i.e. TCL1 in 14q32 and MTCP1 in Xq28. In 43 cases with features of T-PLL acquired over the last 25 years we identified TCRAD breaks in 93%, TCL1 breaks in 74% and MTCP1 breaks in 14%. TCL1 and MTCP1 breaks were mutually exclusive. Two cases contained a TCRAD break with a partner other than TCL1 and MTCP1 and 3 cases did not show breaks in any of these three loci. To determine the pattern of secondary genetic changes on a base pair level we have performed custom full genome sequencing of flow-sorted T-PLL cells and corresponding non-T-cells of the same patients using Complete Genomics* technology. Initial analyses of the first three patients suggest the genomic landscape of T-PLL to be highly complex with a mean number of 150 protein-changing somatic single nucleotide mutations and in-dels. Validation of these findings and extension into the full cohort is ongoing and supplemented by DNA-methylation profiling using Illumina 450K Methylation BeadArrays. We are confident that the combined genomic and epigenomic profiling of T-PLL will identify potentially druggable pathways in this still poor-prognosis disease.

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P06.200

Genetic replication study of susceptibility loci for testicular germ cell cancer in the Croatian population

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Testicular germ cell tumour (TGCT) is the most common cancer in young men showing a pronounced degree of heritability. Recent genome-wide association analyses in British and US samples have identified six susceptibility loci of genome-wide significance. The goal of our study was to perform a genetic replication analysis of these loci in an independent European population. We therefore analyzed six single nucleotide polymorphisms [rs2900333 (ATF7IP), rs210138 (BAK1), rs755383 (DMRT1), rs995030 (KITLG), rs4624820 (SPRY4), and rs4635969 (TERT/ CLPTM1L)], each representing one of the published susceptibility loci, in a Croatian case-control sample consisting of 331 tumour-free male controls (> 50 years of age) and 325 cases. Indeed, five of these SNPs were found to be associated in the Croatian population: rs995030 (OR 2.94, p=1.835e-10), rs755383 (OR 1.53, p=0.00023), rs210138 (OR 1.68, p=0.00031), rs4624820 (OR 1.50, p=0.00041) and rs4635969 (OR 1.35, p=0.01739), a finding which is still significant after conservative correction for multiple testing. Similar to previous studies, the association was comparable for different histological subtypes. Moreover, we evaluated if any SNP was associated with aggressiveness of TGCT measured by different staging categories. Interestingly, while rs2900333 near ATF7IP just showed borderline association with all-TGCT [OR 1.26, p=0.062], it showed significant association with the more aggressive forms of the tumour [OR 1.55, p=0.0067]. In summary, our data provide further evidence that the previously identified loci are involved in the susceptibility for TGCT and suggest a possible role of ATF7IP in regulating the progression of TGCT, although it has to be confirmed in independent samples.

P06.201

Comprehensive mutation screening of miRNA loci in testicular germ cell tumours

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MicroRNAs (miRNAs) are endogenous small non-protein coding RNAs which regulate basic cellular processes. There is considerable evidence that expression of miRNA genes is deregulated in human cancer, and specific over- or underexpression has been shown to correlate with particular cancer types.

Testicular germ cell tumours (TGCTs) are the most common malignancy affecting males between the ages of 15 and 45 years, and are associated with significant morbidity including infertility. These cancers are most commonly formed from undifferentiated fetal germ cells contained within the testis. There are several methods for identifying variants in DNA. High-resolution melting curve (HRM) analysis and multiplex ligation-dependent probe amplification (MLPA) are two sensitive, cost effective and high throughput techniques for rapidly screening a large number of DNA samples. To identify point mutations we performed HRM analysis on eight miRNA loci implicated in either testis cancer and/or pluripotency. To identify deletions and duplications we developed an MLPA mix containing probes that recognize 50 miRNA sequences that have been identified as being deleted or duplicated in tumours.

We have carried out a pilot study using these two approaches on 48 TGCT samples. To date we have identified a number of potential variants that we are currently confirming with independent techniques. Given the success of this initial screen we plan to study an additional 100 DNA samples. Our findings will give a better understanding of the genetic basis of testis cancer, and may lead to improved diagnostic or therapeutic protocols.

P06.202

Whole exome analysis of testicular germ cell tumours

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Testicular germ cell tumours (TGCTs) are the most common cancer in adolescents and young men, with a median age of diagnosis of approximately 30 years. Although the majority of cases can be successfully treated with radical surgery and/or chemotherapy, these treatments have significant undesirable side effects including renal, vascular, neural toxicity and negative effects on fertility. There is also compelling evidence for long term increased risks of secondary malignancies, cardiovascular disease and metabolic syndrome.

Despite the frequency with which TGCTs occur, little is known about the genetics underlying TGCT initiation and progression. In order to obtain a better understanding of this condition we have performed whole exome analysis on DNA from two TGCT samples, plus matched non-tumour material. Exome capture was carried out with the SureSelect Human All Exon kit from Agilent, which targets 44 Mb of exonic regions, and sequencing was performed on an Illumina HiSeq 2000 instrument.

Data analysis identified several potentially de novo variants that we are currently validating, and we are planning to screen a larger cohort of TGCT samples for specific genes. This study will improve our understanding of the genetic basis of TGCT, with implications for diagnosis and therapy.

P06.203

Nuclear localization of human homeodomain TGIFLX gene and its mRNA expression in stable cancer cell lines

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Background and Aim: Homeodomain transcription factors play a central role in expression of genomic information in all organisms. TGIFX homeobox gene was originally discovered in the human adult testis. Our previous study suggested that TGIFLX could be involved in prostate cancer and azoospermia. The main aim of our study is to analyze the function of TGIFLX protein. One way to analyze transcription factors in eukaryotic cells is to study their nuclear localization, as reported for various organisms such as human and land plants using pEGFP-N1 vector, a eukaryotic expression vector encoding EGFP.

Methods: We cloned entire coding sequence of *TGIFLX* gene into above plasmid and subsequently, SW48 and Caco2 colorectal cancer cell lines was transfected with the recombinant vector harboring *TGIFLX* cDNA. Gene expression analysis of TGIFLX verified using RT-PCR and western blot techniques.

Results: The TGIFLX expression was confirmed by microscopic analysis and RT-PCR technique. Following molecular cloning and characterization of *TGIFLX* transcription factor in stable cell lines , we then for first time studied the nuclear localization and expression of TGIFLX in colorectal cancer cell lines (SW48, Caco2) by means of imaging and tracking of GFP molecules. Interestingly, we found aberrant expression of *TGIFLX* mRNA in SW48 but not in the Caco2 cell line.

Conclusion: This is the first report to perform visualization of nuclear localization of TGIFLX transcription factor by establishing new colorectal cancer cell lines with this gene and potential involvement of *TGIFLX* gene dysregulation in human colorectal cancer cancers.

P06.204

Analysis of frequency CHK2 gene sequence variants: R145W and I157L in Polish patients with differentiated thyroid cancer and Polish population

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Differentiated thyroid cancer (DTC) originate from thyroid follicular C cells and belong to group of slowly progressing benign tumors with good prognosis. Very serious problems are recurrences and regional or remote metastasis. Numerous cases of osteolytic, cerebral and pulmonary metastasis were observed. Progression from well differentiated thyroid cancer to malignant anaplastic carcinoma is possible.

Very important seems to be searching for molecular markers of disease course, good or poor prognosis and response on medical treatment. SNP polymorphisms research in genes associated with neoplastic diseases will be helpful in understanding of molecular mechanisms of thyroid gland tumors development and allow to better diagnosing.

Mutations in CHK2 gene are thought to predispose to sarcomas, breast cancer, and brain tumors. Protein product of this gene is a cell cycle checkpoint regulator and tumor suppressor and is a member of the CDS1 subfamily of serine/threonine protein kinases.

We examined two sequence variants in CHK2 gene in group of 516 Polish patients with differentiated thyroid cancer and 500 individuals from population group. I157T and R145W variants were analyzed by pyrosequencing. There were differences in allele and genotype frequencies in analysis of I157T variation. Allele C was present with frequency 0,05 and allele T - 0,95 in patient with thyroid cancer, compared with 0,03 and 0,097 in control individuals respectively. The differences in allele frequencies were statistically significant (p=0,0072).We didn't observed any variability in R145 position neither in DTC patients nor population group. Project supported by Polish National Science Center grant NN402287436.

P06.205

Evaluation of CYP2C9, CYP2C19 and CYP2D6 gene polymorphisms in thyroid cancer

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Thyroid cancer incidence has increased worldwide during the previous years. Incidence of thyroid cancer is 33599 in European Union countries. It is 1,4 % of all cancers. Mortality of tyhroid cancer is 0,3%.

CYP2C9, CYP2C19 and CYP2D6 genes have been reported to be coding the enzymes responsible for the metabolism of many drugs, including warfarin and other drugs with a narrow therapeutic index. Realising the importance of inter-individual differences in the genetic profile in determining the outcome of a drug therapy. This study was conducted to explore the types and frequencies of CYP2C9, CYP2C19 and CYP2D6 alleles in healthy controls and thyroid cancer patients.

Total genomic DNA was isolated from peripheral blood samples with EDTA and spin column method. A total of 103 subjects including 49 healty control and 53 thyroid cancer patients were recruited into the study. CYP2C9 allele *1, *2, *3, CYP2C19 allele *1, *2, *3 and CYP2D6 allele *1, *2, *3, *4,*5 have been studied by real time PCR method for both two groups.

In thyroid cancer group allel frequency of CYP2C9 *2 was 4,62%, *3 was 19,44%. CYP2C19 *2 and *3 allel frequencies were 17,59% and 0% respectively. Those ratios were 9,18%, 6,12%, 11,22% and 5,10% respectively.

According to our study, genes have roles in drug methoblism like CYP genes may act an important role in cancer ethiopathogenesis and further studies including larger control and patient groups are needed.

P06.206

The possible role of the xenobiotic transporter P-glycoprotein polymorphism that encoded by the MDR1 3435 C>T gene in the susceptibility of differentiated thyroid cancer

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P-glycoprotein (Pgp), encoded by the multidrug resistance 1 (MDR1) gene is an efflux transporter and plays an important role in pharmacokinetics. The current preliminary study was designed to determine association between germ-line polymorphism in MDR1 gene with differentiated thyroid carcinoma (DTC). In the current case-control study of 60 thyroid carcinomas (TC); 45 papillary TC (PTC), 9 follicular TC(FTC) and 6 differentiated TC(DTC) of well-differentiated TC of uncertain malignant potential were examined. Genomic DNA was extracted from peripheral blood with EDTA, target gene was genotyped by multiplex Real-time PCR and PCR-based reverse-hybridization StripAssay method. Carriers of the variant allele of MDR1 exon 26 polymorphism were at 2.8-fold higher risk of DTC than the control group(odds ratio [OR]: 0.3805, 95% confidence interval [Cl]: 0.1597-0.9065.There was an association between DTC and MDR1 C3435T polymorphism in the presented results(p> 0.046). Presented results showed that the homozygous MDR1 3435TT genotype increases the risk factor to develop differentiated thyroid cancer and large-scale studies are needed to validate this association.

P06.207

Exploring the methylome of thyroid cancer at single C resolution: From screening to clinical diagnostics

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Thyroid nodules are endemic in iodine deficient areas, like Europe's alpine regions, where they have a prevalence of 10-20 %. Since 5% of these nodules are malignant, all thyroid nodules have to be clinically evaluated to discern benign from malignant cases. The current method of choice for diagnosis is based on fine needle aspiration (FNA) followed by cytological evaluation. Despite many advances in the diagnosis and treatment of thyroid nodules this method has a high rate of suspicious or indeterminate (e.g. follicular neoplasia) diagnosis. Patients with such a suspicious diagnosis will undergo surgery. The consequence is an extensive overtreatment of patients, as only approximately 20% of the indeterminate cases will be identified as malignant at surgery.

In the present study we address the call for minimally invasive diagnostics based on state of the art molecular techniques to clearly discriminate between the different benign and malignant thyroid entities. Therefore we employed 48 thyroid nodules, consisting of follicular thyroid adenomas/carcinomas (FTA/FTC), papillary thyroid carcinomas (PTC) and struma nodosa (SN) to a whole genome methylation screening using Illuminas Infinium 450k BeadArrays. The data gave first insights in the methylome of thyroid nodules at single C resolution at a whole genome scale. We could clearly differentiate between the different histological groups based on their methylation patterns. We present a bioinformatics analysis and assessment of the discriminatory power for the differentially methylated genomic regions.

P06.208

miR-106b is down-regulated in follicular carcinomas and may modulate the expression of C1orf24 in thyroid carcinoma cell lines

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We have previously showed that C1orf24 (alias NIBAN) is highly expressed in thyroid carcinomas compared to benign lesions. However, the molecular mechanism underlying its expression remains unclear. One of the post-transcriptional mechanisms of gene regulation is the action of the microRNA. microRNA expression varies according tissue, development stage and cancer types. Therefore, we used miRBase Sequence database to identify microRNAs that potentially regulate C1orf24 expression. miR106b was selected for further validation by quantitative PCR in 64 thyroid nodules (09 CVPTC, 18 FVPTC, 11 FTC, 11 HCA and 15 FTA). The data obtained by qPCR showed that the expression of miR106b was down-regulated in malignant lesions compared to benign lesions (p value 0.0060), while C1orf24 expression presented high levels in malignant ones (p value 0.0018). Therefore, to investigate whether miR106b modulates the C1orf24 expression, miR106b was transiently transfected into a follicular thyroid carcinoma cell line (WRO), which we have previously show to have high levels of C1orf24 expression. The results showed that the ectopic expression (30nM at 72hr) of miR106b decreases both C1orf24 mRNAs (p value < 0.05) and proteins levels when compared to negative control. These finds suggest that miR106b may modulate C1orf24 gene expression and may play an important role in thyroid tumorigenesis. Additionally, it may help understand the molecular mechanisms underlying its activation in other tumor subtypes where it was found highly expressed.

P06.209

Comparison of genetic changes in schistosome-related transitional and squamous bladder cancers using fluorescence in situ hybridization.

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In western countries, more than 90% of primary bladder carcinomas are transitional cell carcinoma (TCC), whereas squamous cell carcinoma (SCC) comprises less that 10%. Carcinoma of the urinary bladder is the most common malignancy in many tropical and subtropical countries due to endemic infection by *Schistosoma hematooium*. Compared with non-schistosome bladder cancer, schistosome-related bladder cancer has different clinical and pathological features.

In this study, dual-color FISH cytogenetic analysis was performed using two oncogenes (HER-2/neu and MCY) and a tumor suppressor gene (p53) in relation to chromosomes 8 and 17 centromeres in a group of patients presenting with schistosomal associated squamous and transitional cell carcinoma.

To the best of our knowledge, this is the first report to compare genetic alterations in both transitional and squamous subgroups of schistosomal bladder cancers in Egyptian patients using the FISH technique.

Thirty-sex percent of SCC cases showed gene amplification for **Her2/neu**. On the other hand, for the gene p**53**, 68% had gene deletion. Whereas 29% of the cases show gene amplification for c-myc gene. In TCC cases, 8 cases (23.5%) had gene amplification for Her2/neu gene. On the other hand, 50% of cases had two copies of the gene **P53**, and the other (50%) had gene **P53** deleted. While for the gene **C-myc**, 9 cases (26.4%) show gene amplification.

Our data showed that different histologic subgroups of bladder tumors are characterized by distinct patterns of genetic alterations. The genetic changes found in the transitional cell group are differing from tumors exhibiting squamous differentiation.

P06.210

TSPY and TSPX gene copy number assessment in patients with gonadal tumours, prostatic cell lines and control groups

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Background: TSPY gene is localized on Y chromosome and have a homologue TSPX on X chromosome. TSPY is specific expressed in testes. TSPX is normally expressed in ovaries and testis. Over expression of TSPY was discovered in tumour tissues. Product of TSPY accelerates a pass trough G2/M phase via cycline D2 and positively affects the cell proliferation. Over-

expression of TSPX or SET leads to cell retaining in G2/M.
Aim: Quantification of TSPX/X gene copy number and study of potential changes in the TSPY/X copies and their mutual ratios.
Method: There were assessed 10 women and 8 men patients with gonadal tumours, 5 prostatic cell lines (DU-145, LAPC-4, PC-3, RWPE-I, LNCeP), 80 woman and 80 man controls in the study. The study was supported by IGA UPOL LF_2011_004.
Relative copy number of TSPY/X genes was quantified by capillary electrophoresis in comparison to one-copy genes AMELY/X.
Results: We observed more TSPY gene copies in patients with seminomas compare to TSPX gene than in control group. Number of TSPX gene copies in men control group is higher than in patients with seminomas. More variability in TSPX gene copies was indicated in women with ovary carcinoma compare to controls. The women control group has more TSPX gene copies than patients with tumours in average. In prostatic cell lines DU-145, LAPC-4 and LNCeP was significantly increased amount of TSPY copies compare to control group.
Conclusion: Obtained data could contribute to understanding of TSPY/X gene role in tumor-genesis process in gametogenetic tissues.

P06.211

Evidences of the association between *UCP2* gene expression with Obesity, Family history of cancer and Metabolism

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Increased risk of cancer is one of the consequences of obesity. UCP2 has been implicated in free radical scavenging relevant to pathological processes, including obesity and has a unique role in energy balance and their responses to inflammatory stimuli. UCP2 expression was changed in human cancer and may correlate with the degree of oxidative stress. The aim of study was measurement of *UCP2* and *PGC1α* gene expressions *in vitro* in samples of PBMCs derived from all participants in a cellular model and to investigate their potential correlation with family history of cancer.
A total of 220 obese subjects were included in study. The PBMCs were separated, total cellular RNA was extracted and the cDNA was synthesized. Real-time PCR using specific primer pairs was performed. We analyzed our findings according to categorized group: group with a family history of cancer and Individuals without a family history of any cancer.
Of the 220 participants, 20 (9.09 %) had a family history of cancer and 200 (90.91%) hadn't family history of any cancer. The mean of age and BMI were 36.99±9.02years and 34.99±4.13kg/m^2 respectively. We found significantly lower relative *UCP2* gene expression in group with a family history of cancer. The estimated family history of cancer relative risk attributable to UCP2 gene expression was 1.46%. We found significantly lower *PGC1α* gene expressions in low *UCP2 gene expression* group.
It seems that the relative expression of involved genes in energy balance may have important role in cancer risk in obese subjects.

P06.212

Detection of *GNAQ* and *GNA11* mutations in plasma of metastasized uveal melanoma patients by deep amplicon sequencing

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Elevated levels of DNA are frequently found in the cell free plasma of cancer patients. Activating mutations in *GNAQ* and *GNA11* are highly specific for uveal melanoma. To establish a reliable assay which might allow for early detection and monitoring of metastatic disease we determined the proportion of *GNAQ* or *GNA11* mutant reads in DNA from cell free plasma of uveal melanoma patients by ultra-deep sequencing. We isolated cell-free DNA from 23 blood samples from patients with metastasized uveal melanoma. *GNAQ* and *GNA11* regions of interest were amplified on 6 ng DNA. To detect even low proportion of mutant sequence reads ultra deep sequencing of amplicons was performed (Roche GS Junior).Levels of DNA ranged from 20 to 1550 ng per ml of plasma. On average about 2600 sequence reads were obtained for each amplicon (range: 281 to 6191). We detected either GNAQ Q209 or GNA11 Q209 mutations in the plasma from 9 out of 23 patients. The proportion of mutant reads ranged from 2 to 38 %. However, the background noise (0.5% at any given nucleotide position) limited the sensitivity of detection. We found no correlation between amount of cell-free DNA in plasma and the proportion of mutant reads. This suggests that at least in some patients elevated DNA levels in the plasma do not originate from tumor cells. Ultra-deep amplicon sequencing can be used to detect low proportion of tumor DNA in plasma. This biomarker might thus be helpful to measure tumour burden and to monitor anti-metastatic treatment.

P06.213

Valproic acid achieves its anticancer activity by re-expression of cyclin D2

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Histone deacetylase inhibitors (HDACi) are widely known as remedies against epilepsy. But in the last years, HDACi research in the field of cancer expanded. In this study we demonstrated that the treatment of primary murine prostate cancer (PCa) cells derived from the well-established TRAMP (transgenic adenocarcinoma of mouse prostate) model with the HDACi valproic acid (VPA) has an anti-proliferative, anti-migrative and anti-invasive effect on the cells.
To our knowledge this is the first study that identified that treatment of PCa cells with VPA leads to the re-expression of cyclin D2, which is known to be frequently inactive in patients with PCa. Additionally, we could demonstrate that VPA specifically induces re-expression of cyclin D2 in human colorectal and mammary gland adenocarcinoma cell lines, whereas VPA treatment has no effect in NIH/3T3 fibroblasts. Moreover, the intensity of re-expression is dependent on the inhibition of proliferation, because for NIH/3T3 cells no inhibition of proliferation after VPA treatment was observed.
The re-expression of cyclin D2 can also be achieved by other HDACis. The conclusion that cyclin D2 re-expression observed in cancer cells after the treatment with VPA is activated by an increase of histone acetylation was shown by chromatin immunoprecipitation studies for the promoter region of the cyclin D2 gene. However, the re-expression seems not to be due to changes in the methylation status of the cyclin D2 promoter region. In summary, our results propose VPA as an anticancer therapeutic option in tumors with epigenetically repressed cyclin D2 expression.

P06.214

Current variant of uncertain significance rates in *BRCA1/2* and Lynch Syndrome testing (*MLH1, MSH2, MSH6, PMS2*)

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Laboratories that provide full gene sequencing frequently detect Genetic Variants of Uncertain Significance (VUSs). VUSs present a challenge to the clinician in how to appropriately guide the medical care of their patient in the context of an inconclusive test result. While the majority of VUSs are eventually discovered to be non-disease causing, some are pathogenic. Myriad Genetic Laboratories, Inc. has pursued protocols to collect sufficient data to appropriately reclassify VUSs. Statistical techniques that lead to VUS reclassification have been developed on a large *BRCA1/2* dataset. While the basis of these statistical techniques has been published, they have been further refined such that they are now applicable to not only *BRCA1/2*, but also Lynch Syndrome genes as well as *APC*. We report the current VUS rate in *BRCA1/2* as 3.0%, *MLH1+MSH2+MSH6* as 7.3% and *PMS2* as 4.4%. The continual drop in VUS rate through time reflects the success of these VUS reclassification techniques and Variant Classification Programs. In recent years the application of these techniques has also led to significant drops in VUS rates in non-European ethnic groups, with the most prominent change being in *BRCA1/2*. Characterizing VUSs gives the clinician the required information to appropriately manage their patient.

P06.215

Investigation of the VDR gene polymorphisms and expression association with Family history of cancer in obesity

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Population-based study demonstrated that VDR gene variation, especially *FokI* SNPs owing to its functional significance, may influence risk of some cancer. The aim of this study is to measure *VDR* expression *in vitro* in samples of PBMCs derived from all participants in a cellular model and to investigate their potential correlation with family history of cancer. We analyzed

VDR FolkI polymorphism (*rs* 10735810) and its correlation with VDR gene expressions in obese subjects with and without a family history of cancer separately.
A total of 190 obese subjects were included in study. *FOKI* was genotyped and the PBMCs were separated by Ficoll-hypaque technique. Total cellular RNA was extracted and Real-time PCR performed. We analyzed our findings according to categorized group: group with a family history of cancer and Individuals without a family history of any cancer.
23 (12.1 %) had a family history of cancer and 167 (87.89%) hadn't family history of any cancer. The mean of age and BMI were 35.32±8.31years and 33.32±3.24kg/m^2 respectively. The frequency of ff genotype was significantly higher in subjects with family history of cancer. We found significantly lower relative *VDR* gene expression in group with a family history of cancer ($p<0.05$). However *VDR* expressions were low in ff genotype in group without a family history of cancer, but its expression were low in every three genotypes in group with a family history of cancer.
It seems that the having ff genotype and lower expression of *VDR* could be associated with risk of cancer.

P06.216
Overexpression of VEGF isoforms generated by alternative splicing in head and neck cancer

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Vascular endothelial growth factor (VEGF) is a potent mitogen for endothelial cells and its overexpression is associated with tumor growth and metastasis. However, the selection of a alternative splicing site at the end of the exon 8 of *VEGF* gene results in a sister-family of isoforms, VEGFxxxb, that seems to have anti-angiogenic proprieties. The aim of this work was to quantitatively analyze the expressions of *VEGF* gene isoforms generated by alternative splicing in samples of head and neck squamous cells carcinoma and adjacent normal tissues, and to determine the effect of regulatory proteins (SRp55, SRp40, ASF/SF2 and SRPK1) in the control of *VEGF* gene splicing. The overexpression of both VEGF isoforms was observed in head and neck squamous cells carcinoma related to normal tissue samples. Positive correlation between VEGFxxx and VEGFxxxb expression was observed in head and neck tumors. SF/SF2 presented higher expression in tumors when compared to normal tissues. Pharynx tumors presented overexpression of VEGFxxx. VEGFxxxb was underexpressed in oral cavity tumors. Overexpression of both VEGF isoforms was observed in aggressive tumors. There was a positive correlation among ASF/SF2, SRp55 and SRp40 proteins and both VEGF isoforms, and among SRPK1 protein and ASF/SF2, SRp55 and SRp40 in tumor tissues. The results suggest that both VEGF isoforms play a role in angiogenesis promotion in head and neck tumors. VEGF isoforms present differential expression related to the anatomic sites of tumor and tumor aggressiveness. ASF/SF2, SRp55 and SRp40 proteins are involved in the regulation of the VEGF gene splicing mechanism.

P06.217
The additional effect of VHL and PTEN mutations in tumour development

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The von Hippel-Lindau (VHL) protein is well described as an ubiquitin E3 ligase for HIFalpha subunits but plays also a role in microtubule stability, ECM formation and cilia assembly. Due to germline mutations in the VHL gene patients develop retinal and CNS haemangioblastomas (HBs), pheochromocytomas (PCCs) and clear cell renal carcinomas (ccRCCs). Interestingly, cystic lesions of VHL patients possess an increased activation of phosphoinositide-3 kinase (PI3K) pathway and downstream mTOR signaling. The PI3K pathway is negatively regulated by PTEN which is one of the most frequently inactivated tumour suppressor genes. Partial deletion of Pten in mice results in tumour formation in a tissue specific manner. As PCCs are also part of the PTEN associated tumour spectrum, we hypothesized that VHL and PTEN may interact functionally in tumour suppression.
Here, we present two Vhl knockin mouse models with endogenous tumour formation. The effect of Vhl type IIB (V2B) and type IIC (V2C) germline mutations was analyzed over several generations. Additionally, Vhl knockin mutations were also combined with hemizygous inactivation of Pten, and the impact on tumour spectrum, incidence and tumour progression was investigated in over 300 mice of the distinct genotypes at the ages of 3 to 12 months. Pten knockout mice developed various tumours at 9 to 12 months independent of the Vhl genotype. Furthermore, V2B mice displayed a clear Vhl genotype dependent effect on the development of renal cysts. Additionally, we observed a significantly increase in incidence and tumour mass of PCCs in V2B and V2C compound hemizygous mice.

P06.218
Variability in von Hippel-Lindau disease-Manifestation rate throughout life

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Background
Clinical management and counseling of von Hippel-Lindau (vHL) families is complicated by variable phenotypic expression. Natural history of disease progression is not fully understood, and better knowledge of specific factors' influence would greatly benefit vHL management. We aim to describe the variability in vHL-manifestation development throughout life and to assess association to gender and genotype.
Methods
Full medical records were collected from 52 *VHL* mutation carriers, 26 female and 26 male. Patients were followed from birth, and age-dependent manifestation rates were analyzed using Poisson regression. Relative rates between age groups were compared using robust standard errors which allowed for heterogeneity between patients. Effects on manifestation rates of gender and genotype (truncating mutation versus missense mutation) were determined.
Results
Overall, 381 manifestations were diagnosed in 42 subjects, while 10 were asymptomatic mutation carriers. Maximum manifestation rate was reached in the 30-34 year age-group with 0.880 manifestations per year ((95% CI: 0.57-1.36). Compared to the 30-34 year group, the relative rates of especially the younger, but also of older groups were lower. We found a trend of a higher relative manifestation rate among patients with truncating *VHL* mutations compared to those with missense mutations ($p= 0.060$) and less when comparing men and women ($p= 0.58$).
Conclusions
Rate of manifestation development is associated to age, increasing from birth to the 30'ies. Also, truncating *VHL* mutation carriers seem to have a higher rate of tumor development. Better knowledge of factors influencing phenotypic variability will facilitate surveillance targeting and counseling of affected families.

P07. Cancer Cytogenetics

P07.01
CIZ Gene Rearrangements in Pediatric CD10-Negative Acute Lymphoblastic Leukemia

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The *CIZ (ZNF384)* gene, located distal to the *TEL (ETV6)* gene at 12p13.31, is a putative zinc finger transcription factor which is recurrently rearranged in acute leukemia. Rearrangements of the *CIZ* gene result in attachment of various 5' partner gene sequences to form *CIZ* fusion genes. The *CIZ* gene has three known partners: *TAF15* at 17q12 (16 cases), *EWSR1* at 22q12 (4 cases), and *E2A* at 19p13 (3 cases). We present seven new pediatric pre-B ALL patients with *CIZ* gene rearrangement. Our patients had lymphoblasts with a CD10-negative immunophenotype, similar to the antigenic profile seen in *MLL* gene-rearranged ALLs. Followup on the patients ranges from 3 to 5 years, and none of the patients have relapsed. Identification of the rearrangements was facilitated using dual-colour breakapart probes for the *E2A*, *CIZ*, and *EWSR1* loci. Four of the patients had *E2A-CIZ* gene rearrangement and one had *EWSR1-CIZ* gene rearrangement. Two patients had *CIZ* gene rearrangement involving regions on chromosomes 6 and 22, suggestive of additional *CIZ* partner genes. Copy number and expression microarray analyses have been performed, and results comparing the genomic and gene expression changes in *CIZ* rearrangement ALL to that of other ALL subtypes will be presented. Our data suggests that *CIZ* gene rearrangement may have an incidence of ~3% in pediatric ALL, with an incidence of at least 18% in CD10-negative pre-B ALL. *CIZ* gene rearrangement may be associated with a more favorable prognosis than *MLL* gene rearrangement, and *CIZ* FISH analysis is recommended in patients with CD10-low/negative ALL.

P07.02

Chromosome 11 as a target of breakpoint mapping in acute myeloid leukemia

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In acute myeloid leukemia (AML), chromosome 11 breakpoints are typically localized at chromosomal band 11q23.3 where a proto-oncogene *MLL* (myeloid/lymphoid leukemia) is frequently altered. However, recurrent aberrations of other regions of chromosome 11 without identification of altered gene have been described. The aim of this study was characterization of chromosome 11 aberrations in bone marrow and peripheral blood cells of AML patients and mapping of recurrent breakpoints using conventional and molecular cytogenetic methods (FISH with BAC probes, mFISH, mBAND and aCGH).
During years 2006-2011, chromosome 11 aberrations were proved in 55 (17%) out of 318 newly diagnosed AML patients. We identified approximately 30 different chromosome 11 breakpoints, 13 as recurrent (in chromosomal bands 11p15, 11p13, 11p12-p11.2, 11q13.2, 11q14.2, 11q21, 11q22.3, 11q23.3) and two altered genes: *MLL* (11q23.3) and *NUP98* (11p15).
In conclusion, chromosome 11 aberrations are the most frequent cytogenetic abnormalities in AML with many repeated as well as sporadic breakpoints on both short and long arm. The *MLL* gene is famous for the great number of partner fusion genes. We identified two new *MLL* gene translocations. The breakpoint localization in chromosomal regions without known gene associated with leukemogenesis predicts their involvement in malignant process. However, the reason for breakage and role of genes localized at the breakpoint for cancer origin and progression is still under discussion. Therefore, the significance of new chromosomal breakpoints could be revealed by further studies only.
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P07.03

Do vessel-forming endothelial cells in B-cell lymphomas carry the hallmark chromosomal translocation and belong to the tumor cell clon?

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Angiogenesis is one of the hallmark features of cancer. Recently it has been suggested that vessel-forming endothelial cells in B-cell lymphomas could be derived from clonal tumor-cells (Streubel et al., 2004). This hypothesis was based among others on the observation that the hallmark chromosomal translocations in these lymphomas, i.e. t(14;18) in follicular lymphoma, t(8;14) in Burkitt lymphoma and t(11;14) in mantle cell lymphoma, were detectable in microvascular endothelial cells of various B-cell lymphomas. To independently corroborate these findings we here investigated vessel nuclei by means of interphase FISH for the presence of t(8;14)(q24;32), t(14;18)(q32;q21) or t(11;14)(q13;q32) in 28 B-cell lymphomas known to carry these characteristic translocations. Simultaneous immunofluorescence staining of endothelial cells using a vWF-antibody and of B-lymphocytes using a PAX5-antibody was performed to accurately distinguish these cell populations from each other. All investigated samples carried the hallmark translocations in a significant number of B-lymphocytes. Additionally, we observed FISH patterns suggesting presence of these translocations in cells surrounding blood vessels as well as in some vascular intraluminal and intramural nuclei. Nevertheless, we failed to unambiguously distinguish nuclei from endothelial cells from lymphocyte nuclei. Thus, we investigated whether clonal B-lymphocytes invading blood vessel carrying the entity typical hallmark translocation could be mistaken for endothelial cells. Indeed, immunofluorescence staining showed intramural and intraluminal PAX5-positive cells in vessels and vessel walls. In summary, we failed to unambiguously assign clonal cells carrying the lymphoma-specific translocations to endothelial cells. Instead, nuclei with lymphoma-specific translocations in vessels could exist due to vessel-invading neoplastic lymphocytic cells.

P07.04

Determination Of Genotoxic Effects Of Boric Acid In Cervical Carcinoma Cell Lines

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Aim: It has been shown that boron could be anti-carcinogenic effects in limited number of epidemiological and in vitro studies. In this context, particularly its preventive and therapeutic potential for prostate and cervical cancer gaining power by the day. In this study that planned, investigated the cytogenetic effects of boron in cervical carcinoma cell lines.
Methods And Materials: In our study, HTB-32 (from ATCC) and CCL-62 (HeLa contaminant, from ATCC) that cervical carcinoma cell lines were used. On this cell lines were treated 250 µM, 500 µM, 1000 µM doses of Boric acid. For estimate of genotoxicity while chromosome abnormalities in shape (CAs) were evaluated in terms of frequency, also Micronucleus (MN) frequency were calculated. Number of MN calculated for each boron doses in 1000 pieces binucleotid cells. For chromosome analysis, numbers of break and gap were evaluated for each dose in 50 pieces metaphase. The data that obtained, compared with the control group by applying the chi-square in SPSS 16.0 program.
Results And Discussion: In conclusion, statisticaly we didn't find significant genotoxic effects of boric acid when compaired with control group in human servical carcinoma cell lines ($p>0.05$). Our data suggested that boric acid no effects that increase or decrease on incidence of MN and CAs in human cervical carcinoma cell lines.

P07.05

A functional assay for the identification of DNA double strand break (DNA-DSB) repair deficiency in heterozygous carriers of *BRCA2* and *RAD51C* mutations

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Mutations in breast cancer gene 1 and 2 (BRCA1/2) account for 50% of the familial aggregation of breast and ovarian cancer. Numerous allelic variants are of unknown clinical relevance (unclassified variants, UCV). We recently identified RAD51C as a third high penetrance gene. Like BRCA1/2, RAD51C is also involved in homologous recombination repair (HRR) in response to DNA-DSB. Patient lymphocytes carrying a pathogenic BRCA1/2 variant exhibit an increased level of chromosomal damage after irradiation. We aimed at developing a reliable functional test system which allows the evaluation of HRR deficiency in heterozygous patient lymphocytes. Carriers of pathogenic BRCA2 mutations, pathogenic RAD51C mutations, healthy controls as well as BRCA2 UCV carriers were γ-irradiated in G2 phase to introduce DNA-DSB. Repair capacity was subsequently assessed on metaphase chromosomes stained by multicolour fluorescence in situ hybridisation. Chromosomal translocations and breakages were counted per mitosis and referred to total chromosomal number. Lymphocytes from carriers of pathogenic BRCA2 and RAD51C mutations revealed a significantly higher mean aberration frequency than from controls ($p<0.001$, student's t-test). Patients carrying an UCV in BRCA2 could be allocated to either the pathogenic or the control group. In summary, our assay may enable the identification of HRR deficiency irrespective of the underlying gene defect and may also serve as a biomarker for sensitivity to PARP inhibition.

P07.06

Chromosomal aberration in cervical cancer: FISH or chips?

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Cervical tumorigenesis is linked with numerous chromosomal aberrations. Different studies investigated alterations in this cancer mainly by fluorescence *in situ* hybridization (FISH) or metaphase comparative genomic hybridization (mCGH). Currently, high throughput methods such as array-comparative genomic hybridization (array-CGH) together with single nucleotide polymorphism (SNP) arrays are available to study genome-wide aberrations.

We identified highly complex large-scale alterations of 13 cervical tumors (spinocellular carcinoma stage IA-IIIB) using genome-wide microarrays (Agilent). The most common regions of copy number gain were 3q22qter (6/13), 20q11q13.3 (5/13) and 8q21.3qter (4/13) and of copy number loss 13q11q21.2 (4/13), 3q and 4q (3/13). Patients with lymph nodes positive for metastases had often duplications in regions 2p, 5p and 19p and deletion at 3p compared to patients with lymph nodes negative for metastases.

However, array-CGH was not able to detect gains at 3q26 and 8q21 in four patients as compared to results obtained by Cervical FISH Probe Kit (Abbott). This kit allows simultaneous identification of HPV-infected cells and copy number aberration of the *hTERC* (3q26) and *MYCC* genes (8q21). Failure of array-CGH in detecting these aberrations can be probably explained by their presence in HPV-infected cells only whose abundance was very low in the four patients.

Array-CGH represents a perspective method for whole-genome screening and identification of potential biomarkers, which may enable a better risk stratification of HPV-positive women.

Nevertheless, reliable determination of DNA copy number aberrations requires either tissue sections from representative regions of tumor or FISH analyses of selected genes.

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P07.07

Mapping of the minimal deleted region in 13q in chronic lymphocytic leukaemia with concomitant translocation-deletion of 13q14 by mBAND technique

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Background: We have previously demonstrated by interphase fluorescence *in situ* hybridization (I-FISH) submicroscopic 13q deletion in chronic lymphocytic leukaemia (CLL) with 13q14 translocation. In this study, we employed multi-colour banding technique (mBAND) to further elucidate the translocation breakpoint and map the minimal deleted region in chromosome 13.

Methods and Result: 6 cases of CLL with 13q14 translocation diagnosed during 1997-2011 were available for study (Table 1). mBAND hybridization with direct fluorochrome-labeled region-specific partial chromosome paints on chromosome 13 (mB13) was performed on fixed cytogenetic preparations. Metaphases were captured on Carl Zeiss Z2 microscope using Isis/mFISH imaging software (MetaSystems, Altlussheim, Germany) after overnight hybridization. Analysis was based on fluorescent banded profile along the length of individual chromosome. Metaphases showing 13q translocation provided positive identification of the tumour clone. Deleted 13q was shown in 5 cases with the deleted 13q breakpoints unambiguously identified. The minimal deleted region was mapped to 13q14.3q21.1.

Conclusion: mBAND is a high-resolution tool for genetic study at single-cell single-metaphase level with a high sensitivity and specificity. It can refine the breakpoints for a more definitive karyotype. Our study has helped confirm the frequent occurrence of deleted 13q in CLL with 13q translocation, and map the minimal deleted region to 13q14.3q21.1.

Table 1: CLL with 13q14 translocation

Case	G-band karyotype	I-FISH for 13q14	mBAND for chromosome 13
1	46,XY,t(11;13)(q12;q14)[10]/46,XY[10]	deleted	del(13) (q14.3q21.3)
2	46,XY,t(7;13)(p15;q14)[4]/46,XY,inv(14)(q11q32)[2]/46,XY[18]	deleted	del(13)(q14.2q22) and del(13)(q31q34)
3	46,XY,-10,t(10;13;14)(q26;q14;q24),+12,der(17)t(10;17)(q11;p12)[17]/45,XY,idem,dic(4;6)(p12;:?:;p23)[2]/46,XY[1]	no deletion	del(13)(q21.3q22) no deletion
4	46,XY,del(2)(p21),t(11;17)(q13;q25), t(13;15)(q14;q26)[15]/46,XY[5]	deleted	del(13)(q14.1q21.1)
5	46,XY,t(10;13)(p11.2;q14)[2]/46,XY, t(14;22)(q11.2,q13)[1]/46,XY[17]	deleted	del(13)(q14.3q21.3)
6	46,XY,?ins(21;13)(q11.2;q12q14)[2]/46,XY[17]	deleted	del(13)(q14.1q21.3)

P07.08

Independent coexistence of clones with 13q14 deletion at reciprocal translocation breakpoint and 13q14 interstitial deletion in chronic lymphocytic leukemia

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13q14 deletion is the most frequent chromosomal aberration in chronic lymphocytic leukemia (CLL), occurring in more than 50% of CLL cases. It is found predominantly as an interstitial deletion, less frequently in a form of reciprocal translocation with deletion at 13q14 breakpoint. The parallel presence of two clones with the different forms of 13q14 deletion has been noticed only once so far. By detailed metaphase analysis (G-banding, FISH) on IL2 and DSP30 stimulated CLL cells, we revealed the translocation form of 13q14 deletion in 13 of 135 patients with 13q14 deletion (13/135; 10%). The coexistence of a clone with the deletion at reciprocal translocation breakpoint and another clone with the interstitial deletion was found in 5 of the translocation cases (5/13; 38%). In one of them a subsequent clonal analysis proved an independent origin of the both coexisting clones, giving the evidence of their purely coincidental presence. We showed the coexistence of clones with the translocation form of 13q14 deletion and clones with the interstitial deletion not to be rare. As a mark of clonal evolution it could signify an increased risk of disease progression. Based on our presented results we assume that 13q14 reciprocal translocation with deletion at the breakpoint could be formed independently from formation of more common interstitial deletion also in other translocation cases.

P07.09

Detection of chromosomal abnormalities in chronic lymphocytic leukemia (CLL): FISH or MLPA?

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Chronic lymphoid leukaemia (CLL) is a genetically heterogeneous disease with recurrent chromosomal aberrations of prognostic significance. Current strategies for detecting chronic lymphocytic leukemia (CLL) include Fluorescence In Situ Hybridisation (FISH) and cytogenetics. Multiplex Ligation-dependent Probe Amplification (MLPA) is a multiplex PCR method detecting abnormal copy numbers in genomic DNA.

We studied a cohort of bone marrow samples from suspected leukemia patients and compared the results of FISH with cytogenetics and MLPA. We used a panel of 7 FISH hybridisation probes known to be of diagnostic relevance in CLL. Concordance between MLPA and FISH was excellent, when the abnormal clone was present more than 50% of the cell population. The use of MLPA allowed the identification of small alterations undetected by FISH, but e.g. translocations remain undetected. MLPA additional with cytogenetics represents a useful technique for the characterization of genomic changes in CLL. It is easy to use, faster and less cost intensive than FISH.

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P07.10

Correlation between interphase FISH and real-time quantitative PCR for the therapeutic monitoring of chronic myeloid leukaemia

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Background

Both interphase FISH (I-FISH) and real-time quantitative PCR (RQPCR) have been used for the monitoring of patients with chronic myeloid leukaemia (CML) on tyrosine kinase inhibitor (TKI) therapy. We compared the international standardized ratio (ISR) from the RQPCR test with I-FISH on patients with standard 9;22 reciprocal translocation and variant 9;22.

Methods and Result

104 samples from 54 patients with CML on TKI were studied during 2008 - 2011. 14 patients have ≥ 3 serial samples. ISR was derived from ratio of [*BCR-ABL1*]/*ABL1* transcript. I-FISH was performed with dual-fusion *BCR-ABL1* probes. ISR and I-FISH results were analyzed according to the cytogenetics response (Table 1). ISR showed wide inter-individual variation for the same tumor load as shown by I-FISH. The time to reach major molecular response (ISR $\leq 0.1\%$) ranged from 152 - 744 days after complete cytogenetic remission as shown by I-FISH. The falling trend of I-FISH correlated with ISR except in 2 patients, with one of them having a variant 9;22 translocation.

Conclusion

I-FISH is a better indicator of the tumour load especially in patients with variant 9;22 translocation while RQPCR is sensitive to detect *BCR-ABL1* fusion. Further study is required to determine the relationship between ISR values and cytogenetic responses as previous studies were mostly based on the latter.

Table 1. Correlation of ISR and I-FISH in classical and variant 9;22 translocation.

Group	N =	I-FISH, %	ISR
CCgR, FISH: 0%	36		
CCgR, classical 9;22	31	0	0 - 1.481, median = 0.141 (1/31, ISR = 10.243)
CCgR, variant 9;22	5	0	0.006 - 1.962, median = 0.126
PCgR, FISH: <35%	31		
PCgR, classical 9;22	25	1 - 33.5	0.303 - 18.669, median = 2.02
PCgR, variant 9;22	6	2.5 - 29.5	1.431 - 10.337, median = 3.548
No CgR, FISH >= 35%	37		
No CCgR, classical 9;22	22	49 - 100	3.58 - 63.583, median = 26.497
No CCgR, variant 9;22	15	41.5 - 99.5	10.823 - 56.845, median = 18.962

P07.11

Intermittent Dosing of Imatinib Mesylate in the Treatment of Childhood Chronic Myeloid Leukemia: a Case Report.

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Introduction: Chronic myeloid leukemia (CML) is rare in childhood (less than 5% of all childhood leukemias). The main characteristic is the Philadelphia chromosome and the tyrosine kinase inhibitor imatinib mesylate is the treatment of choice.
Purpose: To investigate whether intermittent dosing of imatinib is also an effective treatment in minimizing the adverse effect.
Material-Method: A 6-year-old girl presented with abdominal pain, leukocytosis ($27.8x10^9$/L) and thrombocytosis ($1.072x10^9$/L). Bone marrow studies suggested the diagnosis of CML. Cytogenetic studies revealed 46,XX,t(9;22)(q34;q11). The translocation was confirmed by FISH and RT-PCR. Treatment with imatinib was initiated at 400mg/m^2/day, followed 200mg/m2/day when minimal residual disease (MRD) was undetectable. MRD reappeared positive, so the dose was adjusted to 400mg/m^2/day but the patient experienced dry skin and significant hair loss. To avoid toxicity, the drug was given on alternate months (one on/one off).
Results: MRD is undetectable and the patient is in stable remission three years following the end of the treatment.
Conclusions: No guidelines exist regarding dosage and duration of treatment with imatinib, especially for childhood CML. In this report, intermittent administration of imatinib proved efficient, both in achieving complete stable remission and in minimizing toxicity.

P07.12

Clonal Evolution in Chronic Lymphocytic Leukaemia

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INTRODUCTION: Chronic lymphocytic leukaemia is characterized by variable clinical course. The most frequent chromosomal abnormalities are trisomy 12, deletion of 13q14.3, 11q22.3 and 17p13; all of these are a relevant prognostic factor. Clonal evolution (CE) is defined as acquired aberration during the disease course.
The goal of this study is to determine the frequency of CE in CLL patients using conventional and molecular cytogenetics and its relationship with prognostic markers such as CD38 and ZAP70.
MATERIAL AND METHODS: Seventy-three patients with CLL were analysed by conventional and molecular cytogenetics (FISH) at diagnosis and during follow-up. Median time of observation after first analysis was 32 months (range 5-116). The second study was undertaken at follow-up (n=34), at clinical progression before treatment (n=24), and at relapse after treatment (n=15).
RESULTS: CE was observed in 38/73 (52%) of the patients using both techniques. The 70% (14/20) of patients with CE by conventional cytogenetics had normal karyotype at diagnosis. On the other hand, the 63% (19/30) of patients with CE by molecular cytogenetics had FISH abnormalities at diagnosis.
CE was detected during follow-up (14/34); at progression before treatment (12/24); and relapse after treatment (12/15) which was correlated with CE (p=0.042). In relation to ZAP-70, it was differentially expressed in patients with CE (p=0.039).
CONCLUSION: Clonal evolution is a significant event in CLL patients. Conventional and molecular cytogenetics were necessary to detect clonal evolution. We believe that CE could be a new prognostic factor and an indicator of change in clinical course of the disease.

P07.13

Cytogenetic studies on a chronic myeloid leukemia case developing into acute myeloid leukemia after treatment with imatinib.

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A 34-year-old male was diagnosed with CML in April 2006. At the time of diagnosis all metaphases in the bone marrow showed t(9;22)(q34;q11.2). After one year of daily treatment with 400 mg imatinib no Philadelphia-chromosome positive metaphases were found in the bone marrow. FISH showed 2.5% of the 200 interphase nuclei positive for BCR/ABL1. Surprisingly, a monosomy 7 was detected in 15 out of 20 metaphases, whereas at this time no clinical signs of MDS or AML were present. Three months later, cytogenetic studies of bone marrow demonstrated 3 clones: 46,XY,t(9;22)(q34;q11.2)[2]/45,XY,-7[13]46,XY[5]. There were still no morphological signs of MDS or AML. After three more years, monosomy 7 was detected in all metaphases and by FISH in 90% of 200 interphase nuclei. Again FISH for BCR/ABL1 was negative. This time bone marrow cytology showed AML. The patient received one cycle of induction therapy with cytarabine and daunorubicine till now.
Transformation of a Ph-negative clone with monosomy 7 and no clinical signs of MDS into overt AML is a rare but recurrent phenomenon. Therefore patients showing monosomy 7 during imatinib treatment should be closely monitored both cytogenetically and clinically for signs of MDS or AML.

P07.14

Effect of new tubulin inhibitors drugs in colon cancer cells

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Introduction: Cell cytoskeleton is composed of microtubules formed by polymerization of tubulin. Antitumor drugs that perform on the microtubules are divided in three groups according to the zone of interaction: Vinca, Colchicine and Taxan-binding domain. First and second one prevent tubulin polymerization. Paclitaxel is a microtubule stabilizing agent. We have studied the effect of three new molecules that bind on the colchicine domain in two cell lines of colon cancer.
Experimental design: Cell lines were treated with drugs A, B and C, derived from the isocombretastatin and Paclitaxel with a concentration of 50 nm. The cells were collected at times 24, 48 and 72h after incubation with the drug, using as negative control untreated cells (0h). Protein were removed from each culture and we prepared cultures for immunofluorescence analysis. Expression studies of tubulin was performed by Western Blot. We performed a study of cell death by flow cytometry. Finally, we made a study of immunolocalization and stability of microtubules by immunofluorescence.
Results: Our results show that none of the new drugs reduce or increase the amount of tubulin. The viability study showed that Drug A and B not induce apoptosis, however, Paclitaxel and Drug C significantly increased apoptosis after 24 hours of exposure. Drug A does not induce changes in the microtubules at 72h, Drug C and Drug B produce a destabilization of microtubules and inhibit tubulin polymerization.
Conclusion: None of these drugs improve the effect of paclitaxel.
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P07.15

Detection of donor cells with a clonal abnormality 20 years after transplantation: Is this evidence of Donor Cell Leukemia (DCL)?

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DCL is a rare complication following BMT with unknown etiology. We report a patient who showed no cytogenetic evidence of engraftment for 20 years following which donor cells were detected with a clonal abnormality. A 59-year-old female diagnosed with CML in 1986 underwent an allogenic BMT from her brother in 1987. Subsequently, she developed graft-versus-host disease in 1990, suggesting possible rejection of donor cells. Afte rrelapse in 2000 her chromosome analysis demonstrated the karyotype 46,X,-X, t(1;7)(p10;p10), add(4)(p16),t(9;22) (q34;q11.2),-15,+3mar[16]/46,XX,der(9)add(9)(p24)t(9;22)(q34;q11), add(18)(p11.3),-19,der(22)t(9;22)[4]. Since Gleevec treatment was unsuccessful due to intolerance, follow-up studies in 2006 revealed persistence of known Ph+ clones and an additional Ph+ clone. Upon treatment with dasatinib she achieved partial remission and chromosome studies in 2007 showed, for the first time, donor cells with trisomy 8 (T8) and recipient cells with the Ph+ clone; 46,XX,t(9;19)(p24;p13.1),t(9;22)(q34;q11.2),t(14;18)(q24;11.2)[2]

//47,XY,+8[3]/46,XY[20]. Subsequent studies demonstrated donor cells with T8 in 15-35% of metaphases. Constitutional T8 was ruled out as the donor's blood karyotype was normal. T8 is an additional anomaly in 10% of CML cases emerging at the time of acute transformation and after imatinib treatment. Although engraftment is an immediate response, the cytogenetic evidence of donor cells did not become apparent due to the proliferative dominance of Ph+ clones. Dasatinib treatment in our patient effectively removed the dominant Ph+ clones, facilitating the detection of donor cells with T8. Since the etiology of DCL and the significance of the T8 in donor cells in our patient are unclear, the patient is monitored closely for secondary MDS /AML.

P07.16

Variant chromosomal translocation in Ewings Sacoma

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This study reports the cytogenetic findings on a fine needle aspiration (FNA) specimen from ten patients with Ewing's Sarcoma (ES) using G-banding technique. Out of ten ES cases t(11;22)(q24;q12) was observed in 6 cases(60%), only del(22)(q12) in one case(10%) and variant translocation t(1;11;22)(q31;q24;q12), t(11;12;22)(q24;p13;q12) & t(4;22)(q35;q12) in 3 cases (30%). In third variant case only chromosome 22q12 was involved in translocation and del (22) (q12) (EVS gene) in another suggests that the rearrangement of 22q12 is the cytogenetic hallmark of ES. Specific chromosomal abnormalities often correlate with particular morphologic or phenotypic subtypes of tumor and play an important role in prognosis.

P07.17

Gain of the short arm of chromosome 2 in Chronic Lymphocytic Leukaemia

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Background: Chromosomal abnormalities are important prognostic factors in chronic lymphocytic leukaemia (CLL). Interphase FISH (I-FISH) is standard method for detection of important aberrations in CLL. However, progress in cultivation technique such as application of CpG-oligonucleotides and IL-2 as B-cell mitogens allows to reveal another chromosomal aberrations. Recently, gain of the short arm of chromosome 2 (2p) was found as one of recurrent aberrations.

Aims: Metaphase cytogenetic and molecular-cytogenetic analyses were performed in 477 CLL-patients from 2008 to 2011 to find out gain of 2p.

Methods: Conventional cytogenetics was performed on peripheral blood or bone marrow samples cultured in medium with stimulants. I-FISH was performed on unstimulated cells for detection of trisomy 12, del(11q), del(13q), del(17p). Gain of 2p was confirmed by multicolor FISH, the range of the gain was determined by multicolor banding.

Results: Gain of 2p was detected in 20/477 (4,2 %) cases. In 17/20 cases, the partial trisomy of 2p was identified as a result of unbalanced translocation with other chromosomes and direct duplication of 2p was presented in 3/20 cases. Four of all these cases had jumping translocation of 2p (in one case together with direct duplication of 2p). I-FISH revealed aberrations in 19/20 cases. The most frequent abnormalities were del(13q) and del(11q).

Conclusion: Mitotic stimulation using CpG-oligonucleotides and IL-2 is an efficient method that can improve quality of cytogenetic analysis. Detection of another aberrations i.e. gain of 2p provide new prognostic information in CLL. These findings can significantly modify prognosis of patients evaluated by FISH.

P07.18

A pediatric chronic myeloid leukemia case progressing to blast crisis characterized by the deletion of Ikaros.

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Introduction: Rearrangements in the gene IKZF1 (Ikaros) are considered as an additional adverse risk factor, among other prognostic markers, in leukemia.

Purpose: To present the case of a pediatric patient with BCR/ABL1 positive CML who was treated with imatinib and achieved remission, but transformed to lymphoid blast crisis (BC) with detected deletion of Ikaros within 205 days following diagnosis.

Material-Method: A 12,5-year-old girl presented with leukocytosis and bone marrow (BM) studies suggested the diagnosis of chronic phase (CP) CML. Cytogenetic analysis demonstrated 46,XX,t(9;22)(q34;q11). The patient was treated with imatinib (400mg/m^2/day) and achieved remission.

Results: On the 205th day, the blood test revealed a 73% blast count and BM studies suggested a diagnosis of B-cell ALL. Cytogenetic findings revealed 46,XX,der(3)t(1;3)(q12;p26),t(9;22)(q34;q11). FISH analyses were negative for translocations, but three copies of PBX1 were detected. MLPA analysis revealed a deletion in Ikaros, including exons 4-8, and in the EBF1 gene, in exon 1. No copy number variation was found in the BM sample from the CML diagnosis. The patient received chemotherapy according to the ALL IC-BFM 2009 protocol plus imatinib (400mg/m^2/day), but failed to achieve complete remission and the duration of survival from the onset of BC was 75 days.

Conclusions: Abnormalities of Ikaros are associated with a very poor outcome and a high rate of relapse in childhood leukemia. Deletion of Ikaros was not detected in CP-CML, but was identified as an acquired lesion in the BC-CML sample. This finding suggests that alterations in Ikaros contribute to the pathogenesis of BCR/ABL1 ALL.

P07.19

Prognostic Genetic Markers in Glial Tumors

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Glioblastoma is the most common primary brain tumor in adults and the most malignant types of human cancers with median survival time of <1 year detected in a population-based study. Although histopathological diagnosis of GBM is known as a gold standard, the primary and secondary subtypes that have at least two distinct pathways contributing to the tumorigenesis of GBM, are histologically indistinguishable and molecular markers are necessary for prognostic predictions of the GBM cases. Frequent mutations of the genes involved in G1-S cell cycle transition control have been previously reported in both subtypes of the GBM. This study was planned to determine prognostic values of growth-control molecules including MDM2, CDK4 protooncogenes and RB1tumor suppressor gene in primary GBMs. Of 40 cases, 26 were male and 14 were females and the mean age was 55.45+/- 2.25. Since no brain tumor history, the GBM subtype of all was primary GBM. The genomic copy aberrations of the genes were determined by the FISH assay in tumor sections of the cases. Of 40 cases, no gene copy number aberrations was seen in four cases whereas the MDM2, CDK4 gene amplifications and RB1 gene deletion were detected in 62,5%, 60.0% and 47.5% of the cases, respectively. The copy number alterations for all analysed genes were seen in seven cases. However, no significant differences between the worse survival and genetic markers was highlighted. Further detailed study related with genetic and epigenetic markers in larger population is necessary for clinical outcome of patients with primary GBM.

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P07.20

Genotoxicity of hepatitis B virus

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Background: Chronic hepatitis B is one of the major causes of cirrhosis and hepatocellular carcinoma worldwide. An estimated more than 350 million people are chronically infected with hepatitis B virus (HBV), characterized with various clinical states. Although HBV shows mainly pathologic effect on liver, it can also infect peripheral mononuclear cells. Since HBV has the capability of integrating in the human genome and causing chromosomal rearrangements, it might act as a clastogenic factor and exert a direct genotoxic effect on lymphocytes.

Aim: In this study, potential genotoxic effect of HBV was investigated on peripheral lymphocytes in chronic HBV patients ,HBV carriers and normal

controls by the micronucleus (MN) technique, is used as an index of chromosomal damage.
Method: The MN assay was performed on peripheral blood lymphocyte cultures from chronic HBV patients, HBV carriers and controls. The cells with 1-4 nuclei and micronucleated cells were scored. Then frequency of micronuclei and cytokinesis- blocked proliferation index (CBPI) were calculated. The obtained data were compaired between HBV patients, HBV carriers and controls.
Results: There were no differences among HBV patients, HBV carriers and controls in terms of MN frequency and CBPI.
Conclusion: Our findings suggest that HBV does not show genotoxic effect at least as MN formation and CBPI.

P07.22

The detection rate of chromosomal abnormalities in mature lymphocytic neoplasms is increased by adding CpG oligonucleotide DSP30 and IL-2 to the culture medium

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It has been shown that the addition of the CpG-oligodeoxynucleotides DSP30 and IL-2 to the culture medium increases the mitotic activity of chronic lymphatic leukemia cells and consequently the detection rate of chromosomal abnormalities in this malignant haematological disorder. With this study we aimed to investigate the effect of this addition in the diagnosis of a broader group of mature lymphoid malignancies. From January till December 2010, bone marrow samples for staging of lymphoid malignancies were cultured in either a conventional medium or in a medium with DSP30 and IL-2. Between 5 and 20 G-banded metaphases were analysed in each sample.
Classification of the chromosomes and definition of a clone were made according to ISCN (2009). In total, 233 samples were included in the study. In 99% of the samples a successful stimulation for metaphase generation was obtained. In 34/233 samples an abnormal karyotype was observed, suggestive for invasion of lymphoid malignant cells into the bone marrow. In 15 of those 34 abnormal samples, the aberration was only found in the cultures with added DSP30 and IL2. However, in one sample the chromosomal abnormality was only observed in cultures with the conventional medium and not in those with DSP30 and IL2. For this patient, the final diagnosis was not a lymphoid neoplasm but acute myeloid leukemia.
Our findings confirm that stimulation of cultures with DSP30 and IL-2 in case of mature lymphoid malignancies results in an increased detection rate of clonal abnormalities.

P07.23

A new type of karyotype evolution?

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Cytogenetic findings play an important role in the diagnosis and assessment of prognosis of myelodysplastic syndromes (MDS) and are emerging as an important factor in treatment allocation and in selection and monitoring response to therapy. Abnormalities involving chromosome 7 are frequent in MDS and suggest an intermediate to poor prognosis. In 2% of MDS patients we observed a coexistence of monosomy 7 and 7q-deletions at the same time by FISH analysis of CD34+ peripheral blood cells. Two patients showed an increase of -7 clone size parallel to a decrease of 7q- clone size during progress. Now we report a case of a 72-year old man with suspected MDS. Analysis of bone marrow revealed MDS RA. Banding analysis of bone marrow cells showed three clones with different chromosome 7 abnormalities: del 7q in 22%, ring chromosome 7 in 22% and monosomy 7 in 11% of the metaphases.
We hypothesize that chromosome 7 is subject to karyotype evolution during MDS progression. Due to the existence of the third clone with r(7) we assume that r(7) is a transitional stage of karyotype evolution between 7q- and -7 which is resulting of loss of telomere ends in 7q- cells. FISH analysis confirmed deletion of telomeres in r(7). Follow up analyses of this patient will allow further examination of karyotype evolution involving chromosome 7 abnormalities.

P07.24

The molecular background of the medulloblastoma - identification of the most common chromosomal aberrations in Polish patients.

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Medulloblastoma (MB) is a highly malignant primary brain tumor that originates in the cerebellum or posterior fossa and the most common malignant brain tumor in childhood. The exact pathogenesis of MB remains unclear. Up to date multiple molecular dysfunctions are known to be responsible for medulloblastoma formation, including aberrant activation of the Sonic Hedgehog or Wingless signaling pathways as well as mutational inactivation of DNA repair genes results in genome instability. The most common genetic alterations identified in medulloblastoma is isochromosome 17 (currently reported in 30-50% of patients). Others less common chromosomal changes observed in MB include chromosome 6, 8, 9q, 16, 18 and X.
In our project on medulloblastoma's genetic background we determined the frequency of the most common chromosomal aberrations in Polish patients with MB. We screened a total of 21 patients, using a multiplex ligation-dependent probe amplification analysis (MLPA) and identified 11 carriers (52,3%) of chromosome 17 aberrations (including 8 patients with isochromosome - 38%) and 5 carriers (29,4%) with abnormalities within chromosome 6. All the identified changes were somatic mutations. These findings suggest that the identified common chromosomal aberrations are the consequence of another molecular mechanism involved in the pathogenesis of medulloblastoma. The analysis on the genetic background of MB in the Polish population will be continued.
The study was financed by the National Science Centre, project no. 6917/B/P01/2011/40.

P07.25

Comprehensive high-resolution genomic profiling and cytogenetics of two pediatric and one adult medulloblastoma

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Medulloblastoma (WHO grade IV) is a rare malignant, invasive embryonal tumor which mainly occurs in children and represents less than 1% of all adult brain tumors. Systematical comprehensive cytogenetic and molecular biological analyses on medulloblastomas are very limited but necessary to provide more detailed information.
Therefore, we performed comprehensive cytogenetic analyses (blood and tissue) of two pediatric and one adult medulloblastoma, WHO grade IV, using trypsin-Giemsa staining (GTG-banding), spectral karyotyping (SKY, only tissues), and molecular karyotyping using genome wide 6.0 SNP-arrays.
We confirmed frequently detected chromosomal aberrations in medulloblastoma, such as +7q, -8p/q, -9q, -11q, -12q, +17q, and identified novel genetic events. Applying SNP array, we identified constitutional de novo losses 5q21.1, 15q11.2, 17q21.31, 19p12, (pediatric medulloblastoma), 9p21.1. 19p12, 19q13.3, 21q11.2 (adult medulloblstoma) and gains 16p11.1-16-p11.2, 18p11.32, Yq11.223-Yq11.23 (pediatric medulloblastoma), Xp22.31 (adult medulloblastoma), possibly representing inherited causal events for medulloblastoma formation. We show evidence for somatic segmental uniparental disomy in regions 1p36, 6q16.3, 6q24.1, 14q21.2, 17p13.3 and 17q22, not previously described for primary medulloblastoma.
Analyses of tumors and matched normal tissues (blood) with a combination of SNP arrays and complementary techniques will help to further elucidate potentially causal genetic events for tumorigenesis of pediatric and adult medulloblastoma.

P07.26

Analysis of chromosomal abnormalities in phenotypically normal plasma cells detected by I-FISH in monoclonal gammopathy of undetermined significance

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Monoclonal gammopathy of undetermined significance (MGUS) is a premalignant stage of multiple myeloma. MGUS consists of phenotypically normal (nPCs; CD138+19+56-) and abnormal malign plasma cells (aPCs; CD138+19-

56+/-). Combination of fluorescence activated cell sorting (FACS) and interphase fluorescence in situ hybridization (I-FISH) allows monitoring of specific chromosomal abnormalities in separate PCs populations. We hypothesized that there should not be any chromosomal abnormalities in nPCs.

By I-FISH we examined following chromosomal alterations in nPCs and aPCs: del(13)(q14), del(17)(p13), IGH rearrangement, 1q21 gain and hyperdiploidy (+5, +9 and +15). In this study, we chose MGUS patients from whom it was possible to separate nPCs and aPCs and who had IGH rearrangement in aPCs (n=15). In the nPCs we found only IGH disruption. Total of 27% (4/15) and 73% (11/15) MGUS patients have more than 20% and 10% aberrant PCs bearing IGH rearrangement not only in aPCs, but also in nPCs, respectively. Other chromosomal abnormalities were detected only in aPCs: del(13)(q14) was found in 20% (3/15), 1q21 gain in 7% (1/15) and hyperdiploidy in 13% (2/15).

In conclusion, we found IGH rearrangement in phenotypically nPCs defined by CD138+CD19+CD56- and thus we assume that the separation according to this phenotype is not sufficinent to separate genetically nPCs.

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P07.27

Combination of FISH and array-CGH technique provide powerful diagnostic toll in multiple myeloma: single centre experience

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Multiple myeloma (MM) is a hematological disease caused by malignant proliferation of clonal plasma cells (PCs). Identification of copy number aberrations (CNAs) in genome of PCs plays a key role in MM pathogenesis and is supposed to have important prognostic significance for MM patients. Combined utilization of array-CGH technique and FISH proved to be a powerful toll in MM diagnostic. There are two major genetic entities in MM. Hyperdiploid group (H-MM), which include about 50% of MM patients and have is commonly characterized by gains of odd-numbered chromosomes and lower prevalence of *IgH* translocations. Non-hyperdiploid (NH-MM) cases are connected with frequent incidence of one of several recurrent *IgH* translocations: 4p16 (*FGFR3* and *MMSET*), 11q13 (*CCND1*) or 16q23 (*MAF*) and are associated with adverse prognosis. Using combination of array-CGH and FISH technique, we found CNAs in 100% of cases (106/106). Most common CNAs were found in 1p, 1q, 6p, 8p, 13q, 14q, 16q and 22q along with gain of extra copies of odd-numbered chromosomes. Hyperdiploidy was found in nearly half of the cases (48%) with subgroup of cases with +11 and with gain(1q) and del(13q) Translocation t(4;14)(p16;q32) was found in 12% (14/81 cases)and it was more common in non-hyperdiploid cases (P=0.041), deletion *TP53* was observed in 14% (15/106). Most common homozygous deletions were found 1p32.3, 11q22, 13q14 and 16q22. We conclude that simultaneous utilization of FISH and array-CGH technique provides powerful toll for identification of CNAs in MM patients. This study was supported by grants LC06027, MSM0021622434, NS10207, NT11154 and NT12130

P07.28

Balanced translocations in a series of 1,850 patients with myelodysplastic syndromes.

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INTRODUCTION: Myelodysplastic syndromes (MDS) are a group of clonal haematopoietic stem cell diseases characterized by cytopenia(s), dysplasia in one or more of the major myeloid cell lines, ineffective haematopoiesis, and an increased risk of developing acute myeloid leukaemia (AML). Chromosomal abnormalities are detected in half of patients with the novo MDS. The infrequency of balanced translocations in MDS makes their identification and reporting imperative for the recognition of the recurrent ones. This increases the likelihood of identifying which genes are possibly involved in the pathogenesis of the neoplasia.

OBJECTIVE: Identify the frequency of balanced translocations in a series of 1,850 patients with MDS included in the database of the Spanish group of myelodysplastic syndromes (GESMD).

RESULTS: Two hundred and twelve (11%) balanced translocations were diagnosed in 1,850 SMD patients. The chromosomes more frequently involved were chromosome 1 (n=34), 2 (n=31), 3 (n=30) and 5 (n=16). All chromosomes were involved in the translocations, with the exception of chromosomes 19, 20 ,22 and Y. Half of the translocations were found as a part of a complex karyotype (>=3 chromosomal abnormalities). One hundred and seventy-six (83%) translocations were not previously described neither in MDS nor AML. Nine new apparently recurrent translocations were found in our series.

CONCLUSION: Balanced translocations were found in 11% of MDS patients, half of them being involved in complex karyotypes.

P07.29

The low level clones of BM cells with BCR-ABL fusion gene amplification have unfavorable influence on CML imatinib therapy outcome

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Imatinib CML therapy have shown the high effectiveness but primary and secondary resistance is observed in some cases. The aim of this study was to elucidate the influence of low level clones of BM cells with BCR-ABL gene amplification (GA) to the results of CML imatinib treatment.

Bone marrow samples from 174 CML patients (pts) were analyzed by cytogenetic and FISH analysis. 200 interphase nuclei were analyzed after hybridization with dual color/dual fusion BCR-ABL gene probe ("Vysis") in each case. According to the ELN criteria (2009) 51 pts have achieved optimal or suboptimal response, 86 pts have failure and 37 pts have loss of achieved response.

FISH did not revealed BCR-ABL GA in pts with optimal and suboptimal responses. Additional copies of BCR-ABL gene (from 1 to 7) were found in 32 (37,2%) pts with primary resistance and in 12 (32,4%) pts with secondary resistance. The probability of complete cytogenetic response (CCyR) achievement in pts with BCR-ABL GA was significantly lower than in pts without it (31,6% vs. 63,8%, p=0,000025). All Pts with additional copies of BCR-ABL gene were subdivided in 4 quartiles (Q) in accordance with % of BM cells with BCR-ABL GA. The probability of CCyR achievements in pts of Q1-Q2 (1-6% BM cells with BCR-ABL GA) do not differ from pts of Q3-Q4 (7-72% BM cells with BCR-ABL GA) (p=0, 86454).

In conclusion, the pts with low level clones with BCR-ABL GA have the same unfavorable prognosis as well as pts with high level clones.

P07.30

The clone with trisomy 8 in the culturing bone marrow mesenchymal stem cells of healthy donors

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It has been clearly recognized that the hematopoietic microenvironment plays a key role in the physiology of the hematopoietic system. Mesenchymal stem cells (MSC) are key components of the hematopoietic microenvironment. Several studies have shown that MSC play important role in the genesis of some hematological disorders.

We have investigated the bone marrow MSCs from healthy donors (n=20), intended for allogeneic transplantations recipients with hematological disorders. We have analyzed the characteristics of bone marrow MSC including the growth pattern, the immunophenotype and their cytogenetic characterization by GTG-banding and FISH with centromere-specific DNA-probes for chromosomes X and 8.

Despite decrease proliferation possibilities from the third to the 12 passages, cells kept typical MSC immunophenotype. There were two bmMSC cultures with abnormal clones: clone with trisomy 8 and monosomy X. Culture with trisomy 8 was explored three times. In 4th passage 24% of all analyzed

cells had extra chromosome 8. In 6th passage their amount increased to 34% and in 12th passage it decreased to 16%. In other case the culture of bmMSC of healthy woman had clone with one X chromosome. The amount of such cells in 4th passage was 12% and increased to 91% in 10th passage.
Thereby, the cytogenetic analysis revealed the presence of genetic abnormal cells clones in early passages and persisted till late stages of cultivation. In one of the cultures of healthy donor's bmMSC was found the clone with trisomy 8, chromosomal abnormality, which is strongly associated with myeloid malignancies.

P07.31

Micronucleus (MNs) induction and FISH analysis in peripheral lymphocytes of Tunisian pathology and anatomy laboratory workers exposed to Formaldehyde (FA)

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Formaldehyde is an important industrial compound but it is also a naturally occurring biological compound present in all cells and body fluids. The International Agency for Research on Cancer (IARC) classified FA as carcinogenic to humans (group I) based on the studies indicating an increased incidence of nasopharyngeal cancer in populations occupationally exposed to FA.
A genotoxic effect of (FA), in particular micronucleus induction, has been shown in several studies. The aim of our study was to assess the frequency of (MNs) and to identify the type of chromosomal damage in Tunisian staff from pathologic anatomy laboratory of Farhat Hached hospital (Sousse, Tunisia).
Assessment of chromosomal damage was carried out in peripheral lymphocytes of 31 exposed to (FA). We used 31 controls from the administrative department of the same hospital. The clastogenic/ aneugenic effect of FA was evaluated using the standard MN assay in combination with the fluorescence in situ hybridization (FISH) technique.
The results showed a significant increase of the MN frequency in lymphocytes of exposed workers compared with the control group (25.35 ‰± 6.28 versus 7.08 ‰± 4.62).
As assessed by FISH, the frequency of centromeric micronuclei (C+MN) was higher in exposed subjects than in controls (18.38 ‰±5.94 versus 5.03‰±3.64). Among the (C+MN), the frequency of MN containing one centromere was significantly higher in pathologist/anatomists than in controls (15.35 ‰ ± 6.0 versus 3.33 ‰±2.74).
The increase of the frequency of centromeric micronuclei observed in exposed group may suggest a slight aneugenic effect of exposure to FA.

P07.32

Evidence for a pre-malignant cell line in a skin biopsy from a patient with Nijmegen Breakage Syndrome (NBS)

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The chromosomal instability disorder, Nijmegen breakage syndrome (NBS), is characterized by microcephaly, growth retardation, immunodeficiency, hypersensitivity to X-irradiation, and predisposition to malignant tumours, especially lymphomas. Nibrin, the product of the *NBN* gene, is part of the MRE11/RAD50 complex, which is involved, amongst others, in the repair of DNA double strand breaks (DSBs). The majority of NBS patients are of Central and Eastern European origin and carry the common founder mutation in the *NBN* gene, 657del5. Skin fibroblasts, derived from a 9 year old NBS patient showed a mosaic of normal diploid cells and those with an unbalanced translocation, resulting in partial monosomy for 6q and 13q21→qter, and partial trisomy for 20q11.2→qter, as confirmed by G-banding, CGH and chromosome painting. The relative proportion of these aberrant cells increased during propagation of the cell line. This was due to a faster cell cycle, as shown after BrdU labelling and is paralleled by a shorter telomere length, as demonstrated by T/C-FISH (telomere/centromere-FISH). Moreover, after treatment with 0.5 and 1.0 Gy the aberrant cells showed significantly more chromosomal aberrations than the diploid cells in the same flask. This chance observation proves that the aberrant cell line has a selective advantage and thus may represent a first step in malignant transformation.

P07.33

Secondary acute myeloid leukemia after treatment for Neuroblastoma stage III. A Case Report.

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Introduction: Therapeutic advances in the treatment of pediatric neoplasms have improved the prognosis but increased the risk of developing second malignant neoplasms (SMNs). Acute myeloid leukemia (AML) is the most likely SMN to occur during the first 5 years following treatment.
Purpose: To describe the case of a pediatric patient with Neuroblastoma (NBL) stage III, who developed secondary AML at 4 years following cessation of treatment.
Material-Method: A 2,5-year-old boy was diagnosed with stage III NBL on the adrenal and received chemotherapy. No cytogenetic study to NBL was performed. The mass progressively decreased and 1 year later the follow-up was negative. Eight months after the cessation of treatment, a local relapse of NBL was revealed and treated with partial resection, radiotherapy and chemotherapy. One year later, a second local relapse was revealed and a total resectomy of the mass achieved complete remission.
Results: Four years following the cessation of treatment (eight years after NBL diagnosis), the patient was admitted with anaemia (Hb 7,3 gr/dl) and thrombocytopenia (PLTs 5000/μl). A secondary AML was diagnosed. FISH analysis revealed MLL rearrangement in 60% of the nuclei and cytogenetic analysis showed 46,XY,t(11;19)(q23;p13.3). The patient received chemotherapy according to AML-BFM-2004 protocol and MLL rearrangement was detected in 29.4% on the 15th day of treatment.
Conclusions: NBL is the most common solid tumors in childhood. However, little is known about the factors that determine the long-term risk of SMN following this type of cancer. The risk of AML was significantly increased after combined radiation and chemotherapy.

P07.34

T/C-FISH studies on telomere length of derivative chromosomes in HeLa cells

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Telomeres play an essential role in preserving chromosomal integrity and genomic stability. To achive a better understanding of telomere length and its possible impact on development of chromosome aberrations, we analyzed the telomere length of the karyotypically well characterized HeLa cell line. HeLa cells have a hypertriploid chromosome number with specific numerical deviations and clonally abnormal chromosomes known as HeLa signature chromosomes. Karyotypic heterogenity is also present exhibiting 'shared' and 'unique' karyotypic alterations. To measure the telomere length of individual chromosomes we did sequential analysis of metaphase spreads using telomere/centromere fluorescence in situ hybridisation (T/C-FISH) as well as multi-colour-FISH (M-FISH) to identify the marker chromosomes unequivocally. In the present study, individual telomere length was highly heterogeneous. Telomere length was associated with both frequency and type of chromosomal aberrations. The telomere length of derivative chromosomes, which were present in almost all metaphases did not differ from the average telomere length. However, derivative chromosomes appearing only in few cells showed great variability in telomere length (0% up to +643%) compared to the average telomere length. Regarding the type of chromosome aberration small marker chromosomes and isochromosomes showed significantly longer telomeres than deletions and complex translocations.
Our results imply that recurrent aberrations have passed the point of short telomeres and high instability. Their telomeres may be stabilized and even increase in length possibly depending on the type of aberration.

P08. Statistical genetics, includes Mapping, linkage and association methods

P08.01

Tissue inhibitor of metalloproteinases-2 -418 G/C gene polymorphism and abdominal aortic aneurysm

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Objective and design. Pathogenesis of abdominal aortic aneurysm (AAA) is connected with abnormal extracellular matrix remodeling with the assistance of extracellular matrix metalloproteinases (MMPs). Tissue inhibitors of metalloproteinases (TIMPs) inhibit their activity. Any imbalance in the MMPs/TIMPs ratio may cause various disorders. A decrease of tissue inhibitor of metalloproteinases-2 (TIMP2) gene expression was detected in AAA patients. Recently, a -418 G/C (rs8179090) polimorphism of the TIMP2 gene promoter, influencing the transcription rate of the gene has been described. The aim of this study was to investigate whether -418 G/C gene polimorphism was associated with AAA in the Polish population.

Methods. TIMP2 gene promoter polymorphism was evaluated by polymerase chain reaction followed by restriction enzyme analysis and pyrosequencing in 128 patients affected with AAA and 180 individuals treated as references. The control group was directly matched to patients according to common risk factors of vascular diseases.

Results. The genotypes distribution was 17 CC, 5 CG, 106 GG in the 128 AAA cases and 12 CC, 0 CG, 168 GG in the 180 control subjects. The frequency of the C allele was significantly higher in the AAA patients than in the control group (P=.0005, OR=2.516). The distribution of genotypes also differed significantly between the studied groups (CC+CG vs. GG: P=.0037, OR=2.906) or was close to being significantly different (CC vs. GG+GC: P=.0501, OR=2.144).

Conclusion. This study supports the hypothesis that TIMP2 and -418G/C polymorphism located in promoter of TIMP2 gene are important in AAA pathophysiology.

P08.02

Analysis of polymorphisms in GABRA2 and AUTS2 genes in patients with alcoholism from Russia

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Family, twin and adoption studies have provided evidence of a genetic component (40-60%) in the origins of addictive disorders. One of very few confirmed genetic association findings differentiating alcoholics from non-alcoholics is with variants in the inhibitory gamma-amino butyric acid a2 receptor subunit (*GABRA2*) gene. Also according to the recent genome-wide association study variation in autism susceptibility candidate 2 gene (*AUTS2*) was strongly associated with alcohol consumption. Population stratification and ancestry differences within populations may compromise the success of association studies. Therefore analysis of genes variants in homogeneous ethnic groups is of great importance.

We designed a classical case-control association study for two polymorphisms: rs279858 in *GABRA2* and rs6943555 in *AUTS2* that were tested for association with alcoholism. 307 men with ICD-10 diagnosis of alcoholism (112 Russians, 91 Tatars, 100 Bashkirs), and matched control groups were typed for the above-mentioned gene variants using PCR-RFLP technique.

In Russian population the frequency of individuals carrying the **G* allele of rs279858 polymorphism was significantly higher in patients with early-onset alcoholism as compared with the healthy controls (p=0.03;OR=1.40). The carriers of *GABRA2*A/*A* genotype were found to be at low-risk of alcoholism development (p=0.04;OR=0,57). In Tatars the frequency of *AUTS2* rs6943555 **T* allele was higher in group of alcoholics with acute alcohol psychosis (0.74) as compared with controls (0.64). However observed differences were not statistically significant.

Our results suggest that *GABRA2* genetic variation might be involved in the development of alcoholism in Russians. This work was supported by Russian Foundation for Basic Research (#11-04-97032-r_povolzhye_a).

P08.03

Fine-mapping of the *PICALM* locus, CSF biomarker profile analysis and neurofibrillary pathology in a Flanders-Belgian Alzheimer cohort

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Genome-wide association studies (GWAS) have identified significant genetic association of Alzheimer disease (AD) with genetic variations located 5' upstream of the gene *PICALM* gene. We used a high-density single nucleotide polymorphism (SNP) map of the *PICALM* locus in a Flanders-Belgian patient-control cohort and analyzed the effect of SNP genotypes on cerebrospinal fluid (CSF) biomarker profiles in AD patients. We additionaly investigated association with AD related neurofibrillary changes.

We have performed SNP genotyping in the PICALM locus in 1047 patients and 858 healthy control individuals. In addition CSF levels of β-amyloid peptide ($A\beta_{1-42}$), total tau protein (T-tau) and tau phosphorylated at threonine 181 ($P\text{-tau}_{181P}$) were available for 308 patients. Quantitative scoring of tau pathology was performed on immunohistochemical stained brain sections of autopsied AD patients. Our results show

two *PICALM* SNPs (SNP 2, SNP 4), both located in the 5' region, with nominal association with AD. Interestingly, SNP 4 showed borderline significance with T-tau in CSF of AD patients, while SNP 2 was significantly associated with $P\text{-tau}_{181P}$. No significant association could be identified for AD related neurofibrillary tau pathology, probably because of limited statistical power due to a small sample size (N = 24).

We were able to fine-map the GWAS *PICALM* association signal in the Flanders-Belgian cohort. Furthermore, this study strengthens the observation that genetic variations in *PICALM* affect AD risk and that these variations represent genuine AD susceptibility factors. Of specific interest, our study highlights a possible implication of *PICALM* in tau biology in AD.

P08.04

Association of Angiotensinogen M235T polymorphism in Tunisian patients with dilated cardiomyopathy

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Background: Angiotensinogen (AGT) is a liver protein that interacts with renin to produce angiotensin I, the pro-hormone of angiotensin II (Ang II). The aim of this study was to determine the association of Angiotensinogen (M235T AGT) polymorphism with the risk of dilated cardiomyopathy in a Tunisian population.

Methods: A total of 73 patients with dilated cardiomyopathy was compared to 149 ethnically, age- and gender-matched controls.

Results: The frequencies of the TT genotype and T allele were significantly higher in patients as compared with controls, and were associated with increased risk of dilated cardiomyopathy (AGT TT versus MT and MM: OR = 4.1 (95% CI, 2.78-6.24; p = 0.018)); T versus M: OR = 1,3 (95% CI: 1.25-3.27; p = 0.008)). No association was found between the combined genotypes (TT+MT) or T allele and left ventricular end diastolic diameter in dilated cardiomyopathy patients with severe and moderate clinical phenotypes.

Conclusion: TTgenotype and T allele of Angiotensinogen (M235T) gene polymorphism are associated with increased risk of dilated cardiomyopathy in a Tunisian population but do not influence the cardiac phenotype severity

P08.05

Estimating the prevalence of TRIC gene mutations in 144 Iranian families with non-syndromic autosomal recessive hearing loss

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Hereditary hearing impairment is a genetically heterogeneous disorder, which affects about 1 in 1000 newborns. So far, over 90 loci and 40 genes have been mapped for autosomal recessive non-syndromic hearing loss (ARNSHL).

One of the loci related to ARNSHL, DFNB49, is located on the long arm of chromosome 5. The gene responsible for the hearing loss in this locus is *MARVELD2*, which encodes an essential protein called TRIC.

DFNB49 was first described in our neighboring country, Pakistan, and is estimated that approximately 1.06% of the causes of ARNSHL in this population, is due to *MARVELD2* gene mutations.

To determine the contribution of *MARVELD2* gene in Iranian deaf population, one hundred and forty four ARNSHL families with two or more affected individuals originated from different ethnic groups of Iran were chosen for the screening of this gene's mutations. Among these families, which were subjected to homozygosity mapping using flanking STR markers of *MARVELD2*, one family showed linkage to DFNB49. *TRIC* gene sequencing in this family led to the identification of a novel mutation (c.1543delA) in exon 6 which causes a premature stop codon [p. (Lys517ArgfsX16)].

According to our result, it is computed that 0.7% of families with ARNSHL in our country manifest hearing loss due to mutations in the *MARVELD2* gene and it shows the low contribution of this gene in Iranian population.

P08.06

MEFV gene mutations are associated with Behçet's Disease

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Mutations in MEFV, the gene encoding pyrin, play majority role an autoinflammatory disease Familial Mediterranean Fever (FMF). Behçet's disease is a chronic inflammatory multisystemic disorder of unknown cause. In recent years, prevalence MEFV gene mutations in Behçet's disease (BD) has been reported significantly higher compared with general population. We were investigated prevalence of five MEFV gene mutations (M694V, M680I, V726A, E148Q and P369S) in BD patients and comparison with controls.

DNA samples were collected from 207 BD patients and 200 control subjects were used. MEFV gene mutations were detected by PCR-RFLP method. SPSS 16.0 Software was utilized to estimate OR and Chi-square tests.

All investigated mutations were detected higher in BD patients. But only two mutations, M680I and E148Q, were out of significantly higher than controls. Especially E148Q mutation found remarkable way higher in BD patients (14.49% in BD patients vs 4.50% in control; p=0.001; OR, 3.60; 95% CI, 1.66-7.77). This mutation contains approximately 35% of the observed total mutations in BD patients. Additionally, total mutation rate was detected higher in BD patients (p=0.00001; OR, 2.74; 95% CI, 1.75-4.29). On the other hand, compound heterozygosites were found higher in BD patients than controls (2.90% vs 0.50%), but statistically significant not found (p>0.05).

Our study showed that, MEFV gene mutations associated with BD. Especially E148Q and M680I mutations may play a role in BD susceptibility.

P08.07

Breast Cancer Risk Assessment in a High Risk Cohort: Which covariates are associated with differential model performance?

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Background: Clinical prediction models estimating lifetime risk of breast cancer vary widely in their estimates. There is a need to communicate to the medical community this variation and reasons for the differences.

Methods: Using a NYC cohort (N=1,857 women) we evaluated the model performance between two prediction models including non-genetic and genetic risk factors (BCRAT and IBIS), by assessing accuracy through the Hosmer-Lemeshow goodness of fit statistic and discrimination by the area-under-the-receiver-operating (AUC) characteristic curve. We assessed differential model performance (accuracy and discrimination) in subgroups defined by broad covariates.

Results: We observed substantial difference between the two models in estimating lifetime-risk: IBIS was better calibrated and showed greater discrimination than BCRAT. Additionally we compared the discrimination and accuracy of the two models for covariate specific subgroups. The observed/ predicted ratio was better for IBIS than for BCRAT for all subgroups except those defined by race. In addition, IBIS was better than BCRAT in identifying women who went on to develop cancer except for the subgroup of women with at least one breast biopsy.

Conclusion: There is a need for a single accurate model that performs well for all women. In our cohort, we found that overall prediction was better in IBIS for almost all covariate-specific subgroups of women, including women who were not gene carriers and who had a more limited family history. Thus, enhancing existing models such as IBIS with additional risk factors (number of biopsies and race/ ethnicity) may further improve the performance.

P08.08

First genome-wide association study of classic bladder exstrophy

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Classic bladder exstrophy (CBE) is part of the bladder exstrophy and epispadias complex (BEEC), a spectrum of urogenital anomalies in which part or all of the distal urinary tract fails to close. Birth prevalence, including terminated pregnancies, has been estimated to be 1 in 37 000. Isolated BEEC is assumed to be multifactorial involving both genetic and environmental factors.

For the purpose of a genome-wide association study (GWAS) 127 CBE patients of Central European ancestry were genotyped using Illumina's Beadchips and compared to 525 ethnically matched population based controls. After standard Quality Control (QC) procedures the data set was subjected to imputing based on 1000 Genomes Project reference panels. After applying post-imputation QC, single marker analysis was conducted using a logistic regression (additive model). Our analysis yielded promising results, including a genome-wide significant finding ($P = 4.55 \times 10^{-8}$) for a SNP near the *SALL1* gene. Interestingly, mutations in *SALL1* have been reported to cause Townes-Brocks syndrome, a rare genetic disease with (among others) anorectal and urogenital/renal malformations. Currently, replication of the top associated SNPs (n=88) is been carried out in additional 200 cases and ethnically matched controls (n=500).

Our data provide the first GWAS for CBE patients in a reasonable large cohort with this rare malformation. While our top finding *SALL1* represents an excellent candidate for urogenital malformations, further studies are warranted to verify these findings.

P08.09

Catechol-O-methyltransferase Val158Met polymorphism and uterine leiomyoma

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Uterine leiomyoma (ULM) is the most common gynecological benign tumors that affecting around 20% to %50 of women over age of 30 years. Although their molecular pathogenesis is still unknown, ULM has a multifactorial etiology determined by both genetics and environmental factors. The present study was designed to find out whether Val158Met polymorphism in the Catechol-O-methyltransferase (COMT) gene are associated with the risk of ULM.

We analyzed COMT Val158Met polymorphism in 105 ULMs patients and 105 healthy subjects by using polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) assay. We found remarkably similar frequencies in ULM compared with controls for COMT Val158Met genotypes and alleles, and no association was found between ULM and this polymorphism (p=0.482). COMT 158 Met allele in patients with large (≥5 cm) fibroids was higher than in patients with small (<2 cm) fibroids, and significant association was found between fibroid size and COMT 158 Met allele (p=0.021 O.R 0.54 95%CI 0.30- 0.97).

Our results reflect that COMT Val158Met polymorphism is not associated with increased risk of ULMs but Val158Met polymorphism may be risk factor for development of large fibroids in Turkish patients with ULM.

P08.10

Relationship between cytokine gene polymorphisms and graft-versus-host disease after allogeneic stem cell transplantation in an Iranian population

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Despite advances in post-transplant immunosuppressive strategies GVHD is a major complication of allogeneic HSCT, leading to serious morbidity and mortality. In this retrospective, case-control investigation, 91 subjects were recruited, including Iranian patients and their HLA matched siblings.

Donor/recipient pairs were genotyped for cytokine polymorphisms including IL-1α -889, IL-1β -511, IL-1β +3962, IL-1R pst1 1970, IL-1RA mspa1 11100, IL-4Rα +1902, IL-12 -1188, IFN-γ +874(A/T), TGF-β codons 10 and 25, TNF-α -308, TNF-α -238, IL-2+166, IL-2 -330, IL-4 -1098, IL-4 -590, -33, IL-6 -174, IL-6 nt565, and IL-10 -1082, -819, and -592. Cytokine typing was performed by PCR-SSP assay.

Negative association was found between aGVHD and donor IL-10 GCC haplotype or donor IL-4Ra A allele in the whole population studied. When we compared within the leukemia subgroup, we observed positive association between recipient IL-1α -889/C allele and negative association between recipient IL-10 CAA haplotype and donor IL-4Ra A allele and development of aGVHD. We also observed possible positive and negative association for different genotypes and aGVHD; however, on multivariate analysis only donor IL-4Ra and donor IL-12 showed significant association. We conclude that several cytokine polymorphisms are positively and negatively associated with aGVHD in Iranian HLA matched siblings, of which IL-4Ra and IL-12 may play important roles.

P08.11

Identifying genetic determinants of congenital heart defect in Down syndrome

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Congenital heart defect (CHD) is a common developmental defect of Down syndrome (DS) occurring in 40% of cases. While carrying three copies of genes or other functional genomic elements on chromosome 21 increases the risk for CHD, trisomy 21 itself is not sufficient to cause CHD. Thus additional genetic variation and/or environmental factors could contribute to CHD risk. Here we use association studies to identify genomic variations that in concert with trisomy 21, determine the risk for CHD in DS. This case-control GWAS includes 187 DS with CHD (AVSD=69, ASD=53, VSD=65) as cases, and 151 DS without CHD as controls. Chromosome 21 specific association study revealed rs2832616 and rs1943950 (both cis-eQTLs for KRTAP7-1 gene) as CHD risk alleles (adjusted p-values < 0.05). Furthermore rs2183593 and rs7282991 (both cis-eQTLs for ADARB1 gene) were identified as risk factors for ASD. Since DS is likely to be a disorder of gene expression, 2-locus interaction was applied for whole genome eQTLs. A pair of interacting eQTL on chr2 and chr11 was identified. Furthermore, a search for chr21 risk CNVs for CHD was performed using a customized chr21 array of 135K probes across 55 DS-CHD and 53 DS controls. It revealed two CNV regions (FDR=0.04) located in the region previously associated with CHD risk in DS and another CNV region (FDR=0.03) upstream of POFUT2 gene. We propose that the CHD risk of DS is determined by specific SNPs and CNVs variations on chr21 and interaction of non-chr 21 genomic variants.

P08.12

Recommendations for genome-wide search for epistatic loci

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Various strategies to identify interacting SNPs in GWAS studies were evaluated. Series of „realistic“ disease models were defined on a 2-SNP-genotype table by specification of allele frequencies, penetrances, minimal distance between available and causal SNPs. We compared single-marker analysis with multi-marker analysis, investigated performance of tests for interaction and tests including both marginal and interaction effects, so-called „tests allowing for interaction“. We compared case-only with case-control tests for interaction and contrasted the performance of allelic and genotypic models.

A subtle problem is that tests including marginal effects may become significant because of the marginal effect of just one SNP from a pair. Since our goal was to detect both SNPs, we embedded tests allowing for interaction in a two-step strategy: analysis of all pairs with a test with marginal effects, followed up by an interaction test on significant pairs remained after multiple testing adjustment.

For about 5% of settings the most efficient strategy is single-marker analysis, typically when allele frequencies are high or causal variant tagging is poor. For another 5% of models the most powerful strategy is testing for interaction without inclusion of marginal effects provided that a case-only test is used. Genotypic case-only test is typically more powerful than allelic case-only test. In the remaining majority of scenarios, a hybrid strategy is most suitable: genome-wide interaction analysis with a combined case-only-interaction/marginal-effects test; follow-up analysis of the significant pairs with a test for interaction excluding the strongest marginal effect but allowing for marginal effect of the second potential SNP.

P08.13

The power of meta-analysis of RNA-seq datasets for eQTL identification

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Many genetic variants affect gene expression levels, although the exact mechanism through which this works is still mostly unclear. Previously, numerous expression quantitative trait locus (eQTL) mapping studies have been performed using microarray data. Recently, through next generation sequencing gene expression level quantitation has become possible (RNA-seq) and it has shown to be a very powerful approach of quantifying the transcriptome.

However, various RNA-seq strategies have been proposed, but it is still unclear what the best strategy for eQTL mapping is and how to combine eQTL data that has been generated by different technologies.

Here, we used three different types of RNA-seq data: paired-end RNA-seq (56 samples), single-end RNA-seq (64 samples) and deepSAGE data (94 samples). eQTL mapping (FDR = 0.05) on each dataset yielded 1287 unique genes for single-end RNA-seq, 601 unique genes for paired-end RNA-seq and 1188 unique genes for deepSAGE data. We show that a meta-analysis on different types of data can be performed to increase statistical power, permitting us to identify significant associations to 3504 unique genes. We compared the eQTLs that had been identified using RNA-seq and array based data and observed a concordance of 95% in allelic directions, indicating highly consistent results.

Our study indicates that different types of RNA-seq datasets can be well combined and that meta-analysis of RNA-seq is a logical step forward to gain better insight into the genetic regulation of gene expression variation.

P08.14

Alternative splicing in the Fanconi anemia candidate gene FAAP100

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Fanconi anemia (FA) is a rare autosomal or X-chromosomal recessive disease that can be regarded as a model for genomic instability, premature aging and tumorgenesis. Each of the 15 genes, whose products are members of the FA/BRCA pathway for genomic maintenance, have been identified to be causative for FA. FAAP100 is an additional member of the FA core complex but has not yet been found to be mutated in FA patients. It forms a subcomplex with FANCL and FANCB and protects both components for proteolytic degradation. After siRNA depletion or gene knockout FAAP100-deficient cells show features comparable to other FA cells defective of FANCD2 monoubiquitination. Because FAAP100 is a FA candidate gene, we screened seven FANCD2 monoubiquitination deficient cell lines by Sanger sequencing of all exons and adjacent introns portions. We detected eight common SNPs registered in the dbSNP database and a non-annotated heterozygous synonymous single-base substitution in exon 9 which cannot be causative for FA. Additionally, we found an alternative splicing event via sequencing cDNA of the FA cell lines. This event occurs because of the presence of a cryptical splice donor and results in skipping of 414 bp (c.1760_2173del414). In order to test an association with FA we examined FAAP100 transcripts in a control cell line. Since we detected the same alternative splicing event in the control it seems not likely to be causative for FA. The alternative transcripts could be involved in cell cycle-dependent or tissue-specific gene regulation, a role that will be studied in further experiments.

P08.15

Novel method of CNV analysis in FcγR locus and its application to immune-related diseases

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Genetic variants near the FC-gamma receptor (FcγR) locus are associated with several immune-related diseases. However, most FcγR genes are located in complex regions of segmental duplications (SD) and they are therefore not well covered by the genotyping platforms. To be able to identify copy-number variants (CNVs) in this locus, we first developed a method to analyse CNVs using principal component analysis of the raw intensity values of single nucleotide polymorphisms (SNPs) genotyped on the Immunochip platform. This platform includes 1,159 SNPs in the SD block of FcγR genes; of these 1,019 (88%) failed our quality control for SNP analysis but their intensity values are informative for the CNVs estimation. We identified several CNV loci in the FcγR block. Second, we confirmed our results via an independent method - arrayCGH genotyping - and observed a perfect correlation in CNV estimation between both methods. Third, we applied our method to an RA cohort (3,326 cases; 3,397 controls). We found no associations between these CNVs with RA ($p > 0.05$). We are now applying our method to cohorts of celiac disease and inflammatory bowel disease, in total ~20,000 subjects. Fourth, by performing functional studies we observed a correlation between the number of FCGR3A gene copies and FCGR3 (CD16) expression on T-cells.

Conclusion: We have developed a method to accurately estimate CNVs based on SNP intensity data that can be extended to other SD loci in the human genome.

P08.16

Study allelic variants G-2548A of the gene LEP and G223A gene LEPR in individuals with different levels of the main indicators of lipid profile

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It is known that structural and functional properties of lipoproteins are controlled by genetic factors. In this regard, the current association of polymorphic variants of genes with the level of serum lipids are the subject of intense attention. We examined polymorphic variants of the gene G-2548A LEP and leptin receptor gene G223A LEPR leptin in patients with different levels of the major lipid profile.

The material for the study included DNA samples from 434 healthy individuals. Determining the level of the main indicators of lipid profile (total cholesterol, triglycerides, low density lipoprotein, high density lipoprotein, atherogenic index and body mass index) was performed by standard enzymatic methods. Analysis of polymorphic DNA loci of LEP and LEPR was performed by PCR-RFLP.

The study found that significantly more frequent in individuals with high levels of total cholesterol (64,58% vs. 50,82% in the group of individuals with total cholesterol levels within the physiological norm; p=0,0189), triglycerides (55,45% vs. 41,67% in the group of individuals with triglyceride levels in normal; p=0,0408) and body mass index (85,71% vs. 41,67% in group of persons with BMI within the physiological norm; p=0,0524) alleles found LEPR A and LEP A, and in individuals with a level of performance within the physiological norm - alleles of LEP G, LEPR G and the genotype of LEP G/G (23,36% vs. 8,33% in the group of individuals with total cholesterol is normal; p=0,0321).

P08.17

Association of *ACE*, *PPARGC1A* and *PPARA* Genotypes with Footballers Performance

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The aim of this study was to determine the impact of *ACE* (I/D), *PPARGC1A* (G/A), *PPARA* (G/C) polymorphisms on footballers performance among 199 Lithuanian professional footballers and 167 sedentary, healthy men (controls). The football players were divided into groups according to the position in the field: forwards (n=44), defenders (n=63), midfielders (n=75), goalkeepers (n=17). The novelty of the study is association analysis of *PPARGC1A*, *PPARA* polymorphisms in combination with *ACE* which is the gene candidate for footballers' performance research by others. Genotyping was performed using the methods of polymerase chain reaction and restriction fragment length polymorphism. The results showed that the *ACE* genotype distribution was significantly different between the total football players group and the controls (II-23.6%,ID-46.7%,DD-29.6% *vs.* II-24.6%,ID-29.9%,DD-45.5%, P=0.002). We revealed that in defenders (P=0.033) and midfielders (P=0.012) the *ACE* ID frequency was higher although DD genotype frequency was lower than in control. According to the analysis of *PPARGC1A* and *PPARA* polymorphisms, significant differences were determined between forwards and controls (*PPARGC1A*: GG-54.6%,GA-29.5%,AA-15.9% *vs.* GG-49.7%,GA-44.3%,AA-6.0%, P=0.044; *PPARA*: GG-52.3%,GC-40.9%,CC-6.8% *vs.* GG-72.4%,GC-24.6%,CC-3.0%, P=0.034). There were no athletes with *PPARGC1A* AA and *PPARA* CC genotype among the researched goalkeepers. In the whole cohort, the odds ratio of [*ACE* ID+*PPARA* GG] being a footballer was 1.69 (95%CI 1.04-2.74), and of [*ACE* ID+*PPARGC1A* GG] 1.93 (95%CI 1.10-3.37), and of [*ACE* II+*PPARA* GC] 2.83 (95%CI 1.02-7.91) compared to controls. In conclusion, the above data suggest that *ACE* I allele in combination with *PPARGC1A* G allele or *PPARA* G allele is associated with football players' ability.

P08.18

HLA class II (DRB1 and DQB1) genes associated in Tunisian patients with idiopathic dilated cardiomyopathy

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There is growing evidence of an important immunologic/ autoimmune component to idiopathic dilated cardiomyopathy (dCMP) disease, with abnormalities in both humoral and cellular response that is becoming the major pathogenic hypothesis for myocardial damage. Owing to the important participation of the immune system in the development of the CMP, genetic factors implied on its onset could include genes located on the Human Leukocyte Antigen (HLA). The present study was the first report to evaluate the relationship between class II HLA genes HLA (DRB1 and DQB1) and the genetic susceptibility to idiopathic dCMP in Tunisian patients. The HLA (DRB1, DQB1) alleles were analyzed in 76 patients with idiopathic dCMP and 111 ethnically matched healthy controls using polymerase chain reaction-sequence specific primers technique. An increased frequencies of HLA-DRB1*0401 (OR = 2.67, $P < 0.001$), HLA-DQB1*0302 (OR = 3.28, $P = 0.001$) and HLA-DQB1*0401 (OR = 6.26, $P = 0.005$) alleles were found in patients compared with healthy controls. Individuals with HLA-DRB1*1301 (OR = 0.24, $P < 0.001$) and HLA-DQB1*0201 (OR = 0.49, $P = 0.002$) alleles have a protective effect against idiopathic dCMP. Two haplotypes were associated with increased risk of idiopathic dCMP: DRB1*0401/DQB1*0302 (OR = 4.53, $P = 0.002$) and DRB1*0401/DQB1*0401 (OR = 9.42, $P = 0.004$). In conclusion, our data suggest that the variation in class II HLA alleles could be a genetic factor involved in the susceptibility to idiopathic dCMP in the Tunisian population.

P08.19

Genetic data fusion: combining sequence and array data for improved genotype calling

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Data sets containing large cohorts of individuals who have been genotyped on microarrays as well as sequenced at low coverage are now becoming available. Whilst the genotype calls coming from modern arrays are of high quality, accuracy and call rate can still be improved by incorporating sequence data into calls. This is particularly true for low frequency variants where very few observations are available to identify clusters for the rare genotypes. Higher quality genotype calls and better call rates will reduce time spent on quality control and decrease Type I and II error in genome wide association studies.

We present a Bayesian mixture model for genotype calling from array allele signal intensities, with parameter estimation and calls augmented by genotype likelihoods from sequence data. The method successfully identifies and discards spurious data and is capable of handling individuals with missing values, for example, a subset of individuals may have only been assayed on an array and not sequenced. This results in genotype calls with greater accuracy and a higher call rate than standard array-only methods. The implementation requires similar computational time to standard software for array-only based genotyping, providing higher quality genotype calls for minimal extra time and effort. We demonstrate these capabilities on 1000 Genomes Phase 1 data which have been genotyped on the Illumina HumanOmni2.5S chip as well as sequenced at 4X coverage.

P08.20

eQTL analysis of glucocorticoid regulated gene expression: new insight in the genetics of mood and anxiety disorders

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Abnormal hypothalamic-pituitary-adrenal axis regulation is a key neurobiological characteristic of depression. Glucocorticoid receptor (GR) function has been shown to be disturbed in depression, hence polymorphisms altering the transcriptional effects of GR-activation might be interesting candidates for this disorder.

The aim of this study was to identify SNPs associated with glucocorticoid (GC)-induced gene expression changes (*cis*-eQTLs) in peripheral blood.

160 male Caucasians (69 cases, 91 controls) were genotyped using Illumina Human660W-Quad BeadChips. Imputation was performed using IMPUTE-v2 with HapMap III and 1,000 Genomes Project as reference panels. Baseline and stimulated (1.5 mg dexamethasone) gene expression was analyzed using Illumina Human HT12v3 array. Quality control checks, filtering, batch corrections and linear regression analysis was performed in PLINK and R.

Of a total of 4,395 significant *cis*-eQTLs, 2,364 significant response-eQTLs, namely loci associated with GC-stimulated gene expression variation were identified after multiple testing corrections. Over 44% of response-eSNPs were located >200kb from the probe, indicating long-range regulation of gene expression by GCs. This was accompanied by significant enrichment of GR response elements (GREs) within the response-eQTLs. We also observed differences in the affinity of GREs between the opposite SNP alleles. Further, response-eQTLs were significantly more likely to be associated with unipolar depression susceptibility loci from a recent meta-analysis than baseline eQTLs. Interestingly, the majority of these enriched eSNPs alter the gene expression of more distant genes.

In conclusion our data suggest that GC-stimulated eQTLs could expand our understanding of the genetic basis of stress-related disorders, in which GR-function plays an important pathophysiologic role.

P08.21

Atypical haemolytic uraemic syndrome in a large population with a single CFH (complement factor H) gene mutation: modified penetrance data including a newly discovered family, based on mutation testing and family history.

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Atypical haemolytic uraemic syndrome (aHUS) can have high morbidity and mortality. Usually it is due to mutations in the Factor H, Factor I, or membrane cofactor protein genes, which play a role in the alternative pathway of complement activation. The trigger events for an episode of HUS leading to renal failure are unknown. We have previously presented initial penetrance data for four families with over 400 individuals, mainly resident in one region, who carry the same Factor H mutation [c.3643 C>G (p. R1215G)].[1] Previously we knew of three families whom we have been unable to link to date. A fourth branch has now come to light.

We have constructed updated Kaplan-Meier survival curves for this mutation to estimate the penetrance. In the current generation we have a large number of unaffected carriers. We have very few obligate unaffected carriers in previous generations, as consanguinity means we cannot clearly trace the line of descent of the mutation. We have therefore estimated the penetrance by including family members at 50 % and 25 % risk with appropriate weighting. Including such members lowers the penetrance from a lifetime risk of around 2/3 to just under 1/2 (0.45; i.e. 4/9). The penetrance of aHUS by adulthood falls from 1/3 to 1/4. We compare this approach with other methods of estimating historical carrier frequency in less than fully penetrant conditions.

1. Sansbury FH *et al.* J Med Genet 2010:47 (Supplement 1).

P08.22

Analysis of association between age and JAK-STAT signaling pathway gene polymorphism

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Aim of study was to estimate alleles and genotypes frequencies dynamic of STAT5A (rs9889323), JAK1 (rs310216) and JAK3 (rs3212780) genes with age.

Total group (1678 unrelated individuals, from 1 to 109 years, ethnic Tatars from Russia) was divided into young (1-20), middle-age I (21-35), middle-age II (36-55), aged (56-74), senile (75-89) and long-living (90-109) persons. Gene polymorphism was analyzed by PCR-RFLP. For comparison of age groups was used Fisher's two-tailed exact test. Search of genetic markers associations with age was performed using logistic regression analysis (SPSS18.0).

In female there was an increase of JAK3*C/*T genotype frequency in age from 30 until 80 years (p=0.045, OR=1.019). In middle-age I group STAT5A*C/*C genotype frequency was lower than in aged (P=0.005), senile (P=0.005) and long-livers groups (P=0.002). Frequencies of STAT5A*C/*T and STAT5A*T/*T genotypes differed between aged individuals, in one hand, and senile and long-lived persons, on the other (p<0.01). STAT5A*T/*T genotype frequency was decreasing with age in both male (57-98 years, p=0.033, OR=0.971) and female (44-87 years, p=0.005, OR=0.976); also only in female there was increasing of STAT5A*C/*T genotype frequency in 44-87 years (p=0.002, OR=1.029).

Thus, STAT5A (rs9889323) and JAK3 (rs3212780) gene polymorphisms are important for achieve of senile age in both male and female; STAT5A (rs9889323) polymorphic marker may be associated with longevity in male.

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P08.23

Identifying additional variants associated to celiac disease by imputation-based GWAS

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Celiac Disease (CeD) is a complex immune-mediated disorder caused by an unknown number of genetic variants. To date, 39 non-HLA loci have been identified by GWAS that explain 15% of the heritability. Previous GWAS studies in CeD were limited to directly genotyped SNPs. This study aims to boost the GWAS' power through imputation. Genotyping data for four European CeD collections of cases (3,796) and controls (8,154) used in the previous GWAS study will be imputed with the pilot version of the Genome of the Netherlands (GoNL). GoNL consists of whole genome, high coverage (12), sequencing data from 250 Dutch trios. Additionally, we will assess the added value of using a population specific dataset (GoNL) compared with the multi-ethnic 1000 Genomes Project (1KG) reference. We did obtain our first results with the GoNL pilot data of 48 trio's. Preliminary results in chromosome 20 indicate that GoNL contains genotypes for 99.3% of the HapMap550 SNP-set, whereas 1KG covers only 72%. The imputation R2 values were similar between the two reference panels for common markers (MAF > 5%) but exhibit a significant increase for GoNL in rare variants. We also measured the concordance between imputation with HapMap2 and GoNL and a validation dataset consisting of 1,758 samples genotyped on Immunochip in chromosome 3. GoNL imputation showed an average increase from 97% to 99% in the concordance and showed suggestive association for 4 novel variants in the CRR locus (not present in HM2) that are not in linkage disequilibrium with a previous associated SNP.

P08.24

Evaluation of the performance of several imputation strategies in an admixed sample from Mexico City

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Background: We explored the imputation performance of the program IMPUTE in an admixed sample from Mexico City (N=1,310). We evaluated the: (a) impact of different reference panels on imputation; (b) potential differences in imputation performance between single-step and two-step approaches; and (c) effect of different INFO score thresholds on imputation performance.

Methods: The samples were genotyped with the Affymetrix 5.0 array. We randomly masked 5% of the markers directly genotyped on chromosome 12 (n=1,046) and compared the imputed genotypes with the microarray genotype calls. The concordance rates between imputed and observed genotypes reflect imputation accuracy and the proportion of non-missing genotypes indicate imputation efficacy.

Results: Using an INFO threshold of 0.9 to define valid genotypes, the single-step imputation approach produced slightly higher concordance rates than the two-step strategy (99.1% vs. 98.4% - HapMap phase II combined panel), but at the expense of a lower proportion of non-missing genotypes (85.5% vs. 90.1%). The 1,000 Genomes panel produced similar concordance rates to the HapMap phase II panel (98.4%), but increased substantially the proportion of non-missing genotypes (94.7% vs. 90.1%). The average INFO scores of alleles with frequencies <1% was much lower than the scores for alleles >5%.

Conclusions: The program IMPUTE had an excellent imputation performance for common alleles. Genotype concordances were higher than 98.4% using all the imputation strategies. The best balance of imputation accuracy and efficiency was obtained with the 1,000 Genomes panel. However, rare alleles were not captured effectively by any of the panels.

P08.25

Insersion/Deletion Polymorphism of The Angiotensin-Converting Enzyme Gene And Knee Osteoarthritis

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Objective/Aim:

Knee Osteoarthritis (OA) is a multi-factorial disease. Various genetic polymorphisms have been reported that they might be associated with OA. Angiotensin converting enzyme (ACE) is a critical component of the renin-angiotensin system, and a large body of evidence indicates its proinflammatory role. The aim of the present study was to examine the possible role of angiotensin-converting enzyme (ACE) insertion/deletion (I/D) gene polymorphism as a risk factor in the development of knee OA.

Meterial and Method:

In this study, we studied 102 (60 women, 42 men) patients with knee OA and 150 (87 women, 63 men) healty control groups. ACE I/D **polymorphism** were analyzed by using PCR (Polymerase Chain Reaction) method.

Findings: DD, DI and II genotype frequencies of ACE I/D polymorphism was detected 27 %, 58%, and 15 % in patient group and 32 %, 50 %, and 18 % in control group, respectively. There were not significant differences in genotype/allele frequencies of ACE gene polymorphism between patients with knee OA and controls (p=0.24).

Result: Our results reflect that ACE I/D polymorphism does not have a role in susceptibility to Knee OA in Turkish patients. Currently, we continue to testing higher number of people in both patient and control groups to obtain more data.

P08.26

Association analysis of the leptin receptor gene haplotypes with cardiovascular risk phenotypes

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Aims: Leptin is an adipocyte-derived protein with an important role in regulation of food intake, metabolism, reproductive and immune function. It acts through its specific receptor which is predominantly expressed in hypothalamus. Aim of this study was to investigate association of haplotypes of the leptin receptor gene (LEPR) with several cardiovascular (CVD) risk phenotypes, namely, body mass index, waist circumference, serum lipid, fibrinogen and C-reactive protein levels.

Methods: We selected 43 single nucleotide polymorphisms (SNP) in and near LEPR gene from genome-wide association study data (Human Hap300 Illumina platform) of 986 inhabitants of the island of Vis, Croatia. We used Haploview software to assess linkage disequilibrium (LD) structure in genomic region of LEPR gene and Unphased software for haplotype association analysis.

Results: SNPs were grouped into nine blocks according to LD structure. Although none of the single markers in LD block comprised of six SNPs (rs1782754, rs1171269, rs1022981, rs6673324, rs3790426 and rs1049338) was individually associated with waist circumference, haplotype A-C-A-A-G-A of this LD block showed the strongest association signal, p= 7.085 x 10-22. However, after permutation testing the result was found to be only marginally significant.

Conclusion: Haplotype association analysis of CVD risk phenotypes show marginally significant association of LEPR gene only with waist circumference.

P08.27

Allele-based N-Test in linkage analysis

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There are many tests of inheritance based upon sibling information for diseases that have late onset. The N-test (Green et al. 1983) is one of these tests, which utilizes information from affected siblings. The N-test is the count in affected siblings of the most frequently occurring haplotype from the father plus the analogous count from the mother. When applied to haplotypes, the N-test excludes recombinant families from the analysis. In this study we modified the N-test to be based on alleles instead of haplotypes. This modified allele-based N-test can include all families (recombinant as well as non-recombinant). We carried out a simulation study to find the thresholds and powers.

P08.28

A robustness study of parametric and non-parametric tests in Model-Based Multifactor Dimensionality Reduction for epistasis detection

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Model-Based Multifactor Dimensionality Reduction (MB-MDR) is data mining technique that enables the fast identification of epistasis, without the need to make restrictive assumptions about the modes of inheritance. The most commonly used implementation of MB-MDR involves testing one multi-locus genotype cell versus the remaining cells. By construction, this procedure creates two imbalanced genetic groups that subsequently need to be compared. To date, for continuous traits, we have adopted a standard F-test to make such group comparisons. However, when either the assumption of normality or homoscedasticity or both are violated, highly inflated type I errors and false positives are to be expected. In this study, we assess, through simulations, the effects of aforementioned model violations on the performance of MB-MDR to detect epistasis signals, and propose remedial measures in order to maintain efficiency. Since important lower order genetic effects can also give rise to inflated type I errors or false positive epistatic effects, we restrict our simulation study to pure epistasis models. In particular, we consider normal, chi-square and t-distributions with constant and non-constant phenotypic variances. In all simulating settings, we apply the standard F-test , as well as a novel implementation based on the Welch's F-test .The original traits were either left untransformed or first transformed into new traits via rank and logarithm transformation, or via a rank-transformation to normality.

In conclusion, when performing MB-MDR screening for gene-gene interactions with quantitative traits, we recommend to first rank-transformtraits to normality, prior to classical F-testing.

P08.29

Association of TNF polymorphism rs1800629 with Multisomatoform Disorder in a group of German patients and healthy controls

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Causes of MSD are not sufficiently elucidated yet, but genetic factors are suspected to influence MSD pathogenesis. The main symptom MSD patients suffer from is pain. As prior studies have demonstrated that genetic polymorphisms of different proinflammatory cytokines are associated with pain, our goal was to find out whether cytokine polymorphisms are also associated with MSD. Blood from 148 MSD patients and 149 demographically matched healthy controls was used for genotyping of nine polymorphisms located on seven cytokine genes. Thereafter statistical analysis was performed. In addition to the examination of possible associations with MSD, we searched for correlations with individual thermal and mechanical detection and pain thresholds, which were determined by quantitative sensory testing (QST). Association with MSD was found for alleles and genotypes of rs16944 (interleukin 1β), rs1800629 (tumor necrosis factor α) and rs909253 (lymphotoxin α). Due to multiple testing we corrected the results using Bonferroni method, which caused only the association of rs1800629 with MSD to remain significant. The rare A-allele occurred more often in MSD patients while the G-allele could be found more frequently in the control group (p=0.007). Genotype distribution was in line with those results as genotype GG correlated with being healthy (p=0.004) and AG was associated with MSD (p=0.008). We could not detect correlations for any of the tested polymorphisms with the investigated QST parameters. To conclude, we assume the A-allele of SNP rs1800629 (TNF) to be a risk factor for MSD while the G-allele appears to have a protective effect.

P08.30

The association of the MYF6 gene polymorphism with size and composition of muscle fibers

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Transcription factor myf6 regulates expression of many genes involved in development, maturing and work of human skeletal muscle. The results of our previous studies showed that frequency of MYF6 964TT genotype and 964T allele was significantly higher in endurance athletes in comparison with control subjects. The 964T allele carriers had 5% bigger cross-sectional area (CSA) of m. rectus femoris in comparison with 964CC genotype carries.

In the present study the DNA of 8 young healthy physical active males and 26 elite ice-skaters was genotyped using PCR-RFLP method. Histomorphological and immunohistochemical analyses were conducted. Percentage composition and CSA of muscle fibers were measured.

Average CSA of muscle fibers in young males was almost two times larger among TT genotype carriers (41385 ± 14636 mkm^2) in comparison with CC homozygotes (23065 ± 20691 mkm^2) and heterozygotes (25642 ± 20084 mkm^2), (P < 0.0001). Content of fast muscle fibers in ice-skaters was 40.7 ± 2.4% and content of slow fibers was 66.4 ± 2.5%, which did not differ among MYF6 genotypes. In TT homozygotes average CSA of both types of muscle fibers was larger than in CC homozygotes and heterozygotes (TT - 6278.8 ± 1560.4 mm^2, CC - 5500.7 ± 852.7 mm^2, CT - 5195.4 ± 1278.8 mm^2; P = 0.04).

In conclusion, the results of this study in two independent samples have shown the association of 964TT genotype with larger CSA of muscle fibers. It is necessary to conduct repetition studies of the MYF6 C964T polymorphism in greater samples of elite athletes.

P08.31

New genes for normal hearing function and age-related hearing loss by genome-wide association and expression studies

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Little is known about the molecular bases of normal hearing function and age-related hearing loss (ARHL) or presbycusis. Thus, research still needs to study hearing as a complex trait and to understand genetic factors underlying ARHL. To reach this goal an integrated strategy has been designed based on: A) GWAS on hearing quantitative traits as well as on ARHL qualitative traits, and B) expression studies in wildtype mice (at 4 and 5 days postnatal) using immunohistochemistry and confocal microscopy of genes identified by the GWAS. Up to now, for hearing quantitative traits we have run 2 meta-analyses of GWAS data (one with 6 isolated European populations and 1 adding samples from Caucasus and Central Asia), while for ARHL (qualitative traits) a candidate gene analysis has been performed. Matching all the data we defined a list of 27 candidate genes that have been chosen for expression analysis. 5 candidate genes show strikingly specific expression in the cochlea (e.g at the top of sensory hair cells and in the marginal cells of the stria vascularis) while the other 12 are located in multiple cell types in the cochlea. Additional studies now in progress include the identification of variants in genes confirmed by expression analysis, the development of mouse models, and replication of the data.

In conclusion, preliminary results prove the useful combination of GWAS and expression studies in providing new insights into the molecular basis of hearing function and ARHL, and may suggest new targets for hearing impairment treatment and prevention.

P08.32

Association of FOXE1 in nonsyndromic cleft lip with or without cleft palate in Central European and Mesoamerican populations

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Nonsyndromic cleft lip with or without cleft palate (NSCL/P) is one of the most common birth defects. Its etiology is multifactorial, with both genetic and environmental factors contributing to this craniofacial malformation. Several genes have been suggested to play a role in NSCL/P development. However, only the *IRF6* gene has shown a convincing degree of consistency across studies. Recently, the forkhead box E1 (*FOXE1*) gene on chromosome 9q22 has emerged as promising candidate gene (Moreno et al., 2009). In that study, comprehensive genetic analyses in NSCL/P samples from different ethnicities revealed two markers, located inside a 70 kb LD-block containing *FOXE1*, to be strongly associated with NSCL/P. Also, *Foxe1* knockout-mice show a clefting phenotype, providing further evidence for *FOXE1* being a susceptibility gene for NSCL/P. However, so far, the genetic findings on *FOXE1 i*n NSCL/P have not been convincingly replicated.

In order to further elucidate the contribution of *FOXE1* to NSCL/P, we investigated the two most strongly associated markers of the initial study (rs3758249, rs4460498) in two case-control samples of Central European (949 NSCL/P cases, 1,163 controls) and Mayan Mesoamerican (156 NSCL/P cases, 338 controls) descent. We obtained significant associations for both variants in both samples, with rs4460498 providing the lowest *P*-values (P_{Europe}=6.50x10^{-06}, P_{Mayan}=0.0151). Furthermore, we obtained evidence that the effect size increases for homozygous carriers of the risk alleles, suggesting a recessive effect. Our data conclusively identify *FOXE1* as second confirmed candidate gene for NSCL/P and give rise to further investigations into its underlying functional basis.

P08.33

Analysis of association of polymorphic variants of D5S422 and D5S402 receptor gene gamma-aminobutyric acid GABRG2 with the level of intellectual development of man.

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INTRODUCTION: gamma-aminobutyric acid (GABA) is the basic type of inhibitory neurotransmitters in humans, providing a process of inhibition of the central nervous system by using three types of receptors. The gene GABRG2 (5q34) encodes the alpha subunit of the gamma receptor GABA and contains 9 exons. This gene has two polymorphic site (D5S422, D5S402), affecting gene expression and alter the permeability of the membrane for the transmission of nerve impulses. The analysis of polymorphic variants of D5S422 and D5S402 on the GABRG2 gene in individuals with different levels of intellectual development.

METHODS: The polymerase chain reaction (PCR) carried out an analysis D5S422 and D5S402 polymorphisms of the gene receptor gamma-aminobutyric acid GABRG2 in 180 unrelated individuals. The level of intellectual development (IQ) in subjects determined by the method of Cattell. In accordance with the performance IQ subjects were divided into two groups: those with high (above 140 points) and low (below 95 points) level of intellectual development.

RESULTS: Analysis of associations of polymorphic loci studied showed a significant reduction in the frequency of allele D5S422 * 15 (7.61% vs. 21.15% in the group with low IQ; P = 0.0009, x2 = 13.3887), and increased frequency of allele D5S402 * 2 (57.89% vs. 28.84%, P = 0.0031, x2 = 9.3491) in the group with a high level of intellectual development.

P08.34

Comparison of running time of variance-component based methods for whole genome association analysis

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One of the most flexible and powerful methods of accounting for genetic substructures in genetic association testing is the variance component (VC) approach based on the mixed models. To decrease the computational com-

plexity of this method, it was proposed using a two-stage score test instead of the standard likelihood ratio test. Several fast implementations of the score test, including approximate ones, have been developed recently. These methods differ in their computational speed and the accuracy of the SNP effect estimation. We compared the running time of the different implementations of score test (mmscore, EMMAX, FaST-LMM, GRAMMAR-Gamma), using simulated data. The GRAMMAR-Gamma implementation provides the fastest means to run genome wide association study using mixed models. Compared with EMMAX and the FaST-LMM, GRAMMAR-Gamma achieved a speed-up of more than 30 and 10 times, respectively, for the data studied. The more individuals and genetic markers are analyzed, the larger is the expected speed-up of GRAMMAR-Gamma compared to other methods. While the scenario above assumes use of an SNP array, one of the current challenges in statistical genomics is the analysis of whole-genome re-sequencing data. We investigated a scenario in which 36.5 millions of SNPs in 3,000 people were analyzed. Using GRAMMAR-Gamma method, the analysis of this data set was completed in 38 min.
We conclude that GRAMMAR-Gamma is a fast tool for the analysis of human GWA scans. Its role will increase in the future with the availability of larger sample sizes and increased number of genetic markers.

P08.35

Linkage analysis of quantitative traits with a spike in the distribution

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Quantitative data coming from proteomics and metabolomics studies often have irregular distribution, characterized by presence of a proportion of observations in the point-mass (spike) at zero and some continuous distribution of non-negative values. Thus these data contain information about both the binary (zero or not) and continuous components. The general approach to simultaneous analyses of these components was proposed by Broman (2003). However, his method focuses on experimental crosses. We introduced Broman's approach in the parametric linkage analysis of pedigree data, which is applicable to large human pedigrees of arbitrary structure. We developed GADS software, which implements this method. Our software package includes not only the programs for parametric linkage analysis, but also the program for complex segregation analysis, which allows the estimation of the model parameters used in linkage. We tested our method on the real data about vertical cup-to-disc ratio, the important characteristic of the optic disc associated with glaucoma, in a large pedigree from a Dutch genetically isolated population. Significant linkage signal was obtained on chromosome 6q23-q24 (LOD = 3.33) with the help of GADS, whereas the analysis of the continiously distributed values demonstrated only a suggestive linkage to this chromosome. Our results support the feasibility of the simultaneous analyses of the point-mass observations and continuous measurements for the QTL mapping. The software GADS is freely available at http://mga.bionet.nsc.ru/soft/index.html

P08.36

Implication of two transforming growth factor-beta1 (TGF-beta) gene polymorphisms in TGF-beta serum levels and susceptibility to acute myocardial infarction

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Purpose: Transforming growth factor beta-1 (TGF-β1) gene plays an important role in acute myocardial infarction (AMI), however little is known about the relation of variations within the gene and risk of cardiovascular diseases. In this study, we evaluated the influence of TGF-β1 polymorphisms on the onset and progression of AMI.
Methods: Genomic DNA and peripheral blood mononuclear cells (PBMCs) of 900 enrolled patients with AMI and 900 control subjects were extracted. The -509 C/T and 913G/C TGF-β1 polymorphisms as well as mRNA expression and serum levels of TGF-β1 were detected.
Results: The frequency of 'T'allele in -509 C/T and 'C' allele in 913G/C polymorphisms were significantly higher in the patients than control subjects (P<0.001). There were significant differences in circulating levels of TGF-β1 in the patients than in control subjects (34.96±1.74 via 30.46±1.46 respectively, P<0.001) which these concentrations are associated with its gene polymorphism. There was a significant increase in serum levels in the patients who carry the 'T' allele in -509 C/T and 'C' allele in 913G/C, respectively (P<0.001). The mRNA expression levels of TGF-β1 were significantly higher in the patient serums compared with controls (TGF-β1/β-actin, 2.86±1.02 via 1.28±0.89, P<0.001).
Conclusions: Our results confirmed the association between the TGF-β1 polymorphisms and risk of AMI which suggest that genetic polymorphisms in TGF-β1 might be helpful for determining susceptibility to AMI in Iranian patients. There are also significant relationship between serum TGF-β1 and occurrence of AMI and susceptibility to AMI might be related to TGF-β1 gene expression which affects serum levels.

P08.37

Genetic causes of Primary microcephaly in Iranian population

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The volume of human brain through evolution has been tripled since the divergence from chimpanzees. This change has resulted in much higher complex wiring and physiology of the human brain. In primary microcephaly, reduction in brain size-without gross abnormalities in brain architecture or gyral formation-results in intellectual disabilities in majority of cases. So far, seven genetic loci (MCPH1-7) with their genes have been mapped and additional four novel genes (CAPN10, CNKSR1, HIST1H4B, and ZBTB40) have been identified by our group for this disorder. Identification of these genes, which result in microcephaly, can explore the understanding of evolution of human brain size.
Total of 114 families with two or more affected individuals with ID and primary microcephaly have been recruited at Genetics Research Center since 2004 of which 18 families had ataxia or other minor neurological symptoms. Short stature was observed in 12 families and the remaining families did not show any additional features. In addition, all the affected individuals with MCPH 5 gene regardless of their mutation had short stature. All the known genes have been excluded and the causative mutation in MCPH genes was detected only in 20% of the families. For the rest large families, autozygosity mapping was performed and one affected from each familiy has been subjected to exome sequencing. So far, we have identified number of novel loci on chromosome 2, 4, 14, 17, and 21. Our results indicate that there are additional genes involved in microcephaly and there is high heterogeneity among the microcephaly families.

P08.38

Analyses of MEFV Mutations in Patients with The Rheumatoid Arthritis

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Objective/Aim: Rheumatoid Arthritis (RA), a systemic, inflammatory, autoimmune disorder, is called a complex genetic disease, meaning that several genes and enviromental factors act in concert to cause pathological events. Immum system genes including MEditerranean FeVer (MEFV) gene may affect the phenotype of RA. Therefore, we aimed to investigate the relationships of MEFV gene mutations (M694V, M680I, V726A, P369S and E148Q and) and Rheumatoid Arthritis in Turkish population.
Material And Method: In this study, we studied 110 (63 women, 47 men) patients with RA and 140 (77 women, 63 men) healty control groups. MEFV mutations were analyzed by using PCR (polymerase chain reaction) and RFLP (Restriction Fragment Length Polymorphisms) methods.
Findings: The frequencies of MEFV gene mutations were detected 27/110 (24.5%) and 15 (10,7 %) in patient group and control group, respectively. Our results showed that there were no significant differences between MEFV gene mutations and Rheumatoid Arthritis (p=0.006).
Result: Our results reflect that MEFV mutations have a role in susceptibility to Rheumatoid Arthritis in Turkish patients. Currently, we continue to testing higher number of people in both patient and control groups to obtain more data.

P08.39

Genome-wide, permutation-based rare variant analysis with INTERSNP-RARE

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Due to growing accessibility to comprehensive, genome-wide data, systematic investigation of disease association with rare variants (MAF<5%) becomes increasingly appealing.

We present INTERSNP-RARE, a software for genome-wide rare-variant testing using different testing procedures: CMAT (cumulative minor allele test), COLL (collapsing test, a version of CMC) and FR (Fisher_rare, a version of the Fisher combination test). We offer an implementation of corresponding extensions to variable-threshold (VT) tests using a method based on permutations. Combined with permutation-based determination of p-value, this approach promises maximized power without overcorrection for multiple testing while accounting for LD structure.

All rare-variant tests operate on bins, physically continuous chromosomal segments. Bins can be created algorithmically, using on distance or number of (rare) SNPs. Additionally, creating bins from on user-supplied data in various formats is supported, facilitating binning strategies based on a priori information like LD block structure or genomic function. Various functions for bin modification, like merging and flanking are supported.

Results from our power study using simulated data offer insights into strengths and shortcomings of implemented tests under different conditions. Using 20 to 60 causal, protective or neutral rare SNPs per bin, we find that the single-marker analysis outperforms other approaches in some scenarios, in particular for relatively large MAFs and few causal markers (~10%), while CMAT and COLL have excellent power in models with ~30-50% damaging and up to 20-30% protective variants. FR is well-powered even for a low fraction of causal SNPs (upwards from 10%) and highly robust with increasing number of protective markers.

P08.40

Explore the association between cytokines, cytokine related genes and antidepressant in major depressive disorder: a Bayesian approach

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With the recent advance in pharmacogenetics, how to combine data of genetic markers and biomarkers to predict treatment response in diseases has become an important issue in population health sciences. This kind of studies generally contain data from a number of subjects, each of whom has been observed on one or more times, with a binary or continuous response and possibly some covariates recorded for each subject on every time. Thus, the issue of the correlation of repeated measures in a single subject has to be taken into account in statistical analysis. For binary outcomes with repeated measurements, one of the most commonly used analysis methods is generalized linear mixed model (GLMM) and the parameter estimation in GLMM typically involves maximum likelihood. However, for small sample sizes, likelihood-based estimation can be unreliable and their variance components are difficult to estimate. To overcome such problems, we apply the Bayesian framework in the GLMM by assigning prior distributions for the fixed effects, random effects as well as for variance components. After deriving posterior distributions of these parameters, we can generate posterior samples by MCMC to make inferences. The performance of the proposed procedures was compared with likelihood-based methods by simulation studies. Finally, we applied our proposed model to a case-control major depression study with twelve week treatment of antidepressants to evaluate whether cytokines and their related genes might play some role in susceptibility to depressive disorders as well as in the treatment response of antidepressant.

P08.41

Analysis of IL-17 A and IL17F Genetic Polymorphisms as Risk Factors for Allergic Rhinitis

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Background and Aims: The development of allergic rhinitis entails a complex interaction between genetic predisposition and environmental exposure to different factors that allergens are the most important. Responding molecules are; chemokine's and their receptors, interleukins and their receptors, eosinophil peroxidase and leukotriene's, among others. The interleukin-17 cytokines (IL17A and IL17F) are emerging as critical players in host defense responses and inflammatory diseases. This study investigated the association between single-nucleotide polymorphisms (SNP) in IL17A gene promoter (rs2275913, IL17 G152A) and IL17F exon 3(rs763780 IL17F 161His-Arg) and Rhinitis-related traits among the patients in Iran.

Methods: DNA was extracted using standard phenol-chloroform method. The screening of mentioned polymorphisms was performed using PCR-RFLP procedure. A case- control association study was performed (rhinitis group; n=300 and control group; n=160). Chi- square test was performed to compare proportions of subjects with different clinical features among subjects with different genotypes.(All statistical analyses were performed using SPSS).

Result: There was significant association between rs2275913; IL17A and allergic rhinitis (p=0.025) but no association between rs763780; IL17F and cited disease in Chaharmahal va Bakhtiari province was found (p=0.468).

Conclusions: Our data indicated that the IL17A may play an important role in the inflammatory response and promoting allergic rhinitis and rs 763780; 1L17F have no role in rhinitis in Iran.

P08.42

Contribution of APO E allleles and ACE I/D polymorphism in the development of hypertension (HT) in Sleep Apnea-Hipoapnea Syndrome patients

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Sleep apnea/hypopnea syndrome (SAHS) is a common condition affecting approximately 0.3-4% of the middle-aged population and is defined on the basis of symptoms of daytime sleepiness and objective measures of disordered breathing

during sleep. . Several studies have identified SAHS as a risk factor for hypertension, but a direct etiologic link between these disorders has not been established definitively.

Aims: Evaluate the influence of polymorphisms on the APO E gene and the I/D polymorphism on ACEI in the presence of hypertension (HT) In Sleep Apnea - Hipoapnea Syndrome patients.

Methods: APO E and ACEI I/D genotypes were obtained from 99 controls and 114 patients with a diagnosis of sleep apnea-hipoapnea sindrome after polysomnography in the Sleep unit of the Rio Hortega Hospital.

Results: There were not any difference in the APO E alleles frequency between patients and controls, but SAHS patients carrying the APO E ε4 allele showed an increased frequency of HT 3,145 higher than ε3 homozygous and ε2 carriers(CI 1.269-7.79). These findings keep significant even after correction for sex . The ACE I/D genotypes were in Hardy-Weinberg equilibrium (p<0.05) and they seem don´t have any influence on the development of HT in these patients (DD OR 0,478 (CI 0,21-1,08)

Conclusions:. Our results demonstrate that the presence of the ε4 allele increases the probability to develop HT in Sleep Apnea patients. We suggested that this allele could be useful as a biological marker for identification of a subgroup of SAHS patients who are more likely to have HT.

P08.43

SVM-based generalized multifactor dimensionality reduction approaches for detecting gene-gene interactions in family studies

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Gene-gene interaction plays an important role in the etiology of complex diseases, which may exist without a genetic main effect. Most current statistical approaches, however, focus on assessing an interaction effect in the presence of the gene's main effects. It would be very helpful to develop methods that can detect not only the gene's main effects but also gene-gene interaction effects regardless of the existence of the gene's main effects while adjusting for confounding factors. In addition, when a disease variant is rare or when the sample size is quite limited, the statistical asymptotic properties are not applicable; therefore, approaches based on a reasonable and applicable computational framework would be practical and frequently applied. In this study, we have developed an extended support vector machine (SVM) method and an SVM-based pedigree based generalized multifactor dimensionality reduction (PGMDR) method to study interactions in the presence or absence of main effects of genes with an adjustment for covariates using limited samples of families. A new test statistic is proposed for classifying the affected and the unaffected in the SVM-based PGMDR approach to improve performance in detecting gene-gene interactions. Simulation studies under various scenarios have been performed to compare the performances of the proposed and the original methods. The proposed and original approaches have been applied to a real data example for illustration and comparison. Both the simulation and real data studies show that the proposed SVM and SVM-based PGMDR methods have great prediction accuracies, consistencies, and power in detecting gene-gene interactions.

P08.44

The frequency of common α-deletions among β-thalassemia minor individuals and the importance of it in blood indexes change by comparing data in an Iranian population.

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β-thalassemia is the most common monogenic disorder in Iran, and one of the challenges in the screening of the carriers is the coinheritance of alpha thalassemia mutations. Alfa-thalassemia acts as a secondary modifier in clinical manifestations of beta thalassemia. In the view of high prevalence of alfa - thalassemia mutations in many parts of the country, its coinheritance with beta-thal may cause misdiagnosis.

The aim of this study was to determine the carrier frequency of alpha deletions in carriers of beta-thalassemia with known mutations in beta-globin gene and compare some data of them to interpret the importance of these coinheritances.

The study includes families referred from different primary health care centers with microcytic hypochromic anemia [MCV<80fl; MCH<27pg] and A2> 3.5. After providing informed consent, genomic DNA was extracted from peripheral blood leukocytes by salting out method. Allele-specific PCR was exp;oited for common β-mutations. Then common alpha deletions (-α3.7, -α4.2, -α20.5 and

- MED) were screened by multiplex gap PCR.

Among 227 β-thalassemia minor individuals we found alfa thal mutations in 43 cases: 37 heterozygote -α3.7(16.3%), 5 homo -α3.7 (2.2%)and 1 -MED (0.44%).

The results show the high prevalence of the coinheritance of alfa- and beta-thal in a selected Iranian population and the changes are different. Therefore, it is highly recommended that physicians and genetic counselors involved in the screening program of beta-thalassemia major in the country consider this phenomenon.

P08.45

A novel gene-gene interaction: the role of SPRY2 and SPRY4 in tooth agenesis susceptibility

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Tooth agenesis affects 20% of the world population and agenesis of maxillary lateral incisors (MLIA) is one of the most frequent subtypes, characterized by the absence of formation of deciduous or permanent lateral incisors. Odontogenesis is a complex mechanism regulated by sequential and reciprocal epithelial-mesenchymal interactions. Disturbances in FGF signalling can lead to abnormalities in odontogenesis, resulting in alterations in the formation of the normal number of teeth. Sprouty family members function as a negative feedback regulator of FGF signalling.

Therefore, our aim was to study for the first time the involvement of SPRY2 and SPRY4 genes in MLIA susceptibility and to explore a possible gene-gene interaction.

A case-control study, in a total of 306 individuals, is underway; a case-control ratio of 1:2 was achieved in order to increase the study statistical power. We selected 10 tagging single nucleotide polymorphisms (SNPs), which were genotyped by SNaPshot, using a multiplex approach.

We found that the GA genotype of rs504122 in SPRY2 gene leads to an increased risk in individuals with MLIA (p=0.008). Noteworthy, we uncovered a strong synergistic interaction between these two genes and MLIA liability.

Although the molecular mechanisms involved in tooth agenesis remain unknown, our results provide the first evidence of the involvement of sprouty genes in MLIA susceptibility, leading to a better understanding of the genetic mechanisms underlying this trait.

P09. Complex traits and polygenic disorders

P09.001

Polymorphisms in genes involved in the hypoxia-related signaling pathway are associated with abdominal aortic aneurysm and aortoiliac occlusive disease

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The four SNPs in genes involved in the hypoxia-related signaling pathway: *VEGF +405G>C* (rs2010963), *HIF1A 1771C>T (*rs11549465), *1790G>A (*rs11549467) and *HMOX1 -413A>T* (rs2071746) were analyzed in search for the functional differences predisposing either to the aneurysmal or to the occlusive type of arterial diseases.

The case-control study was designed, in which the series of 535 patients with abdominal aortic aneurysm (AAA), 365 patients with aortoiliac occlusive disease (AOID) and 316 persons without symptoms of vascular diseases were analyzed. Associations between studied alleles/haplotypes and the intermediate traits related to the vascular diseases were also examined.

The frequency of *VEGF +405C* allele carriers in the AAA (50,6%) and AIOD (49,3%) groups was 1,4-fold higher than that in the control group (41,6%; p=0,01 and p=0,048, respectively). AIOD patients significantly differ from the other groups in the frequency of *HMOX1 -413T* allele carriers; the observed frequencies were: 61,8% (AIOD) << 71,3% (AAA, p=0,003) < 74,8% (controls; p=0,0004). In patients, weak positive correlations between dose of *HMOX1 -413T* allele and fasting glucose (β=0,074; p=0,037) and triglyceride (β=0,070; p=0,04) levels were found (adjusted for age, gender and type of vascular disease). However those associations should be considered carefully because there was a deviation in *HMOX1* genotype distribution from HWE in two out of three studied groups.

In conclusion, 1) *VEGF +405C* allele is the risk factor of large arteries diseases, 2) *HMOX1 -413T* allele may be involved in AAA pathogenesis by increasing the predisposition to diabetes and dyslipidemia. Supported by the National Science Centre grant NN403_250440.

P09.002

ICAM-1 and CCR5 gene polymorphism and susceptibility to abdominal aortic aneurysm or aortoiliac occlusive disease

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Abdominal aortic aneurysm (AAA) is a life-threatening condition affecting 4-9% of population with a risk increasing with age. Aortoiliac occlusive disease (AIOD) is characterized by aortoiliac obstruction and is caused by advanced atherosclerosis. Both of these common vascular disorders are considered to have multifactorial etiology with both genetic and environmental risk factors involved. Destructive remodeling of extracellular matrix and histological signs of chronic inflammation associates both of these pathologies.

ICAM1 and chemokine CCR5 are mediators of inflammatory process. The CCR5_32 deletion genetic variant was found to be associated with AAA in Italian population. The ICAM1 (K469E) genetic variant was characterized as potentially affecting the autoimmunity process.The purpose of the present study was to determine if there is an association between the ICAM1 (nt+469) or CCR5_32 deletion gene polymorphisms and susceptibility to AAA or AIOD in Polish patients. Genotyping was performed by PCR and gel electrophoresis for CCR5 and by pyroseqencing for ICAM1 in three selected groups: 300 patients with AAA and 305 patients with AIOD who underwent surgery; 310 individuals from control group. Genotypes were compared with demographic and clinical data of subjects and analyzed in relation to risk factors. No significant differences in genotype distribution and allele frequencies of ICAM1 (nt+469) or CCR5_32 deletion were found between patients with AAA or AIOD and control group. Conclusion: We found no evidence of association of ICAM1 (nt+469) or CCR5_32 gene polymorphisms and AAA or AIOD in Polish patients.

P09.003

Correlation between polymorphism of ACE gene and Insulin-like Growth Factor-I (IGF-1) in malnourished children

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Malnutrition is a clinical problem caused by inadequate intake of one or more nutritional elements, and it is one of the most important health problems in developing countries. The aim of this study is to determine the relationship among body weight, concentrations of IGF-1 and ACE gene polymorphisms in malnourished children.

Material and method: The study group consisted of 50 children diagnosed with malnoutrition. Fifty healthy children were enrolled as the control group. All children were genotyped for I/D gene ACE polymorphism.

Results: Three genotypes of 16th intron of ACE gene(D/D,D/I,I/I) were detected. The distribution of ACE DD, ID, and II genotypes in malnourished patients were 32; 50 and 18%, respectively; while for the control group were 44; 44 and 12% respectively. Compared with control subjects, I-allele frequency and I/D+I/I frequency of ACE gene were higher in malnourished children (43% and 68%, respectively; P>0.05).

Malnourished children had significantly lower body weight, height, mid-arm circumference, skinfold thickness, mean serum IGF-1 levels compared with healthy subjects (P<0.05).

Conclusions: There is polymorphism of the ACE gene in healthy and malnourished children. Malnutrition is characterized by the important decreases in the IGF-1 level. ACE polymorphism is not a significant factor for nutritional disorders and does not contribute to the odds of malnoutrition in children.

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P09.004

First systematic association study for achalasia points to a strong involvement of the HLA region within the disease process

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Ideopathic achalasia is a rare esophageal motility disorder with a lifetime prevalence of 1:10,000. It is a neurodegenerative disorder in which the neurons of the myenteric plexus are lost, leading to dysfunction of the lower esophageal sphincter (LES) and to a derangement of esophageal peristalsis. In the final stage of achalasia, esophageal motility is irreversibly impaired, and complications ensue because of the retention of food that is no longer transported into the stomach. The cause of achalasia is mainly unknown, but autoimmune processes appear to be involved in individuals with a genetic susceptibility. In the present study, we performed the first systematic association analysis for achalasia using Illumina´s Immunochip (Trynka, G. *et al.*, Nature Genetics 2011), enriched for immune-relevant loci. To the best of our knowledge, we analyzed the largest achalasia case-control sample that has been studied so far. The sample consisted of 633 cases with ideopathic achalasia and 2,653 population-based controls from Germany, Belgium, and The Netherlands. After quality control 126,899 markers from the Immunochip remained for association testing and the analysis yielded a total of 24 markers reaching genome-wide significance. All of the associated markers are located within the *HLA* region and the most significant finding (rs1794265; P=7.53x10^{-15}) yielded a stronger signal in females (P=2.83x10^{-15}) than in males (P=1.94x10^{-3}). Further analyses to determine how many independent findings are present in the *HLA* region and to assess the *HLA* alleles that may be involved in the disease process are ongoing and will be presented at the congress.

P09.005

Adipophilin (ADRP/ADPH/PLIN2): Variability in Exonic Regions and Secretion of Adipophilin into Breast Milk

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Introduction: Adipophilin (ADRP/ADPH/PLIN2), a member of the perilipin (PAT) family of lipid droplet proteins, is believed to play a crucial role in both formation and secretion of milk lipids in mammals. The aim of our study was to determine whether adipophilin is secreted into human breast milk and to associate adipophilin levels in breast milk with the exonic variations of the adipophilin gene.

Material and methods: The total of 20 pregnant women with physiological pregnancy, originating from the Caucasian Central-European population, were enrolled into the study and serum-milk sample duos were collected at the time of birth and at the days 1-3, 12-14, 28-30, 88-90 and 178-180 postpartum and investigated using ELISA-based methodology. The exons 3-7 of the PLIN2 gene were directly sequenced.

Results: Adipophilin was constantly secreted into breast milk during the whole period of the study. The maternal serum circulating levels were extremely low (<0.8 ug/l), while the adipophilin levels in breast milk substantially exceeded serum levels at the given timepoints (p = 0.03). Two SNPs in exonic sequence of PLIN2 gene were identified, synonymous rs2228416 and missense rs35568725 that were not associated with maternal serum / breast milk levels of adipophilin.

Conclusion: This is the first study to demonstrate that adipophilin is secreted into human breast milk during the whole 6 months after the birth. We do not report major association of investigated exonic variations of PLIN2 gene with adipophilin levels in maternal serum / breast milk in the Central-European population.

P09.007

The impact of CFH, ARMS2 and APOE gene polymorphisms in Greek age related macular degeneration patients

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Age related macular degeneration (ARMD) is a degenerative ocular disease, which may lead to serious loss of central vision. Genetic background seems to have an impact on the onset and progress of the disease. The aim of the present study was to assess the association between ARMD and the single nucleotide polymorphisms (SNPs) Y402H (rs1061170) in the CFH gene, A69S (rs10490924) in the ARMS2 gene and APOE2 (rs7412) in the APOE gene. Genotyping was performed on isolated DNAs from peripheral blood samples from 27 patients with ARMD and 34 age-matched controls, all of whom were clinically evaluated with optical acuity measurement and funduscopy. A real-time PCR-melting curve analysis methodology in the LightCycler (Roche) was developed for the Y402H SNP genotyping. Genotyping of the A69S and APOE2 SNPs was performed with PCR-RFLP methods. Statistically significant association was found for Y402H SNP (OR=2.68, p=0.012). The association between ARMD and the A69S and APOE2 SNPs did not reach levels of statistical significance. The present study confirmed the reported association between Y402H SNP and ARMD in the Greek population as well. The potential association of the A69S and APOE2 SNPs with the disease amidst Greek population needs further evaluation on larger samples.

P09.008

Modelling the genetic risk in age-related macular degeneration

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Age-related macular degeneration (AMD) is a common sight-threatening disease of the central retina affecting approximately 1 in 30 Caucasians. Besides age and smoking, genetic variants from nine gene loci have reproducibly been associated with this condition and likely explain a large proportion of disease. Here, we genotyped 16 AMD risk variants from 8 gene loci in 986 late stage AMD patients and 796 controls. We developed a risk model for AMD based on a genetic risk score (GRS) calculated from a parsimonious set of thirteen variants from eight AMD loci. The model exhibited good discriminative accuracy (area under the receiver-operating curve = 0.820). We present a five-category risk classification with the relative risk for AMD of individuals in the highest category (GRS >3.44, about 0.5% of general population) being 70.4 compared to subjects with the most common genetic background (GRS -0.05-1.70, 40.2% of general population). Noteworthy, younger AMD patients with an age below 75 years had a significantly higher genetic risk score (1.87, 95% CI: 1.69-2.05) than patients aged 75 and above in the same risk groups (1.45, 95% CI: 1.36-1.54). Our findings underscore the large proportion of AMD explained by genetics particularly for younger AMD patients. Our risk classification could be useful for therapeutic stratification or as screening test once preventive treatment is available.

P09.009

Epigenetic alteration of the dopamine transporter gene in alcohol dependent patients is associated with age

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Chronic alcohol abuse and dependence are associated with dysfunctional dopaminergic neurotransmission in mesocorticolimbic circuits. Genetic and environmental factors have been shown to modulate susceptibility to alcohol dependence, and both may act through epigenetic mechanisms that can modulate gene expression, e.g. DNA methylation at CpG sites. Recent studies have suggested that DNA methylation patterns may change over time. However, few data are available concerning the rate of these changes in specific genes. A recent study found that hypermethylation of the promoter of the dopamine transporter (DAT) gene was positively correlated with alcohol dependence, and negatively correlated with alcohol craving. The aim of the present study was to replicate these findings in a larger sample of alcohol dependent patients and population-based controls matched for age and sex. No difference in methylation level was observed between patients and controls, and no difference in methylation level was observed before and after alcohol withdrawal in patients. However, patients with more severe craving showed a trend towards lower DAT methylation levels (p=0.07), which is consistent with previous findings. Furthermore, in our overall sample, DAT methylation levels increased with age. Interestingly, a separate analysis of patients suggested that this finding was mainly driven by the patient group. Although the present data do not clarify whether chronic alcohol abuse is responsible for this phenomenon or merely enhances an aging specific process, our findings suggest that hypermethylation in alcohol dependent patients is a consequence, rather than a cause, of the disorder.

P09.010

Follow-up study of a genome-wide association scan in alopecia areata: IL13 and KIAA0350 as new susceptibility loci supported with genome-wide significance

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Alopecia areata is a common genetic complex hair loss disorder which affects approximately 1-2% of the general population, including both sexes and all age groups. Although the scalp is the most commonly affected site in AA, all hair-bearing areas of the skin, including the eyebrows and eyelashes, may be affected. Recently, the first genome-wide association study (GWAS) of alopecia areata (AA) was performed in a North American sample, and this identified eight susceptibility loci surpassing genome-wide significance. The aim of the present follow-up association analysis was to determine five of these eight loci (SNPs from the *CTLA4*, *IL-2RA*, and the HLA regions were not included due to previous own findings) and to test 12 other loci from the GWAS which did not surpass the threshold for genome-wide significance. Twenty three SNPs from the 17 loci were investigated using a sample of 1,702 Central European AA patients and 1,723 controls. Of the five loci with previously reported genome-wide significance, association was confirmed for all of these: *ULBP3/ULBP6*, *PRDX5*, *IL-2/IL-21*, *STX17*, and *IKZF4/ERBB3* (P-value <16 0.05). To detect robust evidence for association among the 12 other loci, a meta-analysis of the present association data and the data of the recent GWAS was performed. Genome-wide significant association was found for rs20541 (Pcomb=7.52*10-10; OR=1.30 [1.23-1.38]) and rs998592 (Pcomb=1.11*10-1119; OR=1.28 [1.21-1.36]), thus establishing *IL-13* and *KIAA0350/CLEC16A* as two new susceptibility loci for AA. Interestingly, *IL-13* and *KIAA0350/CLEC16A* are susceptibility loci for other autoimmune diseases, supporting the hypothesis of shared pathways of autoimmune susceptibility.

P09.011

Association of CALHM1 Gene Polymorphism with Late Onset Alzheimer Disease

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Late-Onset Alzheimer disease (LOAD) is the most common form of AD that affecting people over 65 years old. The etiology of LOAD is complex that has strong genetic heterogeneity. The studies show that the different regions on chromosome 10(CALHM1) has concordant to LOAD in linkage analysis. CALHM1 encodes a transmembrane glycoprotein that controls cytosolic Ca2+ concentrations and Aβ levels. The previous studies reported that that P86L polymorphism in this gene is significantly associated with LOAD.

Our main objective was to determine the relationship between this polymorphism and the risk of LOAD in 160 AD patients and 163 healthy controls of Azerbaijan population. DNA was extracted of blood specimens that collected of these groups. The genotype and allele frequencies were determined in test and control goups using PCR/RFLP method.

Analysis of acquired data with statistical methods of allele and genotype frequency distribution of P86L genotype showed slight significant difference between the two study groups, that we concluded that T allele (mutant) has a protective role and is nearly significant between two groups (p= 0.026).

P09.012

Genetic testing of Alzheimer's Disease associated polymorphisms using biochip-based assay

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Late-onset Alzheimer's disease (AD) is the most common form of dementia in the elderly. The presence of an *APOE* epsilon4 allele is a well-established genetic risk factor for AD, with a higher percentage of epsilon4 allele in patients with AD comparing to general population. Recent genome-wide association studies also have identified the other loci of interest including *CLU*, *TOMM40*, *EXOC3L2*, *GAB2*, *A2M*, *CR1*, *BIN1* and *PICALM*, as putative genetic determinants of the late-onset form of AD. The aim of the work was to develop biochip for simple and rapid genetic tests of allelic variations in these genes in population and AD cohorts. The genotyping assay has been developed employing multiplex PCR and allele-specific hybridization of the amplicon probes on low-density gel-based biochips. In total, set of 235 case-control subjects of Russian origin have been tested to validate the sensitivity and specificity of the biochip. The genotype data are in agreement with described associations in both Russian and other European origin populations showing association of *APOE* epsilon4 allele and *CLU* C-allele (rs11136000) in AD (OR = 2.34, 95%CI= 1.22-4.47, p=0.01 and OR = 1.6, 95% CI=1.06-2.4, p = 0,024, respectively). Additionally, protective effect for the *APOE* epsilon2 allele has been observed (OR = 0.28, 95% CI=0.13-0.63, p = 0,001).

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P09.013

Association study between *APOE* and *TNF- α* gene variations and sporadic Alzheimer's disease in Iranian population

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Introduction: Amyloid ß (Aß) peptide deposits and Neurofibrillary tangles have key roles in pathogenesis and progression of the late-onset Alzhimer's disease. Likewise it has been shown that inflammatory reactions play a significant role as well. Inflammatory mediators such as complement, chemokines and cytokines activators and inhibitors can release from activated microglia and astrocytes, causing neuronal dysfunction and death. One of the most important cytokines is tumor necrosis factor-α (TNF-α). This study was designed and carried out to determine the association between sporadic Alzheimer's disease and the human *TNF- α* and *APOE* gene variations in Iranian population.

Materials and Methods: In this case - control study, the role of *TNF-α* gene polymorphism was determined in 167 sporadic AD patients and 163 healthy controls. Genomic DNA was extracted and *TNF-α* -850C/T promoter polymorphism was genotyped using PCR/RFLP technique. Comparing the genotype and allelic frequencies were analyzed using chi-square and logistic regression tests by SPSS 11.5.

Results: The obtained results indicated that the frequency of *TNF-α* -850 heterozygote genotype (CT) was significantly higher in AD patients comparing healthy controls (p=0.038). Although no significant difference were observed in *TNF-α* -850 homozygote genotype (TT) and T allele between the

studied groups. No interaction was shown between *TNF-α* -850 and *APOE* gene polymorphisms as well.
Conclusion: These data suggests the role of *TNF-α* -850 TC genotype as a risk factor for AD in Iranian population. Although to show the effects of homozygote genotype (TT) and T allele, a study with a larger sample size maybe indicated.

P09.014

The effect of AVPR1B gene polymorphisms on personality traits in healthy individuals from Russia

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Since the role of arginine vasopressin in modulation of social behaviors was established, we aimed to define a single genotype and haplotype effect of AVPR1B (rs28632197 and rs33911258) gene polymorphisms on personality traits assessed with TCI-125.
We recruited1018 healthy individuals (68% women) of Caucasian origin (Russians-357, Tatars-549, Chuvashs-112) from Russia (mean age: 19.53±2.24 years) without any history of psychopathologies. Genotyping of two polymorphisms was performed using PCR-RFLP. Statistical analysis was conducted with SPSS 13.0, PLINK v.1.07, Haploview 4.1.
ANOVA demonstrated an association of AVPR1B rs33911258 and Cooperation in males (p=0.003;F=8.77) occurred mainly due to the lower Cooperation in Tatar males bearing G-allele (p=0.028;F=4.90) compared to A/A-genotype-carriers. Subsequent haplotype analysis revealed an association of AVPR1B A*G-haplotype (rs28632197 and rs33911258, respectively, D'=0.87) and higher Self-transcendence (ST) (p=0.008; R2=0.6%) and G*A-haplotype and lower scores on ST (p=0.003; R2=0.7%) in the total group. The same effect of G*A-haplotype on ST was observed in Chuvashs (p=0.007; R2=6.6%), mainly caused by haplotype effect in Chuvash females (p=0.002; R2=12.1%).
Our findings indicate that AVPR1B gene has larger impact on character- than on temperament traits (according to TCI-125). Moreover,ethnicity mediated the main and haplotype effect of AVPR1B gene polymorphisms on sociability-related character traits.
Study was supported by Russian Foundation for Basic Research (#11-04-97032-r_povolzhye_a).

P09.015

Array comparative Genomic Hybridization (array-CGH) as a clinical diagnostic tool in syndromic and nonsyndromic Congenital Heart Defects

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•**Aim:** Congenital Heart Diseases (CHD) are often associated with other congenital anomalies, peculiar facies and developmental delay and only few cases of chromosomal abnormalities are detected by conventional cytogenetic techniques. The microarray Comparative Genomic Hybridization analysis (array CGH) allows the identification of submicroscopic genomic rearrangements. This study describes for the first time in Greece, the application of array CGH, as a diagnostic tool for the investigation of patients with congenital heart disease.
• **Materials- Methods:** During the last 3 years, a total of 330 patients were studied, of whom 55 had CHD of unknown aetiology plus at least one additional indication of abnormal chromosomal phenotype but with normal conventional karyotype. High resolution 1x244K Agilent arrays were used in this study (> 236.000 probes average resolution of 8.9 Kb).
• **Results** Submicroscopic genomic rearrangements (CNVs) ranging in size from 0.08 to19.01 Mb were detected in 37/55 patients (67%). In 29 of these (52.7%) the following genes associated with heart disease were identified:*DVL1, CHRD, DISP1, ETS1, KCNJ5, SCN3B, WNT7B, SCO2, ELN, GATA5, COL4A1, ADAMTS1, ADAMTS5, TBX1, DGCR8, KCTD13, TBX6, EMILIN2, MRCL2, MRCL3, MYOM1, LPIN2, LAMA1, CIDEA, KCNG2, TOP3B, TOP3B2, HIC2, CACNA1B, EHMT1, BAG3, NKX1-2, RBFOX1, EDN1, DTNBP1, MYLIP, KCNT1, NOTCH1, MAML1, FLT4, PRKAG3, WNT6, CYP27A1, NEXN, KCNQ1, ADAMTS13, CTNNA3, CACNB2, IGLL3, SLC29A.*
• **Conclusions** In patients with CHD and / at least one additional indication of abnormal chromosomal phenotype array CGH analysis is mandatory to detect possible submicroscopic chromosomal abnormalities and provide proper genetic counseling.

P09.016

The challenges of molecular genetic testing in Arrhythmogenic Right Ventricular Cardiomyopathy

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Arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D) is characterised by fibrofatty replacement of the RV myocardium and a marked predisposition to ventricular arrhythmias; it is clinically and genetically heterogeneous. ARVC/D is generally considered to be an autosomal dominant trait. The incomplete penetrance and variable disease expression seen in this condition suggests a complex aetiology. Approximately 50% of patients are reported to have variants in genes encoding components of the cardiac desmosome; with 4-8% of index cases having more than one desmosomal gene variant. The difficulties in interpreting the clinical significance of variants in these genes are highlighted in the literature and the utility of genetic diagnosis in this condition the subject of debate.
Since 2008, Oxford Medical Genetics Laboratory has provided a diagnostic service for the four most commonly associated desmosomal genes: *PKP2*, *DSP*, *DSG2*, and *DSC2*. >175 individuals referred with a clinical or probable clinical diagnosis of ARVC/D have been analysed to date. Probable/possible disease causing variants were identified in ~46%. The majority of variants were detected in *PKP2* (27%), followed by *DSP* (12%), *DSG2* (11%) and *DSC2* (6%). 12% of index cases who had variants thought highly likely to be pathogenic also harboured at least one other desomosomal gene variant.
The results of genetic testing in our cohort will be compared to the literature. The challenges in unravelling the molecular aetiology of ARVC/D in a family will be discussed and the utility of genetic testing in this disorder examined.

P09.017

Anti-leukotriene therapy with Montelukast reduce expression of *IL12B* genes, which are overexpressed in children with asthma

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Single nucleotide polymorphisms (SNPs) in the cytokine gene cluster on 5q31-33 region, that includes genes *IL12B* and *IL13*, was associated with asthma development. However, the role of those SNPs in asthma behavior and response to therapy is poorly characterized. The aim of our study is to analyze the role of *IL13*, *IL12B* and *IL23R* genes in asthma pathogenesis and determinate their role in response to diferent therapies. We have included 288 children with asthma and 186 healthy individuals. Genotyping was performed by PCR-RFLP technique. Gene expression was measured by TaqMan assay using real-time PCR. The data were statistically analyzed. We were the first to found that *IL12B* gene is overexpressed in asthmatics compared to the control group (p<0.001). After treatment with anti-leukotriene drug montelukast the expression of *IL12B* was reduced (p=0.015). Montelukast affect the expression of *IL23R* gene, which significantly increased after the therapy. In eQTL analysis, we found that the expression of *IL12B* is significantly higher in carriers of at least one allele of SNP rs6887695 G, which was in preliminary studies identified as a risk factor for chronic immune-mediated diseases. In our study, we were the first to found an impact of allele G on airway obstruction in asthma (p=0.006). The finding of a higher *IL12B* expression in asthmatics may in future serve as diagnostic marker for asthma and the finding that montelukast decrease its expression may become an important fact in choice of therapy.

P09.018

Association of GRIA1 gene polymorphisms with asthma in Bashkirs from Russia

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Asthma is a complex disease that is influenced by many genes and environmental factors. Genome-wide association studies have identified many candidate loci for a number of complex traits, including asthma. For the first time, we performed GWAS of asthma in the Volga-Ural region of Russia. The study involved 358 unrelated patients with asthma and 369 control subjects of different ethnicity. DNA samples were genotyped using an Illumina Human 610 quad array as a part of the GABRIEL project. After QC filters, 550 915 SNPs genotyped in 330 cases (141 Russians, 120 Tatars, 69 Bashkirs) and 348 controls (145 Russians, 111 Tatars, 92 Bashkirs) were used for testing the association of SNPs to asthma. Some markers on chromosome 5q33 showed the most significant association with asthma in Bashkirs (p<5xl0-7). The maximum association was at the SNP rs9324750 (p=4,25x10-7) in the second intron of GRIA1. Eight linked SNPs (rs495703, rs480726, rs726877, rs726876, rs1463747, rs17114771, rs9324750, rs10041179) located within second intron GRIA1 were selected for haplotype analysis. The haplotype GTGCGGAT was found to be overrepresented in Bashkirs with asthma (p=3,0x10-4). GRIA1 encodes a subunit of the AMPA receptor, a tetrameric ligand-gated ion channel that transmits glutamatergic signals in the brain. It has recently been shown that glutamate not only has a role as a neurotransmitter, but also as an immunomodulator. These results suggest that GRIA1 gene play an important role in susceptibility to asthma in Bashkirs from the Volga-Ural region of Russia. Supported by a contract from the European Commission (018996).

P09.019

Immune-response modifying gene polymorphisms and susceptibility to asthma and *Opisthorchis felineus* helminth invasion

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It has been shown that common genetic variants of cytokine and cytokine signal transduction genes, especially *IL13* and *STAT6*, predict risk of asthma and allergy, as well as the intensity of helminth invasion by *Ascaris* and *Schistosoma* species.

Opisthorchis felineus is a common helminth infection in Siberian region of Russia. So far, no information on genetic component of susceptibility for this infection was published. To investigate whether the same genes are involved in predisposition to asthma and O. *felineus* invasion, we analyzed 10 single-nucleotide polymorphisms (SNP) of immune-response modifying genes in 107 asthma patients, 103 individuals infected by O. *felineus*, 100 persons with combination of asthma and O. *felineus* infection, and control group of 126 healthy people.

The *PIAS3* rs12756687:G/G genotype was associated with symptomatic O. *felineus* infection (P = 0.02), while the C/C genotype was associated with asthma (P = 0.033). The *SOCS5* rs6737848:C/C genotype and the *IFNG* rs2069705:C/T genotypes were associated with asthma (P = 0.006 and 0.032, respectively) alone.

Thus, the *PIAS3* rs12756687 polymorphism demonstrates the inverse association between asthma and *O. felineus* invasion clinical manifestation. This suggests that the gene-environmental interaction between the *PIAS3* and *O. felineus* modifies the risk of development of asthma in the helminth endemic region. At the same time, the studied *SOCS5* and *IFNG* polymorphisms are likely independent risk factors of asthma susceptibility.

P09.020

Evaluation of gene expression normalization strategy for real-time qPCR in leukocytes from asthmatic patients before and after treatment.

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The aim of this study was to identify the most suitable reference genes to normalize gene expression data obtained by qPCR from asthmatic patients. We analyzed 7 candidate reference genes (*18S rRNA*, *ACTB*, *B2M*, *GAPDH*, *POL2AR*, *RPL13A* and *RPL32*) previously reported as being the most stable in blood samples, in asthmatic patients before and after anti-asthma treatment, and control subjects. Variance of Cq values was analyzed and gene stability determined with geNorm. The influence of normalization strategy on *ORMDL3*, *PSMD3*, *GSMD3*, *MAP3K2*, *SLC22A5*, *TRIM35* and *EPHX2* gene expression was analyzed. Cq values of *ACTB*, *B2M* and *GAPDH* were shown to be stable across samples obtained before and after treatment and also the top-ranking genes determined by geNorm. These produced the most consistent results of the target gene expression. When samples obtained before treatment were analyzed alone, *POLR2A* and *B2M* where shown to be the best selection. Gene expression of *ORMDL3* was shown to be significantly increased, and *PSMD3*, *SLC22A5*, *MAP3K2* and *TRIM35* were decreased in asthmatic patients before and after treatment compared to healthy controls. *GSDMB* was significantly decreased only in asthmatic patients after treatment. When comparing samples obtained before and after treatment, gene expression of *PSMD3* and *MAP3K2* was significantly decreased. In conclusion, a different combination of reference genes should be used according to whether changes in gene expression are being analyzed in samples from asthmatic patients before receiving treatment or together with samples obtained after anti-asthma treatment.

P09.021

Association between *VEGF* polymorphisms and asthma treatment response

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Background_ Asthma affects around 300 million people worldwide. Vascular endothelial growth factor (VEGF), a regulator of angiogenesis, is elevated in asthma patients. VEGF contributes to airway responsiveness and remodelling. Treatment of asthma patients decreased VEGF levels and inhibiting VEGF in mice diminished asthma symptoms. Therefore, polymorphisms in *VEGF* might be associated with asthma treatment response.

Methods: This study enrolled 131 children with asthma. They were treated with inhaled corticosteroid (ICS) fluticasone propionate or leukotriene receptor antagonist (LTRA) montelukast. We analyzed association between improvement of lung function, assessed by measurement of FEV1 – % of predicted, FEV1/FVC after 6 and 12 months of treatment and asthma control after 12 months of treatment, and polymorphisms, rs2146323 and rs833058, in the *VEGF* gene.

Results: Polymorphism rs2146323 A>C in *VEGF* was associated with response to ICS. Patients with the AA genotype had a greater improvement in FEV1 – % of predicted compared to the AC or CC genotype (*p* = 0.01). Conversely, the AA genotype in rs2146323 was associated with uncontrolled asthma in patients regularly receiving LTRA (*p* = 0.02). Polymorphism rs833058 C>T was associated with treatment response to episodically used LTRA. A subgroup of patients with the TT genotype had improvement in FEV1 – % of predicted compared to no improvement in patients with the CT or CC genotype (*p* = 0.03).

Conclusions: Our results showed that treatment response to commonly used asthma therapies, ICS or LTRA, is associated with polymorphisms rs2146323 and rs833058 in *VEGF*, what makes *VEGF* a potent pharmacogenetic marker.

P09.022

Genetic polymorphisms of glutathione S-transferases M1, T1 and P1 and susceptibility to oxidative stress and atherogenesis

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A persistent oxidative stress has been implicated in the pathogenesis of various diseases, among others atherosclerosis. Glutathione S-transferases (GSTs) provide protection against oxidative stress by detoxifying the end-products of lipid peroxidation. Polymorphic deletion variants in the *GSTM1* and *GSTT1* genes produce either a functional protein (non-deletion alleles or heterozygous deletion) or result in the complete absence of the protein (homozygous deletion-null genotype) while *GSTP1 Ile105Val* functional polymorphism influences protein catalytic activity and stability. We investigated the association between these polymorphisms and susceptibility to oxidative stress in 60 angiographically documented patients with manifest atherosclerotic disease and 100 control individuals from Serbia. Genomic DNA was isolated from peripheral blood cells and genotyping was performed using polymerase chain reaction followed by restriction fragment length polymorphism (PCR-RFLP) analysis for *GSTP1* and multiplex-PCR or Real-time PCR methods for *GSTM1* and *GSTT1* gene variants. We observed significant association of *GSTM1* null (OR=2.0, 95%CI=1.05-3.86, P=0.03) and *GSTT1* null (OR=2,26, 95%CI=1,06-4,81, P=0.03) genotypes with atherosclerosis. Combined analysis of the two null genotypes demonstrated significant increase in risk (OR=15, 95%CI=3,09-73,3, P<0.0001), too. *GSTP1 Val105* allele (OR=0,63, 95%CI=0,39-1,03, P=0.06), *Ile/Ile* (OR=0,53, 95%CI=0,27-1,05, P=0.07) and *Ile/Val* (OR=0,41, 95%CI=0,12-1,43, P=0,16) genotypes showed a nonsignificant 1.6, 1.9 and 2,44 fold decrease in the risk of atherosclerosis, respectively. Our data provide evidence that both *GSTM1* and *GSTT1* null genotypes, alone or in combination, are associated with increased oxidative stress and atherogenesis. A larger study group is needed to establish true relationship between potentially protective allele *Val105* and disease.

P09.023

Toll-like receptor genes variants and atopic dermatitis in Volga-Ural region of Russia

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Atopic dermatitis is the common chronic inflammatory disorder with cutaneous hyperreactivity to environmental triggers and is often the first step in the atopic march. Recent studies have shown an association between toll-like receptor genes variations and allergic diseases development. We have screened 4 SNPs in TLR1 (rs4833095, rs5743604, rs5743571, rs2101521) and 6 SNPs in TLR10 (rs4331786, rs4129009, rs11096957, rs11466617, rs10004195, rs4543123) genes, both map closely together on chromosome 4. The AD group consisted of 318 AD patients from Volga-Ural region of Russia (Russians, Tatars, Bashkirs and individuals of mixed origin). The control group included 262 non-atopic individuals of the same ethnic origin. Genomic DNA was isolated by phenol-chloroform extraction. The genotyping of SNPs was performed by real-time PCR.
The AD patients have significantly higher TLR1 gene polymorphisms alleles rs5743604*A, rs5743571*C and rs2101521*G frequencies when compared with control group (p=0.036, p=0.020, p=0.0378 respectively). The frequencies of the TLR10 gene variants alleles rs10004195*T and rs4543123*A was also significantly higher in AD patients than in healthy donors (p=0.0091 and p=0.0156). The haplotype analysis revealed that the most frequent haplotype is TTATAC (rs11466617-rs10004195-rs4543123-rs4833095-rs5743604-rs5743571), with statistically significant difference between patients and controls (69% and 63% respectively; p=0.05), besides AD patients have significantly higher TTACAC (p= 7.0E-4) and lower TAGCGT (p= 0.0083) haplotype frequencies compared with controls.
The results of our investigation show that TLR1 and TLR10 genes polymorphisms are important risk factors of atopic dermatitis in the Volga-Ural region of Russia.

P09.024

Genetic dissection of atrial septal abnormalities: integrating QTL mapping and genomic technology

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The formation of the atrial septum during cardiac development is a complex process being vulnerable to a wide range of dysmorphogenesis. A genetic link and anatomical continuum between secundum atrial septal defect (ASDII) and patent forame ovale (PFO) have been suggested by murine and human studies. While ASDII and PFO occur commonly and represent a significant burden to health resources, the genetic complexity of such conditions is not fully known. PFO incidence in inbred mice, as we reported earlier, is strongly correlated with quantitative parameters of atrial septum. We previously mapped quantitative trait loci (QTL) underlying such parameters using F2 intercross between QSi5 and 129T2/SvEms, parental strains with extremes of septal dysmorphogenesis. Subsequently, breeding of parental strains continued for 12 further generations to establish an advanced intercross line (AIL). We genotyped 150 single nucleotide polymorphism (SNP) markers at an average interval of 2cM in 400 F14 mice. AIL confirmed the F2 QTL and significantly improved confidence intervals of the QTL. Afterward, we performed whole genome sequencing of the parental strains and identified variations between the sequences. The genome was partitioned into high and low SNP rate intervals and the genes within high SNP rate regions of the QTL confidence intervals were identified. As a confirmatory method, we used mouse HapMap imputation genotype resource. The list of candidate genes was prioritized according to sequence and expression profiles. In conclusion, integrating QTL mapping and genomic technology form a powerful approach to dissect genetic complexity underpinning atrial septal abnormalities.

P09.025

The GABAergic hypothesis in the etiology of attention-deficit/hyperactivity disorder in the Portuguese population: a family-based association study

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Attention-deficit/hyperactivity disorder (ADHD) is one of the most common neuropsychiatric disorders diagnosed during childhood and several studies demonstrated that ADHD is a highly heritable disorder with a strong genetic basis. However the identification of genes that may predispose to ADHD has been difficult. Several lines of evidence suggest that changes in genes from GABAergic system and particularly the GABA A receptors might be involved in ADHD, but this hypothesis remains unexplored by genetic studies. Therefore, the aim of this study was to investigate the role of a GABRB2 gene polymorphism (C1412T) in the etiology of ADHD, in the Portuguese population through a family-based association strategy.After obtaining informed consent, blood samples were collected from trios, composed by parents and respective offspring, diagnosed with DSM-IV-TR and genomic DNA was isolated from peripheral leukocytes using an enzymatic method. The GABRB2 C1412T polymorphism was investigated with polymerase chain reaction-restriction fragment length polymorphism technique. We performed both haplotype relative risk (HRR) and transmission disequilibrium test (TDT) and found no association between the GABRB2 C1412T polymorphism and ADHD (HRR: $\chi^2 = 0.199$, df = 1, P = 0.656; TDT: $\chi^2 = 0.182$, df = 1, P = 0.670). The preliminary results obtained with HRR and TDT analyses do not support the hypothesis that the C1412T polymorphism of GABRB2 gene contributes with a minor effect to the expression of ADHD in the Portuguese population. However further studies with larger samples are in course in order to confirm or refute these results.

P09.026

Identifying phenotypes and exploring genetic aetiology of autism spectrum disorders : a 87 patient study.

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Autism is characterized by limited verbal communication, lack of reciprocal social interaction, and stereotypical behaviour affecting preferentially Boys. Mental retardation and or seizures coexists in two-thirds of patients. Autistic spectrum disorders are complex multifactorial disorders involving various genes, and many aetiologic diagnosis remain unravelled.
We report a collaborative study between our Genetic Department and the Autism Center (CRA). A population of 87 children with autism was selected. We identified 5 different phenotypic groups (8-32 children per group), using the behaviour and the intellectual efficiency evaluation criteria from the following tests PER-R, CARST, and ADIR.
Among them 45 patients (6 -14 per group) were negative for FMR1 amplification and then tested by array-CGH (180K) to search for deleterious chromosomal rearrangements. The Array-CGH analysis showed the presence of 49 copy number variants (CNV) not referred as polymorphisms in 28 children with an average of 2 CNV per patient. A deleterious CNV was found in 5 children from 4 different groups. A parental study was done in 10 families for 14 aberrations : 12 rearrangements were inherited and 2 were de novo with a 6q26 deletion (gene PACRG) and an isochromosome Yp (deletion of NLGN4Y) both considered of uncertain clinical significance (VOUS). Five CNVs were previously reported in autism: 1q21.1,3p26.3q26.2 (CNTN4), 15q13.3 (CHRNA7), 16p11.2 and Xp22.31. Three aberrations in 3p26 (dup) and Xp22 (del/dup) seemed more specific to autism as not present in our cohort of 400 patients with intellectual disability.
In conclusion, the aetiologic diagnosis was found in 11% of autistic children.

P09.027

A 3- year- old patient with autism and microdeletion in the *KIAA0442* (*AUTS2*)- gene

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A 2 10/12- year- old boy with bilateral cleft lip and cleft palate as well as developmental delay was presented to our genetics clinic. He is the first child of non-consanguineous healthy parents. He shows behavioural patterns of the autism spectrum which include avoiding eye contact, no response to his name, playing alone and stereotyped movements when listening to music. His postnatal chromosomal analysis revealed a normal male karyotype 46,XY.

Array-CGH analysis showed a microdeletion of 170 kb: arr (7q11.22)(70,077,607-70,247,036)x1 dn. This part of the chromosome contains exons 6-15 of the 19 exons spanning the *AUTS2*- gene. His parents do not carry this microdeletion, indicating that it was a de novo event.

Autism spectrum disorder (ASD, OMIM 209850) encompasses different forms of autism with a broader phenotype. Two-thirds of all patients with ASD suffer from mental retardation. Among the genes involved, *AUTS2*-disruption has been described in 7 patients with ASD and mental retardation. In all of these seven patients translocations with different breakpoints between exon 1 and 7 and different translocation partners were the underlying mechanism of the disruption. Additionally other genes were disrupted according to the breakpoint of the partner chromosome. Our patient shares the same symptoms as the 7 patients with translocation, indicating that disturbed function of *AUTS2* and not the truncated translocation partner causes the clinical presentation of the patients.

P09.028

Contribution of rare and common variants of the *PTCHD1* gene to Autism Spectrum Disorder and Intellectual Disability

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Autism is a severe neurodevelopmental disorder, characterized by impaired verbal communication, limited reciprocal social interaction, restricted interests and repetitive behavior, often accompanied by intellectual disability (ID). Although it is one of the most heritable neuropsychiatric disorders, the underlying genetic factors remain largely unknown.

A recent study reported rare mutations in the X-linked gene *PTCHD1* (patched domain-containing protein 1)in patients with autism spectrum disorder (ASD) and ID (Noor *et al.* 2010), suggesting a possible role of this gene in cognitive development. *PTCHD1* is highly expressed in brain regions and encodes a transmembrane protein containing a patched-related domain. It has been suggested that *PTCHD1* plays a role in the hedgehog signaling pathway.

In this study we aimed to investigate the possible contribution of common variants in *PTCHD1* to ASD through a case-control association study. The study sample consisted of 595 Caucasian autistic patients (270 Spanish, 247 Dutch and 78 German) and 680 gender-matched controls (320 Spanish, 269 Dutch and 82 German). Twenty-eight tagSNPs were selected on the basis of linkage disequilibrium (LD) patterns. A significant association, that overcame the Bonferroni correction for multiple testing and permutations was obtained with the marker rs7052177 (p= 6.13e-4). Furthermore, in order to evaluate the possible participation of *PTCHD1* rare variants in ASD and ID, we are currently performing a mutation screening in the Spanish ASD cohort, and in an additional sample of 200 individuals with ID. The preliminary results of this study support the involvement of this gene in autism and cognitive impairments.

P09.029

Targeted next generation sequencing in Thai families with autism spectrum disorders identifies a novel variant, p.E683Q, in the *CNTNAP2* gene

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Autism Spectrum Disorders (ASD) is a group of neurodevelopmental disorders characterized by distinct patterns of social deficits and communication impairment, rigid ritualistic interests and stereotypical behaviors. Recent genetic studies have shown that some synaptic genes, including *NRXN1, NLGN3, NLGN4X, CNTNAP2* and *SHANK3*, are associated with ASD. To study these 5 genes in 5 Thai multiplex families with ASD, the target regions of these candidate genes were enriched from total DNA samples using NimbleGen microarrays. The enriched DNA fragments from each index case were sequenced using 454 Genome Sequencer FLX Titanium. Sequences were aligned and compared with reference sequence UCSC hg18 using Newbler software. Variant annotations were focused on the exons and exon-intron boundaries, then novel variants were validated by Sanger sequencing. One novel variant, c.2047C>G (p.E683Q) in the exon 13 of *CNTNAP2*, was identified in a boy with ASD. The variant was transmitted from his mother but it was not present in his father and younger brother with ASD. This variant was not found in the 170 control alleles. Bioinformatic analysis showed that the glutamic acid (E) in CNTNAP2 protein was highly conserved across different species and the glutamine (Q) variant affected on secondary structure of CNTNAP2 protein. However, further studies involving a larger set of ASD samples (i.e. association studies) are necessary to determine the potential disease relevance of the p.E683Q in *CNTNAP2*. Otherwise, functional analysis of CNTNAP2 protein with p.E638Q isoform can give us the best conclusion.

P09.030

Mirror effects for Autism Spectrum Disorder due to gene dosage at 10q11.22 affecting GPRIN2 gene, a regulator of neurite outgrowth and PPYR1 gene involved in energy homeostasis.

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We recently reported that a small duplication on 10q11.22 including GPRIN2 gene, a regulator of neurite outgrowth, and PPYR1, a gene involved in energy homeostasis is a candidate modifier for Rett syndrome. Specifically, duplications were found in the Zappella variant, the Rett variant with recovery of speech, and lacking the typical growth delay, underweighting and autistic features. Since PPYR1 knockout mice display underweight and reduced white adipose tissue an overexpression of PPYR1 due to gene duplication may be responsible for the higher body weight characterizing Zappella variant. We concluded that duplication at 10q11.22 may play a role in protecting from both underweighting and autistic features in Rett patients. We now report more convincing evidences that dosage balance at GPRIN2 locus plays a role in autism spectrum disorders (ASD). We observed 6 patients affected by ASD with an overlapping small deletion including the two genes (and extending to MAPK8 in one patient). We then compared a group of 164 ASD patients with a group of 180 syndromic and non syndromic intellectual deficit (SID/NSID) patients and 160 controls. Seven deletions were identified in the ASD group. On the contrary, no deletion has were found in SID/NSID nor in control group (p=0,005 and p=0,008). We are currently extending this study to a second cohort including about 100 ASD patients and 135 SID/NSID. Overall, these data suggest that gene dosage at 10q11.22 affecting GPRIN2 gene may have mirror effects being duplication protective and deletion prone to ASD.

P09.031

Whole-genome methylation profile in BEN patients

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BACKGROUND: Balkan endemic nephropathy (BEN) represents a chronic progressive interstitial nephritis in striking correlation with uroepithelial tumors of the upper urinary tract. The disease has endemic distribution in the Danube river regions in several Balkan countries.

DNA methylation is a primary epigenetic modification that is involved in major processes such as cancer, genomic imprinting, gene silencing etc. Epigenetic tests can prove to be the bridge between environmental factors and genetic background in BEN development.

MATERIALS AND METHODS: Age matched pools of 45 female BEN patients and 45 healthy controls were created. We've performed high-resolution genome-wide methylation array analysis. We've analyzed the methylation status of 27 800 CpG islands of both groups to identify significant methylation profile differences.

RESULTS: Our experiments show significant disparity of the methylation status of several group of genes. Significant hypomethylation in the patient group was discovered for the following genes: ADRA2A, B3GNT4, BTBD6, C1orf183, GCH1, KCNK12,NEO1, REXO1, SHROOM2, SSPO, TTC9B.The genes ADNP, C11orf63, ACTG1, RNF187, C4orf32, MIR153-2, TM6SF1, SSR4, HNRNPH1, EVI5L, TAL1, EBF3, C3orf21, IQSEC1, MSL2, FAM123A, RBMY1B, RBMY2EP showed significantly higher level of hypermethylation in patients.

CONCLUSIONS: Data obtained from our experiments suggest that dysregulation of cytoskeletal proteins, transcription factors, transmembrane ion channels as well as proteins involved in secretion processes, cell adhesion, DNA-splicing and cell proliferation can be key mechanism in BEN pathogenesis. These results are in unison with the key pathological alterations in BEN and further elucidate the precise mechanism behind BEN development.

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P09.032

De novo copy number variation in bipolar affective disorder

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An increased rate of de novo copy number variants (CNVs) has been found in several neuropsychiatric disorders, such as schizophrenia, autism and developmental delay. In this study we wanted to identify de novo CNVs in bipolar affective disorder (BD). We used Illumina OmniExpress microarrays to genotype 119 BD offspring from 114 complete parent-offspring families passing strict QC criteria. CNVs were called by PennCNV. We excluded CNVs <10kb, covered by <10 probes, overlapping segmental duplications and with a frequency >1%.

The initial analysis identified 41 putative novo CNVs. Subsequent validation by a Z-Score calling algorithm, and manual inspection of the logRratios of the trios, reduced this to seven de novos in six probands. This rate of 5% is higher than the reported rates in controls (~1-2%), but similar to studies in schizophrenia and BD. The median size of de novo CNVs was 189kb. Two of the de novo CNVs did not intersect any genes. We find one de novo deletion intersecting an exon of *DLG2,* and one large duplication intersecting 27 genes at 16p11.2, both regions having been implicated in de novo CNV studies of schizophrenia and BD. The remaining three CNVs are also potential novel de novo CNV loci for BD: a 448kb deletion at 2q31.1 that intersects 5 genes including the nicotinic acetylcholine receptor *CHRNA1,* a 160kb duplication intersecting *PCDH15* at 10q21.1, and a 186kb deletion at 9q21.3 intersecting *ELAVL2*.

P09.033

Array-based genome-wide genotyping in 75 patiens with complex/multigenic disorders

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The bladder exstrophy-epispadias complex (BEEC) represents an anterior midline defect with variable expression of phenotype comprising a spectrum of malformations involving the abdominal wall, pelvic floor, urinary tract, genitalia, and occasionally the anus and spine. Most of the BEEC cases are classified as non-syndromic, and the etiology is still unclear. The overall birth prevalence for the complete BEEC spectrum in children of European descent has been estimated to be 1 in 10 000. In this study, we aimed to identify copy number variantions (CNVs) that contribute to BEEC. An array-based molecular karyotyping, utilizing 1,134,514 SNPs (single nucleotide polymorphisms), was performed to screen 75 individuals with BEEC for causative de novo events. SNP fluorescence intensity was analyzed with QuantiSNP using an Objective-Bayes Hidden-Markov model for calling putative CNVs. The SNPs were filtered according to various criteria by use of the Cartagenia Bench software, by in-silico-analysis, and by comparison with 531 healthy controls. A de novo microduplication on chromosome19p13.12 was detected in a patient with isolated bladder exstrophy. The size of the duplication is 0.9 Mb and harbors 23 RefSeq genes. Whole-mount in situ hybridization studies of the genes within the region of rearrangement were performed in order to prioritize genes according to their expression during E9.5 to E10.5. WISH data of mice embryos showed specific expression of CASP14, NOTCH3, WIZ and CYP4F22. According to the function of these genes WIZ becomes the most promising candidate to be systematically sequenced in the complete sample to identify high-penetrance mutations involving small sequence changes.

P09.034

The gene encoding Kit ligand associates with Bronchopulmonary dysplasia

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Background. Bronchopulmonary dysplasia (BPD) is one of the most common chronic lung diseases associated with very preterm birth. Major risk factors are lung immaturity and inflammatory lung injury. As a result of improved treatment methods, increased infant survival has arisen the "new" BPD among the most immature infants. Based on twin studies, genetic factors play an important role in BPD susceptibility. However, the genetic background is still poorly understood.

Aims. Because glucocorticoids are important in the process of lung maturation, the genes encoding glucocorticoid receptor (*NR3C1*) and Kit ligand (*KITLG*, involved in hematopoiesis and cell migration) were investigated as candidates for BPD.

Materials and methods. Total of 259 infants with gestational age <31 weeks born in Oulu University Hospital during 1997-2010 were studied. Of these, 61 were diagnosed with BPD. All infants were of Finnish origin. Eight *KITLG* and 23 *NR3C1* tagging SNPs were genotyped.

Results. Six SNPs of *KITLG* (rs11104906, rs10858753, rs17424193, rs4842477, rs11104948, rs869408) associated with BPD. The frequencies of two haplotypes including all the 8 SNPs were significantly different between infants with BPD and those without BPD. There was no association with *NR3C1* SNPs and BPD.

Conclusion. We are the first to show evidence that the polymorphisms of *KITLG* associate with susceptibility to BPD. This raises the possibility that transcription products of *KITLG,* expressed in endothelial cells or lung fibroblasts, are important in early lung growth. Present results remain to be confirmed.

P09.035

Association of leukocyte telomere length and cardio-vascular fitness: Results of the Austrian Stroke Prevention study.

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Background: Telomeres are short repetitive sequences protecting the ends of the chromosomes. Leukocyte telomere length (LTL) is related to age, inflammation, oxidative stress and life-style factors. Physical activity has been indicated to exert beneficial effects on LTL. This study investigates the effect of cardio-respiratory fitness (CRF) on LTL.

Methods: Relative LTL was measured by quantitative Real Time PCR in 907 participants of the Austrian Stroke Prevention Study, a community-based cohort study on brain aging.

CRF was estimated by exercise ECG in 794 subjects. The measured variables included diastolic and systolic blood pressure, heart rate during resting, peak and recovery phase. VO2max was calculated by the formula

15*weight[kg]*maximum/resting heart rate. The associations between each of the CRF variables and LTL were analyzed using multiple linear regression by adjusting for age and sex (Model 1) and additionally for hypertension, diabetes, cardiac disease and BMI (Model 2).
Results: We observed a significant association between LTL and maximum achieved heart rate (MHR) (Model 1 β=-0.002; p=0.022). Additional adjustment for vascular risk factors did not alter the effect size and the strength of this association (Model 2 β=-0.02;p<0.018). All other CRF variables were not significantly associated with LTL. The association was confined to subjects above the age of 65(Model2β=--0.003;p=0.005), to men (Model2β=-0.002; p=0.043) and to normotensives(Model2β=-0.004; p=0.018).
Conclusion: This is the first study investigating the association between CRF and LTL in a healthy elderly population. Our results suggest a protective role of MHR on LTL, which is present particularly in elderly, men, and normotensives.

P09.036

Expression analysis of celiac disease candidate genes in the 6q22 GWAS peak

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Celiac disease (CD) is an immune mediated, multigenic disorder where HLA-DQ2/DQ8 contributes about 35% to genetic risk. GWAS have found more than 26 regions for CD susceptibility, and several potentially functional candidate genes have been located within. Recently, the IMMUNOCHIP genotyping array discovered additional 13 regions of susceptibility. The GWAS signal in chromosome 6:128.0-128.4 kb pointed to THEMIS and PTPRK as possible candidate genes, both with immune-related function. The signal was narrowed to the PTPRK region in the subsequent study, but functional confirmation is pending.
The aim of this work was to determine the influence of associated SNPs on THEMIS and PTPRK gene expression in the intestinal mucosa of active and treated CD patients and controls.
We assessed the correlation between qPCR expression levels and SNP genotypes of the top SNPs in both studies (rs802734, rs55743914 and rs72975916) and those most strongly associated in our CEGEC population (rs10484718 and rs9491896).
THEMIS showed higher expression in active CD compared to treated patients and controls, while PTPRK showed lower expression. Our study confirmed an association of this region with CD in the local population, although only rs802734 genotype showed any influence in THEMIS expression. Interestingly we found a significant positive correlation between THEMIS and PTPRK mRNA levels in CD patients but not in controls.
Our results suggest a possible role for both candidate genes in CD pathogenesis although further investigation is needed to clarify the impact of the associated SNPs on their expression.

P09.037

Association of *FUT2* (rs601338) with celiac disease in Finnish patients

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Intestinal microbiota plays an important role in human health, and its composition is determined by several factors, such as diet and host genotype. However, host genes determining the intestinal microbiota composition are largely unknown. Recently, we (Wacklin et al. 2011) and Rausch et al. (2011) have showed that polymorphism in *FUT2,* which defines the expression of ABH and Lewis histo-blood group antigens in intestinal mucus and other secretions, affects intestinal microbiota composition. The *FUT2* gene encodes fucosyltransferase 2 enzyme. Non-functional enzyme resulting from nonsense mutation (rs601338, NM_000511.5:c.461G>A) in *FUT2* gene leads to the non-secretor phenotype (AA). Celiac disease is chronic inflammatory enteropathy occurring in genetically predisposed individuals after dietary gluten consumption. It is classically manifested in gastrointestinal tract (diarrhoea, malabsorption), but extra-intestinal symptoms, such as dermatitis herpetiformis (DH) are also common. In spite of several well-known genetic risk factors for celiac disease, environmental factors, e.g. microbiota, are suggested playing role in development of celiac disease. Interestingly, *FUT2* association with celiac disease was detected by Dickey et al. (1994), but not by Heneghan et al. (1996). Thus, it is possible that host genes could indirectly via microbiota composition be involved in manifestation of disease. We studied 1025 celiac disease patients and 2738 controls using Taqman assay for rs601338. We found suggestive associations at level of genotype (Cases and controls: f(AA) = 18.0%, 14.7%; f(AG) = 42.4%, 47.6%; f(GG) = 39.5%, 37.8%; p = 0.006) and recessive model (Cases and controls: f(AA) = 18.0%, 14.7%; f(AG/GG) = 82.0%, 85.3%; p = 0.011).

P09.038

Expression of TLR signalling-related miRNAs (mir-21, mir-146a, mir-155) in celiac disease

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BACKGROUND: MicroRNAs have emerged as important regulators of gene expression by decreasing target mRNA levels. A trio of microRNAs, mir-21, mir-146a and mir-155, have been proven to be of considerable interest in relation to TLR signalling, and their dysregulation may be involved in many inflamatory diseases, including celiac disease (CD)
AIM: To evaluate the expression and relationship with disease status of these 3 microRNAs in the pathogenesis of celiac disease.
PATIENTS AND METHODS: Biopsy specimens from distal duodenum were obtained from 30 paediatric celiac patients at diagnosis and after 2 years on gluten free diet, and 18 non-celiac controls. miRNA expression was determined by RT-PCR, using commercially available mature-microRNA TaqMan assays. Experiments were run in triplicate in an ABI-PRISM 7900HT and results were normalized to RNU48 endogenous control. Groups were compared using parametric and non-parametric tests.
RESULTS AND CONCLUSIONS: mir-21 and mir-146a were upregulated in both groups of CD patients (debut and after 2 years on gluten free diet) when compared to controls, while mir-155 was only upregulated in active CD mucosa. The same tendency has been found in other immune related diseases, such as rheumatoid arthritis and systemic lupus erythematosus, and functional analysis of their target genes and proteins must be done to assess their role in the disease.

P09.039

Exploring the NFkB signalling pathway in celiac disease

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The NFkB transcription factor family regulates a large array of genes involved in immunity, inflammation and cell survival. Previous work of our group showed constitutive activation of NFkB in the intestinal mucosa of patients with untreated celiac disease (CD), suggesting a pivotal role in the perpetuation of the inflammatory process.
Using RT-PCR in a custom Taqman Low Density Array, we analyzed the expression of 93 NFkB cascade genes in biopsies from 16 active celiac patients, 16 treated celiac patients on gluten-free diet and 16 non-celiac controls lakking inflammation of the gut at biopsy.
Twenty-two genes were significantly overexpressed in mucosa from both active and treated celiac patients compared to controls, and only one was downregulated, confirming the overall upregulation of NFkB pathway in CD. Most of those genes have central regulatory functions in the cascade and are clustered in Apoptosis and Toll-like Receptor (TLR) signalling KEGG pathways. In contrast, of the 10 genes upregulated only in mucosa from active CD patients, most were located in the periphery of the NFkB route, including activating ligands and receptors. The expression signature of each patient group, extracted using Correlation-based Feature Selection and Principal Component Analysis highlighted several deregulated genes and a substantial set of TLR-related genes. These preliminary results point to several specific response pathways as key activators of NFkB in CD.

P09.040

Effect of SOCS3 SNP rs4969170 in celiac disease

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BACKGROUND: Celiac disease (CD) is defined as a Th1-mediated gluten-sensitive enteropathy. Recent studies have shown that Th17 cells may also contribute to disease pathogenesis. *SOCS3* is known to be a negative regulator of the Th17 response, and has been considered a candidate gene in CD. A SNP in *SOCS3* (rs4969170) has been recently associated with CD.
AIM: To study the expression of *SOCS3* in affected tissue in relation to rs4969170 genotype and to replicate the genetic association.
PATIENTS AND METHODS: Gene expression was analyzed in 29 CD biopsy pairs, taken at diagnosis and after 2 years on gluten free diet, and 12 controls. SNP rs4969170 was genotyped in 512 celiac patients and 607 controls from the CEGEC collection. TaqMan gene expression and genotyping assays were employed.
RESULTS: Gene expression showed statistically significant differences when comparing celiac patients before and after treatment, and also with healthy controls. *SOCS3* is downregulated in active disease, compatible with an increased Th17 response. No effect of the rs4969170 genotype on gene expression was detected, nor was the genetic association for this SNP replicated in our sample.

	Replication set (CEGEC)	
	CD	**Controls**
	(N=512)	(N=607)
GG	221 (43.2)	236 (38.9)
AG	229 (44.7)	283 (46.6)
AA	62 (12.1)	88 (14.5)

AA genotype; p = 0.266
CONCLUSION: Although SOCS3 could be a functional player in celiac disease, changes in expression are observed only in active mucosa, and seem to be a consequence of the inflammatory response rather than a primary effect of the associated variant.

P09.041
Association of the matrix metalloproteinases, disintegrin and metalloprotease 33 and the tissue inhibitors of metalloproteinases genes polymorphic markers with chronic obstructive pulmonary disease

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The contribution of the polymorphic markers of the matrix metalloproteinases MMP1 (-1607 G>GG, rs1799750; -519 A>G, rs494379), MMP2 (-735C>T, rs2285053), MMP3(-1171 5A>6A, rs35068180), MMP9 (-1562C>T, rs3918242; 2660A>G, rs17576), MMP12 (-82 A>G, rs2276109), the disintegrin and metalloprotease 33 ADAM33 (12418A>G, rs2280091; 13491 C>G, rs2787094), the tissue inhibitors of metalloproteinases TIMP2 (-418G>C, rs8179090), TIMP3 (-1296T>C, rs9619311) genes to chronic obstructive pulmonary disease has been assessed. For this purpose, PCR-RFLP analysis of the gene polymorphisms in case (N=391) and control (N=514) groups has been performed. The 6A6A genotype of the MMP3 -1171 5A>6A polymorphism was associated with significantly high risk of chronic obstructive pulmonary disease (OR=2.490, Padj=0.003979 adjusted for age, sex, smoke pack-years, ethnos). Analysis showed an association of the G-G haplotype of 13491 C>G and 12418A>G ADAM33 gene polymorphisms (OR=0.39, Padj=0.0012) with chronic obstructive pulmonary disease. We found a significant interaction of the smoking status and ADAM33 12418A>G (Pinteraction=0.026) and TIMP3 -1296T>C (Pinteraction=0.044). The relationship between the GG genotype of the ADAM33 13491 C>G and emphysema risk was found (OR=1.74, Padj=0.013, Pcor=0.117). Severity of chronic obstructive pulmonary disease was modified by MMP9 -1562C>T in additive model (OR=1.883, Padj=0.028). The MMP3, MMP9, ADAM33, TIMP3 genes polymorphism may be an important risk factor for the development and progression of chronic obstructive pulmonary disease, important gene and environmental interactions were determined.

P09.042
Colorimetric Assay For Medium-High Resolution HLA-DQ2/DQ8 Typing For Coeliac Disease Predisposition Analysis

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Coeliac disease (CD) is a small intestinal inflammation disorder, triggered by the intake of gluten protein that present in certain cereals [1]. The prevalence of CD is about 1% in the European population [2].
CD has been shown to affect only genetically predisposed individuals; strong relation between this disease and Human Leukocyte Antigens (HLA) has been proved, with 95% of the CD patients carrying HLA DQ2 heterodimer and ca. 5% carrying DQ8 heterodimer [3]. DQ2 and DQ8 negative individuals have been shown to be very unlikely to develop CD[4].
Herein we present a colorimetric assay, based on enzyme-linked oligonucleotide assay (ELONA) technology in combination with reverse dot-blot *Sequence Specific Oligonucleotide Probes (SSOP)* approach, for rapid, easy to use and cost effective HLA typing of CD associated genes.
Multiplex colorimetric assay for medium to high resolution HLA typing of the DQ2 and DQ8 genes is demonstrated. Probes with high specificity for the cd associated alleles (DQA1*02:01, DQA1*03:01, DQA1*05:01/DQA1*05:05, DQB1*02:01/DQB1*02:02 and DQB1*03:02) were designed and tested by ELONA and surface plasmon resonance techniques (SPR).
Assay condition studies, performed by SPR and ELONA, revealed that detection can be performed in 25 minutes if operation temperature was set to 37 ºC.
Finally, the performances of the developed typing platform were validated by the analysis of a series of real patient samples.

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P09.043
Searching for a causative mutation in a four-generation family with celiac disease using next-generation sequencing

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Celiac disease (CeD) is a common food intolerance, caused by a dysregulated immune respone to dietary gluten. It is strongly associated to the HLA-DQ2/DQ8 genes, which explain 35% of the heritability. Genome-wide association studies for CeD identified a further 39 non-HLA loci that explain another 5%. To identify more risk genes, we performed whole-genome linkage in a four-generation Dutch family segregating for CeD. We mapped a dominantly inherited linkage region at chromosome 9p21-13 and another region at 6q25 using a model-free approach.
We hypothesize that these regions may contain causal mutations for CeD and applied whole-exome sequencing to two affected individuals from the family to look for mutations. As CeD segregates in the dominant-like matter, we looked for heterozygous changes present in both individuals. We selected all non-synonymous, nonsense and splice-site changes of unknown or low frequency (MAF<5%) using datasets from 1000 Genomes, an NHLBI exome-sequencing project, and a set of 500 Dutch controls.
Two missense variants, on chromosomes 9p21-13 and 6q25, were further investigated and were both predicted to be damaging. However, the 6q25 variant was also present in one spouse and the family grandmother, both of whom did not carry the risk haplotype. The 9p21-13 variant showed perfect co-segregation in the family and is in a gene of largely unknown function, although it is highly expressed in epithelial tissue. This opens up the exciting possibility that it might be involved in barrier function. Further studies should investigate the gene function and its involvement in CeD pathogenesis.

P09.044
SNP association of *CNTN4, CNTN5, CNTN6, CHL1* and *GRIN2B* corroborates and extends copy number variation data in autism

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Research into the genetics of neurobehavioral disorders such as autism spectrum disorder (ASD) is caught within the dichotomy of rare variants with a high phenotypic impact, such as copy number variations (CNVs), vs. common variants with a low effect size. To overcome this (false) dichotomy and to find missed heritability we performed an association study on candidate genes, which we had previously identified by SNP array-based CNV analyses in ASD patients (Neurogenetics (2011) 12, 315). Within these CNVs, genes with clear expression in the brain were selected. In total we examined 2,042 SNPs, spanning 16 Mb of genomic DNA, which were located within or immediately bordering these genes. We compared a cohort of 74 ASD patients without relevant CNVs with a population-based cohort of 132 healthy individuals that were not related to the ASD families. After Bonferroni correction for multiple testing we found significant association for one SNP within intron 11 of *CNTN4* (rs1420021), two SNPs within intron 7 and 9 of *CNTN5* (rs6590473 and rs11222599), one SNP within intron 1 of *CHL1* (rs17329247), one SNP within intron 1 of *CNTN6* (rs9878022), and 5 SNPs flanking exon 4 of *GRIN2B*. Our data corroborate involvement of contactins in ASD as indicated by our previous CNV study and indicate that certain genes may harbour variants with both high penetrance and with a smaller degree of effect for the same phenotype.

P09.045

An association between JAK3, STAT3 and CCL2 gene variants and myocardial infarction

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Systemic inflammation is considered to be an important factor in the development of coronary artery disease. The aim of the current study was to investigate candidate genes in the inflammatory molecular pathways involved in the progression of atherosclerosis. To achieve this goal, we replicated SNPs in *JAK1*, *JAK3*, *STAT3* and *CCL2* genes in the study group consisting of patients with MI and control subjects.

The group of MI patients of Russian ethnic origin (N=277), when compared to he control group (N=145), has demostrated increased *JAK3*T/*C* genotype frequency (44.23% vs. 32.2% respectively, P=0.032) and decreased *JAK3*C/C* genotype frequency (50% vs. 63.56% respectively, P=0.015). Increased prevalence of *STAT3*C/*C* and *CCL2*G/*G* genotypes was observed in MI patients of Tatar ethnicity (N=220) (16.59% vs. 8.82%, P=0.031, and 5.12% vs. 6.88%, P=0.014 respectively).

In our study, we have detected an association between MI and *JAK3* rs310216 in Russians, *STAT3* rs2293152 and *CCL2* rs3917887 in Tatars. *JAK3**T/*C was associated with increased risk of MI (OR= 1.67, CI: 1.06 - 2.64), while *JAK3**C/*C was found to be protective (OR= 0.57, CI: 0.34 - 0.90). In Tatars, *STAT3**C/*C and *CCL2**G/*G carriers demonsrated higher risk of MI (OR= 2.05, CI: 1.08 - 3.92, and OR= 2.43, CI: 1.21 - 4.90, respectively). Further investigations are needed to confirm these results and to understand the mechanisms underlying the associations.

P09.046

New genes and coronary artery disease

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Genome wide association studies and their replications that have associated DNA variants with myocardial infarction (MI) and/or coronary artery disease (CAD) are predominantly based on populations of European or Eastern Asian descent. Replication of the most significantly associated polymorphisms in populations with distinctive genetic backgrounds and lifestyles is crucial to the understanding of the pathophysiology of a multifactorial disease like CAD.

We have used our Lebanese cohort to perform a replication study of nine previously identified CAD/MI susceptibility loci (LTA, CDKN2A-CDKN2B, CELSR2-PSRC1-SORT1, CXCL12, MTHFD1L, WDR12, PCSK9, SH2B3, and SLC22A3), and 88 genes in related phenotypes. The study was conducted on 2,002 patients with detailed demographic, clinical characteristics, and cardiac catheterization results. One marker, rs6922269, in MTHFD1L was protective against MI (OR=0.68, p=0.0035), while the variant rs4977574 in CDKN2ACDKN2B was associated with MI (OR=1.33, p=0.0086). Associations were detected after adjustment for family history of CAD, gender, hypertension, hyperlipidemia, diabetes, and smoking. The parallel study of 88 previously published genes encompassed 20,225 markers, three quarters of which with imputed genotypes. The study was based on our genome-wide genotype data set, with imputation across the whole genome to HapMap II using HapMap CEU population as a reference. Analysis was conducted on the genotyped and imputed variants in the 88 regions covering selected genes. This approach replicated HNRNPA3P1-CXCL12 association with CAD and identified new significant associations of CDKAL1, ST6GAL1, and PTPRD with CAD.

Our study provides evidence for the importance of the multifactorial aspect of CAD/MI and describes genes predisposing to their etiology.

P09.047

Systematic identification of genetic factors influencing the thickness of the cerebral cortex

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The human cortex is structurally and functionally segregated. It varies more than any other part of the brain between subjects. The identification of gene variants contributing to this inter-subject variability will help to elucidate molecular mechanisms underlying brain functions.

In the present study, we focused on cortical thickness which is a heritable quantitative trait. Moreover, it is undergoing changes in many neuropsychiatric disorders. We analyzed 98 healthy volunteers using a 3T magnetic resonance imaging scanner, obtained T1-weighted images, and performed genome-wide genotyping (HumanOmniExpress/HumanOmni1S). Thickness data were extracted using the FreeSurfer software. To determine those data which explain most of the thickness variability, we applied a principal component analysis (PCA) on the pooled data from all cortical regions. We selected the first 15 principal components which together explained 80% of the total variance and performed genome-wide association studies (GWAS) on each component. Across PCA GWAS, SNP P-values were combined using a meta-analysis approach. Overall, 31 SNPs showed P-values of less than 1E-04. The most significant finding was a SNP on chromosome 16q23.2 (P=9.48E-06). Results of the individual GWAS of the first principal component, which explains 43% of the observed variability in thickness, will also be presented.

Several common genetic variants with suggestive evidence for association with cortical thickness were identified. We are currently analyzing the genes and available knowledge of their function and we have initiated a replication study in two independent samples to follow-up our results.

P09.048

Role for the assemblage of centrosome-derived structures in the pathogenesis of non-syndromic craniosynostosis

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This study attempted to clarify the molecular and cellular mechanisms underlying the premature ossification of calvarial sutures in non syndromic craniosynostoses (NS-CRS), highly prevalent craniofacial malformations with an unclear etio-pathogenesis. Calvarial specimens of patent and fused sutures were collected from 8 patients during surgery. Total RNA and calvarial cells were isolated from tissues. RNAs from matched samples of each patient was used for comparative microarray analysis using exon arrays enabling both gene- and exon-level analyses. Gene-level analysis allowed identifying 114 significantly modulated genes, mainly involved in cell adhesion, cell-matrix interaction, matrix mineralization, osteogenesis, and tissue development. Exon-level analysis produced a list of over 150 genes with significant differentiallly expressed exons suggesting tissue-specific alternative splicing events occurring at the dysmorphic site. These showed selected splice variants of the ciliopaties-associated genes that were differentially expressed in fused-versus-unfused suture calvarial tissues. Interesting the list included IFT88 and BBS9 genes which are associated with ciliopathic conditions in craniofacial disorders.

Calvarial cells isolated in primary culture from the suture specimens were analyzed morphologically using confocal microscopy. Fused suture-derived cells displayed a constitutive tendency towards the osteogenic commitment, increased growth kinetics, reduced primary cilia and abnormally orientated mitotic spindles. These resembled tumor-like features, confirmed in vitro by demonstrating a higher clonogenicity and a higher invasiveness in vitro of fused suture-derived cells. These suggest that a somatic alteration of centrosome-derived structures in the osteo-progenitor cells, could underlie the overworking osteogenic process at the site of calvarial suture fusion.

P09.049

Association analysis of OXTR and AVPR1B genes gene in criminal violence

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Human aggression and violence are, unfortunately, ubiquitous phenomena with substantial costs to society. Aggression - defined as hostile, injurious, or destructive behavior. It is known, that both oxytocin (OXT) and vasopressin (AVP) systems dysfunction is associated with social behavior. The physiologic effects of OXT and AVP are mediated through its specific receptors (OXTR and AVPR). The aim of the present study was to investigate the association OXTR and AVPR1B genes with criminal violence in Russian population. 177 violent offenders (convicted of murder or rape) were included in the study. The control group consisted of 301 voluntaries. Two SNPs (rs53576 and rs2254298) of the OXTR gene and two SNPs (rs33911258 and rs28632197) of the AVPR1B gene were genotyped using PCR/RFLP methods. For pairwise linkage disequilibrium and haplotype analysis, the Haploview 4.1 program was used. Odds ratios (OR) with 95% confident intervals (CI) were calculated. The only association we observed was an allele association between OXTR rs2254298 and criminal violence: G was significantly overrepresented in violent offenders group as compared to controls ($p=0.001$; $OR=2.93$, 95%CI: 1.43-4.03). There were no allele or genotype associations between the AVPR1B SNPs or the OXTR rs53576 and criminal violence. There was an evidence of the strong pairwise linkage disequilibrium (D‘ values between 0.89 to 0.99) between markers within the investigated genes. However, analysis of distribution of the estimated haplotype frequencies revealed no significant differences between violent offenders and control.

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P09.050

Higher post surgical opioid requirement in Crohn’s disease - expression profiling of small intestine biopsies

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Crohn’s disease (CD) is a painful inflammatory bowl disease with complex polygenic inheritance. It has been shown that a number of CD patients require significantly higher post operative opioid doses than patients undergoing comparable abdominal surgery. We recently demonstrated that this is not due to the most common variants in components of opioid metabolism. CD, therefore, may be a suitable model for the identification of novel pain susceptibility genes. In order to further investigate the molecular and genetic basis of this difference in pain perception within CD patients, we focused our attention on the affected tissue. RNA was extracted from sections of inflamed and non-inflamed small intestine tissue of 3 CD patients with high and 3 patients with low postsurgical opioid requirement. Expression profiling of all 12 tissues was performed using Affymetrix U133 Plus2.0 microarrays. Expression profiles were compared between inflamed and non-inflamed tissue of CD patients with high and low postoperative opioid consumption. We identified 18 transcripts (fold change of >2; p-value <0.05) which showed a significantly altered expression in tissue from high versus low consumers independent of the inflammation status. This includes genes already known to be involved in pain perception and/or inflammation as well as genes with yet unknown function. This is the first study investigating intestine tissue from CD patients with respect to their post surgical opioid requirement. Further investigation of the identified genes, their expression in normal and affected tissue, as well as their role in opioid metabolism and/or pain perception is currently in progress.

P09.051

Study of allelic variants in patients with CRPS from Samalanca (Spain)

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Background: Complex regional pain syndrome (CRPS) is a chronic pain condition that is believed to be the result of dysfunction in the central or peripheral nervous systems.

The existence of individual differences in response to painful stimuli suggests that genetic factors can be involved in its modulation. The aim of our study was to investigate the genetic variation of 16 genes involved either in nervous system pathways or in drug metabolism, in patients from Salamanca (Spain), diagnosed CRPS.

Methods: Genomic DNA was extracted from peripheral blood by standard techniques. We selected 16 non-synonymus SNPs. Studies were performed using TaqMan probes (Applied biosystems) for the analysis of the polymorphisms in the following genes: TRPV, GSTP1, CYP2D6, COMT, PTGS2, HTR2A, SLLC6A4, OPRD, OPRM, OPRK, CNR1, DRD2, GABRA1, GABRA6, PPARG, EDN1. Statistical analysis was performed comparing the different allelic variants of the genes in subgroups of patients: patients with a VAS below and over 50.

Results and conclusion: Preliminary analysis has shown significant differences in genotyping distribution ($p<0.05$) comparing both groups of patients in the EDN1 and PTGS2 genes. When we split groups according sex, we find significant differences in EDN1 and IL1B for men, and in PTGS2, GABRA1, TRPV and GSTP1 for women.

That support the hypothesis that genetic variants could be associated with increased susceptibility to suffer pain.

P09.052

Study of the effect of single nucleotide polymorphisms in the mannose-binding lectin gene (*MBL2*) on phenotype in Slovak cystic fibrosis patients

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Cystic fibrosis (CF) is the most common lethal autosomal recessive disease with prevalence of 1:2500 newborns. The cause of death in 90% is respiratory insufficiency due to chronic inflammation caused by bacterial infections.

Mannose-binding lectin (MBL) protein is an important mediator component of the innate immune defense system. It has been shown that *MBL2* variant alleles causing low MBL serum levels are associated with an increased risk of infections. In exon 1, three single nucleotide polymorphisms (SNPs) and one in promoter region cause independently low MBL serum levels. We therefore investigated whether *MBL2* gene variants are associated with pulmonary function or susceptibility to infection in Slovak CF patients.

DNA polymorphisms were typed by single base primer extension assay (SNaPshot) in 91 patients and 100 controls. The concentrations of protein were determined in 34 patients by a sandwich enzyme-linked immunosorbent assay, also spirometric and microbiological data were collected from medical records.

In this study we found that *MBL2* genotypes were associated neither with earlier acquisition of bacterial infection nor with reduced pulmonary function among patients. Although *MBL2* genotypes were associated with the MBL2 protein serum level, results were statistically significant only for polymorphisms in exon 1, with $p=0.0008$.

The role of the *MBL2* gene in lung disease severity in CF patients represents a very complex phenomenon where both genetic and environmental factors play an important role. Understanding this complexity requires further studies based on a broader scale of genetic factors involving both a whole-genome approach and a larger patient cohort.

P09.053

Analysis of *TCF7L2* gene polymorphism in CF Russian patients with and without diabetes.

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Diabetes is an important complication of cystic fibrosis (CF), a multisystem genetic disease caused by mutations in the cystic fibrosis conductance regulator gene (*CFTR*). Diabetes risk increases with age and is associated with a significantly worse CF prognosis. Recent studies indicate that variants in *TCF7L2* gene, transcription factor 7 like 2, or the type 2 diabetes susceptibility gene, modifies risk of diabetes in CF. Analysis of three polymorphisms in *TCF7L2* gene (rs7903146, rs12255372, rs11196205) was carried out in two groups of adult Russian CF patients: 47 patients with diabetes (mean age 22,6 years; 25 males : 22 females); 55 patients without diabetes (mean age 22, 8 years; 28 males : 27 females). The difference of the allele and genotype distribution at rs11196205 polymorphism in *TCF7L2* gene between two analyzed groups of CF patients was revealed. The frequency of C allele at rs11196205 is significantly lower in CF patients with diabetes than in CF patients without diabetes (0,394 versus 0,558; p=0,02). Allele C is associated with decreasing risk of diabetes in CF patients (OR=0,51 (95%CI 0,29-0,91); p=0,02).

P09.054

Changes in methylation of promoters of immune response genes during hemodialysisin patients with diabetic nephropathy detected at the level of cell-free DNA inplasma

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Background: Of patients with diabetes mellitus (DM), 20%-40% develop diabetes nephropathy (DN) which belongs to one of the most frequent causes of hemodialysis therapy. The process of hemodialysis (HD) itself is not without the influence on patient´s immune system, additionally impaired immune functions are documented in DM patients. Elevations of cell-free DNA (cfDNA) concentrations during HD sessions were reported in numerous studies regardless of an applied therapeutic protocol. In this study, we focus on methylation status of promoter of immune response genes at the level of plasma cfDNA and their changes iniciated by the process of hemodialysis in DN patients.
Methods: We isolated plasma cfDNA from 20 patients with DN before and after a HD session. The extent of promoter methylation of 24 genes involved in immune response was examined using the Methyl Profiler DNA Methylation PCR Array System and cluster analysis (SABiosciences, Qiagen).
Results: We discovered significant changes in methylation profiles that were promoted in consequence of patient´s blood contact with artificial surfaces of dialyzer. According to the character of methylation profiles it seems that Th17 cells related immunity may play role in the response of DN patients to HD.
Conclusion: The method provides the new tool for evaluation of immune system activity in HD patients and its results can be further correlated with clinical data to bring new insights in the complex pathogenesis.
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P09.056

Locomotor dysfunction and hypotonia in Down syndrome mouse models for the Stch-App region as a consequence of dosage sensitive genes controlling muscular metabolism and mitochondrial function

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Aneuploidies of human chromosome 21 (Down syndrome and monosomy 21) lead to variable physiological abnormalities, with constant mental retardation and delayed locomotor skills. Delayed motor performance, weak muscle strength and exercise limitation observed in individuals with Down syndrome are not yet understood and remain a challenging issue. They have been attributed to impaired coordinated input due to cerebellar dysfunction, but might have a more complex origin. Phenotypic investigation of mouse models of trisomy (Ts2Yah) and monosomy (Ms3Yah) for the *Stch-App* region revealed the existence in this region of genes sensitive to dosage that are controlling muscle strength, motor function and endurance. A transcriptome analysis of skeletal muscle in Ms3Yah mice revealed up-regulation of genes implicated in mitochondrial function and in the oxidative phosphorylation pathway, a finding that was confirmed by the visualization of increased number of oxidative fibers. The opposite down regulation was observed in Ts2Yah mice, although with less effect. In addition, myopathy-like muscle fiber and mitochondria damage was observed in the Ms3Yah model. Our findings demonstrate that one or more gene(s) present within the *Stch-App* region are implicated in the regulation of muscle energy metabolism and integrity, and point at a defect in the mitochondrial respiratory chain. We propose that locomotor deficits observed in Down syndrome are the results, not only of a central nervous system defect, but also have a peripheral origin ant that the *Stch-App* region contributes to those. Candidate genes for this new phenotype are currently under investigation.

P09.057

Aging alters DNA methylation level in the genes involved in common diseases

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Aging affects various physiological processes and increases susceptibility to diseases. Whether aging alters DNA methylation level and how the aging-related epigenetic change influences disease traits are of great interest. To settle this matter, we performed epigenome-wide association study by analyzing approximate 450K DNA methylation sites using the blood sample of total 288 subjects in Japanese general population. We conducted multistage association analysis between DNA methylation level and age by adjusting with sex and body mass index using general linear model for 96 subjects in each step. We identified 15 methylation sites of 11 genes associated with age which showed P-value < 0.001 in every three sets and showed P-value $1.0 \times 10^{-29} \sim 1.0 \times 10^{-9}$ in combined set. The functional information of these genes are as follows; 3 sites in one of the susceptibility loci of Diabetes Mellitus, 2 sites related to fatty acid metabolism, 2 sites in the coactivator of the androgen receptor, 1 site in proapoptotic pathway, 1 site related to atherosclerosis development, 1 site affected putative deubiquitinase and 1 site of plasma membrane sodium and calcium exchanger. Our results suggest that aging may alter epigentic status in the genes involved in common diseases such as diabetes, dyslipidemia, cardiovascular disorder and cancer.

P09.058

Investigation of variants of diverse hormone receptor genes and the male pattern baldness major genetic susceptibility loci AR/EDA2R and 20p11 in women with female pattern hair loss

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Androgenetic alopecia is a common hair loss disorder which occurs in both sexes. The disorder is termed male-pattern baldness (AGA) in men, and female pattern hair loss (FPHL) in women. Although the precise etiopathogenesis of FPHL remains unknown, one likely hypothesis is that sex steroid hormones are crucial for the development of FPHL. However, we could not demonstrate significant association for any of the 32 genotyped variants of the hormone receptor genes aromatase-gene (CYP19A1), progesterone receptor (PGR), steroid-5-alpha-reductase alpha polypeptide 1 and 2 (SRD5A1, SRD5A2) and estrogen receptors 1 and 2 (ESR1, ESR2). Another likely hypothesis is that FPHL and AGA share common disease-causing mechanisms and a common genetic background. The two major susceptibility loci for AGA are the X-chromosomal locus AR/EDA2R; and a locus on chromosome 20p11. We performed a fine mapping study of AR/EDA2R and genotyped five SNPs from the chromosome 20p11 region that reached genome-wide significance in a recent GWAS of AGA. No significant association was obtained for any of the 20p11 variants, assuming that this locus do not influence susceptibility to FPHL. At AR/EDA2R, seven markers showed significant association in the subgroup of early affected UK patients, suggesting that the AR/EDA2R locus may be specifically involved in the pathogenesis of early-onset FPHL. Enlargement of the collective of 230 patients (145 UK; 85 German) and 329 controls (179 UK; 150 German) as well as a genome-wide association study might help to understand the role of AR/EDA2R and to identify further genes contributing to the development of FPHL.

P09.059

Multi-ethnic fine-mapping reveals potential causal variants for a complex disease

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Genome-wide association studies have identified 26 non-HLA risk loci for celiac disease (CD), a common autoimmune disorder. The SNPs in most of these loci are in very high linkage disequilibrium, which makes fine-mapping difficult. To refine the association signals we used Immunochip, a custom-made array that includes common and rare variants with a dense coverage of known immune-related loci. We were able to fine-map more than 50% of CD loci and identified 13 new risk loci. The most significant signal mapped to an intronic region of 70 kb in the LPP gene (rs2030519, p-value 3x10-49). Upon conditional analysis on this SNP, the association within this locus disappeared, indicating association to a single common haplotype.

Our aim was to refine the associated region in the LPP locus by comparing the risk haplotype across four independent populations of European origin, using the Cross test, a haplotype association algorithm. We have shown that risk haplotypes in the four populations are derived from the same common ancestor and thus might carry the same causal variant in all four populations. By comparing risk haplotypes to non-risk haplotypes, we were able to narrow down the region from 70 kb to 18 kb.

To investigate functional regulatory elements at this region, we used ENCODE annotation to indicate the most likely candidate variants. We suggest that deregulation of transcription factor binding properties might be a causal mechanism underlying the association of the LPP region to celiac disease. These variants should be studied further by functional means.

P09.060

FTO levels affect RNA modification and the transcriptome

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A block of single nucleotide polymorphisms (SNPs) within intron 1 of the *FTO* (fat mass and obesity associated) gene is associated with variation in body weight. Previous works suggest that increased expression of *FTO*, which encodes a 2-oxoglutarate-dependent nucleic acid demethylase, leads to increased body weight, although the underlying mechanism has remained unclear. To elucidate the function of FTO we examined the consequences of altered FTO levels in cultured cells and murine brain. We show that a knockdown of *FTO* in HEK293 cells affects the transcripts levels of autophagy related genes, whereas overexpression of *FTO* affects the transcript levels of genes involved in RNA processing and metabolism. Subcellular localization of FTO further strengthens the latter notion. Using immunocytochemistry and confocal laser scanning microscopy, we detected FTO in nuclear speckles and - to a lesser and varying extent - in the nucleoplasm and nucleoli of HEK293, HeLa and MCF-7 cells. Moreover, RNA modification analyses revealed that loss of FTO affects the 3-methyluridine/uridine and pseudouridine/uridine ratios in total brain RNA, which mostly consists of rRNAs. We conclude that altered levels of FTO have multiple and diverse consequences on RNA modifications and the transcriptome and that the links between autophagy, RNA modifications and obesity need to be further explored. At present, we are trying to identify the RNA(s) that is(are) modified by FTO.

P09.061

The FTO gene polymorphisms are associated with obesity in the Chinese postmenopausal women

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Obesity is an important risk factor for type 2 diabetes and cardiovascular diseases. The fat mass and obesity-associated gene (FTO) was recently identified as a susceptibility locus for both obesity and type 2 diabetes by genome-wide association studies in several European populations. To investigate the association between FTO gene polymorphisms and obesity in the Chinese postmenopausal women, we genotyped three single nucleotide polymorphisms (SNP) of the FTO gene (rs1421085, rs9939609, and rs9930506) in 424 postmenopausal women recruited from a health survey at the clinics of General Medicine and Metabolism in a university teaching hospital. Among these women, 56 of them were obese (BMI>=27), 108 with overweight (24=<BMI<27) and 260 with normal weight (BMI<24). We found the rs9930506 SNP was significantly associated with obesity in our postmenopausal cohort (p = 0.041). The women carrying with the GG or GA genotypes of the rs9930506 was associated with increased risk of obesity as compared with those with the AA genotype (42.9% v.s. 26.9%, *p* = 0.014). After adjustment for age and physical inactivity, the association between rs9930506 and obesity remains statistically significant (OR=2.02, 95%C.I.:1.13-3.62, p-value=0.019). In conclusion, our results suggest the polymorphisms of the FTO gene are significantly associated with obesity in postmenopausal women. We replicated previous finding of the association between FTO gene polymorphisms and obesity in our Chinese postmenopausal women population.

P09.062

Opisthorchis felineus liver fluke modifies genetic risk of bronchial asthma and serum IgE levels

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Gene-environmental interactions (GxE) are in focus of contemporary studies of allergic diseases and traits. Helminth invasion is recognised as an important factor influencing atopic disease risk. Earlier, we revealed that liver fluke *Opisthorchis felineus* invasion has a significant impact on epidemiological portrait of allergic diseases and immunological status in atopy in Siberian populations (*Parasitol Res. 2007;101(4):1165-8*). We now set out to investigate if the helminth invasion is an environmental factor modifying genetic risk of allergy.

Twenty one single nucleotide polymorphisms (SNP) of immune-response genes were analysed in 222 healthy people and 207 bronchial asthma (BA) patients with established status of *O. felineus* invasion. Linear regression models were built using the interaction of the SNPs and *O. felineus* invasion as a predictor of BA and total IgE levels.

Three significant GxE models were found for BA, including rs2069705 (*IFNG*; $P_{int} = 0.010$), rs2066807 (*STAT2*; $P_{int} = 0.015$), and rs673848 (*SOCS5*; $P_{int} = 0.004$) SNPs. Odds ratios for the significant GxE terms indicated approximately 2-times higher or lower risk of BA in corresponding groups. Two significant GxE models were obtained for total serum IgE, and included rs2069705 (*IFNG*; $P_{int} = 0.046$) and rs167769 (*STAT6*, $P_{int} = 0.032$). In both models, the presence of the helminth invasion associated with lower IgE levels. The results lacked the significance after correction for multiple testing, likely, due to small sample size. However, the data is the first indication of the importance of *O. felineus* invasion as an environmental factor modifying genetic risk of BA and atopy.

P09.063

Suicide and glutamatergic system: NMDAR1 gene

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Suicide is one of the leading causes of mortality in many countries and is caused by a combination of genetic and environmental factors. Alterations in glutamate neurotransmission and particularly the ionotropic glutamate receptors have long been suggested to play a crucial role in the etiology of suicide. However, no genetic studies have been performed with NMDAR1 gene in order to explore the hypothesis of glutamatergic system in suicide susceptibility. The aim of this study was to test the potential involvement of single nucleotide polymorphism G1970A of NMDAR1 gene in the etiology of suicide in the Portugese population. Peripheral blood was collected from suicide victims and controls in the National Institute of Legal Medicine, Portugal and using a standard method, genomic DNA was extracted. The polymorphism G1970A in the coding region of NMDAR1 gene was investigated by RFLP-PCR. The PCR product was digested with MspI enzyme at 37ºC and after digestion, the fragments were separated by electrophoresis on a 3% agarose gel.

For the G1970A polymorphism of NMDAR1 gene, there was no significant differences either in allele frequency or genotype frequency between two groups (p> 0,05). In this study no evidence of association was found between the G1970A polymorphism of NMDAR1 gene and suicide in our sample and these results suggest that this polymorphism does not play a major role in the susceptibility to suicide.

P09.064

Genetic predisposition to tuberculosis in native and immigrant Siberian populations

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There is a difference in prevalence of tuberculosis (TB) among ethnically divergent Siberian populations with highest rates of disease among aboriginal Asian populations. Given the similar environment, differential genetic background can be responsible for distinguished TB liability in native and immigrant Siberian populations. We addressed this issue in a study of common polymorphisms of twelve immune-response modifying genes in Siberian populations of Russians (304 patients, 265 controls), Tuvinians (238 patients, 263 controlss), and Yakuts (150 patients, 135 controls). Both ethnic specific and common association between the genes and TB were identified. The rs12756687 (*PIAS3*), rs3760903 (*PIASY*), rs167769 (*STAT6*), rs1024611 (*MCP1*), and rs2069705 (*IFNG*) polymorphisms were associated with TB in Russians. The rs7572482 (*STAT4*), rs3760903 (*PIASY*), and rs167769 (*STAT6*) were linked to disease in Tuvinians. The rs16967593 (*STAT5B*), rs3760903 (*PIASY*), rs17880053 (*IFNGR2*), rs1024611 (*MCP1*) were associated with TB in Yakuts. The *PIASY* gene was associated with TB in all studied populations suggesting it as a cosmopolitan TB gene. However, while in Russians the common allele was protective against the disease (OR=0.67, P = 1E-6), in Tuvinians and Yakuts it increased the risk of TB (OR = 1.41, P = 0.032; OR = 1.67, P = 0.035, respectively). *STAT6* gene common allele was protective in Russians and predisposing in Tuvinians; while in case of *MCP1* gene, common allele was predisposing in Russians and protective in Yakuts. This finding suggests specificity in allelic effects predisposing to TB in Asians and Russians of Siberia. Molecular mechanisms of these inverse effects are to be investigated.

P09.065

Genome-wide association study identifies sequence variants associated with hematological traits in Asian population

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To identify genetic loci influencing hematological traits, we conducted a genome-wide association study (GWAS) of 8,842 subjects recruited from population-based cohorts in Korea. Replication from independent population (N=7,861) to validate GWAS results revealed SNPs reaching genome-wide significance for selected hematological traits. We identified significant associations between platelet count and genetic variants in four regions on chromosome 4p16.1 ($P_{combined} = 1.46 \times 10^{-10}$, in *KIAA0232*), 4q25 ($P_{combined} = 6.68 \times 10^{-12}$, in or near *EGF*), 12q24.12 ($P_{combined} = 1.11 \times 10^{-15}$, in *SH2B3*) and 6p21 ($P_{combined} = 1.69 \times 10^{-7}$, in *BAK1*). GWAS for WBC showed strong evidence of genetic association on 17q21.1a ($P_{combined} = 1.1 \times 10^{-16}$) which contains *CSF3* gene. Blood hemoglobin concentration was significantly associated with one region on 22q12.3d ($P_{combined} = 2.2 \times 10^{-8}$) localizing to *TMPRSS6* that is required to sense iron deficiency. Two SNPs were detected for their association with RBC from GWAS (RBC data not available in replication subjects). One ($P = 8.6 \times 10^{-9}$) is located on 6p21.1f in *MED20* encoding a component of the mediator complex. The other ($P = 3.3 \times 10^{-12}$) localizes to 4q12 surrounded with several genes (*CHIC2/GSX2/PDGFRA/KIT*). Interestingly, One locus located on 6q23.3a near *MYB* showed strong association with WBC ($P_{combined} = 5.7 \times 10^{-7}$), RBC ($P = 3.8 \times 10^{-25}$) and hematocrit ($P_{combined} = 5.1 \times 10^{-9}$). This locus is known to play an important role in proliferation and differentiation of hematopoietic progenitor cells. Our findings might enhance to unravel molecular mechanisms underlying these hematological traits.

P09.066

The estimation of Heritability analyses for BMI of Genome-wide Association studies based on Korean cohort

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Many Single nucleotide polymorphisms were significant for various risk factors, but these SNPs account for small fraction. We estimated heritability from the number of risk alleles related on BMI and analyzed linear model. For population and twin-family based on cohort in Korea, we predicted and compared the heritability for BMI using various methods. The aim of this study was to estimate variation and their heritability for BMI including genotype information. We have constructed community and twin-family based on cohort, which is an ongoing prospective studies and surveyed samples were drawn from the Korean Genome and Epidemiology Study and Korea Genome Analysis Project in Korea. From Twin-Family cohort, we selected 2,473 subjects in twin-family cohort and surveyed their zygosity using the self-report questionnaires about 2,000 items and genotyped using Affy 6.0. From community-based cohort(KARE; Korea Association REsource), we selected 8,842 subjects and surveyed their self-report questionnaires about 1,400 items and genotyped using Affy 5.0. We estimated heritability for BMI using SOLAR, GCTA, GENABEL. These were estimated and optimized Quantitative genetic analysis adjust age and sex. The estimation value of heritability for BMI based on twin-family cohort, was 0.67(p=5.21E-86) using SOLAR(including only epidemiological data) and was 0.44(p<0.000) using GCTA and was 0.44(p<0.000) using GENABEL(including epidemiological and genotype data) . The estimation value of heritability for BMI based on KARE, was 0.15(p=1.13E-04) using GCTA and was 0.18(p<0.000) using GENABEL. The estimation difference of heritability between Twin-Family and KARE cohort, it depends on sampling error and related/unrelated structure.

P09.067

Estimated heritability of the metabolic syndrome components in the Tehranian families: Tehran Lipid and Glucose Study (TLGS)

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Introduction: Growing evidence suggests that the metabolic syndrome has both genetic and environmental bases. To evaluate the possibility of further genetic analysis, this study estimated the heritability of the metabolic syndrome (MetS) components in the families with metabolic syndrome among from Tehran Lipid and Glucose Study.

Methods: We investigated 904 nuclear families with two biological parents and at least one offspring (1565 parents and 2448 children), aged 3-90 years of Tehran Lipid and Glucose Study, whom metabolic syndrome information was available and had at least two members of family with metabolic syndrome. MetS was defined in adults according to the Joint Interim Statement (JIS) criteria and for offspring as Cooks guide lines. Variance component methods were used to estimate age and sex adjusted heritability of the metabolic syndrome component using SOLAR software.

Results: The heritability of waist circumference (WC), HDL-C, triglyceride (TG), fasting blood sugar (FBS), systolic blood pressure, and diastolic blood pressure as continuous traits after adjusting for age and gender, were 27, 46, 36, 29, 25, and 26%, respectively. When the metabolic syndrome components were analyzed as discrete traits, the estimates of age and gender adjusted heritability for abdominal obesity, low HDL-C, high TG, high FBS, and high blood pressure varied to 22, 40, 34, 38, and 23%, respectively (p <0.05).

Conclusion: We clearly demonstrated a significant heritability of MetS components among TLGS families. The results strongly encourage efforts to identify the underlying susceptibility genes.

P09.068

Association of HIF1A gene Pro582Ser polymorphism with strength athlete status and strength performance in children

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Hypoxia-inducible factor 1-α (HIF-1α; encoded by HIF1A gene) controls a number of genes that are implicated in various cellular functions including glycolysis, cell proliferation and differentiation. The 582Ser allele of the Pro582Ser (rs11549465 C/T) polymorphism in the HIF1A gene increases protein stability and transcriptional activity, and therefore, improves glucose metabolism. The aim of our study was to investigate the association between the HIF1A Pro582Ser polymorphism, elite strength athlete status and strength related phenotypes in 225 middle school-age children (116 boys and 109 girls; aged 11±0.4). A total of 208 Russian strength athletes (122 weightlifters and 86 wrestlers) of regional or national competitive standard and 1,413 controls were genotyped using PCR-RFLP method. We found that the frequency of the HIF1A 582Ser allele was significantly higher in weightlifters (13.1%, P = 0.0031), as well as in wrestlers (15.7%, P = 0.0002) compared to controls (7.5%). Additionally, the highest (21.1%; P = 0.0052) frequency of the 582Ser allele was found in a group of elite strength athletes. Furthermore, the carriers of the HIF1A 582Ser allele among boys demonstrated the best results of handgrip strength testing than Pro/Pro homozygotes (Pro/Pro - 13.4 (3.1) kg, Pro/Ser - 16.5 (2.5) kg; P = 0.049). Thus, our study provides evidence for the association between the HIF1A gene Pro582Ser polymorphism, elite strength athlete status and strength performance in children. Although more replication studies are needed, the preliminary data suggest an opportunity to use the analysis of HIF1A polymorphism along with other gene variations in athletic talent identification.

P09.069

Replication of functional serotonin receptor type 3A and B variants in bipolar affective disorder: a European multicenter study

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Serotonin type 3 receptors (5-HT3) are involved in learning, cognition, and emotion, and have been implicated in various psychiatric phenotypes. However, their contribution to pathomechanisms remains elusive. Three SNPs in the HTR3A and HTR3B genes have been associated with bipolar affective disorder (BPAD) in pilot studies, and all of them are of functional relevance. We performed a European multicenter study to confirm previous results and provide further evidence for the relevance of these SNPs for neuropsychiatric disorders. This involved analysis of the distribution of the three SNPs among 1804 BPAD cases and 2407 healthy controls. A meta-analysis revealed a pooled odds ratio of 0.881 (P = 0.009, 95% CI = 0.802 - 0.968) for the non-synonymous functional SNP HTR3B p.Y129S (rs1176744), thereby confirming previous findings. In line with this, the three GWAS samples BOMA-BD, WTCCC-BD and GAIN-BD, including more than 3500 patients and 5200 controls in total, showed an over-representation of p.Y129 in patients. Remarkably, meta-analysis revealed a P-value of 0.048 (OR = 0.934, fixed model).

Expression analyses to gain further insights into the distribution of HTR3A and HTR3B mRNA in the human brain detected HTR3A and HTR3B in all investigated brain tissues with the exception of the cerebellum, and large differences in the A:B subunit ratio were observed. Interestingly, expression of the B subunit was most prominent in the brain stem, amygdalae, and frontal cortex, regions of relevance to psychiatric disorders.

In conclusion, the present study provides further evidence for the presence of impaired 5-HT3 receptor function in BPAD.

P09.071

Novel common mechanism of hyperuricemia by decreased extra-renal urate excretion

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Hyperuricemia has been generally classified into uric acid (urate) “overproduction type,” “underexcretion type,” and “combined type” based on only renal urate excretion, without considering an extra-renal pathway such as gut excretion. We recently showed that ABCG2/BCRP is a high-capacity urate exporter and that its dysfunction is a major cause of hyperuricemia and gout. Here we evaluated how ABCG2 dysfunction affects urate excretion pathways.

Clinical parameters for urate handling including urinary urate excretion were examined in 644 male outpatients with hyperuricemia. Severity of ABCG2 dysfunction in them was estimated by genotype combination of two common *ABCG2* variants, nonfunctional Q126X (rs72552713) and half-functional

Q141K (rs2231142). We investigated the relationship between ABCG2 dysfunction and urinary urate excretion, and evaluated urate excretion pathways between Abcg2-deficient and wild-type mice treated with uricase inhibitor, oxonate.

Unexpectedly, urinary urate excretion was inversely associated with ABCG2 excretion function. Mild, moderate and severe ABCG2 dysfunctions significantly raised the risk of overproduction hyperuricemia (overproduction type and combined type). In abcg2-deficient mice, serum urate levels and renal urate excretion were increased, while intestinal urate excretion was decreased, compared to those of wild-type mice.

Together with high extra-renal ABCG2 expression, these results suggested that a decrease in extra-renal urate excretion, especially in intestinal excretion, by dysfunctional ABCG2 is a common mechanism of hyperuricemia, often misunderstood as urate “overproduction.” Thus, “overproduction” hyperuricemia in the current classification should be renamed “renal overload” hyperuricemia, which is caused by two mechanisms, “extra-renal urate underexcretion” and genuine “urate overproduction.”

P09.072

Contribution of Genetic Profiles to the Risk for Inflammatory Bowel Disease Patients in Slovenian Population

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More than 40 genome wide association studies (GWAs) and subsequent meta-analysis in Inflammatory bowel disease (IBD) patients revealed more than 150 IBD associated single nucleotide polymorphisms (SNPs). The single SNP has a limited contribution to disease risk as most of the reported odds ratio (OR) values do not exceed 1.5. This study tested if genetic profiles composed of different numbers of most significant SNPs included in genetic profiles could better describe contribution to disease risk.

We have genotyped 650 Slovenian IBD patients and 400 healthy controls for 34 SNPs previously reported in GWA studies to be most significantly associated with IBD. We confirmed 21 SNPs associated with at least one disease sub-phenotype in our patients, including 5 SNPs associated exclusively with refractory Crohn disease and 4 with ulcerative colits. The highest calculated OR for the genetic profiles reached values between 10-12 for the profiles composed from 10-20 SNPs with more than 90% of disease aleles in tested SNPs included in profiles. The predictive models using combination of 10-12 associated SNPs with highest OR showed above 95% specificity in patients with 90% disease alleles however only a few patients would have 90% disease alleles resulting in low sensitivity usually below 20% of such genetic test. On the other hand, most of the patients would have between 50%-60% of disease alleles however the specificity of genetic test in this case would be less than 15%. These results suggest more complex predictive models need to be developed for better risk prediction in IBD.

P09.073

Association of IRF6 gene polymorphisms with nonsyndromic cleft lip with or without cleft palate

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Background: Cleft lip and palate is most common congenital malformation with prevalence of approximately 1 in 700 live births worldwide, the frequency also differs by laterality - there is a 2:1 ratio of left to right sided clefts among unilateral cleft lip cases. Mutations in *IRF6* gene cause Van der Woude syndrome and it has been reported that polymorphisms in *IRF6* gene are associated with nonsyndromic CL/P.

The aim of the study was to evaluate the relevance of *IRF6* gene in etiology of nonsyndromic clefts.

Materials and methods: Seven SNPs were analyzed for allelic association with nonsyndromic clefts. The data set consisted of 116 nonsyndromic cleft lip/palate samples and 148 control samples. Out of all cleft cases, 28 had CP and 88 had cleft lip with palate or cleft lip only (CLP).

Results: CP patient group showed the stongest association with rs658860 ($p=0.873x10^{-7}$, OR=0.093, 95% CI=0.033-0.264). In patient group with CLP two SNPs showed significant association (rs642961, p=0.011, OR=1.844, 95% CI=1.148-2.962; rs658860, $p=0.24x10^{-5}$, OR=0.376, 95% CI=0.249-0,568). Similar results we found according to cleft lip side: patient group with CL on left side (n=45) showed strong association with rs658860 (p=0.485x10-4, OR=0.332, 95% CI=0.192-0.574) and patients group with CL on right side (n=18) - with rs642961 (p=0.014, OR=2.589, 95% CI=1.187-5.644).

Conclusion: The results of this study suggest that *IRF6* gene contributes to etiology of nonsyndromic clefts. Our data provide evidence that some variations in *IRF6* gene showed decreased risk to nonsyndromic clefts and futher studies have to be made to clear it.

P09.074

The genes in irritable bowel syndrome research network Europe (GENIEUR)

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Irritable bowel syndrome (IBS) is a highly prevalent functional gastrointestinal (GI) disorder with a major impact not only on the healthcare system but also on the patient's quality of life. Genetic factors contributing to the pathogenesis of IBS have not further been specified and knowledge is still poor. The search for genetic factors in IBS is mainly hampered by the fact that only a few groups worldwide have just recently started to perform genetic analyses in small cohorts.

Consequently, contradictory results have been reported due to low statistical power. The GENIEUR network will foster the establishment of a pan-European, interdisciplinary network with the major goal being the creation of guidelines for patient / control recruitment as well as phenotypic characterization by defining quantitative traits as intermediate phenotypes for the following identification of genetic factors in the pathogenesis of IBS. The network will not only focus on genetics but also epigenetics and microbiomics. In line with this, a biobank will be established in which we collect patient material (blood, saliver, colon biopsies, stool samples) for detailed analyses. This represents a solid basis for novel diagnostic and herapeutic approaches and will significantly improve the insight into IBS pathophysiology, and, hence, help identify new targets for treatment with the ultimate goal of increasing quality of life of affected patients.

P09.075

Molecular characterization of the KCNA5 gene in Pulmonary Arterial Hypertension Spanish patients

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Pulmonary arterial hypertension (PAH; OMIM 178600) is a rare and progressive vascular disorder characterized by pulmonary vascular resistance increase, vascular remodelling and right heart failure. Approximately 75% of patients with the familial form of PAH have a mutation in the gene encoding bone morphogenetic protein receptor type II (BMPR2). However, some other candidate genes have been advocated, including the KCNA5 gene that codifies for a potassium voltage-gated channel. This gene is located on chromosome 12p13, with a single exon of 2865 bp and 613 aminoacidic residues.

We included 30 patients with PAH and 50 controls. The DNA extraction was performed with the Qiagen DNA kit FlexiGene. The KCNA5 gene was amplified by PCR and sequenced.

A total of 10 sequence changes were identified in 11 of the 30 patients with PAH, and none were detected in a panel of 100 chromosomes from normal individuals. From all the mutations and polymorphisms found in this analysis, eight of them had not been previously described. Five of the mutations are missense (p.L42H, p.P46T, p.P169R, p.R184P, p.R577K). Also found a nonsense mutation (p.E208X) and one synonymous change (p.L159L). We found three different mutations in the KCNA5 gene 3´UTR region, which do not produce changes in the structure of the protein (c.2093T>G, c.2123T>G, c.2579A>T).

In conclusion, the mutations in KCNA5 gene indicated that this gene is the second most important gene implicated in the development and worsening of PAH familiar, idiopathic and secondary.

P09.076

Circulating phospholipids are associated with telomere length in a Dutch family-based study

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Telomeres are necessary for both successful DNA replication and maintenance of chromosomal integrity. Telomere length (TL) declines with age and, these decreases are associated with increases in coronary artery disease, obesity and insulin resistance. Both TL and various lipid particles have been associated with longevity. Here, we study the relationship between TL in white blood cells, measured by quantitative PCR, and plasma levels of 24 sphingomyelins (SPM), 9 ceramides, 57 phosphatidylcholines (PC), 20 lysophosphatidylcholines (LPC), 27 phosphatidyletanolamines (PE) and 16 PE-based plasmalogens, assessed using mass spectrometry. A total of 784 persons were included from the Erasmus Rucphen Family Study and analysed using variance component analyses (SOLAR version 4.3.1), adjusting for age, sex and batch effects. After Bonferroni correction, TL was strongly associated with 11 PC species (smallest P-value = 2.31 x 10-09, with PC 40:3), SPM 23:0, SPM 23:1 (P-value = 1.16 x 10-04 and 2.39 x 10-04), LPC 22:0 (P-value = 3.16 x 10-09) and PE 42:7 (P-value = 1.81 x 10-04). Results remained significant after adjusting for plasma HDL-C and LDL-C. Backward regression analyses showed that four of the initial fifteen lipid species (LPC 22:0, PC 38:1, PC O 42:6, PE 42:7) were independently associated with TL. Our findings suggest a link between TL and phospholipid metabolism that may help to explain the role of fatty acids and lipids in longevity as showed in animal and family-based studies.

P09.077

A comprehensive phenotypic and genetic analysis of centenarians from 13 Villages located in the Sardinian region of Barbagia-Mandrolisai

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About 30% of longevity is genetically endowed, and there have been repeated observations that long life clusters in families. Presently, it is still unclear whether long life results

1) from the absence in some individuals of genetic variants that would predispose to a range of diseases;

2) or in addition or instead, from a set of alleles that positively and specifically promote healthy old age.

Genetic studies in cosmopolitan populations have been at best inconclusive in addressing these questions: perhaps the major reason is that "centenarians" are rare, and it is reasonable to suppose that alleles involved in conferring longevity are rare as well. These analyses would be simplified by focusing on isolated founder populations, having a high prevalence of centenarians and hence most likely enriched in powerful pro-longevity and "anti-frailty" alleles.

At the geographic centre of Sardinia is located a mountainous, hilly isolated region named Barbagia-Mandrolisai, where many centenarians live and continue to be active. We focused our attention on about 250 healthy 90-year-olds, from a cluster of 13 villages. Presently, we have been collecting their blood samples and measuring over 300 quantitative traits (endophenotypes or quantitative risk-related genetic or environmental factors). Traits of special interest include cardiovascular risk factors, anthropometric measurements, blood test values, facets of personality and a comprehensive panel of immunological traits. With this cohort, genetic studies with batteries of hundred thousand of single-nucleotide markers - and in selected cases with direct sequencing of complete genomes - will be conducted.

P09.078
Susceptibility variants on chromosome 7p21.1 suggest HDAC9 as a new candidate gene for male-pattern baldness

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Male-pattern baldness (androgenetic alopecia, AGA) is the most common form of hair loss among humans. Numerous studies have unequivocally identified two major genetic risk loci: the X-chromosomal *androgen receptor* (*AR*)/*ectodysplasin A2 receptor* (*EDA2R*) locus and the *paired box 1* (*PAX1*)/ *forkhead box A2* (*FOXA2*) locus on chromosome 20. Although these loci explain a significant fraction of the overall genetic risk for AGA, additional genetic risk factors still await identification. Here, we performed a GWAS using a German sample of 581 severely affected cases and 617 partially unaffected controls. The best association signal was obtained for rs756853, located intronically in the *histone deacetylase 9* (*HDAC9*) gene on chromosome 7p21.1. A fine mapping analysis within the case-control sample and a family-based analysis revealed rs756853 and rs2249817, respectively, as primary associated SNPs. The association finding for rs2249817 was confirmed within an independent Australian sample (P=0.026). A combined analysis of severely affected German and Australian cases (N=639) and unaffected controls (N=384) for rs2249817 revealed a strong association signal of P=9.09x10-8, odds ratio 1.63 [1.36-1.95]. Tissue expression studies demonstrated *HDAC9* expression in various tissues, including AGA relevant tissues. Genotype-specific expression as well as splice studies revealed no strong genotypic effects, although smaller effects cannot be excluded. Pathway analyses however support the hypothesis that *HDAC9* plays a functional role in AGA via interaction with the *AR* gene. The genetic data of the present study thus provide strong evidence that *HDAC9* is the third AGA susceptibility gene.

P09.080
Migraine susceptibility factors: the role of GABA genes in females' liability

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Migraine is a chronic disorder characterized by episodes of headache and autonomic and neurological symptoms. Several studies showed that migraine is influenced by genetic and environmental factors. The female-to-male ratio of migraine prevalence is 3- to 4-fold higher among women than men.
The role of common variants of GABA genes in the X-chromosome in migraine susceptibility was assessed, aiming to explain the differences in disease frequency between males and females.
An association study with 188 unrelated cases and 287 migraine-free controls age- and ethnic matched was performed. The case-control ratio was 1:1.5.
Candidate genes were selected based on their possible role in pathophysiology of migraine. Twenty-one tagging SNPs were selected in three genes (*GABRE, GABRA3* and *GABRQ*) and genotyping was performed by SNaPshot.
Allelic, genotypic and haplotypic frequencies were compared between cases and controls and multiple testing corrections were performed. Also, gene-gene interactions were analyzed.
The results for allelic associations revealed five nominal significant associations and three trends for association. In what concerns genotypic frequencies, four noteworthy results were found in *GABRE* and *GABRA3* genes. After multiple testing correction, two allelic associations remained significant (*GABRA3* and *GABRE*) and one genotypic association resisted to Bonferroni correction (*GABRE*), all in the females group.
No significant results were found in the haplotypic analyses but an additive effect was observed between two SNPs of *GABRA3* and two of *GABRE*.
With this study we show, for the first time, a possible involvement of polymorphisms in GABA receptors in migraine susceptibility and in gender-specific liability.

P09.081
The role of genes involved in neurogenic inflammation as susceptibility factors for migraine

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Migraine is one of the commonest neurological and complex disorders, affecting 11% of the world's adult population. In the pathogenesis of migraine several mechanisms are involved such as neurogenic inflammation, with the release of neuropetides (like substance P, neurokinin A or CGRP).
Our aim was to unravel the role of three candidate genes related to neurogenic inflammation processes - F2RL1, TAC1 and CALCRL- in migraine susceptibility. CALCRL encodes the CALCRL receptor of CGRP and F2RL1 gene encodes PAR2; PAR2 agonists increase SP and CGRP release. Additionally we have also selected TAC1 which encodes protachykinin-1 that can normally undergo alternative splicing to generate both SP and NKA.
A case-control association study was designed and performed, using a sample of 188 unrelated migraineurs and 287 healthy controls, age- and gender-matched with cases.
SNPs on candidate genes were selected based on a data dump from The International HapMap Project. From the three candidate genes 14 tagging SNPs were obtained and genotyped by the SNaPshot technique.
Allelic, genotypic, and haplotypic frequencies were compared between cases and controls and multiple testing corrections were applied. Also, gene-gene interactions were assessed using multifactor dimensionality reduction (MDR) method.
Four SNPs in the CALCRL gene showed nominal significant genotypic associations and one significant haplotype association. Importantly, a significant interaction was found between three SNPs of TAC1 gene.
The results obtained reinforce the importance of neurogenic inflammation in migraine susceptibility. Deepening these genetic susceptibilities will be crucial, contributing to public health research and to develop more effective therapeutic strategies.

P09.082
Mitochondrial Uncoupling protein 2 gene variations (exon 8 insertion/deletion and -866 A/G) are associated with childhood obesity

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Objectives: Uncoupling protein 2 (UCP2) belong to a family of mitochondrial carrier proteins which plays important role in thermogenesis and energy metabolism. UCP2 is releated to pathogenesis of several disease including human cancers, neurodegeneration, cardiovascular disease, metabolic disorders and obesity. Our aim was to investigate whether childhood obesity may be associated with variations of UCP2 gene
Methods: In this study, 80 obese children and 100 age-and sex-matched healthy controls were tested for two variations which were exon 8 (45 bp insertion/deletion) and promoter (-866 A/G) variations in UCP2 gene. Genotyping were performed by PCR and/or RFLP.
Results: Exon 8 variation in UCP2 gene was showed an association with obese patients. The distribution of DD, DI and II genotypes for the gene was 47.5 %, 25 % and 27.5 % in obese compared with 65 %, 30 % and 5 % in the controls. II genotype was found lower in patients ($p < 0.0001$), while DD genotype was higher in controls (p=0.0092). The distribution of UCP2 gene promoter variation GG, GA and AA genotypes was 17.5 %, 46.2 %, 36.3 % in obese compared with 16 %, 63 %, 21 % in the controls. Statistically, AA genotype was found to be increased in patients (p=0.0116).
Conclusion: The present study showed that UCP2 gene variations were associated with childhood obesity. Insertion genotype (II) of exon 8 and AA

genotype of promoter variations may influence the susceptibility to obesity. Identification of genetic backround in obesity may provide clues about etiology and therapeutic targets.

P09.083

The MTHFR gene C677T polymorphism is associated with athlete status and muscle fiber hypertrophy

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Recent findings by Terruzzi et al. (2011) suggest that DNA hypomethylation induces the activation of factors determining proliferation and differentiation of myoblasts promoting muscle growth and increase of muscle mass. The C677T polymorphism of the MTHFR gene (involved in DNA methylation) was shown to be associated with reduced 5,10-Methylenetetrahydrofolate reductase activity. We therefore hypothesized that carriers of the MTHFR T allele may have DNA methylation deficiency, and as a consequence, increased skeletal muscle mass and predisposition for high level athletic performance. To test this hypothesis, we examined the MTHFR gene C677T polymorphism in 1,819 Russian athletes and 1,041 controls. We also investigated the association between the MTHFR polymorphism and muscle fiber characteristics in 47 physically active healthy men. Genotyping for the C677T variant was performed by PCR-RFLP. Muscle fiber characteristics of m. vastus lateralis was determined by immunohistochemistry. The MTHFR TT genotype (9.5 vs. 6.6%; P=0.0088) and T allele (30.3 vs. 26.3%; P=0.0015) frequencies were significantly higher in athletes compared with control subjects. Furthermore, the highest frequencies of the MTHFR TT genotype (13.9%: P=0.0003) and T allele (34.1%; P=0.0009) were found in a group of highly elite athletes (n=223). The carriers of the MTHFR T allele (n=25) had significantly higher cross-sectional area of slow-twitch fibers (5545+/-242 vs. 4900+/-169 square microns; P=0.0175) than CC homozygotes (n=22). Thus, the MTHFR gene C677T polymorphism is associated with athlete status and muscle fiber hypertrophy. Collectively, our data support the hypothesis that the presence of MTHFR T allele has a beneficial effect on athletic performance.

P09.084

Circadian rhythm genes and multiple sclerosis (MS)

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Background : Evidence from epidemiological studies indicates, that, prevalence of MS varies with geographic latitude, increasing with distance from the equator on both hemispheres. We hypothesized that explanation for the latitude effect might be related to sun exposure which has been shown to impact internal circadian rhythm. This rhytm is controlled by circadian rhythm genes and that could therefore affect MS susceptibility.

Methods: A total of 826 Caucasian patients and 888 healthy unrelated ethnically matched controls without family history of MS, were included in the study. In patients, the diagnosis of MS was established according to McDonald's criteria. Altogether, 8 SNP were included in our study, 4 in CLOCK gene: rs6811520, rs6850524, rs11932595 and rs13124436; and 4 in ARNTL gene: 3789327, rs1481892, rs4757144 and rs12363415. The significance of association for individual SNPs was analyzed using the Chi-Square test (χ2). Odds ratios (OR) and their respective 95% confidence intervals (CI) was also calculated to compare the allelic frequency and genotype distribution in patients and control subjects.

Results: Significant difference in distribution of ARNTL rs3789327 polymorphism genotypes was found in patients with MS in comparison to controls, with P-value of 4.6e-07 and odds ratio equal to 0.56 (95% CI: 0.45-0.71). Other SNPs in ARNTL and CLOCK genes did not display significant association with MS susceptibility.

Conclusion: We provide evidence for association between genetic variation in ARNTL gene and multiple sclerosis. Further studies will be required to substantiate the significance of these genetic variations.

P09.085

Multiple Sclerosis Risk Variant HLA-DRB1*1501 Associates with High Expression of DRB1 Gene in Different Human Populations

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The human leukocyte antigen (HLA) exerts the largest genetic contribution to MS susceptibility) and HLA DRB1*1501 has been consistently associated with multiple sclerosis (MS) in nearly all populations tested, but exactly how it alters the risk of developing MS is not fully understood. The identification of expression quantitative trait loci (eQTL) for genes in the HLA locus raises the question of the role of gene expression in MS susceptibility. We analyzed the eQTLs in the HLA region with respect to MS-associated HLA-variants obtained from genome-wide association studies (GWAS). We found that the Tag of DRB1*1501, rs3135388 A allele, correlated with high expression of DRB1, DRB5 and DQB1 genes in a Caucasian population. In quantitative terms, the MS-risk AA genotype carriers of rs3135388 were associated with 15.7-, 5.2- and 8.3-fold higher expression of DQB1, DRB5 and DRB1, respectively, than the non-risk GG carriers. The haplotype analysis of expression-associated variants in a Spanish MS cohort revealed that high expression of DRB1 and DQB1 alone did not contribute to the disease. However, in Caucasian, Asian and African American populations, the DRB1*1501 allele was always highly expressed. In other immune related diseases such as type 1 diabetes, inflammatory bowel disease, ulcerative colitis, asthma and IgA deficiency, the best GWAS-associated HLA SNPs were also eQTLs for different HLA Class II genes. Our data suggest that the DR/DQ expression levels, together with specific structural properties of alleles, seem to be the causal effect in MS and in other immunopathologies rather than specific antigen presentation alone.

P09.086

Genetic burden analysis of common associated variants in large Finnish MS families and isolate population of Southern Ostrobothnia

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Multiple sclerosis (MS) is a complex autoimmune disease of the central nervous system. Recent GWAS have discovered over 50 common variants associated with MS. An isolated population in Southern Ostrobothnia in western Finland has two-fold prevalence and familial clustering of MS. Our aim was to study whether the accumulation of common predisposing variants in the isolate and multiplex families contributes to the increased prevalence. We used a weighted log-additive model to calculate a genetic burden score in all samples based on previously identified 51 SNPs. We evaluated the differences in the genetic score distribution between the sample groups by using a non-parametric Kolmogorov-Smirnov test. As in previous reports, the genetic burden score was increased in MS patients: mean 6.73 in the sporadic (n=522) and 6.78 in the familial cases (n=64) compared to IBS-matched population controls (n=1198) mean 6.23 (p <0.0001). When we divided the MS cases according to region of origin, both the isolate (n=111, mean 6.64) and the general Finnish cases (n=497, mean 6.77) had significantly higher score compared to the population controls (p<0.0001). However, we could not detect significant differences in the genetic score distribution between familial and sporadic cases (p=0.16) or between isolate cases and cases from elsewhere in Finland (p=0.26). Since the common variants do not seem to explain the increased familial prevalence in these multiplex families or in the isolate, we hypothesize that there are specific variants that contribute to MS predisposition in familial cases and in the Southern Ostrobothnian region.

P09.087

Investigation of relationship Between ATP5β gene expression and multiple sclerosis disease

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Multiple sclerosis (MS) is an inflammatory, demyelinating, neurodegenerative disorder of the central nervous system (CNS) with unclear exact cause; However studies confirmed the role of environmental and genetic factors in MS etiology. Disease onset usually occurs in young adults and it is more common in women. A number of studies have reported mitochondrial defects in MS and implied a pathogenic role for mitochondria in axonal degeneration. Mitochondria respiratory chain are the most efficient produces of ATP and defects in ATP synthesis may be an important factor in neurodegenerative disease such as Parkinon, Alzhimers and MS. Analysis of the mitochondrial proteomin experimental autoimmune encephalomyelitis (EAE) mous model of MS reveal decrease expression in 5 protein mitochondrial respiratory chain (COX5b, COX5a, ATP5b, NDUFS8, NDUFV2) . One of these proteins was β subunit of complex V with ATP synthase activity. ATP5β encodes a subunit of mitochondrial ATP synthase. In this study, we tested the altered expression of ATP5β in LCLs derived from 30 patients with MS and 31 controls. A significant reduction of ATP5β mRNA expression was seen in MS patients (P> 0.05). According to our results, we suggest that ATP5β can be used as a biomarker for early diagnosis of MS. However, more researches are necessary to reach this goal.

Keywords: Multiple Sclerosis, Mitochondria respitoray chain, EAE model, ATP5β, Real Time PCR

P09.088

Does miR-634 play a role in multiple sclerosis predisposition?

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Multiple sclerosis (MS) is a neurological disorder characterized by chronic inflammation, demyelination, and axonal damage, probably caused by an altered immune response.

The protein kinase C alpha gene (PRKCA, encoding a protein critical for T-lymphocyte activation) was the first non-HLA gene demonstrated to be involved in MS both by linkage and association studies, and subsequently replicated in 4 populations. This gene produces at least 2 alternative transcripts with different 3'UTRs, and hosts a microRNA, miR-634, in its intron 15. Since several miRNAs have recently been implicated in MS pathogenesis, and our preliminary results suggested that both PRKCA and miR-634 are differentially expressed in MS patients vs healthy controls, we decided to better characterize miR-634 expression and function.

The PRKCA and miR-634 discordant expression profiles in different human tissues suggested the presence of an independent miRNA promoter and/or the possible miR-634-mediated silencing of PRKCA. Transfection experiments followed by both luciferase-based and RT-PCR assays demonstrated the existence of a miRNA-specific promoter. Co-transfection of a vector expressing the wild-type pre-miR-634 hairpin into HeLa cells, together with a construct containing the luciferase cDNA coupled to each of the two PRKCA 3'UTRs, showed that miR-634 does target the PRKCA shorter 3'UTR. The specificity of this interaction was confirmed by site-directed mutagenesis of the miR-634 target site.

In conclusion, we demonstrated that miR-634 expression is driven by an independent promoter and we suggest that this miRNA, by modulating PRKCA expression, may be involved in MS pathogenesis.

P09.089

Modulatory influence of vitamin D receptor genotype on OPG/RANKL System and Pathogenesis and Clinical Manifestations in Multiple Sclerosis

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The OPG/RANKL has identified role in immune system via T-cell-activating cytokines. Considering that immune mechanisms play a key role in the pathogenesis of MS, OPG/RANKL might be importance in the underlying mechanism of the disease. The aim of this study is to measure plasma levels of OPG and RANKL as well as to analyze VDR *FokI* polymorphism (rs2228570) in MS patients and healthy individuals to detect any potential correlation. We included a total of 397 participants, 105 of them suffering from two different types of MS, namely relapsing and remitting and secondary progressive multiple sclerosis.

The results showed differences in the plasma levels of OPG and RANKL between patients and the healthy control group that were statistically significant. We found higher plasma levels of OPG and lower RANKL concentrations in RRMS patients in comparison with PPMS and SPMS types of the disease. We detected higher plasma levels of OPG and lower levels of RANKL in subjects with F allele compared to those with f allele in healthy subjects. However, contradicting results were observed when patients with MS were analyzed. We detected lower plasma levels of OPG and higher RANKL concentrations in patients with F allele in comparison with those with f allele.

This might define a role for *FokI* polymorphism and OPG/RANKL system in the pathogenesis and progression of multiple sclerosis with further practical applications.

P09.090

Association of *MMP-8* promoter gene polymorphisms with myocardial infarction: A hospital based study

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Matrix mettalloproteinases (MMPs) are the family of peptidase enzymes responsible for the degradation of extracellular matrix (ECM) proteins. MMP-8 cleaves collagenotype I three times more potently than two other interstitial collagenases, MMP-1 and -13. Therefore, MMP-8 plays a important role in atherosclerosis. The aim of the study was to investigate the association of two MMP-8 polymorphisms, rs11225395 (-799C/T) and rs1320632 (-381A/G). 319 patients were studied out of whom 152 individuals were documented angiographically for coronary atherosclerosis without any evidence of previous MI. 167 patients with normal coronary arteriograms were included as healthy controls. PCR- based restriction fragment length polymorphism (RFLP-PCR) method was applied. MMP-8-799C/T gene polymorphism was observed with CC 93/152 (61.1%), CT among 34/152 (22.3%) and TT in 25/152 (16.4%) among the patients with MI; while in control group, 151/167(90.4%) had CC genotype, 13//167 (7.7%) CT and 3/167 (1.7%) carried TT. The prevalence of TT was 9.0 times higher among the case patients than the controls (16.4% vs. 1.7%, p = < 0.0001). MMP-8-381 A/G gene polymorphism was observed with AA 89/152 (58.5%), AG among 36/152 (23.6%) and GG in 27/152 (17.7%) patients with MI; while in control group, 144/167 (86.2%) had AA genotype, 21//167 (12.5%) AG and 2/167 (1.1%) carried GG. The prevalence of GG was 15.0 times higher among the case patients than controls (17% vs. 1.1%, p = < 0.0001).Our preliminary data indicate that MMP-8-381A/G ,-799C/T gene polymorphisms could be a risk factor for MI.Further studies may explore more to confirm the role of these polymorphisms

P09.091

Long-term outcome of anti-VEGF treatment in patients with neovascular age-related macular degeneration is influenced by the initial response and the genotype of complement factor H (CFH)

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The outcome of anti-VEGF treatment in patients with neovascular age-related macular degeneration (AMD) is influenced by several factors. The aim of this study was to assess the impact of three parameters: (i) loading phase, (ii) initial response for the long-term outcome and (iii) the effect of the complement factor H (CFH) polymorphism (p.His402Tyr). Patients treated with ranibizumab for neovascular AMD were analyzed over a period of 24 months. Visual acuity (VA) was recorded at each visit, effects of loading phase and initial response were analyzed, and the genotype of CFH rs1061170 (c.1204C>T, p.His402Tyr) was determined. The study included 204 eyes. A change of +5.0 [-1;+11] and +1.5 [-5.5;+9.5] letters was observed with a median of 4 [3;7] and 10 [7;14] ranibizumab injections during 12 and 24 months, respectively. Loading phase was no significant predictor for treatment as VA outcome in eyes with and without loading phase was similar (p=0.846 and p=0.729) at 12 and 24 months. In contrast, initial response was a significant predictor for improving vision of 5 or more letters at 12 (p=0.001; OR=6.75) and 24 months (p=0.01; OR=4.66). Furthermore, the CT genotype at CFH rs1061170 was identified as a significant predictor for a favorable VA outcome at 12 and 24 months (OR= 6.75, p=0.001 and OR=4.66, p=0.01). Our data suggest that an initial response as well as the genotype of SNP rs1061170 at CFH present critical criteria that influence treatment outcome. Clinicians may include these when designing a treatment regimen using ranibizumab injections.

P09.092

Nuclearcytoplasmic Shuttling of Disease Protein in Autosomal Dominant Machado-Joseph Disease

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Neurodegenerative disorders are a large category of conditions all caused by a loss of structure or function of neurons, often resulting in progressive neuronal death. Of these conditions, Machado-Joseph Disease, or Spinocerebellar Ataxia Type 3 (SCA3) is an autosomal dominant, late onset neuromuscular disease associated with a coding expansion of a CAG repeat in the *Ataxin-3* gene. The expanded unstable CAG repeat encodes for an abnormally expanded polyglutamine (polyQ) track which in turn causes protein misfolding and aggregation. The most recent research in polyQ disorders has focused on protein transport mechanisms responsible for the traffikking of disease proteins. The ability of proteins to be shuttled between the cytoplasm and the nucleus confers on them the capability to affect cellular transcription in the nucleus and avoid the cellular clearance machinery of the cytoplasm. For disease proteins such as expanded Ataxin-3, localization of protein aggregates to the nucleus has been shown to worsen disease phenotype and increase symptoms in SCA3 animals. In this work we use microscopy, cell viability studies and the filter trap assay to analyze the possible partners of Ataxin-3 which affect the localization, aggregation, pathogenicity, and protein trafficking of this protein. With further understanding of the pathways involved in the progress of SCA3, it will be possible to determine potential targets of therapy to mitigate the course of the disease in patients.

P09.093

The relationship of the COMT gene polymorphism rs4680 with the components of nicotine dependence in a central Romanian population

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Through dopamine release nicotine activates the mesocorticolimbic reward pathway which mediates reinforcement of continued use. Thus, in the multifactorially determined addiction, enzymes responsible for dopamine metabolism and polymorphisms of their genes may be involved. The rs4680 functional polymorphism of COMT (catechol-O-methyl-transferase) due to a Val→Met substitution results a high (H, G1497) and a low activity allele (L, A1497). It has been suggested that carriers of the H allele might present an increased risk to develop dependence, however, results have been contradictory, and no study in this respect has been carried out in our population. In a case-control study of 113 smokers and 84 non-smokers, we assessed nicotine dependence by NDSS, HSI and FNDS adapted for the local population, and genotyped for rs4680 by PCR-RFLP using NlaIII.

Allele frequency in the smoker and non-smoker study group was 53.1 and 46.9 versus 44.4 and 55.6, respectively. Though genotype associated risk for smoking was not statistically significant (GG vs AA: OR = 1.96, CI95%: 0.5-8.2, p = 0.16; GG vs GA+AA:1.54, CI95%: 0.57-4.34, p = 0.2), the overall scores of dependence and its certain components, namely drive and priority, showed significant differences across the genotypes (p<0.05).

In conclusion, COMT might be a risk modifier involved in nicotine dependence rather than a susceptibility gene. Studying the various traits of dependence on a higher number of subjects would be meaningful and could have direct practical implications in cessation management.

P09.095

Association between a promoter SNP in MUC5B and idiopathic pulmonary fibrosis in the Newfoundland population

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Idiopathic pulmonary fibrosis (IPF) is a late-onset, complex genetic disease characterized by inflammation/scarring of the lung parenchyma. To date, heterozygous causal variations in *TERT*, *hTR*, *SFTPC*, *SFTPA2* that account for 2-20% of IPF in various populations have been documented. Recently, Seibold et al identified a promoter variant (rs35705950) upstream of *MUC5B* that is associated with IPF in US populations.

A TaqMan SNP Genotyping assay and a 7900HT Real-time PCR analyzer were used to genotype rs35705950 in our cohort. A case-control analysis was carried out using 110 affected individuals and 277 healthy controls from the Newfoundland population. Our results showed that there was significant association between rs35705950 genotypes and IPF. The odds ratio for individuals affected with IPF who were heterozygous for the variant allele of this promoter polymorphism was 5.4 (95% confidence interval, 3.3 to 9.6, P < .001). The odds ratio for individuals affected with IPF who were homozygous for the variant allele was 12.2 (95% confidence interval, 3.3 to 44.7, P < .001). Furthermore, some of our cases displayed familial segregation of the variant allele with the phenotype.

This study supports the suggestion that the minor T allele of rs35705950 is a contributor to the pathogenesis of IPF. The *MUC5B* gene encodes for a major gel-forming mucin macromolecule in respiratory secretions and is upregulated in some other lung diseases. Further evidence of association is provided by tissue expression studies done through previous research.

P09.096

Common variants in *HFE* and *TMPRSS6* are strongly associated with iron parameters but not with serum hepcidin, regulator of systemic iron homeostasis

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Introduction: Genome-wide association studies have convincingly shown that single nucleotide polymorphisms (SNPs) in *HFE* and *TMPRSS6* are associated with iron traits. However, the role of hepcidin, central regulatory molecule of systemic iron homeostasis, in these associations is not clear. Here, we investigated the associations between common variants in *HFE* and *TMPRSS6* with hepcidin and iron traits, including interaction and haplotype analysis.

Methods: A total of 103 SNPs in *HFE* and *TMPRSS6* were extracted from genome-wide SNP data of 1832 individuals from the general population (Nijmegen Biomedical Study). Associations with serum iron parameters and hepcidin (sHep) were studied using linear regression analyses, adjusted for age, gender and time of blood sampling.

Results: The associations between rs1800562 in *HFE* and rs855791 in *TMPRSS6* with iron and transferrin saturation (TS) were confirmed (p<1E-10) and were independent of sHep. sHep was not statistically significantly associated with any of the SNPs, but sHep/ferritin and sHep/TS ratio were (p between 1E-7 and 1E-3). Our data suggested the presence of an interaction between rs1800562 and rs855791 in relation to sHep and a haplotype effect of rs1799945, rs198853 and rs1800562 in *HFE*, independent of rs1800562.

Conclusion: SNPs in *HFE* and *TMPRSS6* influence iron parameters independent of sHep and affect sHep/iron parameter ratios. This unexpected finding suggests that there might be other, yet unknown, hepcidin-independent mechanisms which play a role in these associations. Larger sample sizes are needed to draw definite conclusions about the additional effect of combinations of *HFE* and *TMPRSS6* SNPs on sHep and iron traits.

P09.097

Association of the FTO variant with obesity in two ethnic groups in Slovakia

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Obesity is a global epidemic, arising from the interaction between environmental factors and genetic variants. Recently, common genetic variants in the FTO gene have been found to be associated with obesity phenotype in multiple ethnic groups. The aim of this study was to test the association of the rs9939609 polymorphism with obesity indices in two ethnic groups in Slovakia. Genotyping was performed in 294 Roma and 560 Slovak ethnic subjects, using the TaqMan assay. The minor allele A frequency at rs9939609 polymorphism in the FTO gene was 0.46 in Roma and 0.44 in Slovak ethnic group. The genotypes distributions in Roma (AA 21.1%; AT 50.7%; TT 28.2%) and Slovak (AA 18.9%; AT 50.4%; TT 30.7%) ethnic groups were compatible with the Hardy-Weinberg equilibrium. We observed that the mean values of obesity indices in Roma subjects with AA genotype were significantly higher than in ethnic Slovaks with the same genotype (BMI=28.7±6.6 kg/m^2

vs. BMI=24.5±4.1 kg/m^2, $P^{BMI}<0.001$; WC=99.8±17.8 cm vs. WC=86.2±13.9 cm, $P^{WC}<0.001$; WHR=0.94±0.1 vs. WHR=0.85±0.1, $P^{WHR}<0.001$). On ANOVA analysis, we found significant differences in mean values of BMI, waist circumference (WC) and waist to hip ratio (WHR) between different genotypes in Roma (P=0.001, P=0.004 and P=0.008, respectively) as well as in ethnic Slovak group (P=0.042, P=0.007 and P=0.017, respectively). In conclusion, our genotype-phenotype analysis showed significant association of genetic variation in the FTO gene with obesity in Roma and Slovak ethnic population.

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P09.098

Analysis of the MC4R in relation to obesity in population of Latvia

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Melanocortin 4 receptor (MC4R) regulates feeding behaviour, therefore, genetic studies are trying to associate genetic variation in MC4R locus with obesity related phenotypes. In this study we preformed evaluation in both non-coding and coding part of MC4R in cohort of extremely obese subjects (n=380) of the population of Latvia. We found no association of common polymorphism rs17782313 in promoter region of MC4R in severely obese individuals and matched normal weight controls with obesity or related phenotypes. Additionally, we explored three SNPs in another obesity related fat mass and obesity-associated protein (FTO) gene locus for association with any available phenotype, two of the studied polymorphisms being in complete linkage disequilibrium, were associated with obesity in our study group, but the third demonstrated p-value close to the border of significance. The sequencing of the MC4R coding region revealed four heterozygous nonsynonymous substitutions - V103I, S127L, V166I and I251L. Two subjects had double V103I and S127L mutations. Noticeably, V166I is a novel substitution that has not been reported before. S127L, V166I and double V103I/S127L mutant receptors had significantly decreased quantity in cell surface compared to wt MC4R. Intriguingly, despite the low abundance in plasma membrane newly discovered V166I variant demonstrated higher cAMP response upon αMSH activation than wt receptor. The results of this study incorporate an influence of the common polymorphisms and rare genetic variants in relation to obesity and suggest that in the study group of morbidly obese cases both SNPs and rare mutations could affect the outcome phenotype of the individual.

P09.099

Whole transcript expression microarray profiling in oesophageal tissue of neonates with oesophageal atresia - preliminary results.

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Etiology of oesophageal atresia and tracheoesophageal fistula (OA/TOF) is not clearly understood and no single, specific genetic factor for its etiology was confirmed. OA/TOF may occur as isolated (IOA) or syndromic anomaly (SOA). Combination of multigenic factors and epigenetic modification of genes can play a role in its pathogenesis.

Methods: Total RNA was extracted from 26 esophageal tissue collected during thoracoscopic esophageal atresia repair in neonates with isolated and syndromic form. Control tissues were taken during autopsy from aborted fetuses and stillborn neonates without OA/TOF. The study was accepted by the University Ethical Committee. We used Agilent One-Color Microarray standard protocol to determined gene expression profiling in IOA and SOA vs control. Multiplicative detrending background subtraction method (Agilent software) was performed to quantify the signal. Quality analysis and scale normalization for results were performed. Analysis of differential expression (DE) was done with the R limma package (multiple testing correction and 0.05 threshold for the adjusted p-value). We also performed pathway analysis using the globaltest method (Hochberg multiple testing p-value adjustment).

Results: We identified about 2300 down- and 2700 up-regulated probes between SOA and controls, and about 2600 down- and 2100 up-regulated probes between IOA and controls. None of the probes showed DE between IOA and SOA. We also identified (0.02 threshold) 74 pathways DE between the SOA and controls and 42 IOA vs controls. No DE pathways were found between the IOA and SOA.

We hope that these observations could suggest an excellent candidates genes and pathways for OA/TOF etiology.

P09.100

Identification of genes involved in the initiation of osteoarthritis

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Objective: The aim of this study is to select small areas from osteoarthritic cartilages and subchondral bone of different severities to represent different stages of disease to provide a more complete picture of the molecular alternations in OA pathogenesis as well as to identify genes involved in the initiation of OA.

Methods: Joint tissues were collected from the knee tibia plateau from primary OA and non-OA patients undergoing total knee arthroplasty. Severity of destruction was estimated based on histopathology assessment (OARSI grading system). Each tibia plateau was divided into three parts: outer lateral tibia (oLT) regions defined as undamaged stage (OARSI score: OA=5.23±1.95, n=67), inner lateral tibia (iLT) regions defined as intermediate stage (OARSI score: OA=5.23±1.95, n=71), and medial tibia (MT) regions defined as damage stage (OARSI score: OA=16.8±2.56, n=52). Expression profiling analysis was performed using Agilent microarray on these regions and real-time quantitative PCR using a second cohort of patients were performed for replication.

Results:Our results revealed that 958 transcripts were significantly up or down regulated at least 2-fold between these three stages. These genes were related to the cell matrix interaction, extracellular matrix remodeling, bone development, inflammation, cytokine, cell proliferation, WNT signaling.

Conclusion: This study revealed some novel genes which have not been reported in cartilage to play a role in the pathology of OA. These results identify molecular targets that can be further investigated in the search for therapy or as biomarker for OA.

P09.101

The study of polymorphisms P447L of calcitonin receptor gene (CALCR) and A986S of calcium response receptor gene (CASR) in women with postmenopausal osteoporosis from Volga-Ural region of Russia

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Osteoporosis characterized by reduced bone mass, and disruption of bone architecture, resulting in increased risk of fractures. The aim of this study was to examine any associations of polymorphisms c.1377C>T (P447, rs1801197) in calcitonin receptor gene (CALCR) and c.2956G>T (rs1801725; A986S) in the Ca+ receptor gene (CASR) with fractures and BMD level in Russian postmenopausal women. As the object of the research were 828 DNA samples (366 with fractures and 462 without fractures). According to the literary data *T allele of polymorphism P447L gene CALCR in homozygotic state is associated with fractures. As a result of our research in women with fractures *T allele frequency was lower (0,669) compared with the control group (0,687) the difference doesn't measure up the statistic significancy. With the arrangement according to BMD level in women with osteoporosis *T allele frequency was lower compared with the control group (0.66 and 0,68, respectively). The study of polymorphism A986S of Ca+ receptor gene (CASR) revealed no certain difference arrangement allele frequency and genotypes between women with fractures and women without factures. In our research the tendency of rising frequency *T*T genotype in women with low level of BMD (0,034) was found compared with the control group (0,005) the difference doesn't measure up the statistic significancy. Our results show no associations of polymorphisms A986S Ca+ receptor gene (CASR) and polymorphism P447L in calcitonin receptor gene (CALCR) with the fractures and BMD level in postmenopausal women from the Volga-Ural region of Russia.

P09.102

Associations of polymorphic variants of OPG gene with male osteoporosis in the Volga-Ural region of Russia

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Osteoprotegerin (OPG), a secreted member of the tumor necrosis factor receptor family, is a potent inhibitor of osteoclast activation and differentiation.

We examined the effect of polymorphisms c.1217-15 C>T (rs3102734), c.6890-8 A>T (rs7844539), c.950 T>C (rs2073617), 163 A>G (rs3102735), 245 T>G (rs3134069), 1181 G>C (rs2073618) in the OPG gene with fractures and level of BMD in 131 Russian men with osteoporosis, 149 with osteopenia and matched control (n=152).

The study of polymorphic locus c.1217-15 C>T localized in 1 intron of the OPG gene revealed that frequency *T allele was 0.14 in male patients with osteoporosis, in control group - 0.16, the differences were not significant.

Frequency of allele *C of locus c.6890-8 A>C in men was higher (0.32), compared with the control group (0.11), the differences did not reach statistical significance.

The study of polymorphic loci c.950 T>C (rs2073617) and 163 A>G (rs3102735) located in the promoter of the osteoprotegerin gene, also revealed no associations with fractures and BMD level in men from Russia.

The analysis of polymorphism 1181 G>C (rs2073618) in the first exon revealed highly significantly differences among patients with osteoporosis and controls. It was found heterozygous genotype *GC associated with increased risk of osteoporosis ($\chi2=7,11$; $p<0,007$; OR=1,95(1,19-3,19)), homozygous genotype *CC was protective for the development of osteoporosis ($\chi2=6,65$; $p<0,009$; OR=0,49 (0,28-0,84)). This locus was not associated with fractures.

Thus, we found association of 1181 G>C (rs2073618) locus of OPG gene with decreased BMD level in male from Volga-Ural region of Russia.

P09.103

Morphogenetic gene Meis1 and bone characteristics in 1975 patients

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Bone mineral density, an important parameter in the diagnosis of osteoporosis, is determined by heredity in about 50 to 80 percent, dependent on its measuring site. Many candidate genes are known to be involved in the inheritance of bone characteristics. We investigated a polymorphic morphogenetic gene called Meis1 which belongs to the HOX (homeobox-containing genes) family and is an evolutionarily highly conserved gene. It is involved in the regulation of the development of segmental vertebral structures and a variety of other tissues.

We investigated the effect of two SNPs in the MEIS1 gene in 1975 patients either routinely referred to our outpatient clinic or investigated in nursing homes. Life style factors, fracture incidence, routine laboratory and parameters of bone turnover and bone mineral density at 3 sites (DXA Hologic 4000 plus) or bone heel ultrasound were compared between genotypes.

Genotype frequencies of MEIS1 SNP rs2049019 were 45,5% (wildtype, WT), 44,7% (heterozygotes, HE) and 9,9% (homozygotes, HO) and 31,2% (WT), 50,6% (HE) and 18,3% (HO) of rs6716792. We detected a significant association of SNP rs2049019 with bone mineral density, body size and testosterone levels in young males. Old males homozygous for one of the two SNPs, showed a significantly higher bone ultrasound values at all sites measured compared to WT or HE persons.

The morphogenetic gene Meis1 is known to influence a variety of bone characteristics during development. We show associations with bone parameters in adults, this gene might therefore be of importance for diagnostic and therapeutic aspects in osteoporosis.

P09.104

Identification of novel genetic markers that predict disease severity and complications in Paget's Disease of Bone

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Paget's disease of bone (PDB) is a skeletal disorder affecting 2% of the UK population over 55. Some patients show deafness, bone deformity, fractures and osteoarthritis. Mutations of SQSTM1, found in 10% of patients, are significantly associated with disease severity (Rios-Visconti et al;JBMR, 2010). We analysed seven SNPs (rs10494112, rs4294134, rs2458413, rs1561570, rs10498635, rs5742915, rs3018362) associated with PDB in a recent GWAS in 771 patients without SQSTM1 mutations from the PRISM study (Albagha et al;Nat Gen, 2011). The study population was divided by the number of risk alleles carried (<14, n=254;14-16, n=254;>16, n=259). Total disease severity score was significantly increased in patients carrying >16 risk alleles (6.24±0.10vs5.57±0.16, $p<0.0001$). They had a greater number of affected bones (1.45±0.04vs1.23±0.07, $p<0.009$); and received previously a greater number of treatments for PDB (2.01±0.04vs1.74±0.07 $p<0.001$). Deafness due to skull involvement was also increased (10% vs. 5.8%, $p=0.037$). There was no difference between the groups in quality of life scores. We found that the novel risk alleles interacted with SQSTM1 mutations to affect disease severity. Patients with SQSTM1 mutations carrying >16 risk alleles had a 44% increase in disease severity score, compared with SQSTM1 negative patients carrying <16 alleles (7.82 ± 0.50 vs. 5.40 ±0.11, $p<0.001$). We conclude that the novel risk alleles identified recently not only predispose to the development of PDB but also significantly influence disease severity both alone and in combination with SQSTM1 mutations. They could identify patients at high risk of complications and this, in turn, could target therapy more effectively.

P09.105

Genetic pathways for ADHD show association to hyperactive/impulsive behavior

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Finding genetic risk factors for Attention-Deficit Hyperactivity Disorder (ADHD) has been challenging. As multiple genes with small effect size are assumed to play a role, considering multiple SNPs within the same analysis might increase the explained phenotypic variance, thereby boosting the power of genetic studies. We investigated whether a pathway-based analysis, considering SNPs within the same biological pathway simultaneously, could bring us closer to unraveling the underlying genetic component of ADHD.

Biological pathways involved in dopamine, serotonin and noradrenalin neurotransmission as well as genes involved in neurite outgrowth, were investigated for pathway-based association to ADHD using data from the International Multicentre ADHD Genetics (IMAGE) study. The DSM-IV inattention and hyperactivity/impulsivity scales of the Conners' Parent Rating Scale were used to assess ADHD symptom severity in the 931 probands. From the imputed and pruned genome-wide data 6501 SNPs were selected and used for the association analysis.

Combined analysis of the four pathways showed significant association with the hyperactive/impulsive score ($p=0.0049$), but no association for inattentive or combined scores ($p>0.05$). Post-hoc analyses showed contribution of three pathways ($p<0.05$) to the hyperactive/impulsive score, only the dopamine pathway showed less nominal significance ($p=0.07$).

The current analysis specifically finds association to the hyperactive/impulsive component of ADHD, suggesting similar underlying mechanisms for the studied pathways. Other mechanisms may be involved in the inattentive component of the disorder. These findings show that pathway-based association analyses may overcome power problems in association testing by taking into account allelic heterogeneity.

P09.106

Genetic variants of interferon-g and periodontitis in patients with coronary heart disease

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Background: Periodontitis and coronary heart disease are inflammatory diseases. Both are influenced by genetic predisposition. The c.-874T>A polymorphism of the gene encoding for interferon-γ (IFN-γ) has been associated with altered cytokine production.

Patients and methods: A total of 960 consecutive patients with angiographic proven coronary heart disease (no or mild periodontitis: n=493, severe periodontitis: n=447) were prospectively included in the study entitled "Periodontitis and Its Microbiological Agents as Prognostic Factors in Patients with Coronary Heart Disease" (ClinicalTrials.gov identifier:NCT01045070).

In this subanalysis, the c.-874T>A polymorphism of the gene encoding for IFN-γ was analyzed by CTS-PCR-SSP Tray kit (Heidelberg, Germany). Subgingival bacterial colonization (11 bacteria) was assessed using a polymerase chain reaction (PCR)/DNA probe test (micro-Ident®).
Results: The genotype (p=0.987) and allele frequencies (p=0.860) of the c.-874T>A polymorphism were not risk indicators for the severity of periodontitis in patients with coronary heart disease. However, AA-genotype and A-allele carriers had a decreased risk for subgingival occurrence of *P. intermedia* (genotype: p=0.006, allele: p=0.01) and *E. corrodens* (genotype: p=0.034, allele: p=0.013). These associations remained also significant after forward stepwise binary logistic regression analyses considering age, gender, smoking, diabetes, plaque index as well as other potential confounders.
Conclusions: Despite the c.-874T>A polymorphism of the gene encoding for IFN-γ could be shown to be associated with subgingival colonization of *P. intermedia* and *E. corrodens* there was no evidence that it is a risk indicator for severity of periodontitis in coronary patients.

P09.107

Genetic polymorphism and mRNA levels of *TNFα* and *TGFβ* genes in patients with chronic leg ulcers

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TNFα and TGFβ mediates a number of biological processes, including lipid metabolism, coagulation, endothelial functions and also have an essential function in healing of pathological wounds such as venous leg ulcers. The purpose of this study was to investigate frequencies of single nucleotide polymorphisms in *TNFα* and *TGFβ* genes and evaluate expression of mRNA levels in chronic leg ulcers. The study population consisted of 65 patients with chronic leg ulcers and 95 healthy control subjects. Polymorphisms were investigated by PCR-RFLP method. The level of *TNFα* and *TGFβ* genes expression was performed by Real-Time PCR with GAPDH as internal control. There were differences in frequency of genotypes TNFα G-308A in both groups. Patients showed higher frequency of AA (43%) and lower of GG (15%), GA (42%) genotypes than controls (2%, 22% and 76% respectively). TGFβ 29T>C heterozygotic genotype was similar in both groups (53% in patients, and 46% in controls). TT genotype was lower (19%), however polymorphic CC genotype was more often (28%) represented in patients than in controls (44% and 10%, respectively). TGFβ 74G>C GG genotype reached 80% whereas GC 20%. The polymorphic CC genotype was not detected both in study and control groups. The level of *TNFα* gene expression was higher than in control group whereas in case of TGFβ the level of expression was similar in both groups. Analyzed polymorphism of *TNFα* gene could be a probable risk factor in patients with leg ulcers and should be taken into account during medical treatment.

P09.108

Association of PON1 and APOA1 genes polymorphism with men's life expectancy in Russian population

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Paraoxonase 1 (PON1) is a high-density lipoprotein (HDL)-associated enzyme that prevents oxidation of low-density lipoproteins. Apoprotein A1 (APOA1) is the key protein of HDL. PON1 and APOA1 play important role in the prevention of atherosclerosis. So PON1 and APOA1 are plausible candidate genes for human longevity due to its modulation of cardiovascular risk. In our previous studies we demonstrated that PON1 192Q/R polymorphism is associated with higher risk of premature atherosclerosis and myocardial infarction and APOA1 83C/T polymorphism is associated with reduced risk of atherosclerosis in Russian population. We conjectured that polymorphism of PON1 and APOA1 genes can be associated with longevity in Russian population. In this study we investigated 192Q/R and (-108)C/T PON1 and 83C/T and (-75)G/A APOA1 gene variants in the group of elderly men which we observed during 8 years (75-104 years old, mean age 84.1±5.7 in the moment of the beginning of investigation, N=144). There was no association of (-108)C/T PON1 and 83C/T and (-75)G/A APOA1 gene variants with life expectancy for men. Unexpectedly, the 192R allele was overrepresented in the group of elderly men when compared with population (df=1, p<0.01). Also we analyzed distribution of 192Q/R PON1 genotypes in group of men that lived 85 years or longer (age of death 85-105, mean age of death 91.0±4.1, N=114) and found significant differences when compared with population (df=2, p<0.05). Men heterozygous for 192Q/R PON1 have 2 times higher chances to live till 85 (p<0.01).

P09.109

Allelic imbalance in retinal expressed disease genes: a common phenomenon?

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In retinal dystrophies, reduced penetrance and variability in disease expression with respect to onset, course, and severity is a well-documented feature. This makes reliable genotype/phenotype correlations as well as individual disease prognosis difficult. Although the basis of this variability is largely unknown, it is commonly accepted that secondary genetic factors (modifier-genes) are key factors for this phenomenon.
We hypothesize that *cis*-acting variants governing gene expression levels play a crucial role in phenotypic variation and disease penetrance in hereditary retinal disorders.
The aim of this project is the identification of such *cis*-acting gene variants and the determination of their impact on disease expression.
To demonstrate if and how common allelic imbalance (AI) in retinal expressed genes are, experiments were done in crossbreeds of five different inbred mouse strains as a proof-of-principle experiment.
Up to now more than **25** different retinal genes were screened for heterozygous cSNPs applying PCR and sequencing. We applied Pyrosequencing assays on RT-PCR amplified cDNAs generated from retinal RNA to determine allelic expression differences based on the identified cSNPs.
Using the Pyrosequencing technology, we identified an AI in **7** retinal disease genes. In two of those genes we can see the AI already on genomic level suggesting a copy number variation. Screening of the *Pde6c* gene revealed a 116-bp insertion on cDNA level that results in a premature termination codon leading, due to the nonsense mediated mRNA decay, to a downregulation of the mutant transcript. For the remaining genes the cause of the AI has to be verified.

P09.110

Lack of association between CFH and ARMS in psoriasis and psoriatic arthritis

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Psoriasis (Ps, OMIM 177900) is a chronic hyperproliferative inflammatory disease of the skin, scalp, nails, and joints. About 30% of affected develop psoriatic arthritis (PsA, OMIM 607507). Psoriasis and psoriatic arthritis have a multifactorial etiology, involving environmental (infections, drugs, stress, smoking and climate) and genetic factors. It's well known that inflammation plays a central role in the development of many multifactorial diseases, as in age-related macular degeneration (AMD, OMIM 603075). As psoriasis and psoriatic arthritis share with AMD a multifactorial etiology and the inflammation as central process, we analyzed the some replicated genetic variations associated with AMD in our cohorts of Ps and PsA. A number of 400 Ps cases, 510 PsA cases and 400 healthy controls were tested for rs1061170 (Y402H) in the CFH gene and rs10490924 (A69S) in the ARMS2 gene. Our results suggest that variations in these genes are not associated with the development of Ps and PsA.
The work was supported by ADIPSO, (Italian Association of psoriatic subjects)

P09.111

Genome-wide analysis of copy number variants suggests common and rare copy number variants contributing to psoriatic arthritis

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Not only single nucleotide polymorphisms (SNP) but also copy number variants (CNV) contribute to variability of the human genome and can therefore be involved in genetic disease. In order to identify CNVs for psoriatic arthritis (PsA), we investigated our previously described SNP-based GWAS dataset (*Hüffmeier et al. Nat Genet 2010*) for CNVs using the *Birdsuite* algorithm optimized for the array system used and performed a CNV GWAS. Primarily, we detected significant differences at 33 gene loci at the 5% level, while a first *in silico* analysis revealed "batch effects" at 16 loci - false-positive findings due to erroneous CNV-determination of single batches. Of the remaining 17 loci, *in silico* association analysis showed association at 15 loci (n=9: MAF>5%, n=6: MAF<5%), 11 loci with $p<1x10^{-4}$. Validation of those 15 CNVs with an alternative quantitative method (MLPA) confirmed CN-variability at 11 loci. For four common CNVs, perfect concordance between array system and MLPA was observed in 136-157 individuals. Genes located in or nearby CNVs are reported to be involved in immune regulatory pathways, maintenance of extracellular matrix or signal transduction and are therefore plausible candidates. These four validated common variants fit a dosage-additive model with a p-value of $1.11x10^{-4}$, suggesting the presence of simple additive effects towards disease susceptibility. Ongoing studies aim to replicate associations in independent case-control studies, if replicated to identify the break points and to functionally analyze the CNVs.

P09.112
Too much, but also too little of calreticulin in major psychiatric disorders

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Evidence on population association studies support the hypothesis that the high heritability of major psychiatric disorders is a combination of relatively common alleles of modest effect, and rare alleles some with relatively larger effects. We have recently reported three mutations in the proximal promoter of the human calreticulin (CALR) gene at positions

-48C, -205T and -220A that co-occur with the spectrum of psychosis, including schizophrenia, schizoaffective disorder, and bipolar disorder type I. The frequency of those mutations was estimated at <0.0007, and none of those mutations have been detected in the control population (p<0.005). Mutations -48C and -220A were found to increase the expression of the CALR gene. The third mutation at -205C>T was detected in an isolate case of schizoaffective disorder.

In this paper, the functional implication of mutation -205T was studied in the human neuroblastoma cell lines BE(2)-C and LAN-5, and HEK-293 cell lines. In contrast with other mutations in the promoter region, which increase expression of the gene, the -205T mutation significantly decreased gene expression in those cell lines in comparison with the wild-type -205C-nucleotide (p<0.0005, p<0.001, and p<0.017, respectively). Treatment of the cells lines with the anti-psychotic drug, valproic acid, showed synergistic increase in gene expression in the cell lines with the mutant -205T allele vs. wild type -205C constructs (p<0.001). We propose that a deviation from normalcy in the level of CALR in either direction is associated with major psychiatric disorders.

P09.113
Cannabis and psychosis: a systematic review of genetic studies

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Though the basic pathophysiology of psychosis is largely unknown, the role of synaptic dysfunction and altered neural connectivity that origin early in neurodevelopment is currently recognised. There is reliable evidence that genes contribute to the aetiology of psychosis and recent findings provided consistent clues for an overlap in genetic susceptibility across the traditional psychosis categories.

Genetic variations can influence disease risk through the interaction with environmental factors (gene-environment interaction). Epidemiologic studies suggested that chronic use of cannabis is a risk factor for the development of psychosis. Recent researches have focused on the identification of genetic variants that moderate the effect of cannabis on psychosis risk under the gene-environment interaction paradigm.

We performed a systematic literature search to identify genetic studies that explored the association between cannabis and psychosis. We included genetic studies that reported the direct measures of genetic risk in the association between cannabis and psychosis.

Out of 184 articles identified in the screening phase, 14 articles met the inclusion criteria. We report a structured summary of populations studied, methodology, genetic variations used as predictors, evaluations of cannabis use, outcome measures and main results.

The current state and limitations of genetic researches on interplay cannabis and psychosis is discussed. We address how the application of new genetics technologies and the harmonization of the data between the studies could allow to consistently identify genetic risk variants that in conjunction with exposure to cannabis may explain the occurrence of psychotic symptoms.

P09.114
Matrix metalloproteinase genes on chromosome 11q22 and range of motion assessments in physically active individuals.

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INTRODUCTION: A recent heritability study demonstrated that human range of motion (ROM) has a substantial genetic component. The objective of this study was to investigate if variants within the *MMP10*, *MMP1*, *MMP3* and *MMP12* genes associate with ROM measurements, including sit-and-reach (SR), straight leg raise (SLR) and total shoulder rotation (ShTR), in physically active individuals.

METHODS: Three hundred and thirty-four physically active Caucasians were genotyped, using a Taqman assay, for the *MMP10* C/T rs486055, *MMP1* 1G/2G rs1799750, *MMP3* A/G rs679620 and *MMP12* A/G rs2276109 variants. Genotype effects on SR, SLR and ShTR ROM measurements were determined. Significance was accepted p<0.05.

RESULTS: There were no significant differences between the MMP10 (SR, mm: CC 267±114mm, n=222; CT 273±102, n=70; TT 252±125, n=16; p=0.923), MMP1 (SR, mm: 1G1G 267±110, n=79; 1G2G 265±116, n=170; 2G2G 282±110, n=64; p=0.507), MMP3 (SR, mm: AA 265±114, n=81; AG 277±102, n=160; GG 257±120, n=88; p=0.078) or MMP12 (SR, mm: AA 265±110, n=147; AG 270±110, n=61; GG 331±61, n=7; p=0.321) genotypes and the SR ROM measures. Furthermore there were no significant genotype effects on SLR and ShTR. Interestingly, individuals with the minor *MMP12* rs2276109 GG genotype were much more flexible for all measurements (SR, mm: GG 331±61, n=7; AA+AG 266±110, n=308; p=0.135), however, due to sample size, this finding was not significant.

CONCLUSION:

The *MMP10*, *MMP1*, *MMP3* and *MMP12* genes were not associated with ROM in this study. Due to the observed trends and rarity of the *MMP12* GG genotype, further analysis with a larger sample size is required.

P09.115
Genome-wide association study for refractory celiac disease (RCDII) in Europeans

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Celiac disease (CeD) is a common autoimmune disorder triggered by dietary gluten in genetically susceptible individuals. The only treatment is a lifelong, gluten-free diet (GFD). However, about 5% of CeD patients do not respond to GFD and some develop refractory celiac disease (RCD), which is characterized by persistent symptoms, severe malabsorption, and intestinal damage despite a strict GFD. RCD patients with clonal T-cells, referred to as 'RCDII', have a high mortality and poor prognosis due to the development of lymphomas.

To discover genetic risk factors associated with RCDII, we performed a genome-wide association study (GWAS) in a Dutch cohort (38 patients, 846 controls), validating the top-associated variants in an independent French cohort (33 patients, 787 controls).

This revealed 21 potential susceptibility variants in 15 independent loci (p < 1x10-5); we genotyped the top-15 variants. Using the Fisher Exact test, we found evidence for association with two variants in our replication cohort: SNP4 in the KLF12 gene at chromosome 13 (p = 0.04, OR = 1.703) and SNP7 in the WWOX gene at chromosome 16 (p = 0.007, OR = 2.139).

None of the known celiac disease susceptibility variants showed association with RCDII, suggesting that the RCDII phenotype is due to different genetic factors. To further validate our findings, we are currently performing replications in other European RCDII cohorts.

P09.116

Investigating the association of rs1800801 and rs1800802 single nucleotide polymorphisms on the promoter of MGP gene with Renal Stone

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The Matrix Gla Protein (MGP) is an important inhibitor of calcification. Polymorphisms of MGP have been shown to be associated with stone formation in Kidney. Mainly two single nucleotide polymorphisms (SNPs) G-7A (rs1800801) & T-138C (rs1800802) on MGP gene have been suggested to play a role in susceptibility towards calcification which may affect gene expression due to their location in promoter region of the gene.

This work is investigating the possible association of these two SNPs with the formation of renal stone in Iranian population using a family based (Parents-Affected Child Trios) association study.

So far 100 trios were recruited. After obtaining their written consent, blood samples were taken and DNA was extracted. For genotyping, the target region was amplified by PCR and the products were sequenced. Genotypes of both SNPs were confirmed for 40 trios so far. Analysis of the preliminary data shows over transmission of A allele for rs1800801 and T allele for rs1800802, although the differences statistically were not significant. Transmission Disequilibrium Test (TDT) value was 2.45 (*p-value* $\approx$ *0.1)* for rs1800802, and 0.25 (*p-value* $\approx$ *0.6)* for rs1800801. It is very likely that adding the genotypes of the remaining trios, reveals a significant over transmission of T allele in rs1800802, confirming the association of this allele with the disease.

This work is being continued by genotyping the rest of samples and the full data will be presented at the meeting.

P09.117

Functional study of Peptidylarginine deiminase type 4 as genetic risk factor for RA

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Rheumatoid arthritis (RA) is well-known as an autoimmune disease and is a chronic inflammatory disorder characterized by the destruction of multiple joints. Many genome wide association studies were performed and multiple RA-susceptibility loci and autoimmune-susceptibility loci have been identified. These studies suggested that multiple genes and its functions were related with disease causing and development.

Previouly, peptidylariginine deiminase type 4 **(**PADI4) was identified as a susceptibility gene for RA in a Japanese population by case-control association study. PADI4 is a member of the PADI gene family and converts arginine residue (peptidylarginine) to citrulline residue (peptidylcitrulline). PADI4 is highly expressed in bone marrow, macrophages, neutrophils and monocyte. Peptidylcitrulline is an interesting molecule in RA, because it is an antigen of ACPA and only PADs (translated protein from PADI genes) can provide peptidylcitrullines, via modification of protein substrates. To evaluate the importance of PADI4 gene in the progression of RA, we generated Padi4 -/- C57BL/6 (B6) mice by speed congenic method. We used Padi4-/- mice to show that PAD4 is affected to development and progression of collagen induced arthritis (CIA), well known as an RA model animal. Padi4-/- B6 and WT mice were immunized with Chicken type II collagen (CII) for CIA. Clinical disease score was reduced and the incidence of WT and Padi4-/-mice were 56% and 44%, respectively. Padi4-/- mice also significantly reduced concentrations of serum anti-CII IgM and IgG compared with WT mice. Resulting from these studies, we suggested that Padi4 enhanced collagen-initiated inflammatory responses.

P09.118

Copy number variants in RB1CC1 and OR4C46 are overrepresented in a German case-control sample of schizophrenia

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Schizophrenia is a severe and debilitating neuropsychiatric disorder with an estimated heritability of 60-80%. Recent studies have shown that rare, highly penetrant variants account for a fraction of the overall genetic risk.

Some of these disease-associated rare variants have been identified several times in sporadic cases as independent de novo mutations and are obviously subject to negative selection. These findings support the hypothesis that the recurrent de novo occurrence of risk variants may compensate for risk variants that constantly disappear from the gene pool by negative selection.

It is reasonable to assume that schizophrenia susceptibility genes identified by de novo mutations in patients might carry additional risk variants, such as other rare single nucleotide mutations but also rare copy number variants (CNVs). We tested this hypothesis by starting from 34 potential schizophrenia susceptibility genes that were reported to carry de novo mutations by Xu et al. (2011). Using Illumina BeadArray data of 1,637 patients with schizophrenia and 1,627 population-based controls, we investigated whether CNVs at these loci might contribute to the allelic spectrum in schizophrenia. Each individual's SNP-chip information was analyzed with QuantiSNP and PennCNV.

In two genes, RB1CC1 and OR4C46, we found CNVs overrepresented in patients compared to controls providing further support for an involvement of these genes in the development of schizophrenia. We are currently extending our analyses to large, independent case-control datasets. From a functional point of view, it is noteworthy that RB1CC1 insufficiency causes neuronal atrophy. This may provide a link to pathophysiological considerations in schizophrenia.

P09.119

Unraveling the implication of de novo mutations in the genetics of schizophrenia

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Recently, a wave of studies has shown that de novo mutations play a very important role in the genetic mechanism of psychiatric disorders. Our group has shown that there is an enrichment of pathogenic de novo mutations in schizophrenia. Other groups have made the same observation in schizophrenia, but also in autism and mental retardation. We are now trying to characterize this paradigm using two methods. First, we are looking at healthy twins to evaluate the rates of germ line and somatic de novo mutations using exome capture. This is the first report of an exonic de novo mutation rate that can be used as a direct comparison to the previous studies. We are also doing a follow up of the genes identified in the first study using an enrichment solution in order to evaluate the rare variants burden of each gene in a schizophrenia cohort. We are resequencing approximately a hundred genes that were found to harbour a de novo mutation in previous studies, in a cohort of 250 schizophrenia patient and 250 healthy individuals.

P09.120

Analysis of copy number variants (CNV) and gene expression in monozygotic twins discordant for schizophrenia

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Schizophrenia has a lifetime prevalence of 1% and is a severe psychiatric disorder characterized by positive symptoms (e.g. hallucinations, delusions), negative symptoms (e.g. apathy, poor social functioning) and cognitive deficits (e.g. poor working memory). There is strong evidence that a polygenic component and environmental factors contribute to disease development. A powerful strategy to identify etiologically relevant genetic, epigenetic and gene expression patterns is the investigation of phenotypically discordant monozygotic (MZ) twins. Recent studies have identified differences in copy number variation (CNV) between MZ twins with both concordant and discordant phenotype suggesting that somatic mosaicism may not be uncommon. These findings suggest that CNV analysis in phenotypically discordant MZ twins may provide a powerful tool for identifying disease susceptibility loci.

In the present study, we utilized 20 MZ twins discordant for schizophrenia (n=18) or schizoaffective disorder (n=2) collected in 3 medical research centers located in Germany and The Netherlands. All 20 pairs are currently being genome-wide genotyped on Illumina HumanOmni1S beadchips to analyze CNV discrepancies. In addition, for 7 of these discordant MZ twin pairs peripheral mRNA is available and will be subjected to whole genome expression profiling by use of llumina HumanHT-12 Expression BeadChips to determine potentially differentially expressed regions and to enable integration of the genetic and expression data.

P09.121

Genetic association of RGS2 gene variants with schizophrenia and antipsychotic response

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Schizophrenia is a common and complex psychiatric disorder with a strong genetic component. As regulators of G protein signaling and regulators of G protein signaling-like proteins play a pivotal role in dopamine receptor signaling, genetically based, functional variation could contribute to interindividual variability in therapeutic and adverse effects

The present study is aimed at exploring whether two single nucleotide polymorphisms (SNPs) rs2746073 and rs2746072 within RGS2 gene could be associated with schizophrenia and whether they could predict clinical outcomes in 300 patients of two ethnic groups (158 Russians and 142 Tatars) from the Volga-Ural region of Russia treated with antipsychotics. Baseline and final clinical measures, including PANSS and SAS scales were recorded. No significant association was found with the diagnosis of schizophrenia. However analysis of variance revealed that genotype RGS2*T/*T to be a marker of reduction of Negative symptoms (P=0.04) in the overall sample comprised of Russians and Tatars on the 21 day from the baseline.

Our findings provide no evidence for an association between SNPs within RGS2 gene under investigation and schizophrenia susceptibility. However, taking into account the several limitations of our study, further research is needed to draw more definitive conclusions.

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P09.122

Influence of GRIN2B gene polymorphic loci on antipsychotic treatment response and susceptibility to schizophrenia

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One major problem in the schizophrenia (SZ) treatment is finding the right drug for the right patients.

We aimed to examine the effect of five polymorphic loci (rs1805502, rs7301328, rs1805247, 5806A/C, rs1805482) of GRIN2B gene on treatment response to typical antipsychotics (TA) in schizophrenia patients from Russia.

Study sample comprised of 300 drug naive patients with the first episode of SZ (158 Russians and 142 Tatars) from the Volga-Ural region of Russia. Clinical response and severity of Extrapyramidal symptoms (ES) were determined by administering the PANSS and SAS scales at base line and at days 21 and 45. Differences between groups were tested by using unpaired t-test (two-tailed), analysis of variance (ANOVA), and chi-square test.

Analysis of variance detected genotype GRIN2B *T/*T (rs1805247) to be a marker of reduction of Negative (P=0.03) and General Psychopathology symptoms (P=0.04) in Tatar patients on the 21 assessment day.

ANOVA revealed GRIN2B *T/*T (rs1805502) genotype to be a marker of low risk of ES development (P=0.003) in Russian patients on the 21 assessment day.

Analysis of association educed allele GRIN2B *T (rs1805247) to be a risk marker for schizophrenia in Tatars (OR=2,39). A CTC haplotype encompassing rs180552, rs1805247, rs1805482 of GRIN2B gene was significantly overrepresented among Tatar patients (OR=2,32).

Our results are preliminary and suggest that the SNPs in GRIN2B gene may influence the treatment response to TA and susceptibility to schizophrenia. Further studies are necessary to confirm the reported associations.

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P09.123

Association study of candidate gene polymorphisms with paranoid schizophrenia susceptibility in Russian population

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Schizophrenia is a multifactorial disorder (about 1% in the most populations studied) and is characterized by the contribution of multiple susceptibility genes that could act in conjunction with epigenetic processes and environmental factors. Based on previous molecular genetic studies the following polymorphisms: *HTR2A (rs6311, rs6313, rs6314* and *rs7997012), BDNF (rs6265), SLC6A4 (rs28914832), DISC1 (rs3737597), ZNF804A (rs1344706), RELN (rs7341475), COMT (rs165599), SLC18A1 (rs2270641)* and *PLXNA2* (*rs1327175*) have been chosen and analyzed in 198 patients with schizophrenia and 192 healthy individuals. The genotyping procedure included multiplex PCR with fluorescently labeled nucleotides and allele-specific hybridization of labeled PCR products with a biochip. The statistically significant association between *rs6314* (p = 0.014, OR = 2.26) of *HTR2A* gene and risk of paranoid schizophrenia was found. Also it was shown that genetic variants *rs6311* (p = 0.011, OR = 2.33) and *rs6313* gene *HTR2A* (p = 0.008, OR= 2.40) were associated with suicidal behavior in schizophrenic patients.

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P09.124

Association study of class II cytokine genes with psoriasis in three ethnic groups from the Russia

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Background: The molecular basis of pathogenesis of psoriasis remains unclear, but one unifying hypothesis of disease aetiology is the cytokine network model. The class II cytokines (CF2) and their receptors (CRF2) are all involved in the inflammatory processes and single nucleotide polymorphisms (SNPs) in respective genes have been associated with psoriasis in a previous study of the Estonian population.

Objective: We performed a replication study of 47 SNPs in CF2 and CRF2 genes in independent cohorts of psoriasis patients of three ethnic groups (Russians, Tatars, and Bashkirs) from the Volga-Ural region of Russia.

Methods: DNA was obtained from 499 psoriasis patients of three ethnic groups from the Russia and 581 ethnically matched controls. 47 SNPs in the loci of CF2 and CRF2 genes were selected by SNPbrowser version 3.5. Genotyping was performed using the SNPlex™ (Applied Biosystems) platform.

Results: Comparison of allele frequencies between cases and controls using chi square test revealed differences for seven SNPs rs276466, rs30461, rs3795299, rs1342642, rs10784680, rs4896227, and rs2834117. Only the SNP rs30461 in the IL29 gene displayed a statistically significant association with psoriasis in the cohort of Russians when adjusted for multiple comparisons (corrected P-value (Pc)=0.008, OR=0.44).

Conclusion: Our results suggest that polymorphisms rs30461 of the IL29 gene may contribute to a protection to psoriasis in Russian population. In addition, there might be a probability that other variations in CF2 and CRF2 genes influence susceptibility to psoriasis, but further investigations are needed to provide more conclusive evidence theirs exact contribution.

P09.125

Additional Patients and an Association Study support a Role of SOX9 in CD-ACD-PRS Phenotypic Continuum and in CPO.

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Sox9 has an essential role in chondrogenesis. Nonsense mutations and deletion of Sox9 suggested haploinsufficiency to underly campomelic dysplasia (CD), a rare autosomal dominant disease characterized by campomelia, skeletal defects and Pierre Robin sequence (PRS). Translocations in the 350kb region upstream or downstream of SOX9 were reported in less severe CD patients. Some translocations and deletions further upstream have been identified in patients with acampomelic campomelic dysplasia (ACD) and PRS, suggesting that these three syndromes form a continuum of phenotypes. We report the identification of a translocation 600kb upstream of SOX9 in a patient with classical features of ACD. In parallel, we identified a deletion in a PRS family that overlaps a deletion reported in a patient with the same phenotype. These data and the literature lead us to test whether or not there is a genetic association between the SOX9 locus and cleft palate (CP) and/or PRS. We used three SNPs in a cohort of case-parent trios analyzed using TDT. While two SNPs, tagging a conserved region upstream of SOX9, achieved borderline significance (p=0.045 and p=0.052) in the PRS cohort, significant over-transmission of an intragenic SNP was detected in the combined CP+PRS cohort (p=0,026), with a relative risk of 0.43. The association has been replicated in an independent Italian CP-cohort. Our data are in agreement with the hypothesis that removal or disruption of cis-regulatory elements upstream of SOX9 can lead to phenotypes of gradual severity. We show for the first time an association of SOX9 with CP and PRS.

P09.126

Investigating copy number variants within a cohort of individuals with specific language impairment

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Specific language impairment (SLI) is a developmental language disorder that, in the absence of any comorbid neurological deficits, affects an individual's spoken and/or receptive language despite adequate intelligence and accessibility to learning. SLI is a common childhood disorder with an estimated prevalence in pre-school children of up to 7%. It is a complex genetic disorder that is closely related to autism, dyslexia and ADHD. SLI has a high genetic component with twin studies finding a monozygotic concordance rate of up to 70%. Recent studies of neurodevelopmental disorders have implicated copy number variants (CNVs) in conditions such as autism, intellectual disability and ADHD. Therefore a study of CNVs within families containing individuals with SLI is currently being performed. The SLI consortium has collected a cohort of samples from across the UK that have been phenotypically well characterised for language. 176 of these families containing 186 individuals with SLI have been genotyped using the Illumina HumanOmniExpress beadchip that contains more than 700,000 SNPs. The SNP data is being used to identify CNVs across the genome using the copy number detection algorithms QuantiSNP and PennCNV. Data will be presented, for example, of the relative burden of CNVs in cases compared to their unaffected siblings and of novel variants. CNVs of interest are to be validated using quantitative PCR. To our knowledge this will be the first genome-wide CNV analysis performed within a cohort of samples with SLI.

P09.127

Association study of the functional polymorphisms in candidate genes with ischemic stroke in residents of central Russia

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The frequencies of 34 SNP in candidate genes associated with ischemic stroke (*F12, PON1, PON2, NOS2, PDE4D, HIF1a, GPIba, CYP11B2, REN, AGT, AGTR1, AGTR2, BKR2, ADRB2, ACE, FGB, F2, F5, F7, GPIIIa, PAI-1, MTHFR, APOE, NOS2, NOS3, LTA, ALOX5AP, ADRB3*) have been studied in ischemic stroke (IS) patients and healthy controls, the residents of Central Russia*.* Also polymorphism in *TUB* gene (rs4578424) and rs2881013, rs4459584 in non-coding regions revealed in genome-wide association study in Russian population were analyzed. The genotyping procedure included the amplification of selected gene sequences following by hybridization of fluorescently labeled fragments with SNP-specific DNA probes immobilized on a biochip. An analysis of allele and genotype frequencies for each SNP in IS patients (n=300) and controls (n=300) did not reveal any significant difference. The pair-wise comparison of genes demonstrated that the frequency of genotype combination *PON1* A/- *x PON2* GG was higher in the group of IS patients *(*p=0.044, OR=3.4, 95% CI 1.06-10.4) compared to controls and, thus, was associated with higher risk of stroke. Further the analysis using MDR (Multifactor Dimensionality Reduction) algorithm has been performed. The statistically significant association with stroke for several genotype combinations was revealed, for example for *FGB* G/- *x ACE* I/- *x MTHFR* C/- *x PAI-1* 5G/5G (p=0.018, OR =2.6, 95% CI 1.2-5.6).

P09.128

Association analysis of CRH, CRHR1 and CHRM2 genes in suicidal behavior

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Dysregulation in the stress response of the HPA axis, involving the corticotrophin-releasing hormone (CRH) and its main receptor (CRHR1), is considered to play a major role in suicidal behavior (SB). Cholinergic mechanisms are also implicated in stress.

The aim of the present study was to investigate three genes involved in stress response, the corticotrophin-releasing hormone gene (CRH), the CRH receptor 1 gene (CRHR1) and the muscarinic cholinergic 2 receptor gene (CHRM2), for association with suicidal behavior (SB) in Russian population.

Cases were 288 suicide attempters hospitalized in the Clinical Republic Hospital (Ufa, Russia). Controls were 348 individuals without a personal or familial (first degree) history of neuropsychiatric disorders and SB.

Two SNPs (rs6159, rs1870393) of the CRH gene, five SNPs (rs242941, rs878886, rs242938, rs1876831, rs1876828) of the CRHR1 gene and four SNPs (rs1824024, rs2061174, rs2350786, rs324650) of the CHRM2 gene were genotyped. For pairwise linkage disequilibrium and haplotype analysis, the Haploview 4.1 program was used. Odds ratios (OR) with 95% confident intervals (CI) were calculated.

The only association we observed was an allele association between CRHR1 rs878886 and SB: C was significantly overrepresented in patients with SB as compared to controls (OR=2.65, 95%CI: 1.12-2.02). Haplotype analysis showed a significant overrepresentation of the CHRM2 (rs1824024, rs2061174, rs2350786, rs324650) C-G-G-T haplotype (OR=12.48, 95%CI: 1.24-2.01) in suicide attempters as compared to controls. These results may help understand better the pathophysiology of suicidal behavior, its prevention and treatment.

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P09.129

Copy number variations (CNVs) in the androgen receptor gene (AR) in British patients with systemic sclerosis - a pilot study

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Systemic sclerosis (SSc) is an autoimmune disorder characterized by excessive fibrosis and vascular abnormalities in various organs. It is the end result of a complex interaction of genetic factors and unknown environmental influences. Three subgroups can be distinguished, morphea, limited and diffuse SSc. SSc is 3 (diffuse SSc) to 9 (limited SSc) times more frequent in females than in males which has been discussed to be due to elevated ratios of skewed X-chromosome inactivation (XCI) in female patients.

Previously, we investigated the XCI patterns in a cohort of 205 patients with limited SSc and 97 patients with diffuse SSc using the human androgen receptor (AR) gene (HUMARA) assay. This analysis identified 34 patients (11%) that were homozygous for the CAG polymorphism in the AR gene, thereof 17 patients belonging to the limited SSc subgroup (17 of 205, 8.3%) and 17 patients belonging to the diffuse SSc subgroup (17 of 97, 17.5%). To verify if homozygosity of the CAG polymorphism in these patients is due to a deletion of the AR gene, we determined the gene dosage at the AR locus in these patients as well as heterozygous female and hemizygous male controls using genomic qPCR. Two of the 34 patients belonging to the limited SSc group displayed copy number changes ; one had a deletion and one a duplication at the AR gene. These data let us speculate that changes at the AR gene itself might contribute to the female predisposition for SSc indicated by elevated ratios of skewed XCI.

P09.130

Association of leukocyte telomere length and plasma antioxidants :Results of the Austrian Stroke Prevention Study(ASPS)

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Background: Telomere length plays an important role in the maintenance of DNA and chromosomal stability. Telomeres undergo progressive shortening in replicating cells that lack active telomerase. Ageing, oxidative stress and inflammation are related to shorter telomere length. The objective of the present study was to explore the association between plasma levels of antioxidants and leukocyte telomere length (LTL).
Methods: Relative LTL was measured by quantitative Real Time PCR in 907 participants of the Austrian Stroke Prevention Study, a community-based cohort study on brain aging. Levels of plasma antioxidants including ascorbate, cryptoxanthin, canthaxanthin, lycopene, α-carotene, β-carotene, retinol, γ- & α- tocopherol and zeaxanthin were measured by HPLC in 614 subjects. Association between plasma antioxidants and LTL was analyzed using multiple linear regression by adjusting for age and sex (*Model 1*) and additionally for hypertension, diabetes, cardiac disease and BMI (*Model 2*).
Results: There was a significant association between LTL and ascorbate (*Model 1*:β=0.003;95%CI:0.002,0.005;p<0.001), lycopene (β=-0.192;95%CI:-0.338,-0.045;p=0.01), retinol (β=-0.045;95%CI:-0.084,-0.005;p=0.029) and β-carotene (β=-0.052; 95%CI:-0.104,0.000;p=0.050). After adjusting for vascular risk factors (*Model 2*) the significance of the associations and the effect sizes remained unchanged.
Conclusion: This is the first study investigating the association between plasma antioxidants and LTL in a healthy elderly population. Our results suggest a protective role for ascorbate and an opposite role for lycopene, retinol and β-carotene in maintaining telomere length. The effect of these antioxidants on LTL is not mediated by vascular risk factors.

P09.131

A variant in the carboxyl-terminus of connexin 40 alters GAP junctions and increases risk for tetralogy of Fallot

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GJA5 gene (MIM# 121013), localized at 1q21.1, encodes for the cardiac gap junction protein connexin 40. In humans, copy number variants of chromosome 1q21.1 have been associated with variable phenotypes comprising congenital heart disease (CHD) including isolated tetralogy of Fallot (TOF). In mice, the deletion of *Gja5* can cause a variety of complex CHDs, in particular of the cardiac outflow tract, corresponding to TOF in many cases. In the present study, we screened for mutations in the *GJA5* gene 178 unrelated probands with isolated TOF. A heterozygous nucleotide change in exon 2 of the gene leading to a missense variant at the carboxyl-terminus of the protein was found in two unrelated sporadic patients, one with classic anatomy and one with pulmonary atresia. This *GJA5* missense substitution was not observed in 1,568 ethnically-matched control chromosomes. Immunofluorescent staining and confocal microscopy revealed that cells expressing the mutant protein form sparse or no visible gap-junction plaques in the region of cell-cell contact. Moreover, analysis of the transfer of the gap junction permeant tracer lucifer yellow showed that cells expressing the mutant protein have a reduced rate of dye transfer compared to wild-type cells. Finally, use of a zebrafish model has shown that microinjection of the *GJA5*-mutant disrupts overall morphology of the heart tube in the 37% (22/60) of embryos, compared to the 6% (4/66) of the *GJA5* wild-type-injected embryos. These findings implicate *GJA5* gene as a novel susceptibility gene for TOF.

P09.132

Intron5 +104 A/G polymorphism (IVS+104A> G) of TGF-β gene can be considered as a marker for the onset of non-syndromic cleft lip with/without palate in Turkish patients

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Introduction: Non- syndromic cleft lip with or without palate (NS-CL/P) affects about 1/700 births and have diverse causes including environmental factors of either ethnic or socioeconomic origin and genetic factors. The nature of the environmental and genetic factors contributing to this malformation is still unclear. In this study, we aimed to identify the effect of TGF**β** gene IVS+104A> G polymorphism on the onset of NS-CL/P in Turkish patients.
Material- Methods: 68 NS-CL/P patients and 114 healthy controls were enrolled to the study. Study procedure was in accordance with the principles of the Declaration of Helsinki II and all subjects provided written informed consent prior to the enrollment. DNA isolation was carried out from peripheral blood and genotyping was assessed by PCR- RFLP methodology.
Results: The respective frequency of the AA, AG and GG genotypes of the patients were 24%, 29% and 47 %, whereas they were 54%, 36% and 20% for the controls. The frequencies of the patient group showed statistically difference from that of the controls (p<0,05). When AA genotype was used as the reference group, G allele frequency was found to be statistically higher in patients when compared to controls (p<0,05).
Discussion: The risk of NS-CL/P was statistically increased as the number of the G allele of the TGF-β IVS5+104 increased. The results of this study are in agreement with the previous studies. This gene locus suggested to be an informative screening marker for NS-CL/P in Turkish patients.

P09.134

A rare variant on chromosome 11 is associated with total mortality in a European case-cohort study

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Aims
The aim of our study was to find genomic regions which associate with total mortality and to study the molecular mechanisms behind the detected associations.
Methods
6535 individuals from five European countries in the MORGAM prospective study were genotyped with Illumina Cardio-MetaboChip capturing nearly 200,000 common and rare genomic variants. The 2458 individuals who died during the follow-up of 7-18 years were selected as cases.
A Cox proportional hazards model was used to estimate the association of the genotyped variants with total mortality. The genomic region within 1Mb of the top mortality variant was studied further for association to other disease end points and to 22 mortality and cardiovascular risk factors in MORGAM and in two subcohorts of the FINRISK 2007 Study (n=3950+694).
Results
A rare SNP (MAF=0.0022 in MORGAM) on chromosome 11 was associated with total mortality (p=2.93*10-8, HR = 4.6, 95% CI 2.7-7.9). The SNP showed association to mortality also in independently analyzed MORGAM subsamples: men, women, two Finnish cohorts and the combined non-Finnish cohorts. Furthermore, the SNP was associated with CVD, CHD and cancer mortality (p<10-4) and with CVD and CHD events (p<0.05). None of the SNPs studied for association with risk factors survived correction for multiple testing.
Conclusions
The genome-wide significant association of a rare variant on chromosome 11 with mortality is highly interesting as it does not seem to mediate through common risk factors. Replication of the association in multiple subcohorts and disease end points gives strength to the original finding.

P09.135

Screening of first degree relatives of type 1 diabetes patients by using HLA genotyping in combination with regular autoantibodies testing

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Introduction: The most important genes which are related to type 1 diabetes (T1D) are supposed to be genes for HLA class II molecules. First degree relatives (FDR) of T1D patients have an increased risk to develop the diseases as well. In the Czech republic we screen FDR (children under 18 years of age) of T1D patients and we provide HLA genotyping and annual screening for T1D associated autoantibodies (Abs).
Methods: In our programme (established in 2001) 724 children were HLA genotyped (by PCR-SSP method), 170/724 children developed at least one autoantibody during their follow-up, in 37/740 tested persons T1D was dia-

gnosed. We check by using RIA method for antiinsulin (IAA), antiGAD65 (gamaglutamyltransferase) and IA2 (antityrosinphosphatase) autoantibodies and we successfully attended the last international Diabetes Autoantibody Standardisation Programme (DASP).

Results: Within carriers of risky DQA1 DQB1 haplotypes (DQA1*03 DQB1*0302 resp. DQA1*05 DQB1*02) autoantibody(ies) positivity was observed more frequently (55,9% resp.45,3%) in comparison with carriers of protective haplotype DQA1*01 DQB1*0602 where only 7,1% of children were Abs+ at least once during their follow-up. The presence of the most risky genotype (DQA1*03/05 DQB1*02/0302) was connected to shorter period from the occurrence of positivity of the first autoantibody to the disease diagnose (p=0,005).

Conclusion: Screening of FDR of T1D patients allows early disease diagnose which has a protective effect to the resting pancreatic beta cell mass and also it is useful for designing of future immunointervention strategies (for the selection of their suitable candidates).

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P09.136

Association of polymorphisms representing obesity-related loci comprising FTO and TMEM18 with type 2 diabetes and age at diagnosis of the disease in the population of Latvia

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GWA studies have revealed a number of common obesity susceptibility variants with the strongest signals observed in the loci comprising FTO and TMEM18. Variants predisposing to obesity have been demonstrated to contribute to the risk of type 2 diabetes (T2D), although this has been mostly explained by BMI mediated effects. Association between polymorphisms in the FTO and T2D has been asserted across different populations, however impact of the variation downstream TMEM18 on T2D risk remains elusive. Our aim was to investigate relationship between variants in or near FTO and TMEM18 genes and T2D in the population of Latvia.

For this study we selected SNP rs7561317 located near TMEM18 and rs9939609 and rs57103849 along with other two in the FTO. SNPs were genotyped in 1080 controls and 983 cases by performing TaqMan SNP genotyping assays.

Three of the SNPs in the FTO and rs7561317 showed positive association with BMI and T2D. Furthermore association between the FTO variants and T2D remained significant after adjustment for BMI. We found no correlation between rs57103849 genotype and BMI or T2D, however rs57103849 and rs7561317 were strongly associated with younger age at diagnosis of T2D in a BMI independent manner.

In the current study we confirm that genetic variation in the loci comprising FTO and TMEM18 contributes to higher risk of obesity and T2D in the population of Latvia. Our data support the evidence that obesity susceptibility variants may have an impact on T2D risk through the mechanisms unrelated to those involved in the BMI regulation.

P09.137

Genetic determinants of IL-1-receptor antagonist in the circulation

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Introduction: Interleukins play an essential role in human immunity system acting as mediators of inflammation and tissue damage. The interleukin-1 superfamily consists of two agonists, two receptors, and a receptor antagonist, IL-1Ra. *IL1RN* in chr 2 has been carefully studied, since IL-1Ra concentration in circulation shows a clinical significance and association for example with AMI. Systemic elevation in IL-1Ra concentrations has been previously shown to enable the better control of type 2 diabetes. Recombinant form of IL-1Ra is also commonly used drug in a treatment of rheumatoid arthritis and gout. We wanted to perform GWAS for IL-1Ra concentrations in circulation in independent Finnish population- and case-control-cohorts. We also wanted to test the association of resulting top SNPs with leukocyte transcriptome data.

Methods: We performed GWAS in a sample consisting in total of 7169 adults.

Results: As a result, 17 SNPs were associated with IL-1Ra at p < 10-^{12}, while adjusting with age, sex, BMI and WHR. Furthermore, 29 SNPs were associated with IL-1Ra at p < 10$^{-\ 8\text{-}11}$. While testing the association of the top SNPs with leukocyte transcriptome data, one polymorphism in *IL1F10* was shown to act as cis-eQTL to *PAX8* and trans- eQTL to *ALDH2* in chr 12 and *LILRB4* in chr 19.

Conclusion: IL-1Ra has shown a clinical significance and association with a number of medical conditions. Identifying genetic variants associated with IL-1Ra level in circulation provides further information about related pathophysiological pathways and enables the evaluation of its potential effects on cardiovascular disease and diabetes.

P09.138

Genetic characteristics of Lithuanian and Latvian patients with ulcerative colitis

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Introduction. Genetic susceptibility is known to play a large part in the predisposition to ulcerative colitis (UC). The past years have witnessed remarkable success in the identification of low-penetrance, high-frequency susceptibility variants in inflammatory bowel disease (IBD). However, a large part of the genetic variance in IBD is still unaccounted for. Thus, we aimed to investigate the role of the IBD associated genetic variants in a

Methods. We performed a replication study in 447 Lithuanian and Latvian UC patients and 1,154 controls. In total, 77 SNPs that showed moderate or strong association in five GWAS were studied. Single marker case-control, genotype-phenotype association analysis, and SNP-SNP epistasis analysis were performed.

Results. After correcting for multiple testing, we confirmed associations at 21q21.1 (rs1736135, P = 8.01E-06), 6q21 (rs7746082, P = 6.41E-05), *JAK2* (rs10758669, P = 8.08E-06), *RNF186* (rs3806308, P = 2.40E-06), and *ORMDL3* (rs2872507, P = 1.24E-06). No association with any disease subphenotype was found. SNP-SNP interaction analyses showed significant epistasis between SNPs in the *PTPN22* (rs2476601) and *C13orf31* (rs3764147) genes and increased risk for UC (P = 1.64E-06, OR = 2.44). *In silico* prediction of the interactive network of these genes further validated a possible interaction.

Conclusions. We confirmed the association of five loci (21q21.1, 6q21, *JAK2*, *RNF186*, and *ORMDL3*) with UC in the Lithuanian-Latvian population. SNP-SNP interaction analysis showed that the combination of the SNPs in the T-cell processes involved gene *PTPN22* (rs2476601) and gene participating in *Mycobacterium* infection clearance *C13orf31* (rs3764147) increase the risk for UC.

P09.139

Association study for HLA Class II genes (HLA-DQA1, HLA-DRB1 and HLA-DQB1), CIITA gene polymorphisms (-168A/G and +1614G/C) and multiple sclerosis, in a sample from Rio de Janeiro, Brazil.

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The aim of this study was to evaluate the relationship between HLA Class II HLA-DQA1, HLA-DRB1 and HLA-DQB1 loci, CIITA gene polymorphisms and susceptibility to multiple sclerosis (MS). Peripheral blood samples were taken from 52 MS patients registered with the outpatient clinic of neurology at Clementino Fraga Filho University Hospital (UFRJ, Brazil), as well as from 116 healthy control subjects, who were matched for ancestry, sex and age with the 52 MS patients. The patients were classified according to the criteria laid out by McDonald et al. (2001). After DNA extraction, the alleles of HLA-DQA1, HLA-DRB1 and HLA-DQB1 loci were identified by sequencing and PCR-SSP. Sequencing of polymorphisms -168A/G and +1614 G/C at gene CIIPA was carried out by PCR, followed by capillary electrophoresis (ABI PRISM® 3500 Genetic Analyzer Applied Biosystems). Our results have indicated that the relative risk (RR) associated with the allele HLA-DRB1*15:01 was 3.11. For allele HLA-DQB1*06:02, RR was equal to 2.54. For the haplotype HLA-DRB1*15:01-DQB1*06:02, the RR was 3.35. The polymorphism -168A/G (rs3087456) at CTIIA gene was not associated with susceptibility to MS, but the polymorphism +1614G/C (Rs4774), together with HLA-DRB1*15:01+, increased the RR to 4.46. These findings reinforced the multifactorial and polygenic trait of the disease, indicating that when both the polymorphism +1614G/C of the CIITA gene and the HLA-DRB1*15:01+ allele were present, susceptibility to MS was increased. In addition, our results suggested that HLA-DRB1*08:01, HLA-DRB1*11:01, HLA-DRB1*11:02 and HLA-DRB1*13:03 contributed to resistance against MS, with RR of 0.5, 0.7, 0.27 and 0.27, respectively.

P09.140

Polymorphism in IL12RB1 Contributes to the Risk for Uterine Leiomyomas

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Uterine leiomyoma (ULM), a common female pelvic benign tumour, occurs in ~40% of women. The mechanism of ULM development is believed to be the result of complex interactions between genes and environment. The aim of this study was to investigate the role of single nucleotide polymorphisms (SNPs) in genes IL12B (rs6887695), IL12RB1 (rs11575934) and IL23R (rs7517847) as the potential risk factors for ULM. The association study was performed in 169 women with clinically and surgically diagnosed ULM and 92 women with verified absence of myomas used as the control group. Women with three or more (multiple) ULM had higher prevalence of positive family history (40,0% vs. 19,5%; p=0,029), higher number of miscarriages (60%; p=0,005), higher percentage of smoking (57,4% vs. 21,1%; p=0,001), higher prevalence of GG genotype with G allele frequency in *IL12RB1* rs11575934 polymorphism (p=0,036; OR=0,13 and p=0,029; OR=0,46 respectively), lower age at menarche (0,8 years; p=0,015), lower age at first sexual intercourse (17,7 vs. 19 years; p=0,003), lower number of pregnancies (48%; p=2x10^{-6}) and lower parity (46%; p=1x10^{-6}) compared to healthy controls. Women with solitary ULM had lower parity (27%; p=0,006) and higher prevalence of AG and GG genotypes in *IL12RB1* rs11575934 polymorphism (p=0,037; OR=2,54) compared to healthy control subjects. Our results clearly show an association of GG genotype and G allele frequency in rs11575934 with prevalence of multiple ULM and AG + GG genotypes with solitary ULM. The rs11575934 polymorphism, together with epidemiological factors can contribute to a higher risk for development of ULM.

P09.141

Allele specific hemizygosity in Velocardiofacial syndrome as a risk determinant of schizophrenia

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Velocardiofacial syndrome is caused by a microdeletion in 22q11.2 ranging between 1.5 and 3 Mb, containing about 60 genes, with an incidence of 1 in 4,000 live births. One third of VCFS patients develop schizophrenia during early adulthood. The incidence of schizophrenia in patients with VCFS is thirty times higher than in the general population; thus making the 22q11.2 deletion an important risk factor for schizophrenia.

Based on the high incidence of schizophrenia in these individuals, we hypothesized that some patients with VCFS develop schizophrenia due to allele-specific hemizygosity of one or more critical regions in the 22q11.2 locus.

Paired-end libraries were prepared from 37 VCFS patients, 21 with schizophrenia, schizoaffective disorder or psychotic symptoms, and 16 without. A custom-made SureSelect target enrichment library was used to capture the 3Mb non deleted allele on chromosome 22q11.2 plus 200kb of upstream and downstream genomic sequences. The enriched region was then sequenced on an Illumina HiSeq2000. Bioinformatics analysis revealed that 98% of the targeted region was covered at least 8x and on average 2500 variants were detected per sample.

The complete haplotype of the non deleted 22q11.2 region was created using approximately 3500 unique variant sites (including on average 16 non-synonymous sites per sample). A candidate region that showed allele-specific enrichment in VCFS patients with schizophrenia was identified. Validation with an independent sample is ongoing.

This study suggests that the remaining hemizygous region in microdeletion syndromes could be a further risk determinant for certain variable phenotypes of such syndromes.

P09.142

Association of single nucleotide polymorphism in TGFB1 and acute radiation-induced mucositis in patients with nasopharyngeal carcinoma

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Radiation therapy (RT) is the standard primary treatment of nasopharyngeal carcinoma (NPC), which is particularly common in southern China. Radiation-induced side effects may affect treatment outcomes and lead to postponement of RT. Studies have shown that genetic variations in genes that control cellular processes may act as potential markers to detect radiation hypersensitivity in normal tissues.

Blood samples were collected from local Chinese NPC patients treated with RT to extract genomic DNA. Patients in control (n=34) and case groups (n=34) were matched based on age, sex, staging, treatment regimen, and severity of mucositis. Severity of mucositis was graded by oncologists based on radiation morbidity scoring criteria published by Radiation Therapy Oncology Group (RTOG). Eleven tag single nucleotide polymorphisms (SNP) were selected from *TGFB1* and *XRCC1*.

Four out of six tag SNPs (rs12983047, rs11466345, rs4803455, rs2241716) in *TGFB1* were genotyped. The major allele (A) of rs12983047 at the 3' flanking region of *TGFB1* was found to be associated with increased risk of developing radiation induced mucositis with an odd ratio 2.16 (95% confidence interval 1.06-4.41, p=0.0328, chi-square test). No significant difference was found in all tag SNPs of *XRCC1* (rs25487, rs3213344, rs1001581, rs12611088, rs3213282).

XRCC1 did not show association to radiation-induced mucositis while *TGFB1* maybe a potential marker for exploring individual variation of radiation sensitivity in normal tissues. The remaining tagSNPs (rs1800469, rs1800470) of *TGFB1* will be genotyped for haplotype analysis. Complete analysis of all tag SNPs in *TGFB1* may provide support for our hypothesis.

P10. Evolutionary and population genetics, and Genetic epidemiology

P10.01

Investigation of the effect of polymorphic variants of genes ACE and BDKRB2 on hemodynamic parameters of power and strength athletes

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In the course of the experiment was to study the functional state of central and peripheral hemodynamics (noninvasive tetrapolar impedancemetry) of power and strenght athletes (n = 119, in the following age groups: 13-16 years - 56 people., 17-21 years - 30 people., 21 and over - 23 people). During a survey of athletes at rest (horizontal position) were recorded 16 indicators of central and peripheral hemodynamics and oxygen transport for 500 kardioitervals.

Statistical analysis using the Statistica 6.1 software package includes: Z-transformation (standardization) and T-test for independent variables.

The study has been shown in a group of athletes with genotype ACE * I / I a statistically significant increase in the rate AfPG (amplitude photoplethysmogram) (p = 0,044709), reduction in vivo (stroke volume) (p = 0,031834) and chdREO (respiratory raterheovasography) (p = 0,021320) compared with ACE * I / D. As well as lowering chdREO index (p = 0,046426) and rising OPS (total pepefericheskoe resistance) (p = 0,039986) compared with ACE*D/D.

The study can make a preliminary conclusion that the athletes with genotypes ACE*I/I and BDKRB2*-9/-9 adaptation of the cardiovascular system more efficient at high loads and moderate power. And in the types of endurance, they will have an advantage and a lower risk of pathological conditions, compared to athletes with genotypes ACE*D/D and BDKRB2*+9/+9, and the speed and power modes do not realize their „genetic potential".

P10.02

Differential selection for the 3.7-type and 4.2-type single alpha-globin gene deletions within the same population

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Since the 1950s, the relationship between high carrier rates for α- and β-thalassemia mutations and selective survival advantage in tropical and sub-tropical "malarial belt" regions has been well established. Mechanistically, the -$\alpha^{3.7}$ and -$\alpha^{4.2}$ α-globin single gene deletions arise from non-allelic recombination between the homologous α1- and α2-globin genes during meiosis, with concomitant production of reciprocal $\alpha\alpha\alpha^{anti3.7}$ and $\alpha\alpha\alpha^{anti4.2}$ triplicated alleles, respectively. A characteristic signature of positive selection for the -$\alpha^{3.7}$ deletion has been its significantly higher frequency compared to its reciprocal $\alpha\alpha\alpha^{anti3.7}$ allele frequency within the same population. Much less is known about the relative frequencies of the -$\alpha^{4.2}$ and $\alpha\alpha\alpha^{anti4.2}$ crossover alleles. Using simple, multiplex PCR strategies, we genotyped 1,285 unselected anonymous cord blood DNAs from the 3 major ethnic groups in Singapore for the presence of α-globin deletions and triplications. The frequency of the -$\alpha^{3.7}$ deletion was significantly higher than its reciprocal $\alpha\alpha\alpha^{anti3.7}$ triplication, consistent with the hypothesis of positive selection for -$\alpha^{3.7}$ in malarial endemic regions. In marked contrast, there was no significant difference between the -$\alpha^{4.2}$ and reciprocal $\alpha\alpha\alpha^{anti4.2}$ allele frequencies in the same population groups, suggesting an absence of positive selection for the -$\alpha^{4.2}$ allele. Similar frequencies of the $\alpha\alpha\alpha^{anti4.2}$ and $\alpha\alpha\alpha^{anti3.7}$ triplicated alleles suggest negligible difference in crossover frequencies at the ~1.3kb X-homology and ~1.6kb Z-homology boxes. The factor(s) underlying preferential positive selection for the -$\alpha^{3.7}$ allele but not for the -$\alpha^{4.2}$ allele, within the same population groups, merits further investigation.

P10.03

Ancestry Informative Markers Set in Brazilian Amerindians and Ancestry Estimates in Brazilian Quilombo Remnant Communities

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Individual and population ancestry can be estimated by AIMs (ancestry informative markers with high allele frequency differentials between founder populations). Ancestry estimates of Brazilian population depend on the knowledge of the frequencies of genetic markers in the three founder populations (Portuguese, Amerindian and African). The main objective of this study is to determine the frequencies for 24 AIMs (FY, RB, LPL, AT3, Sb19.3, APO, PV92, CKMM, DRD2, MID93, MID52, MID575, MID154, MID187, TSC, DRD2-TAQI, OCA, WI-161857, WI-11153, GC*1F, GC*1S, WI-17163, WI-14319, WI-7423) in Amerindias for use in estimates of ancestry in three Brazilian urban population samples (Teresina, Jequié and São Paulo) and in four (Mimbo, Sítio Velho, Gaucinha and São Gonçalo) *quilombo* remnants (african-derived communities founded by fugitive slaves 150-200 years ago). The 24-AIMs were sufficient for an adequate discrimination among the considered ancestral populations. All ancestry estimates were trihybrid. African estimates ranged from 45.1% (Sítio Velho) to 68.7% (Gaucinha). *Quilombo* remnants of Mimbo, Sítio Velho and Gaucinha showed a higher Amerindian contribution in comparison with São Gonçalo (15.5%, 33.8%, 14.3% and 3.5%, respectively). A higher European contribution was observed in urban populations from different Brazilian regions (Northeast or Southeast). This finding was expected and had been previously reported by mtDNA, Y and autosomal AIMs. Population differentiation (F_{ST}) showed significant differences between all quilombo remnants. Our findings could reveal the different founding histories of the quilombo remnants studied, as well as generate reliable ancestry estimates of urban populations from different Brazilian regions.

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P10.04

Analysis of C9orf72 repeat expansion in an Italian cohort with amyotrophic lateral sclerosis

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Amyotrophic Lateral Sclerosis (ALS) is an adult-onset and fatal neurodegenerative disease characterized by the selective loss of upper and lower motor neurons. Although most cases are sporadic (SALS), approximately 10% of patients are classified as familial forms (FALS). ALS is a genetically heterogeneous disorder and 12 genes, including *SOD1, FUS* and *TARDBP,* are associated with the disease, accounting for about 30% of FALS cases. Recently, a hexanucleotide repeat expansion in the non-coding region of *C9orf72* gene has been identified as a major cause of ALS (23-47%) and fronto-temporal dementia (FTD) (12-29%), which can often occur together with ALS in the same individual or familial pedigree.

We assessed the frequency of the *C9orf72* repeat expansion in a large cohort of ALS Italian patients, including 35 FALS and 487 SALS cases. The genetic analysis was performed by a two-step protocol including a standard PCR with primers external to the expanded region and a fluorescent repeat-primed PCR. In our cohort, 20% of FALS (7/35) and 4.1% of SALS (20/487) patients carried a pathogenic repeat expansion (more than 40 repeats). We also observed *C9orf72* repeat expansion in 0.2% (1/382) of controls included in the study.

Survival time was shorter in patients carrying the repeat expansion and a prevalence of bulbar onset was also observed.

Our findings suggest that *C9orf72* hexanucleotide repeat expansion can be considered the most common cause of FALS in Italian population with large impact even on sporadic forms, according to previous studies conducted on cohorts of different origin.

P10.05

Molecular characterization of the ancestral centromere of chromosome 2

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Human chromosome 2 is the product of a head-to-head fusion of two acrocentric ancestral chromosomes, IIp and IIq, which remained separated in chimpanzee and gorilla. The dicentric chromosome originated from the fusion reached stability by inactivating one centromere corresponding to the IIq, through the loss of alphoid DNA, via poorly understood mechanisms. Unlike the fusion point, the ancestral centromere mapping at 2q21.1-2q21.2 has been poorly investigated.

Here we performed comparative in silico and molecular analyses in chimpanzee, gorilla, orangutan and macaque genomes in order to shed light on the genomic organization of the 2.1 Mb region encompassing the ancestral centromere. This approach allowed us to track precisely the evolutionary history of the ancient centromere and the corresponding pericentromeric region, whose assembly is still complicated by segmental duplications. In particular our data invalidated the hypothesis of the neocentromere formation occurred in macaque lineage and confirmed the pericentric inversion occurred in the common ancestor of human, chimpanzee and gorilla. In this study we provide the patterns of segmentally duplicated regions among chromosomes for each analyzed species and propose a two-steps model to explain the rearrangements occurred in the region flanking the ancestral centromere, highlighting species-specific deletions and duplications.

P10.06

Using ancestry-informative markers to identify fine structure across 15 populations of European origin.

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The Wellcome Trust Case Control Consortium 3 anorexia nervosa genome-wide association scan includes 2,907 cases from 15 different populations of European origin genotyped on the Illumina 670K chip (UK, Dutch, Swedish, Finnish, German, Austrian, Polish, Northern Italian, Southern Italian, Greek, USA, Canadian, Czech, French, Norwegian). This offers a unique opportunity to study genomic variation within and across these populations, and establish genomic relationships with other publicly available populations of European ancestry. We have examined the allele frequency spectrum of common variants, and compared genomic characteristics across these populations and also with populations from the 1000 Genomes Project. It is usual to identify population structure in such studies using only common variants with minor allele frequency (MAF)>5%; we find that this may result in highly informative SNPs being discarded, and suggest that instead all SNPs with MAF>1% should be used. We have established informative axes of variation identified via principal component analysis and highlight important features of the genetic structure of diverse European-descent populations (Novembre et al. 2008), some studied for the first time at this scale. We identified ancestry-informative markers using a method novel to the human genetics field, which may correct for sample size bias in smaller population sizes (following the bias-corrected entropy estimator proposed by Panzeri and Treves, 2007) and which allows for more efficient use of these SNPs.

Finally, we investigated substructure within these 15 populations and identified SNPs that help capture hidden stratification. This work can inform the design and association results interpretation of trans-ethnic studies.

P10.07
Five novel mutations in the GDAP1 associated with Charcot-Marie-Tooth disease in Iranian families

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Autosomal-recessive forms of Charcot-Marie-Tooth (ARCMT) are more common in the Mediterranean basin and the Middle East because of more widespread consanguinity. CMT disease caused by mutations in the ganglioside-induced differentiation-associated protein 1 (GDAP1) gene is a severe autosomal recessive neuropathy resulting in either demyelinating CMT4A neuropathy or axonal neuropathy with vocal cord paresis. Total genomic DNA was extracted from whole peripheral blood of the patients and their families by using of standard procedures. PCR- sequencing method were used to analysis the whole coding regions of the GDAP1 gene in all samples. Five novel mutations (c.100_101ins T, c.802_803 del TG, c.254C>T (P85L), c.102G>C (S34S) and IVS5+25C>T) was identified in GDAP1 gene. In order to show that these found novel mutations are pathogenetic 50 normal controls were sequenced for all of these genes for whom no such mutations were found. We are going to discuss about clinical and molecular aspects of our patients. We need further investigation to prove these mutations as founder effect in Iranian population.

P10.08
Array painting of Gibbon chromosomes enables access to 44 evolutionary breakpoints compared to the human genome

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Gibbon species (Hylobatidae) have accumulated an unusually high number of numerical and structural chromosomal changes. Recently we were able to describe 71 evolutionary conserved changes/ breakpoints compared to the human genome in the white handed gibbon (Hylobates lar, HLA, Mrasek et al., 2003). Until now there are no molecular breakpoint data available for this species. The aim of this study was a molecular characterization of 44 of these euchromatic breakpoints compared to the human genome. Therefore we combined glass needle based microdissection with high resolution array CGH; i.e. HLA chromosomes of interest were painted by specific labelled DNA-probes and FISH-microdissection of the chromosomes or chromosomal regions encompassing breakpoints was done. The obtained DNA-libraries were DOP-PCR amplified and used in array CGH. Applying this method we were able to narrow down the breakpoints to a mean resolution of 26 kb; they were analysed in detail and as a result we could show that most of the breakpoints are localized in copy number variant and/or segmental duplcation regions. Additionally, we were able to identify novel submicroscopic chromosomal changes that were not described before. Supported in parts by DLR RUS 09/008 and the China Scholarship Council.

P10.09
Sequence variations in non-human primate orthologs suggests role for ZNF41 and ATRX in evolution of higher cognitive functions in man

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A comparatively larger relative brain size and the cognitive abilities associated with it are among the most pronounced features that distinguish man from non-human primates. To investigate the molecular background for these differences, particularly focusing on cognitive aspects, we have sequenced the coding region and splice sites of 16 X-chromosomal genes (ARX, KDM5C, DLG3, GDI1, PAK3, IL1RAPL1, MECP2, TSPAN7, ACSL4, PQBP1, ZNF41, NLGN4, FTSJ1, ATRX, PHF8 and PHF6) in five female non-human primates (Pan troglodytes, Pan paniscus, Gorilla gorilla, Pongo pygmaeus and Pongo abelii). These genes play a role in human cognition, as loss-of-function mutations were identified in patients with non-syndromic intellectual disability.

For PCR amplification of our gene set from human genomic DNA we used previously generated primers for a total of 149 amplicons. We then applied these primers also for investigating the non-human primates and were able to amplify and sequence between 79,2% (Pongo pygmaeus) and 90,6% (Pan troglodytes) of the amplicons from their genomic DNA. Alignment of the sequences to a human reference sequence showed that all di-nucleotides flanking each exon were conserved and we detected no insertions or deletions. Of the total of 85 missense changes we found, 21 were located in ZNF41 and 23 in ATRX. A PolyPhen2 analysis of all 85 missense changes showed that 11 had a potentially damaging effect on the gene product. Interestingly, 10 of the latter were located in ZNF41 or in ATRX. This suggests that ZNF41 and ATRX were relatively important in the development of higher cognitive functions.

P10.10
Distribution of eleven autosomal markers in middle age population from South Romania

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The South part of the Romanian population presents minor cultural and linguistic differences but isn't well studied at the genetic level.

The aim of the present study is to asses the distribution of eleven common di-allelic markers associated with some common human diseases in healthy population living in South Romania. Materials and methods. Healthy Caucasian subjects (n=648, 18-66 years old, M/W: 1/1) living in Bucharest and eight districts from South Romania were selected for this study. PCR or PCR-RFLP protocols were used for genotyping rs1801133, rs3767140, rs2229569, rs1805087, rs5186, rs3842752, rs680, rs2228570, rs4646994, rs1800469 and eNOS ID polymorphisms. PowerMarkerv3.25 and Arlequin3.11 software were used to calculate summary statistics and to compare genotypes distribution between districts.

Results. No deviations from Hardy-Weinberg equilibrium were observed. The polymorphisms were similar represented in booth gender. The observed heterozygosity was found to be in the range of 0,31 (rs2229569) and 0,49 (rs2228570). Average PIC was 0,31 and the overall theta was 0,003. The distribution of genotypes, theta, PIC and gene diversity indicated no significant differentiation of analyzed population.

In age grouped subjects the T allele of rs1801133 increases progressively from subjects of 59-66 years to those with 18-26 years (21,7% vs 41%, p=0,003).

Conclusions. On the bases of eleven di allelic markers we found no significant differentiation of Caucasian population from South Romania.

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P10.11
Carrier frequency of the splice site mutation IVS1+1G>A in GJB2 gene in several indigenous populations of Eastern Siberia

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Extremely high prevalence of splice site mutation IVS1+1G>A in *GJB2* gene, observed in homozygous state in Yakut deaf patients, allowed us to propose that IVS1+1G>A may also be a common pathogenic mutation among other North-East Asians. However, Siberian populations are significantly distinguished by anthropologic and linguistic affiliations, as well as by their population genetic history. We studied the IVS1+1G>A carrier frequency in six indigenous populations of Sakha Republic (Eastern Siberia): Turkic-speaking Yakuts and Dolgans, Tungusic-speaking Evenks and Evens, and Yukaghirs with uncertain (Paleo-Asiatic or Uralic) linguistic affiliation, and also Slavic-speaking Russians inhabiting the Sakha Republic. Among 423 individuals with normal hearing, originated from investigated populations, mutation IVS1+1G>A in heterozygous state was found in 20 subjects: Yakuts (14), Dolgans (2), Evenks (3), and Evens (1), and this mutation was absent in Yukaghirs and Russians. Carrier frequency of IVS1+1G>A is apparently associated with specific linguistic affiliation of studied ethnical groups. Highest carrier frequency of IVS1+1G>A was revealed in Turkic-speaking Yakuts (11.7%) and Dolgans (4.7%). Lower rate of this mutation was found in Tungusic-speaking Evenks (3.8%) and Evens (2.0%), and this mutation was not found at all in Uralic or Paleo-Asiatic-speaking Yukaghirs and Slavic-speaking Russians. Patterns of the IVS1+1G>A mutation prevalence among the studied populations, in general, correspond to the data from mtDNA and Y chromosome lineages studies in Sakha Republic populations that provides an evidence of most reliable distinctions between Yakut's and Yukaghir's gene pools.

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P10.12

A bi-directional Mendelian Randomization analysis in 42,024 individuals of European ancestry identifies a causal relationship between obesity and low vitamin D status

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25-hydroxyvitamin D [25(OH)D] is associated with body mass index (BMI) but the direction of causality is uncertain. We explored the causal direction of the relationship between obesity and vitamin D using genetic markers as instrumental variables in bidirectional Mendelian Randomization analysis. We tested associations of 12 obesity-related SNPs with BMI (for validation) and with 25(OH)D (for causal association) individually and in combination as an allelic score in 42,024 individuals based on a meta-analysis from 21 studies. Also, we examined associations of four vitamin D-related SNPs with 25(OH)D (validation) and with BMI (association) individually and in combination using separate allele scores for SNPs involved in either synthesis or metabolism of 25(OH)D. Each 1 kg/m^2 higher BMI was associated with 1.15% lower 25(OH)D. The BMI and 25(OH)D scores showed strong associations with BMI (P=6.30x10^{-62}) and 25(OH)D (synthesis, P=8.07x10^{-57}; metabolism, P=1.07x10^{-118}), respectively. The BMI score was associated with a lower 25(OH)D (P=0.004), but no association was seen between 25(OH)D scores and BMI (P≥0.08). A 10% increase in genetically instrumented BMI was associated with a 4.2% lower 25(OH)D (P=0.005). No association was seen for genetically instrumented 25(OH)D with BMI, a finding that was confirmed using data from GIANT consortium (n=123,864, P≥0.57 for 25(OH)D scores). Based on a bidirectional genetic approach that limits confounding, our study suggests that a higher BMI leads to lower 25(OH)D, while there was no evidence that lower 25(OH)D contributes to elevated BMI. Hence, population level interventions to reduce BMI are expected to decrease the prevalence of vitamin D deficiency.

P10.13

Evolutionary aspects of centriole associated proteins

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Introduction. We have reported earlier the centriole staining with the anti-titin monoclonal antibody, named MAb Titl 5H1.1 (Mikelsaar et al, 2010). This antibody was developed using the synthetic peptide N-AVNKYGIGEP-LESDSVVAK-C corresponding to an amino acid sequence in the A-band of the titin molecule as immunogen. Now we have further studied the binding of the antigen of MAb Tit1 5H1.1 (titin) with centrioles in association with some other relevant proteins and in connection with the evolution of titin molecule.

Results. We have restricted the epitope of MAb Tit1 5H1.1 by subpeptide mapping to a hexapeptide. According to the data in protein databases this amino acid sequence is located in the COOH-terminus of several different Fn3 domains in the A-band of titin molecule both in human and several other organisms. Our immunohisto- and cytochemical studies with MAb Tit1 5H1.1 in human, mice and zebrafish showed a striated staining pattern in muscle cells and also staining of centrioles, cytoplasm and nuclei in non-muscle cells.

Conclusions. The data prove our previous findings that titin has in addition to the known roles in muscle cells also an important role in non-muscle cells as a centriole associated protein, and this phenomenon is highly conserved in the evolution . Acknowledgements. This work was partly supported by target financing SF 0188096s08 of the Estonian Ministry of Science.

P10.14

Genetic epidemiology of Charcot-Marie-Tooth disease in the Cypriot population

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Charcot-Marie-Tooth disease (CMT) or hereditary motor and sensory neuropathy (HMSN) is one of the most common inherited neuromuscular disorders, affecting approximately 1 in 2,500 people. According to electrophysiological criteria, HMSN is classified into two main subgroups: demyelinating type (HMSN I or CMT1), characterized by decreased motor nerve conduction velocities (MNCV), and axonal type (HMSN II or CMT2) that is characterized by normal or slightly reduced MNCV(s). Further subdivisions within those two types are based on the inheritance pattern and the results of molecular genetic investigations. Inheritance in CMT can be autosomal dominant (AD), X-linked, or autosomal recessive (AR). CMT is associated with more than 30 loci and about 25 causative genes are thus far known. We performed clinical, neurophysiological and molecular genetic studies in thirty six Cypriot families with CMT. The molecular genetic investigation revealed thirteen families with the most frequent mutation the *PMP22* duplication, six families with the S22F point mutation in the *PMP22* gene, four families with *CX32* gene mutations, two families with *MPZ* gene mutations, two families with *MFN2* gene mutations and one family with a *GDAP1* gene mutation. Seven families were excluded from the common CMT genes and are still pending molecular genetic diagnosis and one family is under further investigation for a candidate novel *MFN2* mutation. In conclusion, the PMP22 duplication which is the most frequent CMT mutation worldwide is also the most frequent CMT mutation in the Cypriot population. Five out of the eight other mutations are novel, not reported in other populations.

P10.15

Marriage and population genetic structure in southern Morocco

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The choice of spouse has direct consequences on the distribution, structure and heterogeneity of a population's gene heritage.

The aim of this study is to evaluate geographical endogamy rate among the population of Souss-Massa-Drâa region in southern Morocco in order to estimate the degree of reproductive isolation (or openness) of the population studied.

The study was conducted within a randomly selected sample of Souss-Massa-Drâa population. Various types of endogamous marriage were measured, based on the place of birth, place of residence and geographical origin of the spouses and their parents (600 couples).

The results show a strong tendency towards geographical endogamy of nuptiality (78%). This tendency is important among couples within parental generation. The results of intergenerational comparisons show decrease in endogamy rates from parental generation to current generation. However, this decrease is not statistically significant (p>0,05).

The homogamy index method confirms these results and indicates the importance of this marital behavior among the younger generation.

P10.16

Evolutionary dynamism of the primate *LRRC37* gene family

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Core duplicons in the human genome represent ancestral duplication modules shared by the majority of intrachromosomal duplication blocks within a given chromosome. These cores are associated with the emergence of novel gene families in the hominoid lineage but their genomic organization and gene characterization among other primates is largely unknown. Here, we investigate the expression and potential function of the core duplicon on chromosome 17 that led to the expansion of *LRRC37* during primate evolution. A comparison of the *LRRC37* gene family organization in human, orangutan, macaque, marmoset, and lemur genomes shows the presence of both orthologous and species-specific genes in all primate lineages. Expression profiling in mouse, macaque, and human tissues reveals that the ancestral expression of *LRRC37* was restricted exclusively to the testis. In the human lineage, the pattern of *LRRC37* became increasingly ubiquitous, with significantly higher levels of expression in the cerebellum and thymus, and showed a remarkable diversity of alternative splice forms. Transfection studies indicate overexpression of the product can induce filipodia formation in HeLa cells. Subcellular localization of FLAG-tagged recombinant LRRC37 protein indicates that the protein product is secreted after cleavage of a transmembrane precursor.

P10.17
Deep analysis of human single nucleotide polymorphisms in the Cytochrome P450 superfamily

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Cytochrome P450 (CYP450) superfamily comprises enzymes involved in the cytochrome electron transfer chains. Several studies highlighted that single nucleotide polymorphisms (SNPs) in human CYP450 genes affect disease risk and drug efficacy. Furthermore, CYP450 genes showed a significant variability in the human populations, suggesting that the investigation of inter-ethnic differences in CYP450 genes may be useful to understand individual gene specific properties and then to provide personalized and optimal clinic therapies. This study deeply analyzes SNPs in the fifty-seven CYP450 genes, investigating the differences in human populations and evaluating the functional aspect of the variants.

Using the HGDP and HapMap databases, we analyzed the genetic differences of 449 SNPs in the fifty-seven CYP450 genes evaluating the data from 62 human populations. The HapMap Linkage Disequilibrium (LD) data were used to examine the LD in CYP450 genes: 1,033 SNPs were in perfect LD ($r2=1$) with the previously investigated SNPs. Bioinformatic analyses were performed to predict the functional impact of the CYP450 SNPs.

The analyses of SNPs among human populations highlighted that ethnicity is an influencing factor of CYP450 variability. Considering the functional impact of the variants, we provided an analysis of the functional inter-ethnic variation of CYP450 superfamily. Furthermore, we also considered some SNPs with a high functional impact in order to explain inter-ethnic differences in various health aspects.

In conclusion, our study supplies a deep investigation of CYP450 superfamily highlighting how the population demographic history affects human variation of CYP450 genes and how this variation influences human health.

P10.18
Population analysis of deafness in Mexico in the last century: effects of the genetic background

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BACKGROUND: Deafness in Mexico is currently the 2nd leading cause of disability. Internal migration and Mexican revolution caused population and geographical redistribution of this disability. OBJECTIVE: To determine geographical distribution of hearing loss focused to congenital sensorineural hearing impairment. METHODS: Analysis of the Mexican population censuses (1895 to 2010) about deaf people and their geographical distribution. RESULTS: The proportional increasing in general population and people with hearing loss between 1900 and 2010 was 730.87% and 5,450.93% respectively. The Mexican Revolution produced a demographic declination that required nearly three decades to return to the previous level. Nevertheless, deafness showed a few changes with persistence in some areas. DISCUSSION: Genetic composition, founder effects and genetic drifts seem to have decisive influence on the persistence of such problems where the inheritance is recognized. More detailed studies are necessary to clarify these findings.

P10.19
Mitochondrial DNA mutations are not a common cause of non-syndromic hearing loss in Republic of Macedonia

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Mutations in mitochondrial DNA (mtDNA) are found to contribute to sensorineural deafness, including both syndromic and non-syndromic forms. Hot spot regions for deafness mutations are the MTRNR1 gene, encoding the 12S rRNA and the MTTS1 gene, encoding the tRNA for Ser(UCN). Nucleotide changes are observed with a variable frequency among different populations of deaf persons. Among the known mtDNA mutations, the A1555G is the most common genetic cause of deafness, with variable frequency of 0.4 up to 5.4%, described both among nonsyndromic sensoryneural hearing loss (SNHL) patients and aminoglycoside induced SNHL. The aim of this study was to determine the presence and frequency of the most common mtDNA mutations among 130 Macedonian patients with nonsyndromic hearing loss. A SNapShot analysis for screening of the five mitochondrial mutations associated with deafness (A827G, 961delT+Cn, T1095C, C1494T and A1555G) was performed. None of analyzed deafness-associated mutations were identified in the studied patients. Additionally MTRNR1 gene of 10 patients with only one *GJB2* mutation was sequenced in order to detect other variants that could influence the pathologic effect of the *GJB2* mutation. Mutational screening revealed the presence of one potentially pathogenic substitution T961G in one patient and a G709A polymorphism in two patients. An unpublished variant G1303A was found in a patient with only *GJB2* mutation 35delG. In conclusion, our result suggests that mitochondrial DNA mutations do not represent a significant risk factor for sensorineural deafness in Macedonian population.

P10.20
APCS and RBP4: possible modifiers of age-at-onset in familial amyloid polyneuropathy (FAP ATTRV30M)

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Familial amyloid polyneuropathy (FAP ATTRV30M) is an AD inherited disease, due to a point mutation in the TTR gene. Remarkable differences in mean age-at-onset (AO) have been described in different clusters, including within Portuguese population.

Among Portuguese families, FAP shows a wide variation in AO (17-82 yrs) and asymptomatic carriers aged 95 can be found; this variation is also often observed between generations.

A previous study in Portuguese patients (Soares et al., 2005) found a modifier effect in AO for APCS and RBP4, when comparing classic and late-onset patients with controls. However, variation between generations was not taken into account.

Our aim was to investigate if these two candidate-genes have a modifier effect in AO variation from parent to offspring.

We collected a sample of 36 FAP families with at least 2 generations. We selected 5 tagging SNPs and also the 5 SNPs previously described. These SNPs were analysed by SNaPshot and RFLP, respectively. Samples' genotyping is currently underway and results are being analyzed with the Gene-Mapper v.4.0 software.

Preliminary results in 5 FAP families showed that although for RBP4 we found different genotype's frequencies in patients for rs7079946 and rs17484721 from HapMap, no striking differences were found between generations in the families analyzed for the two genes.

In the total sample, we expect to find or exclude the potential role of these candidate-genes as modifiers of FAP ATTRV30M, in order to better understand the mechanisms involved in AO variability between generations.

P10.21
Variability in age-at-onset of familial amyloid polyneuropathy (FAP ATTRV30M): an extended haplotype effect?

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FAP ATTRV30M is an AD systemic amyloidosis, due to a point mutation in the transthyretin (TTR) gene. A wider variability in age-at-onset (AO) has been uncovered, including among Portuguese patients [17-82 yrs]. Early (less than 40) and late-onset(greater than 50) cases are not separate entities, often coexisting in the same family, with offspring showing anticipation - a much earlier AO than their affected parent. The 'protection' seeming to exist in late-onset cases may be lost in just one generation, raising the hypothesis of a closely linked modifier. Therefore, our aim is to identify genetic modifiers closely linked to the TTR locus that may in part explain the observed AO variability.

Haplotype analysis is on-going in 100 families, using intragenic SNPs and flanking STRs for extended haplotypes.

Fifteen tagging SNPs were selected based on a data dump from the HapMap Project and using Haploview v4.1, with a minor allele frequency (MAF) of 0.1% and covering 60 Kb around the TTR locus. SNP genotyping is currently underway by SNaPshot, using a multiplex approach.

Eight microsatellite markers were also selected, encompassing 11.4 Mb. STRs genotyping is being performed by PCR, using fluorescent-labelled primer pairs and genotypes is being determined using GeneMapper v4.0 software.

In a preliminary group of five families analysed so far, no differences were found in the disease extended haplotypes. In the total sample of 100 families, we expect to find some variants or regions that may confer protection to some TTR V30M carriers (late-onset patients or aged asymptomatic carriers).

P10.22

The spectrum of Familial Mediterranean Fever (*MEFV*) Mutations in the North- west of Iran

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This is the first comprehensive profile of *MEFV* mutations in the North- west of Iran. FMF is an auto-inflammatory autosomal recessive disorder characterized by recurrent and self-limited attacks of fever, abdominal pain, synovitis and pleuritis which are caused by altered pyrin due to a mutated MEFV gene. The most severe complication is amyloidosis, which can ultimately lead to renal failure. FMF is predominantly found among the Mediterranean population, as well as Armenians, Turks, Arabs and Jews. The MEFV gene majority of mutations found on exons 2, 3, 5 and 10. To date, 180 mutations and polymorphisms have been reported with varying prevalence according to the population studied. Our aim was to identify the distribution and the frequency of the MEFV gene mutations in FMF patients in the North- west of Iran .

Five hundred unrelated patients with clinical manifestation of FMF were screened for MEFV mutations in exons 2, 3, 5 and 10 using direct sequencing. The most frequent mutation, M694V, represented only 26.25% of the alleles examined, followed by E148Q in 24.75%, V726A in 11.25% and M680I in 10.75%, respectively. Two novel missense mutations, P313H in 11.47% and P313S in 5.05% were found in heterozygous state in exon 3.

In conclusion molecular analysis rather than clinical symptoms to diagnosis of FMF patient is very trustful therefore to know variations in mutation frequency according to regions of Iran lead to early and correct diagnosis of patient and prevention of amyloidosis with commence lifelong prophylactic treatment of affected individuals with colchicine.

P10.23

The use of survival analysis to estimate age-at-onset of familial amyloid polyneuropathy (FAPATTRV30M) highlights gender differences in early-onset (o.<40) patients but fails to detect it in late-onset (o.>50) patients

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FAP (ATTRV30M) is an AD systemic amyloidosis due to a point mutation in the transthyretin (TTR) gene. First described in Portugal by Andrade (1952) as a disease of young adults (o.<40yrs), Portuguese patients have been characterized by early onset (35.1), unlike patients from Sweden (56.7) and Balearic Islands (45.7) who bear the same mutation. However, late-onset patients (o.>50) and aged asymptomatic carriers have been increasingly ascertained.

While in Portuguese series women had later onset than men, the same was not found either in Swedish or Balearic samples, what raises interesting questions concerning gender and age-at-onset distribution(s). So far, age-at-onset in Portuguese series has only been analysed using information on patients. For the first time we use survival analysis methods, including in the sample age-at-last-observation of asymptomatic carriers, as censored data. Survival curves of patients and carriers were compared by gender using log-rank test.Our sample consisted of 2424 patients (1283 men) and 433 (144 men) proven asymptomatic carriers regularly followed up by the same group of neurologists.

Conventional t-test for independent samples showed significant differences in mean age-at-onset between men (33.1) and women (37.5) patients ($p<0.001$), whereas in the asymptomatic group no significant age differences were found (37.9 vs. 37.1).

The use of survival analysis showed overall different gender distributions and also when considering individuals with onset/age<40yrs. However, for late-onset cases (onset/age>49 yrs), no differences were found.

Further studies are necessary to correct for possible confounding factors and better understand if we are in presence of different underlying gender distributions.

P10.24

How contemporary human reproductive behaviours influence the role of fertility-related genes: the example of the P53 gene

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Recently numerous polymorphic fertility genes have been associated with reproductive system diseases causing infertility/subfertility. Investigations carried out in populations at natural fertility suggest that some fertility genes have acquired clinical relevance only in the last decades due to the interaction with contemporary reproductive behaviours (birth control, delayed childbearing, and spacing birth order among others). In recent years, a new physiological role in human fertility regulation has emerged for the tumor suppressor p53 gene (P53), and the P53 Arg72Pro polymorphism has been associated with recurrent implantation failure in humans. In order to detect a possible interaction between fertility gene P53 and reproductive patterns, in present investigation we examined the impact of Arg72Pro polymorphism on fertility in two samples of Italian women collected from populations with different (premodern and modern) reproductive behaviours, not selected for impaired fertility. Among the women at near-natural fertility (n=98), the P53 genotypes were not associated with different reproductive efficiency, whereas among those with modern reproductive behaviours (n=68), the P53 genotypes were associated with different mean numbers of children (Pro/Pro <Pro/Arg <Arg/Arg, $p=0.056$) and a significant negative relationship between the number of children and P53 Pro allele frequencies ($p=0.028$) was observed. These results are consistent with those of clinical studies reporting an association between the P53 Pro allele and recurrent implantation failure. These findings seem to support the hypothesis that some common variants of fertility genes may have become "detrimental" following exposure to modern reproductive patterns and might therefore be associated with reduced reproductive success.

P10.25

Functional SHMT1, MTR and MTRR gene variants of folate metabolism in Roma and average Hungarian population samples

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The serine hydroxymethyltransferase 1 (*SHMT1*), 5-methyltetrahydrofolate-homocysteine methyltransferase (*MTR*) and 5-methyltetrahydrofolate-homocysteine methyltransferase reductase (*MTRR*) genes play essential role in the folate metabolism. The aim of the study was to analyze three single nucleotide polymorphisms (rs1979277 in *SHMT1*, rs1805087 in *MTR* and rs1801394 in *MTRR* gene) in the Roma population and to compare the results to the average Hungarian Caucasian population samples. We genotyped 293 (113 males, 180 females, mean age 41.7 ± 16.2 years) randomly selected, unrelated Roma subjects from different locations. Pooled DNA of a group of 276 carefully selected, clinically healthy subjects (153 males, 123 females, mean age 37.1 ± 12.7 years) were also studied. The genotypes were analyzed using real-time PCR method. The prevalence of the minor allele A of *SHMT1* rs1979277 was 25.9% in the Roma group, and it was 33.5% in the controls ($p<0.05$), the frequency of the homozygous AA genotype was 6.10%, while it was 10.5% in the controls. The prevalence of the G allele of *MTR* rs1805087 was 32.1% in the Roma group and 20.5 % in the controls ($p<0.05$). The frequency of the homozygous GG genotype was 8.20%, while it was 2.50% in the controls ($p<0.05$). The prevalence of the G allele of *MTRR* rs1801394 was 64.2% in the Roma group and 43.5% in the controls ($p<0.05$). The frequency of the homozygous GG genotype was 38.2%, while it was 19.6% in the controls (p<0.05). All three variants between the Roma and the Hungarian Caucasian population significant difference was found.

P10.26
Gene x Environment interactions and their impact on the stress response system as studied in space-flight analogs

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Environmental and social cues activate the stress response system and stimulate a series of adaptation processes along the hypothalamus-pituitary-adrenal axis (HPA). An overload of the HPA can lead to decreased well-being and is associated to functional illnesses. We are studying under well-controlled conditions how gene x environment interactions impinge on the stress response system. For this purpose, we are using space flight analogs (SFA) to investigate the effect of gravitational unloading, isolation and confinement on healthy individuals. The conditions of SFA are known to induce stress, which can lead to cardiovascular, muscular, immunological, and behavioral problems. SFA are important to identify the health effects astronauts can experience during stay in low Earth orbit or during space exploration, and results obtained from this research has also direct relevance for a better understanding of the impact of social isolation, sedentary lifestyle, aging, and osteoporosis.

We present an overview of the use of these unique SFA for studying gene x environment interactions. More specifically, we will focus on our ongoing studies in the Antarctic station Concordia. Using an approach of integrated physiology we are studying the impact of isolation and confinement on health. We apply behavioral assays and questionnaires, physiological analyses (blood pressure, and heart rate variability), stress physiology (salivary cortisol and alpha-amylase), neurohormonal markers of social affiliation and stability (oxytocin and testosterone), and gene expression changes in blood and saliva. Genotypes associated with HPA reactivity (e.g. brain derived neurotrophic factor, corticotropin-releasing hormone) and human social behavior (e.g. oxytocin) are determined in saliva.

P10.27
The prevalence rates of hereditary disorders in European part of Russia

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Based on a genetic epidemiological study, the prevalence rates of autosomal dominant (AD), autosomal recessive (AR) and X-linked hereditary disorders (HDs) in 13 regions of European part of Russia (7 ethnic groups) were established: Russian from seven populations, Adygean, Maris, Chuvashes, Udmurt, Bashkirs, Tatars. The size of the investigated populations was more than 3 million inhabitants (about 3000 HDs of OMIM could be identified by this research). Genetic differentiation between populations of different hierarchical levels by estimated load of HDs was established. On the contrary, the differences between the populations by the load of AD and AR diseases appear statistically significant. First, the load of both AD and AR diseases is always 2 times higher in rural populations as compared with that in urban populations. Secondly, the differences are also seen while comparing the load of the autosomal HDs in various ethnic groups. For example, the load of AD and AR pathology among the Maris, Chuvashs, Bashkirs, Tatars and Udmurts is higher than that among the Russians. The load of AD diseases varied between populations from 1.01 to 15.66 per 1000 persons. The load of AR diseases varied from 0.85 to 6.33. The differences between the populations in their load of X-linked diseases are no significant. Analysing the load of HDs in the examined populations of Russia demonstrated that there exists a clear-cut differentiation between various populations both within each of the 13 regions examined and between them by the index concerned.

P10.28
Genetic risk score of NOS genes variation to understand trends in coronary-event rates across European populations.

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Reported population distributions of traditional risk factors, such as obesity, have been applied to understand variation of the incidence/prevalence of complex diseases. In this work we evaluate the ability of a Genetic Risk Score (GRS) to explain the variation in coronary-event rates across European populations. Methodologically, we generated a GRS from 68 SNPs in the nitric oxide synthase (NOS1, NOS2A and NOS3) gene regions that contributed to the discrimination between affected and non-affected from the Mycardial Infarction Genetics Consortium (MIGen) samples and a Spanish case-control study. We also tested the correlation of mean GRS values in 8 populations across Europe (North Spain, South France, North France, Central Italy, North Bosnia-Herzegovina, East Germany, Poland and Scotland) with variation in coronary-event rates in these 8 European populations reported by the WHO MONICA Project. As a result, 8 SNPs in NOS1, 3 SNPs in NOS2A and 2 SNPs in NOS3 contributed to discriminate affected and non-affected people. The AUCs of discrimination in the different case-control samples ranged from 0.538 to 0.584. Mean GRS values of the 8 European populations samples ranged from 3.8 to 4.2, being North Spain and East Germany the samples with the lowest and the highest score, respectively. Mean GRS values correlated with mean CHD mortality rate, non-fatal rate and total coronary events rate with correlation coefficients from 0.3 to 0.6 without reaching statistical significance. These results point towards the ability of GRS to estimate risks to suffer from complex diseases at a population level.

P10.29
Haplotype diversity and reconstruction of ancestral haplotype associated with the c.35delG mutation in the GJB2 (Cx26) gene among the Volgo-Ural populations of Russia

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The spectrum and prevalence of the *GJB2* gene mutations are specific to populations of different ethnic origins. For several *GJB2* mutations, their origin from appropriate ancestral founder chromosome was shown, approximate estimations of "age" obtained, and presumable regions of their origin outlined. This work presents the results of the carrier frequencies' analysis of the major (for European countries) mutation c.35delG (*GJB2* gene) among 2308 healthy individuals from 18 Eurasian populations of different ethnic origins: Bashkirs, Tatars, Chuvashes, Udmurts, Komi-Permyaks, Mordvins, and Russians (the Volga-Ural region of Russia); Byelorussians, Ukrainians (Eastern Europe); Abkhazians, Avars, Cherkessians, and Ingushes (Caucasus); Kazakhs, Uzbeks, Uighurs (Central Asia); and Yakuts, and Altaians (Siberia). The prevalence of the c.35delG mutation in the studied ethnic groups may act as additional evidence for a prospective role of the founder effect in the origin and distribution of this mutation in various populations worldwide. For the analysis of the haplotypes and the estimation age of the mutation c.35delG in the *GJB2* gene, three high-polymorphic microsatellite -markers were used: D13S175, D13S141, and D13S143, flanking the DFNB1 locus, which contains the *GJB2* gene. The haplotype analysis of chromosomes with the c.35delG mutation in patients with nonsyndromic sensorineural hearing loss (N=112) and in population samples (N =358) permitted the reconstruction of an ancestral haplotype with this mutation, established the common origin of the majority of the studied mutant chromosomes, and provided the estimated time of the c.35delG mutation carriers expansion (11,800 years) on the territory of the Volga-Ural region.

P10.30
Genotype distribution of GLCCI1 rs37972 in Roma and Hungarian Caucasian samples

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Asthma is a specific respiratory disease which is widespread in most ethnics. The rate of the asthma incidence is 3.3%, which means 235 million people suffer from this disease in worldwide (WHO data from 2012). Often the first choice of treatment is glucocorticoid derived, however in numerous cases therapy resistance can be observed. We investigated the rs37972 SNP, allele distribution in Roma samples of the glucocorticoid induced transcript 1 (GLCCI1) gene. Kelan et al. reported that certain naturally occurring functional variants as rs37972 cause a decrement response to glucocorticoids in asthma patients[1]. In our study we investigated the distribution of allele and genotype frequencies of rs37972 in average Roma and the Hungarian population. We genotyped 295 Roma (113 males, 182 females, mean age: 41.6±16.1) and 278 control patients (157 males, 121 females, mean age: 37.9±12.7, with real time PCR method. We found that the frequency of the TT genotype is nearly two times higher in the Hungarian Caucasian population than in the Romas (31.7% vs. 16.6%; p<0.001). Regarding the T allele frequencies a similar association can be found (55.15% vs. 41.86%;

$p<0.002$). The data presented here strongly suggest marked difference between the corticosteroid response on Roma in average Hungarians, that can have therapeutic implications.
[1]*Kelan G. et al.; N Engl J Med. 2011; 365:1173-1183.*

P10.31
The role of ethnicity in prevalence of glutathione S-transferases (GSTs) polymorphisms in a healthy Tunisian population: The example of GSTM1 *0/*0, GSTT1 *0/*0, GSTP1 Ile105Val, and GSTA1 *A/*B

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Genetic polymorphisms in glutathione S-transferases (GSTs) genes might influence the detoxification activities of the enzymes predisposing individuals to cancer risk. Owing to the presence of these genetic variants, inter-individual and ethnic differences in GSTs detoxification capacity have been observed in various populations. Therefore, the present study was performed to determine the prevalence GSTM1*0/*0, GSTT1*0/*0, GSTP1 Ile105Val, and GSTA1*A/*B polymorphisms in 154 healthy individuals from South Tunisia, and to compare them with those observed in North and Centre Tunisian populations and other ethnic groups. GSTM1 and GSTT1 polymorphisms were analyzed by a Multiplex-PCR approach, whereas GSTP1 and GSTA1 polymorphisms were examined by PCR-RFLP. The frequencies of GSTM1*0/*0 and GSTT1*0/*0 genotypes were 53.9% and 27.9%, respectively. The genotype distribution of GSTP1 was 47.4% (Ile/Ile), 40.9% (Ile/Val), and 11.7% (Val/Val). For GSTA1, the genotype distribution was 24.7% (*A/*A), 53.9% (*A/*B), and 21.4% (*B/*B). The combined genotypes distribution of GSTM1, GSTT1, GSTP1 and GSTA1 polymorphisms showed that thirty one of the 36 possible genotypes were present in our population; eight of them have a frequency greater than 5%. To the best of our knowledge, this is the first report of GSTs polymorphisms in South Tunisian population. Our findings demonstrate the impact of ethnicity and reveal a characteristic pattern for Tunisian population. The molecular studies in these enzymes provide basis for further epidemiological investigations in the population where these functional polymorphisms alter therapeutic response and act as susceptibility markers for various clinical conditions.

P10.32
Whole-genome genotyping of saliva-extracted DNA from participants of an Internet based survey: reliability and success rate

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Whole-genome genotyping (WGGT) demands high quality DNA, usually obtained with whole blood-extracted DNA. Self-administered collection protocols can make it feasible to obtain biological material without meeting survey participants. Additionally, noninvasive, painless sources of biological samples, such as saliva samples are indicated to improve participation rate. With the intent to conduct a genome-wide association study with subjects phenotyped within an Internet-based epidemiological survey, we mailed participants self-collecting saliva sample-kits. To test the reliability of the method, we evaluated the concordance rates between a few (N = 10) saliva- versus the respective individual blood-extracted DNA samples with a high-density genotyping array (Illumina Human OmniExpress 700K). Genotypes were consistent across sources of biological material; concordance rates between genotype calls of saliva- versus blood-extracted DNA samples from the same individuals were > 99.9 ± 3%. Regarding genotyping efficiency, average genotype call rates were also similar for saliva- (97.3 ± 5%) and blood-extracted (99.5 ± 0.2%) DNA. Among the returned self-collected saliva-kits, 378 high-quality DNA samples were genotyped with an average call rate of 99.0 ± 3%. However, approximately 14% of the saliva-extracted DNA did not meet quality control criteria (inspection of absorbance scans and gel electrophoresis) - even after using a re-purification protocol - and could not be used for WGGT. Although blood-extracted DNA provides higher quality DNA, saliva-extracted DNA has proven to be a reliable method to obtain biological material for high-density genotyping arrays.

P10.33
Genetic determinants of IgG synthesis in the cerebrospinal fluid of patients with multiple sclerosis

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Multiple Sclerosis (MS) is considered a chronic inflammatory disease of the central nervous system of autoimmune origin involving T and B cells. Intrathecal IgG synthesis is observed in the majority of patients with MS. Whereas the amount of intrathecal IgG synthesis varies largely between patients, intrathecal IgG remains rather constant in the individual patient. Based on this observation it seems reasonable to assume that genetic factors may impact on the extent of intrathecal IgG synthesis.

To investigate the genetic determinants of intrathecal IgG synthesis in MS patients, we performed a genome-wide association study (GWAS) based on 526,014 SNPs of the Human660-Quad chip in 233 MS patients. For replication, we genotyped 18 SNPs, showing an association with intrathecal IgG synthesis ($p<1x10-5$), in an independent validation cohort of 279 MS patients using Sequenom. Five of the 18 SNPs, which could be replicated in the first validation cohort, were additionally analyzed in a second validation cohort containing 152 MS patients genotyped on the Illumina Human660-Quad chip. Patients of all three cohorts are of European descent.

All five SNPs showing a significant association with intractheal IgG synthesis in the discovery and replication cohorts ($p=2.61x10-7$; $p=3.38x10-8$; $p=1.2x10-2$) are clustered in one locus on chromosome 14 and are in linkage disequilibrium.

The results of this study suggest that intrathecal IgG synthesis in MS patients is genetically determined by a region located on chromosome 14. Further investigation of this region will identify the responsible gene and its role in the regulation of IgG levels in MS patients.

P10.34
Genetic history of "Yakut" diseases

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The phenomenon of accumulation of rare genetic diseases in isolated populations with founder effect is well known. French Canadians, Ashkenazi Jews, Finns, Afrikaners are among the examples. In Siberian Russia the Yakut population is characterized by the accumulation of several monogenic disorders with the prevalence in Yakuts more than ten times higher that anywhere in the World. Such diseases as spinocerebellar ataxia 1, myotonic dystrophy, inherited methemoglobinemia, oculopharyngeal muscular dystrophy (OPMD), Yakut short stature syndrome (3M syndrome) and recently described SCOP syndrome belong to the list of "Yakut" diseases.

We have investigated the genetic variability in Yakuts using Y-chromosomal, mtDNA, X-chromosomal markers and genome-wide SNPs, and found reduced genetic diversity associated with the bottleneck effect. This effect, according to phylogeny of specific Yakut Y-chromosomal lineages, is dated back to 11th - 12th centuries.

Haplotype analysis of CUL7 gene in 3M syndrome, NAG gene in SCOP syndrome and PABPN1 gene in OPMD suggested that the accumulation of the disorders in Yakuts was driven by two major events: a bottleneck about 1000 years ago, associated with the initial migration of ancestors of modern Yakuts from south to north; and population expansion approximately 350 years ago when Yakuts extended from the central part into the territory of their modern settlement.

We suggest that research of Mendelian and common diseases in isolated Siberian populations may provide a new source of understanding of disease genetics, as well as improving the quality of health care to indigenous peoples.

P10.35
Criminality in Klinefelter syndrome and 47,XYY, a Danish registry study

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Background: Four to five decades ago several reports showed an increased frequency of criminality among men with Klinefelter syndrome (KS) and 47,XYY, but these studies were hampered by an excessive risk of selection bias. We therefore wanted to study criminality in KS and 47,XYY using linkage of Danish nationwide registries, trying to avoid the selection bias and thereby to give a more realistic picture of the criminality.

Design: Register-based cohort study comparing the incidence of convictions among men diagnosed with KS (N=934) and 47,XYY (N=161) with an age and calendar time matched sample (1:100) from the general population (N=88,979 and 15,356, respectively), in Denmark, from 1978 to 2006. Crime was classified in eight types (sexual abuse, homicide, burglary, violence, traffic, drug-related, arson, and "others").

Results: In KS, The incidence of convictions was significantly increased of sexual abuse, burglary, arson and "others", but significantly reduced of traffic. Adjusting for socio-economic factors reduced the HR's, but convictions of sexual abuse and arson remained significantly increased.

In 47,XYY, the incidence of convictions was significantly increased of all types, except of traffic and drug-related. After adjustment for socio-economic factors, convictions of sexual abuse remained significantly increased.

Conclusion: This large study, covering all diagnosed individuals with KS and 47,XYY in Denmark, demonstrates that KS and 47,XYY are convicted of a number of specific offences more frequently than the background population. The study also demonstrates that unfavorable socio-economic conditions are part of the explanation, since adjustment for socio-economic factors reduced the hazard ratios in both cohorts.

P10.36

Further Development of the Malta Biobank

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The Malta Biobank forms part of the Laboratory of Molecular Genetics at the University of Malta and is a founding member of EuroBioBank (EBB) and Biobanking and Biomolecular Resources Research Infrastructure (BBMRI). It is designated as the BBMRI-Malta node by the Government of Malta.

"Further Development of the Malta Biobank" is a new biobank project which is being setup with the aim of developing a research resource to discover the genetic causes of disease states in the Maltese population thus increasing medical knowledge of diseases locally. Based on Hb quantitative epidemiology it is assumed that 2-3 alleles at 2-3 loci could generate a braod range of quantitative complexity in phenotypes and account for trans-selective pressures on the regional shaping of genomes.

A new "identifiable" collection of biological samples and associated health and lifestyle information from approximately 1% of the population, i.e. 4200 individuals, will be collected. The collection will be representative of the Maltese population in both age and gender and will be based on Maltese family structures. One newborn cord blood sample will be collected together with its family of approximately 30 to 40 members. Informed parental consent will be obtained from ante-natal classes. Immediate family members will be asked to take part in the study and other family members interested to participate in the study will also be sampled with informed consent. Clinical analysis will include: anthropometric measurements; a complete blood count (CBC); a haemoglobin profile; a lipid profile; urinary metabolites and SNPlotyping.

P10.37

Genetic variants associated with lipid metabolism and cognitive ability at 8 years of age: a Mendelian randomization analysis from the Avon Longitudinal Study of Parents and Children (ALSPAC)

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Brain development occurs most rapidly in utero and in young children. Consequently, it places a high demand on the supply of nutrients from the diet, since adequate nutrient concentrations are required for cell growth, synapse formation and myelination. Nutrient deficiency during this time could influence a child's cognitive ability later in life. Because lipids are vital for membrane biogenesis during cellular growth, a relationship between lipids and cognitive ability has been suggested. It is not possible to infer causality from conventional observational studies as associations between nutrition and cognitive ability are confounded by lifestyle and environmental factors. To overcome this problem we used Mendelian randomization, a method that exploits genetic variation associated with a modifiable exposure to examine the causal effects of that exposure on the outcome of interest.

We examined six SNPs, that have been previously associated with plasma lipids, in mothers and children from the Avon Longitudinal Study of Parents and Children (ALSPAC). We detected an association of an APOE-linked SNP in the mothers with offspring IQ (mean difference in IQ, GG vs AG/AA: -5.6; 95%CI -9.8, -1.4). Among children we identified effects of SNPs located in APOA5 (mean difference in IQ per minor allele: -1.1; 95%CI -2.3, -0.01) and LIPC (mean difference in IQ per minor allele: -0.9; 95%CI -1.5, -0.3), where alleles associated with higher total cholesterol were risk factors for a lower IQ. Lipid levels might be important for brain development in children, thus the role of this pathway on cognitive function deserves further investigation.

P10.38

Quick, „Imputation-free“ Meta-Analysis with Proxy-SNPs

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Imputation is the conventional approach for avoiding loss of SNPs in meta-analysis (MA) of genome-wide association studies (GWASes) with differing SNP panels. Here we present an alternative, fast method to avoid forfeiting SNPs present in only a subset of studies, without relying on time-consuming imputation. This is accomplished by using reference linkage disequilibrium (LD) data from 1,000 Genomes/HapMap projects to find proxy-SNPs together with in-phase alleles for SNPs missing in at least one study. MA is conducted by combining association effect estimates of a SNP and those of its proxy-SNPs. Our algorithm is implemented in the MA tool YAMAS (Yet Another Meta Analysis Software).

Association results from GWAS analysis applications can be used as input files for MA, tremendously speeding up MA compared to the conventional imputation approach. Furthermore, analysis of sex-chromosomal markers with YAMAS is readily available, while it can otherwise pose difficulties because of lack of universal implementation in imputing tools.

We show that our proxy algorithm is well-powered and yields valuable ad hoc results, possibly providing an incentive for follow-up. In addition, it is more robust if the data contains cohort-specific LD patterns. We propose our method as a standard approach for studies without available reference data matching the ethnicities of study participants. As a proof of principle, we analyzed six dbGaP Type II Diabetes GWAS and found that the proxy algorithm clearly outperforms naïve MA on the *P*-value level.

P10.39

Analysis of mitochondrial DNA haplotypes of old human populations from the Bronze and Iron Age from Romania

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Our genetic study was focused on old human populations from the Bronze and Iron Ages from Romania in order to analysed their genetic variation and their genetic kinship al mitochondrial DNA(mtDNA)level with today´s Romanian populations and other modern European populations.

The ancient DNA(aDNA)was extracted from skeletal remains of 50 individuals from the Bronze and Iron Age by a phenol-chloroform DNA extraction method.MtDNA HVR I and HVR II regions were amplified by PCR and sequenced by the dideoxy chain terminator method.The aDNA data were analysed in comparison with corresponding mtDNA data of modern Romanian people and other 11 European populations.The ancient mtDNA haplotypes were framed into 12 haplogroups. The most frequent mtDNA haplotype identified in the old individual sample from Romania was the CRS-like, and the most frequent haplogroup was H. Significant differences in haplogroup frequencies between the old people and modern Romanians were found. Low values of internal standard genetic diversity indices suggested reduced genetic variability within old human populations from the Bronze and Iron Age from Romania, in contrast to all modern European populations and also modern Romanians, which showed higher mitochondrial haplogroup diversity values. This fact might be the result of social and cultural local organization in small tribes, partially reproductively isolated. Concerning the genetic relationships at mitochondrial level, old human populations from Romania have shown closer genetic relationship to Turks of Thracian origin,while modern Romanians were closer to modern Bulgarian, Italian, Greek and Spanish populations.

P10.40

Complete mitochondrial DNA diversity in Iranians

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The complete sequencing of mitochondrial DNA has contributed a great deal to the understanding of the timing and direction of human dispersals around the world. To elucidate the early stages of human colonization process outside of Africa and to investigate the demographic history of human populations from the Middle East we have completely sequenced the mtDNAs of 275 Iranians represented by Persians (N=105), Mazandaranians (N=4), Azerbaijanians (N=22), Kurds (N=5), Lurs (N=5), Armenians (N=10), Bakhtiarians (N=2), Gilakis (N=2), Indians (N=1), Turkmens (N=10), and Qashqais (N=109). Overall diversity is very high, with 252 different sequences falling into 75 major haplogroups within macrohaplogroups L, N and M. The majority of Iranian mtDNAs (90.9%) belongs to Western Eurasian component composed of haplogroups N1, N2, X, R2'JT, U, and R0, though the impact of African (L2a, L3d, L3f), Southern Asian (R8, M4, M5, M18, M42), and Eastern Eurasian (A4, B4, C4, C5, D4, F1, G2a) lineages is also perceptible being found at frequencies of 1.5%, 2.5%, and 5.1%, respectively. Results of molecular dating of Iranian mtDNA lineages show that macrohaplogroup N and its haplogroups N1, R, U, R2'JT coalesce to the time of 45-60 kya, marking the first stages of modern humans movement out of Africa. The ancient ancestry of Iranian gene pool is also confirmed by revealing of the unique N23 lineage survived both in Persians and Qashqais, albeit at low frequencies. This study was supported by Russian Foundation for Basic Research (11-04-00620) and by Far-East Branch of the Russian Academy of Sciences (12-III-A-06-101).

P10.41

mtDNA haplogroups in the population of Lithuania

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Two major ethno-linguistic groups exist in Lithuania, Aukštaiciai and Žemaiciai, that consist of six dialectal subgroups. Individuals from both groups speak Baltic languages belonging to the Indo-European family. Neighbouring Finno-Ugric (Northern and Eastern Europe), Slavonic (Eastern Europe) and Germanic (Northern Europe) populations which are situated in the Baltic Sea region may have influenced the historical formation of Lithuanian ethno-linguistic groups, which we investigate here by mtDNA analysis.

We used multiplex sequencing on the *Illumina GAII* platform after in-solution capture enrichment to obtain complete mtDNA genome sequences from 276 samples with an average of 352-fold coverage. Haplogroups were determined with *Haplogrep* and in total 116 different haplogroups were identified. The most frequent haplogroups are H1 (19.6%), U5 (12.7%), J1 and V (5.8% each); less frequent haplogroups are T2, U4, H6, H, W (<5% each). AMOVA based on haplogroups frequency distribution showed no statistically significant differences between the two major ethno-linguistic groups (Aukštaiciai and Žemaiciai). The percentage of variation among groups was -0.34 (p-value=0.897), among populations within groups 0.36 (p-value=0.188), and within populations 99.98 (p-value=0.313) based on 10100 permutations. A multidimensional scaling plot based on Fst calculated from haplogroups frequencies depicted ethno-linguistic groups in the first dimension according to their East to West geographical position within Lithuania (stress=0.06).

This study is a part of LITGEN Project (VP1-3.1-ŠMM-07-K-01-013).

P10.42

New NADPH-cytochrome P450 oxidoreductase genetic variants in Czech and Jewish populations.

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Human NADPH-cytochrome P450 oxidoreductase (POR) is a membrane-bound flavoprotein that catalyzes the transfer of electrons to a wide variety of enzymes. It is a member of a small group of proteins containing two flavins, FAD (flavin adenine dinucleotide) and FMN (flavin mononucleotide). As indicated by its name, the main protein partners of POR are the microsomal cytochrome P450 enzymes (CYPs). By interactions with CYPs, POR participates in xenobiotic and drug metabolism, as well as steroidogenesis.

In order to estimate the differences in POR allele frequencies between two ethnic groups, we have analyzed coding sequences and flanking intronic regions of the *POR* gene in 301 healthy unrelated individuals from Ashkenazi and Moroccan Jewish populations (92 males and 209 females) and in 322 healthy persons from the Czech population (137 males and 185 females).

A total of 11 *POR* missense single nucleotide polymorphisms (SNPs) were identified in the Israeli group. Beyond the known *POR* variants, we have reported also 6 previously undescribed missense SNPs (p.Ser102Pro, p.Val164Met, p.Val191Met, p.Asp344Asn, p.Glu398Ala, p.Asp648Asn). In the Czech population, we have identified 6 missense genetic variations, four of which are newly described (p.Thr29Ser, p.Arg371His, p.Pro384Leu, p.Thr572Met). Globally, a comparison of the two groups revealed a higher heterogeneity of the *POR* genetic variations in the Jewish population.

The data collected in this study on missense POR SNPs are interpreted in light of the recently published crystallographic structure of human POR.

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P10.43

Distribution of PON1 and P2RY12 polymorphisms in Roma and Hungarian population samples

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Clopidogrel is a widely used thrombocyte aggregation inhibitor, which has recently been in the focus of a debate because of its serious side effects. There are many factors that affect its efficacy, like SNPs in genes of specific receptors and enzymes. The pharmacogenomic investigation of these factors might also leave clinical benefit. In our study we investigated variants of paraoxonase 1 (PON1) and purinergic receptor P2Y, G-protein coupled, 12 (P2RY12) genes and their distribution in average Hungarian and Roma samples. For the PON1 gene we chose rs662 (Q192R) and rs854560 (L55M), for the P2RY12 gene we analysed 3 SNPs, rs2046934, rs6798347 and rs6801273 as the most frequently investigated naturally occuring variants. We genotyped 491 Roma and 477 Hungarian samples with PCR-RFLP method. For the Q192R variant the frequency of the GG genotype is more than 2.5 times higher in the Hungarian group compared to the Romas (11.3% vs 4.3%, p<0.001). In the G allele frequencies similar significant difference could be detected (24,8% vs 31.7%, p<0.001). For the L55M variant the frequency of the TT genotype was more than 2.5 times higher in the Roma group than in the Hungarian (10.0% vs 3.8%, p<0.001). For the 3 P2RY12 variants we could find significant differencies only in rs2046934: the frequency of the CC genotype is 7 times higher in Hungarians than in Romas (1.4% vs 0.2%, p<0.05). The data presented here confirm major differencies between the distribution of PON1 and P2RY12 variants in Hungarian and Roma patients that might have clinical implications.

P10.44

Analysis of polymorphism at eight nuclear genome DNA loci in Tatars

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Population genetic survey of the indigenious populations of the Tatarstan Republic (Russian Federarion) belonged to the two Tatar ethnographic groups: Kazan (Arskiy, Atninskiy districts) and Mishari (Buinskiy, Drojjanovskiy districts), was carried out. DNA samples of 450 individuals from four districts were examined at eight polymorphic DNA loci of nuclear genome, diallelic: *CCR5 (del32)*, *ACE (del/ins)*, *D7S23 (KM19)*, *NOS3 (VNTR)*, and polyallelic: *THOI (STR)*, *FABP2 (STR)*, *CFTR (IVS6aGATT)*, *PAH (VNTR)*. Allele and genotype frequency distributions were obtained for individual samples and districts as well as for the ethnic group overall. Analysis of allele's frequency of autosomal DNA markers in Tatar subpopulations shows considerable genetic differentiation between them. The highest level of genetic diversity in diallelic system was established at locus *ACE (del/ins)*, H_{obs}=0,4687, in multiallelic system - at locus *THOI (STR)*, H_{obs}=0,8089. The index of mean heterozygosity is 0,4784. The analysis of dendrograms, based on correlations between the matrix of genetic distances, and multidimentional scaling analysis prompted us to conclude that Arskiy and Atninskiy subpopulations of Tatar are genetically closer to each other than to Buinskiy and Drojjanovskiy subpopulations. Our findings are consistent with evidences on Tatar ethnogenesis and historical facts. Analysis of genetic distances between populations of the Volga-Ural region shows that the population of Tatars joined the cluster of Chuvash, Udmurt and Mari populations before the population of Bashkirs.

P10.45

Population isolates from Greece offer potential for powerful disease gene mapping: the HELIC-Pomak and MANOLIS studies

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The study of low-frequency and rare variants can be empowered by focusing on isolated populations, in which rare variants may have increased in frequency and linkage disequilibrium tends to be extended. Sequencing is efficient in isolates, because variants are shared in extended haplotype contexts, supporting accurate imputation. Here we assess sample sets collected from two Greek populations: the Pomak villages are a set of religiously-isolated mountainous villages in the North (population size 11,000); Anogia is a mountainous village on Crete, with high levels of longevity (population size 4,000). 747 and 1118 individuals respectively were typed on the Illumina OmniExpress platform. We calculated genome-wide IBS statistics to assess the degree of relatedness and compared it with the general Greek population (707 samples with OmniExpress data, TEENAGE study). We additionally calculated the proportion of individuals with at least one "surrogate parent" as a means for accurate long-range haplotype phasing and imputation, as proposed by Kong et al, Nature Genetics 2008. We find 1-1.4% of individual pairs with pi-hat>0.05, and ~0.4% with pi-hat>0.1 in the isolates compared to 0% in the general Greek population. We also find that ~80%-82% of subjects have at least one surrogate parent in the isolates, compared to ~1% in the outbred Greek population. We have established the HELIC-Pomak and MANOLIS cohorts as genetic isolates and are currently whole-genome sequencing 250 individuals to enable imputation and subsequent association testing. This approach has the potential to identify novel robust associations with disease-related complex traits.

P10.46

Population stratification assessment and genomic control in Brazilian Head and Neck Squamous Cell Carcinoma (HNSCC) patients

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HNSCC is the sixth leading cancer by incidence in the world. In Europe, about 87.5 thousand people died from HNSCC in 2008. In Brazil, the mortality rate is estimated in 12 thousand deaths per year. While the genetic basis of susceptibility to HNSCC has not been defined, an increasing number of studies report its association to genetic risk factors. However, the polymorphism frequencies often vary between ethnic groups. Population stratification can cause spurious relations in population-based association studies. For minimizing spurious association, studies recommend adjusting for population stratification. Ethnic categories are usually based on self-reports or complex phenotypic evaluation, but those are pointed as poor predictors of genomic ancestry. This study intends to evaluate the presence of stratification in a population of 28 patients diagnosed with HNSCC and 74 paired healthy controls from Brazil. A twenty Ancestry Informative Markers (AIMs) set based on InDel's markers were selected based on high allele frequency divergence between different ancestral. The samples were genotyped by High Resolution Melting analysis followed by DNA sequencing. Population structure was inferred using STRUCTURE software. The 20-AIMs set was able to distinguish individuals from different parental populations. The population sample of cases exhibited a proportion of 0.349, 0.226 and 0.425, for Ameridian, European and African contribution, respectively. As for the control population, it was found the proportions of 0.041, 0.822 and 0.137, respectively. The ancestral proportions in cases and control population diverged, confirming the importance of population stratification inference in case-control paired association studies. Financial Support: CNPq, INCTC, FundHerp.

P10.47

Very high frequency of hereditary prosopagnosia among individuals with high intellectual ability

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Prosopagnosia or face-blindness is an impairment in human face recognition. Besides a very rare acquired form which can be triggered by e. g. a stroke or a brain injury, there is a much more common congenital form of prosopagnosia, affecting 2.5 % of the human population. The congenital prosopagnosia is almost always heritable. We therefore coined the term hereditary prosopagnosia (OMIM 610382). When establishing the prevalence data, we came across a high number of prosopagnosic subjects with a high IQ. In an unpublished pilot study 11 % of gifted individuals showed signs of congenital prosopagnosia.

Thereupon we tested the prevalence of congenital prosopagnosia among members of a high-IQ society comprising individuals with IQ ≥ 130. Our methodology consisted of an initial questionnaire-based screening process followed by a semi-structured diagnostic interview.

Among 194 highly gifted participants we diagnosed congenital prosopagnosia with varying levels of severity in 29 cases (15 %). This prevalence is six times higher than that observed in the general population. Among participants and those affected by prosopagnosia, both sexes were equally represented.

P10.48

Towards a central registry for rare disease patients in Belgium

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In 2009 the European Commission asked the Member States to develop a strategy to improve the care of rare disease patients by 2013 (2009/C 151/02). In Belgium, recommendations for measures to fill unmet needs of rare disease patients were formulated by the Fund of Rare Diseases and Orphan Drugs at the King Baudouin Foundation. As a result, for 2012-2013, the government provides a budget to work out the implementation of a central rare disease registry collecting a minimum common dataset. Indeed, registration of rare diseases would be highly beneficial to patients, caregivers, authorities, researchers and the general public. Registries can be used e.g. for epidemiological research; incidence, prevalence and survival calculations; quality of patient care, health-care planning and monitoring.

Belgian data on rare disease patients are scarce and fragmented. The primary goal is to bring together already existing data (e.g. by extraction from established registries), to harmonize the different initiatives and to start up registration of core data for additional rare diseases. The Orphanet nomenclature of rare diseases will be used as this will allow mapping to the ICD11 in the future.

The core dataset will serve epidemiological purposes, including some quality indicators. In the future, also data on orphan drug use will be collected and other administrative or dossier functionalities might be implemented. Finally, and importantly, data from the Belgian registry can be shared at European level, to obtain good statistical power.

The methodology behind the central registry and its progress will be documented.

P10.49

Rett Networked Database: an integrated clinical and genetic network of Rett syndrome databases

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Rett syndrome (RTT) is a neurodevelopmental disorder with one principal phenotype and several distinct, atypical variants (Zappella, early seizure onset and congenital variants). Mutations in MECP2 are found in most cases of classic RTT but at least two additional genes, CDKL5 and FOXG1, can underlie some (usually variant) cases. There is only limited correlation between genotype and phenotype. The Rett Networked Database (http://www.rettdatabasenetwork.org/) has been established to share clinical an genetic information. Through an 'adaptor' process of data harmonization, a set of 293 clinical items and 16 genetic items was generated; 62 clinical and 7 genetic items constitute the core dataset; 23 clinical items contain longitudinal information. The database contains information on 1832 patients from eleven countries (February 2012), with or without mutations in known genes. These numbers can expand indefinitely. Data are entered by a clinician in each center who supervises accuracy. This network was constructed to make available pooled international data for the study of RTT natural history and genotype-phenotype correlation and to indicate the proportion of patients with specific clinical features and mutations. We expect that the network will serve for the recruitment of patients into clinical trials and for developing quality measures to drive up standards of medical management.

P10.50

Genetic variation of six X chromosomal STR loci in Bayash Roma samples from Croatia

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X chromosome STRs are another tool for exploring different populations and their genetic characteristics and have been more and more used in recent years to accompany autosomal and uniparental markers data in population genetics. In the present study, six X chromosomal microsatellite loci (DXS983, DXS1225, DXS8092, DXS986, DXS1066 and DXS8082) were used to analyze 79 samples of unrelated male individuals from two Bayash Roma populations living in Croatia. The number of alleles for the studied loci ranged from 5-8 in Međimurje and 4-9 in Baranja, while the overall gene diversity values varied from 0.9673 to 0.9926, respectively. The most informative marker was DXS8092 (0.80628), whereas DXS1066 (0.18103) was the less informative one. Our results show that there is a statistically significant difference between Baranja and Međimurje samples based on haplotype frequencies, which conforms to our previously obtained data from uniparental markers. The coefficient of genetic differentiation (F_{ST}) is 1.6%, which is lower than in mitochondrial DNA (6%) or Y chromosome (2%). All of these results indicate that although Baranja and Međimurje Bayash Roma populations are part of the bigger Vlax Roma group and thus share similar background and origin, they clearly differ among themselves; however, future investigations should be undertaken to determine whether this difference is a reflection of separation prior to arriving in Croatia or a result of the past two centuries of strong genetic isolation on their current living location.

P10.51

Gorilla genome structural variation reveals evolutionary parallelisms with chimpanzee

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Structural variation has played an important role in the evolutionary restructuring of human and great ape genomes. We generated approximately 10-fold genomic sequence coverage from a western lowland gorilla and integrated these data into a physical and cytogenetic framework to develop a comprehensive view of structural variation. We discovered and validated over 7,665 structural changes within the gorilla lineage including sequence resolution of inversions, deletions, duplications and mobile element insertions. A comparison with human and other ape genomes shows that the gorilla genome has been subjected to the highest rate of segmental duplication. We show that both the gorilla and chimpanzee genomes have experienced independent yet parallel patterns of structural mutation that have not occurred in humans, including the formation of subtelomeric heterochromatic caps, the hyperexpansion of segmental duplications, and bursts of retroviral integrations. We present here a comprehensive overview of inversions, deletions, segmental duplications and retrotranspositions within the gorilla genome. Comparisons with humans and other apes reveal that parallel and independent mutational processes have more dramatically restructured chimpanzee and gorilla genomes.

P10.52

Pharmacogenetic variations of the SLCO1B1 gene in Roma and Hungarian population samples

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SLCO1B1 gene encodes for hepatic transporter protein OATP1B1 that is involved in active cellular influx of statins and many other drugs. The A388G and T521C polymorphisms of the SLCO1B1 gene affect the activity of OATP1B1, which result in muscle myopathy, derangements in hepatic function and psychiatric adverse drug reactions. To improve the predictability of inter-ethnic and inter-individual differences we studied the genetic variability of *SLCO1B1* polymorphisms in Roma and Hungarian populations. Genotypes of 470 Roma and 442 Hungarian healthy subjects for the rs2306283 (A388G) and rs4149056 (T521C) polymorphisms were determined by PCR-RFLP assay. Comparing the genotype and allele frequencies of Roma and Hungarian populations differences were found in the *SLCO1B1* 388 AA (24.5 vs. 45.5%), AG (42.1 vs. 36.6%), GG (33.4 vs. 17.9%) genotypes and in G allele frequency (0.545 vs. 0.362) between the studied groups ($p<0.02$). Futhermore, the frequency of *SLCO1B1* 521 TT (67.0 vs. 65.2%) was higher in Roma than in Hungarian samples ($p=0.05$). Similarly to other Caucasian populations, the 388G allele is the minor allele in Hungarians, while in Roma the 388A was found to be the minor allele. The 388G allele frequency of Roma is similar to that found in populations of Indian origin, however, the minor allele frequency of T521C SNP is almost three times higher in Roma than in Indians. Furthermore, the Roma population differs from Hungarians and Caucasians in common *SLCO1B1* polymorphisms. The results of *SLCO1B1* polymorphisms found in the Hungarian population were similar to that observed in other Caucasian populations.

P10.53

Age-related phenomena in telomere length

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Telomere length (TL) is considered a proxy for biological aging. Studies conducted in the 'oldest-old' often find no significant association with longevity, possibly due to reduced variability in TL with age, leaving these studies underpowered. The most important age-related association for TL is with cardiovascular disease (CVD).

We measured TL in the Erasmus Rucphen Family (ERF) study (n = 2,769) to study several characteristics of TL: first, we estimated the heritability of TL using POLY, second, we tested for the equality of variances to compare TL variability in different age categories, and third, we investigated the relationship of TL with known metabolic risk factors for CVD including adipokines.

TL was highly heritable ($h^2 = 0.65$, *p-value* = $1.45*10^{-61}$). Over the entire age distribution (18 to 88 years), we observed a significant reduction in TL variance with age, ranging from 0.150 in younger individuals to only 0.053 in older individuals (*p-value* = $4.23*10^{-6}$). Descendants of the 'oldest-old' had above average TL, suggesting that the 'oldest-old' themselves had above average TL when they were young. Of the 18 metabolic traits tested, only adiponectin was associated with TL after correction for multiple testing (*p-value* = $5.94*10^{-5}$).

In conclusion, we found that TL has a significant and large heritability and that the variability of TL decreases with age, pointing to a survivor effect. Of all the CVD related traits we tested, only adiponectin was significantly associated with TL suggesting a relationship between adiponectin and TL in the development of CVD.

P10.54

Association of IRGM polymorphisms and susceptibility to tuberculosis in zahedan, southeast of Iran

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Tuberculosis (TB) is a major cause of morbidity and mortality throughout the world. IRGM1 is an important protein in the innate immune system against TB. Indeed by regulating autophagy in response to intracellular pathogens it has a critical role in the innate immune system. Polymorphisms in the IRGM genes are known to influence expression levels and may be associated with outcome of infections. The objective of this study was to determine whether the presence of IRGM polymorphisms −1208 A /G, −1161 C/T and −947 C/T was associated with tuberculosis disease.

We investigated the functional polymorphisms of IRGM −1208 A /G (rs4958842), −1161 C/T (rs4958843) and −947 C/T (rs4958846) in 150 patients with pulmonary tuberculosis (PTB) with an average age of 47.5 years (59 male, 91female; minimum 12 years, maximum78 years) and 150 healthy subjects with a mean age of 44.13 years (53 male, 97 female; minimum 20, maximum 82). Genotype analysis was done using tetra amplification refractory mutation system-PCR (T-ARMS-PCR).

In the present study there was a significant difference in −1161 C/T and −947 C/T IRGM between control and PTB groups, whereas no significant difference in IRGM −1208 A /G was observed between control and PTB groups.

In conclusion significant association was found between the IRGM −1161 C/T (rs4958843) and −947 C/T (rs4958846) polymorphisms and susceptibility to PTB in a sample of Iranian population. Our finding suggests that IRGM −1208 A /G (rs4958842) polymorphism may not be a risk factor for susceptibility to tuberculosis in our sample.

P10.55

Vistafin: Association with Genetic Variability in Vistafin (PBEF) Gene, Anthropometric Parameters and Dietary Composition in Obese and Non-Obese Central-European Population

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Visfatin (PBEF/Nampt) is a recently identified adipocytokine which harbors strong insulin-mimetic activity. However, nothing is known about whether visfatin is related to specific nutritional behavior which may result in obesity development. This is the first study focusing on genetic variability of the visfatin gene and its association with circulating visfatin, anthropometric parameters and dietary composition.

We analyzed a total of 6 exons and adjacent non-coding regions of the PBEF gene in 20 extremely obese Czech individuals (mean BMI 52.2 kg/m2 ± 5.0 SD) using direct sequencing and a frequency of rs2302559 was established in the validation cohort of another 605 individuals with completed 7-day food records and complex anthropometric measurements. Plasma levels of visfatin, leptin and leptin-receptor were measured in all sequenced individuals and in part of the validation cohort.

Three common polymorphisms were identified, two in non-coding regions (rs78411774 A/C, rs71564769 A/C) and one synonymous SNP in exon 7 (rs2302559 A/G). The rs2302559 showed significant correlation with visfatin plasma level throughout the entire study cohort ($p < 0.001$); there was a significant tendency towards higher visfatin levels in G allele carriers with GG homozygotes having the highest visfatin plasma levels. Furthermore, a negative correlation was observed between visfatin and leptin plasma level ($p = 0.01$). No association between investigated SNPs and anthropometric parameters or native dietary composition was observed.

This is the first study to demonstrate that the rs2302559 polymorphism in the PBEF gene is related to circulating levels of visfatin.

P10.56

Allelic and genotypic frequencies of CYP3A5, CYP2C19 and VKORC1 in Bulgarian population

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VKORC1, CYP2C19 and CYP3A5 polymorphisms are frequently studied in pharmacogenetics. VKORC1 is the key enzyme of the vitamin K cycle and a molecular target of coumarins, which represent the most commonly prescribed drug for therapy and prevention of thromboembolic conditions. CYP2C19 and CYP3A5 are members of the cytochrome P450 mixed-function oxidase system and they are involved in the metabolism of xenobiotics.

The goal of this study was to determine the allelic and genotypic frequencies of important variants in CYP3A5, CYP2C19 and VKORC1 in Bulgarian population and compare them with the frequencies in other populations.

We examined 134 unrelated healthy subjects for polymorphisms in CYP3A5, CYP2C19 and VKORC1 by High Resolution Melting on Rotor-Gene Q.

The allelic frequencies for CYP3A5*3 and CYP2C19*2 were 88.43% and 13.81%, respectively, while VKORC1 1173C>T and VKORC1 -1639G>A were found in 51.87% and 45.52% of the subjects tested, respectively. Genotypic frequencies were as follows: 10.45%AA, 2.24%AG, 87.31%GG (CYP3A5); 74.63%GG, 23.13%GA, 2.24%AA (CYP2C19); 26.87%GG, 55.22%GA, 17.91%AA (VKORC1 -1639G>A) and 25.37%CC, 45.52%CT, 20.11%TT (VKORC1 1173C>T). Overall our results showed that the frequencies of allelic and genotypic variants of CYP3A5 and CYP2C19 in Bulgarians were similar to those reported for several other Caucasian populations. Also, a high prevalence of VKORC1 1173C>T and VKORC1 1639G>A polymorphisms among Bulgarians was found.

High-resolution melting analysis provides a simple and accurate method for genotyping of VKORC1, CYP2C19 and CYP3A5. The results of the current study will be useful for clinical pharmacogenetics investigations and for drug dosage recommendations in Bulgarian population.

P10.57

The Homozygosity Index (HI) approach reveals high allele frequency for Wilson Disease in the Sardinian population

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Mutational records make it possible to estimate the allelic frequency (q) of autosomal recessive disorders using a novel approach based on the proportion of homozygotes versus compound heterozygotes born of consanguineous parents or even of apparently unrelated parents (the rarer the disorder, the higher the proportion of homozygotes for the same mutation).

The HI approach, successfully tested for Phenylketonuria and Familial Mediterranean Fever (Gialluisi et al. 2011), has obvious advantages over traditional epidemiological studies. However, the calculation of the coefficient of inbreeding (F) becomes problematic when offspring of apparently unrelated parents is considered. Using inbreeding data from the general Sardinian population (Moroni et al. 1972), we applied the HI approach to a sample of 183 Sardinians (only 3 of whom born of consanguineous parents) affected with Wilson Disease (WD, OMIM #277900) and well characterized for ATP7B mutations.

The estimate of F in this sample, keeping into account the individuals born to consanguineous parents, was equal to 8.9 x 10^{-4} while the HI value was equal to 0.486, which yielded a q of 0.0158 corresponding to a prevalence of 1:3991.

These results confirm that WD prevalence in Sardinia is much higher than previously reported (P=1:10000, Ghiagheddu et al. 1985) and are in line with a recent neonatal screening based on ATP7B mutation analysis (q=0.0192, P=1:2707, Zappu et al. 2008). In conclusion the prevalence of WD, largely underdiagnosed in Sardinia, can be estimated correctly by the HI approach, which is applicable to other autosomal recessive disorders especially in highly inbred populations.

P10.58

A Y -chromosome portrait of modern Bulgarians as viewed from different spatiotemporal aspects

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To address the structure and evolution of the Bulgarian paternal gene pool, we have examined the Y chromosome variation in 809 Bulgarian males. The analysis was performed by high-resolution genotyping of biallelic markers and by analyzing the STR variation within certain haplogroups. The biallelic markers were analyzed by PCR/RFLP and PCR/DHPLC assay. Seventeen fast-evolving Y-STRs were amplified using the multiplex AmpFlSTR Yfiler PCR Amplification Kit (Applied Biosystems) and were read on ABI 310 genetic analyzer with GeneMapper software.

We found that the Bulgarian Y chromosome gene pool is primarily contained within haplogroups common in Europe and surrounding areas. Furthermore, when patrilineal relationships are visualized in a broader context by principal component analysis, Bulgarians are located among European populations. The analysis of molecular variance shows that the genetic variation within the country is structured among Western, Central and Eastern Bulgaria, rather than among the Black Sea coast, the Danubian Plane, Thrace and the Southwest mountainous region; which indicates that the Balkan Mountains have been permeable to human movements.

Y-STR variation ages and median joining networks of haplogroups E-V13, J-M241, R-M458, R-L23 and I-M423 were calculated together with data from other populations. For this purpose, the analyses of STR variation within haplogroups were based on 8 STR loci, with the exception of haplogroup R-M458, for which the STR profiles were further reduced to 7 loci. In general, the Y-STR data reveal that different prehistoric and historic events have left detectable traces in the Bulgarian Y chromosome gene pool.

P10.59
Ethnogenetic Estimation of Baltic ancestry

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Background. Y chromosome is widely used as marker in population genetic studies. The aim of this study was to estimate the possible genetic origin of Balts performing Y chromosome haplogroup (Y-Hg) analysis of Russian individuals that inhabit historical regions of Baltic tribes and to compare Y-Hg frequencies with incidence of Y-Hg in Latvian population.
Material and methods. A study encompassed 192 men that represent four Northern-Western and Central Russian regions and 153 unrelated ethnic Latvians. DNA samples were hierarchically genotyped (using appropriate PCR followed by RFLP or sequencing of corresponding PCR products) by 10 Y chromosomal binary markers (M9, SRY-1532, Tat, P21, M170, P37, M253, M172, YAP, M35) to establish their haplogroup.
Results. Similar incidence of main Y-Hg's - N1c, R1a, and I was found in analysed Russian regions and Latvian population. Significant differences in Y-Hg distribution in comparison to other regions under study were observed only in Mezen (Archangelsk district, Russia). In Mezen the Slavic component representing R1a haplogroup had the lowest frequency (20%) in comparison to other Russian (~55%) and Latvian (~40%) subpopulations. On the other hand the Fino-Ugric speaking population representing haplogroup N1c was the most common in Mezen (51%) in comparison to other Russian (~15%) and Latvian (~45%) subpopulations.
Conclusions: No significant differences in common Y-Hg distribution among analysed Russian and Latvian populations were found. The analysis of Y-Hg genofund in Mezen indicates possible Fino-Ugric ancestry that could be confirmed after Y haplotype (Y-STR) analysis.

P11. Genomics, Genomic technology including bioinformatics methods, gene structure and gene product function and Epigenetics

P11.001
IonTorrent: 2nd generation sequencing in a diagnostic laboratory

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Molecular diagnosis of complex human genetic disorders is still challenging since in most cases multiple genes harboring putative deleterious mutations have to be investigated. Currently Sanger sequencing is applied, however capillary sequencing is excessive time-consuming and expensive for the screening of multiple genes. In the last years next generation sequencing technologies have been developed, but because these technologies are especially made for large sequencing projects it is not easy to scale them down for screening a set of disease causing genes in a diagnostic setting. In order to fill this gap IonTorrent recently introduced a sequencing device with mean throughput utilizing sequencing technology based on the detection of hydrogen ions that are released during the polymerization of DNA.
Based on our established sequencing approach we tested the performance of this technology especially in respect to usability, software requirements and accuracy. As templates we used exclusively PCR amplicons left over from the routine analysis without further normalization. Per sequencing run (314 chip) we analyzed simultaneously 600-800 fragments using barcode technology generating ca 40.000.000 bp of sequence. Although results might be preliminary and improvements should be expected our data will be crucial for the decision whether this technology can be implemented for diagnostic purposes.

P11.003
Screening of 50 Cypriot patients with autism using 400K custom array-CGH

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A standard method of whole-genome screening for subtle genetic defects is array-based Comparative Genomic Hybridization (array-CGH) with a variety of platforms applied in diagnostic and research centers. One of the arrays that combines high resolution and relatively low complexity of analysis is the Agilent 400K custom array (Agilent Santa Clara, CA), which can reliably identify deletions and duplications as small as 13 kb. Here we exploit the potential of the above platform to enter the little understood area of genetic basis of autism, limiting our sample heterogeneity by focusing on the population of Cyprus.
A cohort of 50 patients, their parents and 50 ethnically matched normal control samples were tested using aCGH with Agilent 400K custom array, after chromosomal imbalances and Fragile X syndrome were ruled-out. Microarray results were confirmed with real-time PCR and Fluorescence in situ Hybridization (FISH). As a result, 18 patients were found to carry potentially causative aberrations, one of which was de novo and 17 were inherited from unaffected parents. Four aberrations reside within genes, known to cause autism susceptibility and six are associated with schizophrenia and/or developmental delay and/or mental retardation. None of the above aberrations is found in copy number variation databases or normal ethnically matched population. Moreover, population comparison revealed an increased rate of rare disease-associated variants in normal parents of children with autism. The above data supports the multifactorial model of autism aetiology and the sum of genetic and environmental factors that lead to the disease are yet to be identified.

P11.004
Explore thefeatures of brain-derived neurotrophic factor in mood disorders

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The neurotrophic hypothesis for major affective disorders (MD) has been proposed over a decade. Brain-derived neurotrophic factor (BDNF) is one of the most studied, which plays an important role in neuronal survival and differentiation. However, the features and underlying mechanisms of BDNF for MD is yet clear. Previous studies using different designs often reported inconsistent results for the relationship between BDNF and MD. This study aims to explore the features of BDNF for its role in MD, including aspects in molecular evolution, literature review of genetic association studies, and pathway analysis. Results of sequence alignment among different species revealed that BDNF is a highly conserved gene, having 75% identity in chordates with human and 85.9%-100% in vertebrates. Molecular evolutionary analysis found no signs of recent positive selection. Literature review and meta-analysis exhibited inconsistent association results for rs6265 in MD, which is the most studied marker that locates in the coding region of precursor BDNF (pro-BDNF). Mature BDNF was produced from pro-BDNF and the two proteins have opposite biological functions. Studies in European seabass showed that stress changed the ratio of pro-BDNF and BDNF, implicating the necessity to study pro-BDNF for MD. We identified proteins that interact with BDNF and mapped these genes to genome-wide association datasets of MD. Pathway analyses identified possible biological pathways that involved with BDNF for MD. We concluded that examining the features of BDNF systematically can provide opportunities to have a better understanding for the mechanisms underlying mood disorders.

P11.005
Anticipation in Beckwith-Wiedemann syndrome: Gradual increase in maternal H19/ICR1 methylation associated with tall-statured mothers and BWS in their children

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Beckwith-Wiedemann syndrome (BWS) was diagnosed in two sisters and their male cousin. Both sisters had classical BWS features including Wilms tumour. Their male cousin (DZ twin) died from medical complications after a caesarian section in week 29. Birth weight was 2,1 kg and he had visceromegaly, macroglossia and general subcutaneous oedema. The children's two mothers and their sister were tall statured (178, 185 and 187 cm) and one had mild BWS features as a child. Their parents had average heights of 173 cm (mother) and 180 cm (father). This 2nd generation's increased stature and 3d generation BWS correlated with increased methylation of the maternal H19-locus, from 0.49 (normal range 0,50±0.20) in the grandmother to on average 0.70 in the next generation and 0.85 in the affected children. This data was reproduced by bisulphite treatment and subclone sequencing to quantitate the degree of CpG-methylation in a part of the H19 imprinting control region (ICR1). Long-range PCR did not detect any microdeletions in ICR1 that could explain the lack of maternal allele demethylation. However, ICR1 sequencing revealed the same maternal point variant g.1979595T>C that had been described previously as a cause of BWS in three brothers (Demars et al., Hum Mol Genet 19, 803-14, 2010). This point variant was on the paternal allele in the non-affected grandmother. Mutations in this region affect OCT binding, and our data suggests that this interferes with gonadal switching from paternal to maternal imprinting and that H19 interallelic interaction might play a role in this process.

P11.006

Methylation index as a prognostic marker in bladder cancer patients

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Bladder cancer (BC) is a common malignancy worldwide. At the time point of diagnosis 70% of BCs present as superficial BC (SBC), which do not penetrate the muscle layer of the bladder. The rest 30% of cases present muscle-invasive BC (MIBC). SBC has better prognosis, though recurrences happen in 30% of cases after the primary tumor removal. MIBC has much worse prognosis and survival. Therefore it is of a prior importance to reveal possible markers of recurrences or progression of SBC into MIBC. It is proposed that methylation pattern might reflect the ability of BC to recur and/or to progress.

We examined 119 tumor samples from 108 SBC patients and 11 MIBC patients. Recurrence status after 1 year was known for 39 SBC patients: 31 patient developed relapses, 8 did not. Genomic DNA was extracted from fresh tissue. We investigated the status of promoter hypermethylation of *RASSF1A, RARb, P16, p14, CDH1* using methyl-sensitive PCR. Methylation index (MI) (or mean frequency of methylation) was defined as the ratio between the numbers of methylated genes to total number of examined genes in each sample. Statistical significance was evaluated using the Mann Whitney U-test.

SBC had a significantly lower extent of methylation (median MI 0.1) than MIBC (median MI 0,25) (p 0,017). Recurrent within 1 year SBCs showed median MI 0,0 while non-recurrent tumors had median MI 0,2 (p 0,047). Our results show that MI might be used as a sensitive marker for the assessment of recurrence and progression potential of SBCs.

P11.007

Identification of an integrated molecular network for congenital anomalies of the kidney and urinary tract

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Congenital anomalies of the kidney and urinary tract (CAKUT) form a spectrum of developmental malformations, including multicystic dysplastic kidney, renal hypoplasia, and duplex collecting system. CAKUT are the most common cause of end-stage renal disease in children. Based on previous disease modelling, it is anticipated that variants in genes expressed during embryonic kidney development play an important role in CAKUT aetiology. To gain insight into protein signalling cascades contributing to CAKUT and to visualize these molecular pathways, an analysis of 185 CAKUT candidate genes was conducted using Ingenuity Pathway Analysis software. The 185 candidate genes were selected based on experimental evidence implicating them in CAKUT pathogenesis. Extensive literature mining was subsequently performed to identify the biological mechanisms through which the CAKUT candidate genes interact during embryonic development. The bioinformatics analysis revealed a significant enrichment of molecular pathways regulating Wnt/beta-catenin signalling (28/185 genes, $p=9.72*10^{-26}$), basal cell carcinoma signalling (18/185 genes, $p=1.31*10^{-20}$), and stem cell pluripotency (22/185 genes, $p=1.62*10^{-20}$). In addition and based on the subsequent and extensive literature search, we built an integrated protein signalling network for CAKUT. Within this network, glial cell line-derived neurotrophic factor (GDNF)-dependent signalling, which is essential for growth, maintenance and differentiation of epithelial and mesenchymal cells during kidney and urinary tract development, plays an important role. Our results provide important clues for improving our understanding of the molecular background of CAKUT, which in turn is essential for identifying novel treatment targets for these disorders.

P11.008

Identification and functional characterization of trans-acting factors involved in the post-transcriptional regulation of CDK5R1

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CDK5R1 encodes p35, an activator of CDK5, a proline-directed serine/threonine kinase that phosphorylates proteins involved in CNS development and maintenance. CDK5 and p35 were found to show an important role in neuronal migration and differentiation during CNS development and were also implicated in some neurodegenerative and cognitive disorders. Both the CDK5R1 3'-UTR remarkable size and its conservation during the evolution are strongly indicative of an important function in post-transcriptional regulation. We recently reported that CDK5R1 3'-UTR contains regulatory elements affecting transcript stability. In particular, a 138 bp region, that does not contain known miRNA binding sites, has been identified as the most destabilizing portion of the 3'-UTR by luciferase assays. UV cross-linking and site directed mutagenesis experiments allowed us to delimit potential binding site for RNA binding proteins (RBPs), among which we identified the nELAVs, showing a stabilizing activity on CDK5R1 transcript after overexpression and silencing experiments. To search for putative destabilizing factors, pull-down experiments have been carried out, allowing us to identify further binding factors, among which hnRNPA2/B1. The validation of hnRNPA2/B1 binding and the study of its silencing/over-expression might help to disclose the possible role of this protein on CDK5R1 post-transcriptional regulation.

This study will help to define the functional role of the gene, addressing studies on the CDK5R1 implication in the pathogenesis of neurodegenerative and cognitive diseases.

P11.009

Fast and cost effective ChIP-Sequencing with PGM™ system reviews epigenomic landscape change in the MCF-7 cells upon estrogen stimulation

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ChIP-Seq provides a digital high resolution map of genome-wide protein and DNA interactions. With continuing evolving of next generation sequencing technologies, more and more sequencing platforms become well suited for the ChIP-Seq application. PGM™ system is a revolutionary semiconductor based low cost next generation sequencing system, offers long read length and the fastest sequencing turnaround time. It provides a new tool for ChIP-Seq application. We evaluate PGM™ system performance on ChIP-Seq application in comparison with SOLiD system. Human breast adenocarcinoma MCF7 cells were treated with estrogen. Chromatin immunoprecipitation with RNA pol II and ER-alpha antibodies were performed with MAGnify™ Chromatin Immunoprecipitation System, which enabled fast enrichment of chromatin complexes and efficient DNA recovery from small number of cells. The ChIP DNA samples were further constructed into ChIP-Seq libraries with an efficient ChIP-Seq library construction procedure, which enabled us to construct a library using as low as 1 ng ChIP DNA. Barcoded ChIP-Seq libraries were prepared for SOLiD system, while non-barcoded individual ChIP-seq libraries were constructed for PGM™ system. Both types of libraries were sequenced on SOLiD and PGM™ systems, respectively. The ChIP-Seq data from both systems showed expected response upon estrogen treatment in the MCF7 cells. The effect of different sequencing read length, ranging from 50bp to 200bp, on the ChIP-Seq profiling was investigated. In depth analyses of the ChIP-Seq data from both systems will be presented.

P11.010

Comparative Analysis of CHO Cell Transcriptional Dynamics under Different Cell Culture Conditions using Next Generation RNA Sequencing Technology

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Next Generation RNA-Sequencing (RNA-Seq) is a methodology for comprehensive measurements of cellular transcription at a scale, accuracy and precision never seen with previous technologies. We conducted a comparative RNA-Seq study of basic cell growth conditions to understand and improve high quality therapeutic protein production in CHO cells. In this study, we examined global changes in gene expression in CHO cells across differing cell subtype and media- specific parameters. Eight CHO RNA samples from different cultures were sequenced on two full slides of a SOLiDTM System resulting in approximately 760 million, 50 base pair sequence reads. These reads were mapped to multiple reference sequences including CHO ESTs, mRNAs and well as mouse chromosomes with annotated genes with cognate functional data. From this, we report estimates of transcript expression levels and use known annotation to infer functional differences that can be associated with changing basic bioproduction growth conditions. These findings may uncover novel genetic mechanisms that could be optimized for improved bioproduction. This analysis of this data set represents the characterization of the CHO transcriptome at an unprecedented depth.

P11.011

Chromatin changes in human mesenchimal stem cells during cultivation and differentiation

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Epigenomics is one of the most exciting branches of modern cell biology. Role of chromosome territories (CT) in cell physiology in norm and disease remains poorly understood though chromatin structure is highly important for epigenetic regulatory mechanisms. Mesenchimal stem cell (MSC) is a useful model for studying CT role in stem-cells and during differentiation. Our aim is to study CT changes in MSC during cultivation and differentiation in vitro.

Human MSC derived from adipose tissue and bone marrow were prepared at early (before 4) and late (after 5) passages, and also after adypogenic and osteogenic differentiation. Over 2000 nuclei were analyzed using FISH with centromeric probes to chromosome 6 and 18. It was found that chromosome 6 holds a more distant radial position than chromosome 18 with medians of 0,65 and 0,47 respectively. Homologues of both chromosomes are always placed at different radial distances keeping medium (0,48-0,67) and outer (0,71-0,87) layers for chromosome 6 and inner (0,35-0,46) and medium layers (0,48-0,67) for chromosome 18. Comparison of cells at early and late passages, adypogenic and osteogenic differentiation revealed significant (p=0,0008 for chromosome 18 and p=0,03 for chromosome 6) distal displacement of both chromosomes in cells at late passages and after both differentiations also. Described particular chromatin pattern in cultivated MSCs differs from CT structure in both differentiated (i.e. lymphocytes) and embryonic stem cells. This pattern could be unique characteristics of MSC necessary for epigenetic regulation of their activity; and observed changes may reflect similar processes during differentiation and prolonged cultivation.

P11.012

A next generation sequencing panel based approach in ciliopathy diagnostics

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Many primary ciliopathies are syndromal autosomal recessive diseases characterized by defects of primary cilia function. Cystic kidneys, brain abnormalities and liver fibrosis are overlapping findings in the often severe ciliopathies as Meckel syndrome (MKS) or Joubert syndrome related disorders (JSRD). To identify the underlying gene defect is often challenging because of the genetic heterogeneity with 10 and 16 known genes respectively.

Our aim was to develop a bench top NGS instrument based approach to efficiently analyze the most relevant MKS/JSRD genes and concurrently achieve the wherewithal coverage. The overall detection rate is about 50%. MKS1, MKS3/JBTS6 (TMEM67), MKS4/JBTS5 (CEP290), MKS6/JBTS9 (CC2-D2A), JBTS3 (AHI1) are the mainly contributing loci. Taking into account own data and data from the literature mutation frequencies divide approximately as follows: MKS1 (7%), MKS3 (7-16%), MKS4 (10%), MKS6 (10%) for MKS and JBTS3 (8%), JBTS5 (7-20%), JBTS6 (9%), JBTS9 (9-10%) for JBTS respectively. Further known genes play only little role for the overall detection rate.

We used the GS Junior system (Roche) and chose the amplicon protocol. We designed gene specific oligonucleotides for the above named genes including adapter sequences. The corresponding PCR amplicons were pooled followed by emulsion PCR and enrichment. The data were analyzed with the SeqNext module (Seqpilot/JSI). We analyzed about 20 patients (including controls) and hereby present our experiences regarding this approach: detection rates (false negative and positive rates), average coverage for the investigated amplicons, influence of homopolymeres on the data quality and number of fragments to be reanalyzed by Sanger sequencing.

P11.013

Comparison of different reference genes used for qPCR-based CNV quantification

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Copy Number Variant (CNVs), the change of the DNA copy number in the genome, has been recently shown to be a widely-spread phenomenon that affects about 10-20% of the human genome. The occurrence of the CNVs has been associated with various diseases such as autism, autoimmune disorders, and cancer.

The most commonly used molecular biology tools for discovery of CNVs are array and next-generation sequencing (NGS). These two high-throughput methods can discover multiple potential CNVs, which normally need to be validated with an independent method. Once validated, the confirmed CNVs can also be examined in a large number of samples to identify the statistically significant association of the CNV and phenotype. Quantitative PCR (qPCR), with its ease of use, sensitivity, and scalability, is often the method of choice for CNV validation and association studies. Relative quantification principle is used to determine any possible change of gene copy numbers. Since the consistent copy number of the reference gene is essential for the qPCR-based CNV quantification, we evaluated the reliability of commonly-used single copy reference genes such as Tert. Our results suggest that, compared to single copy genes, stable multi-copy regions can serve as a more sensitive and reliable CNV quantification reference.

P11.014

Comprehensive cancer gene research panel sequencing using fast, efficient and scalable Ion AmpliSeq technology and semiconductor sequencing

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Targeted enrichment of exon regions from genomic DNA has proven useful for mapping somatic disorders in cells. Whole exome sequencing has adaptation barriers associated with the extensive time needed for hybridization-based capture methodologies. Exon centric sample preparation methods are especially challenged in applications that require gDNA analysis from degraded sources, such as archived FFPE specimens. We have developed a fast, easy-to-use, highly multiplex PCR-based selection procedure for next generation sequencing. This process merges the specificity and speed of highly efficient PCR amplification with extremely scalable single-tube amplicon multiplex ranging from 12 to 3,000. This technology has been used to design primer pools to enrich coding gDNA sequence regions from an array of key cancer genes. The Ion AmpliSeq™ Comprehensive Cancer Panel interrogates exons in hundreds important cancer genes, covered by over 12,000 amplicons which can be sequenced on a Ion318™ chip. This kit is suited for archival (FFPE) samples, requiring only 10 ng of DNA per reaction. When the libraries are processed and sequenced with the Ion OneTouch™ and PGM™ Sequencer, mutations present at low frequency can be quantified from DNA to results within a day. The typical sequencing per base uniformity exceeds 96% for the amplicon panel. Using bi-directional sequencing protocols, per base accuracy exceeds 99.5+%. We report cancer gene somatic variant frequencies from studies using paired normal/tumor samples. This technology enables rapid and efficient focused sequencing in a variety of both basic and translational research settings.

P11.015

Delineation of the reliability of in silico copy number variation (CNV) calls from different Illumina SNP arrays

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Illumina SNP arrays are widely used to identify common and rare susceptibility variants involved in the etiology of multifactorial diseases. Besides SNP-based genome wide association studies, SNP-arrays allow to in silico analyze Copy Number Variants (CNV). There has been a comprehensive discussion in the field to what extent in silico CNV calls are reliable. For individual associated CNVs, validation by quantitative PCR (qPCR) is usually performed which is labour-intense and requires large amounts of DNA. Moreover, it is almost impossible to qPCR-validate CNVs from in silico burden analyses which test the genome-wide frequency of CNVs between patients and controls. In order to get an objective overview of the reliability of in

silico CNV calls and the influence of batch effects, we compared in silico CNV calls from a larger number of technical replicates which were genotyped as internal quality controls in different genotyping projects over the last three years. These data had been acquired on different Illumina arrays such as the Human660W, HumanOmniExpress, HumanOmni1S and HumanOmni1M-Quad.
We compared these replicates on basis of genotypes and copy number variants to evaluate technical artifacts arising from batch effects. We will present data on deviances arising from quality parameters (e. g. gentrain score, logR ratio, B -allele frequency, intensity, chemistry batches) and the implications thereof.

P11.016

Generation of Customized and Read-To-Use Genetically Engineered Mice

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The Institut Clinique de la Souris - ICS- is a research infrastructure that provides extensive services ranging from the development of mouse models to comprehensive phenotyping. The Genetic Engineering and Model validation Department is dedicated to the development and molecular validation of new mouse models.
Mouse model can be valuable models to better understand the molecular processes of monogenic diseases or to test drugs in vivo. We can generate duplications and deletions of defined genomic fragment (CNVs) as observed in human disease. We always work in strong interaction with the scientists and make sure to define their needs. Many publications have already arised from mice generated at ICS.
The department is also driving several internal R&D programs and is involved in several international consortium (EUCOMM, EUCOMMtools, GENCODYS, IMPC, PHENOMIN).
We have generated a CreERT2 zoo (http://www.ics-mci.fr/mousecre/). When bred with conditional knock-out mice these lines allow the generation of time and cell specific knock-out after injection of Tamoxifen.
We will give you an example of a fully characterized mouse line: Insulin1-CreERT2. The Cre expression is observed in the β-cells, the translocation in the nucleus is confirmed in the presence of Tamoxifen as expected for an inducible line. By breeding this line with Rosa26 reporter line, a specific LacZ staining is observed in the β-islet cells. This line was phenotyped (under chow diet) and no glucose intolerance was observed at the difference of the Rat Insulin Promoter (RIP)-Cre line. A comparative study (Ins1-CreERT2 versus RIP-Cre) was performed and will be detailed.

P11.017

Performance of seven mutation pathogenicity prediction methods in the classification of missense variants of the CYP1B1 gene

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Nonsynonymous single nucleotide polymorphisms (SNPs) in the coding regions of genes can lead to aminoacid changes and potentially affect protein function and, therefore, susceptibility to disease. Several computational methods have been developed for the classification of SNPs according to their predicted effect on protein function and resulting pathogenic potential. In this study, we evaluated the performance of seven commonly used pathogenicity prediction methods available on the Internet (SIFT, nsSNPAnalyzer, Panther, pMut, PolyPhen, PhD-SNP, and SNAP). In order to test them, nonsynonymous SNPs in the CYP1B1 gene- which codes for the cytochrome P450 1B1 enzyme-were selected. A total of 129 missense variants in CYP1B1 were identified in the literature, from which 87 could be classified as pathogenic or neutral according to criteria such as segregation with disease phenotype, and effect on function, among others. The algorithms showed significant variation in the assignment of the variants to three categories (non-neutral, neutral, no prediction), with a low 37% prediction rate for Panther. Pairwise concordance between methods in the classification of variants as pathogenic or neutral varied between 37% and 94%. The accuracy in the prediction of the pathogenicity of the variants was higher than 68% with all methods except pMut (47%). The highest false positive and false negative rates were found for SIFT and pMut, respectively. Taking into account the rate of prediction, accuracy of prediction, false positive, and false negative rates, the method with the overall best performance in the present study was nsSNP-analyzer, closely followed by SIFT, Polyphen and SNAP.

P11.018

Fine characterization of the recurrent c.1584+18672A>G deep-intronic mutation in the CFTR gene

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Splicing mutations account for ~12% of the 1,890 CFTR mutations described in cystic fibrosis (CF); however, their impact on pre-mRNA processing frequently remains unclear. An interesting opportunity to study CFTR transcripts in vivo is the use of RNA from nasal brushings. We previously identified through this approach a deep-intronic mutation (c.1584+18672A>G), which activates a 104-bp out-of-frame pseudoexon by creating a donor splice site.
Screening of 481 CF patients identified c.1584+18672A>G in two additional individuals, demonstrating that it is a recurrent, and potentially overlooked, mutation among Italian patients. Haplotype analysis suggests that it originated from at least two independent events.
To further characterize the mutation, a genomic region including the activated pseudoexon and surrounding intronic sequences was cloned into an expression vector and transfected into HeLa cells. RT-PCR analysis identified two alternative splicing products, produced by the activation of two different cryptic acceptor splice sites: one including the 104-bp pseudoexon (78.7% of transcripts), the other leading to the inclusion of a 65-bp pseudoexon (21.3% of mRNAs). Allele-specific measurement of wild-type and aberrant splicings in the probands' (genotype F508del + c.1584+18672A>G) RNA from nasal brushing demonstrated: i) a low level of pseudoexon inclusion in the F508del transcript (not containing the splicing mutation); ii) a residual wild-type splicing in the c.1584+18672A>G mRNA; iii) the allele-specific degradation of aberrant transcripts; iv) the relative strength of the different cryptic splice sites. Interestingly, the residual wild-type splicing detected in transcripts bearing the c.1584+18672A>G mutation well correlates with the milder clinical phenotype of patients.

P11.019

Identification of novel Intellectual Disability genes through detection of *de novo* mutations

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De novo mutations may play an important role in Intellectual Disability (ID). Here we evaluate the frequency and role of *de novo* mutations in an unbiased cohort of 100 ID patients using family-based exome sequencing. To identify the recurrence of mutations in certain genes in the population a targeted sequencing experiment was performed for 17 ID-related genes in 540 additional ID patients.
In the 76 trios sequenced thus far 60 unique coding *de novo* mutations were identified and validated in 58 different genes. On average 0.73 (0-4) *de novo* mutations were observed per patient. 47 of these mutations were non-synonymous, of which 9 introduced a premature stop or frame-shift. Pathogenicity analyses were performed for all non-synonymous variants based on evolutionary conservation (DNA and protein level) and mutation impact on the protein.
Combined with the targeted resequencing we identified 30 *de novo* mutations which likely explain the ID phenotype. Nine involved known ID genes (*RAB39B, SYNGAP1, GRIN2A(n=2), PDHA1, LRP2, TUBA1A, TCF4, GRIN2B*), eight occurred in new, but recurrent, ID genes (*DEAF1, YY1, DYNC1H1, GATAD2B*) and 13 additional events had a high pathogenicity score and were present in genes with a potential biological link to ID.
Our findings provide strong support for a *de novo* paradigm for ID, and further strengthen the causality of several previously identified genes. This study comprises the largest cohort of non-syndromal ID patients and suggests an expected diagnostic yield of approximately 35% for *de novo* mutation detection through exome sequencing.

P11.020

Deep-sequencing of TALENs targeted embryonic stem cells to estimate their efficacy in genome editing

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Engineering of biological systems that recapitulate human genetic disorders relies on efficient manipulation of the genome. Recently, transcription activator-like effector nucleases (TALENs) have shown promising potential in site-specific genome editing. Their modular structure enables the design and simple construction of TALENs that can specifically recognize virtually any DNA sequence. Upon delivery into embryonic stem cells (ESCs), TALENs initiate a double strand break that is repaired by non-homologous end-joining, introducing a large variety of mutations. Since this method lacks a selection procedure the applicability depends largely on its efficacy.

Here we focused on altering the hDMD transgene, introduced into the genome of mouse ESCs. So far, editing by homologous recombination of hDMD transgene has failed. Thus, we engineered a TALENs-pair targeting intron 52 of the hDMD gene. The utility of the assembled TALENs-pair was determined by measuring the variation within the targeted sequence of the hDMD transgene among TALENs-transfected mouse ESCs. The 135bp locus of hDMD was PCR-amplified and sequenced for 100,000 TALENs transfected and non-transfected cells using IonTorrent semiconductor sequencing. The targeted locus was covered ≥450,000x. In TALENs-transfected ESCs, the rate of editing events, mainly small deletions and insertions, was 4-fold higher (~11%) than in non-transfected cells. Furthermore, we assembled a list of the most frequently occurred structural variations and cleavage sites to facilitate follow-up functional studies.

Our data endorse the use of TALENs for modifying the hDMD transgene in ESCs. The TALENs efficacy in genome editing can be further estimated using the Pacific Biosciences Single Molecule Real-Time sequencing.

P11.021

Screening of muscular disease genes with the Access Array™ System of Fluidigm and Roche's GS Junior

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Sequencing of large genes like the *DMD* gene or of a group of genes, e.g. muscular disease genes, for many patients in parallel is a major application in our diagnostic laboratory. In addition to Sanger sequencing, we have established next generation sequencing (NGS) with the 454 GS Junior (Roche). For target enrichment, the Access Array™ System of Fluidigm is used which allows parallel amplification of 48 target regions for 48 samples in one single PCR setup. By the combination of target-specific primers and individual-specific barcode primers with 454 adaptors, sequencer-ready amplicon libraries can be produced in a single step.

The Access Array System is being established for the parallel screening of multiple candidate genes in patients with muscle diseases. In a first step, the *DMD* gene has been resequenced in a total of 80 patients in whom mutations and SNPs had already been identified by classical Sanger sequencing. The sequence coverage was satisfying (at least 15-fold by default) and all known variations could be retrieved. We are now extending the system to other genes for muscular diseases, especially the genetically heterogeneous limb-girdle muscular dystrophies (LGMD).

The combination of Access Array System and GS Junior sequencing has proven reliability and practicability as a time and cost efficient alternative for classical PCR and Sanger sequencing. The system seems to be particularly suitable for diagnostics as it is easy-to-use and requires only small amounts of genomic DNA and PCR reagents in order to obtain a sufficient amount of sequence data within three days.

P11.022

High throughput qPCR DNA methylation marker testing and validation

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Over recent years elucidation of genomewide epigenetic changes has become a routine application. As with other genomewide approaches confirmation of elucidated DNA methylation changes have to be validated using alternate methods and also on additional samples.

In principle several strategies either based on bisulfite DNA conversion or methylation sensitive restriction enzyme (MSRE) digestion are suitable for confirmation of methylation changes. During our biomarker-research efforts on several cancer entities we have found that quantitative methylation analyses are inevitably for analyses. Therefore we have set up a cost efficient MSRE-based qPCR strategy and have qualified 576 methylation markers according MIQE guidelines. For enabling high throughput analyses of 96 samples x 96 different assays performing 9216 qPCRs in a nanoliter scaled microfluidic qPCR array per run, DNA target concentrations several hundreds of copies per 5 nanoliter reaction have to be warranted for proper analyses. Therefore we evaluated preamplification protocols and optimised conditions for high throughput DNA methylation testing.

Here we will introduce this procedure and present performance data as well as data generated on clinical tumor samples. Classifiers derived from these analyses have already been confirmed by

bisulfite deamination based tests (like qMSP, Sequenom's MALDI assay and pyrosequencing) and obtained very good correlation of results. Nanoliter scaled MSRE based HTqPCR outperforms standard qPCRs with respect to material costs, data quality and efficiency. Preamplification enables also paralleled analyses of up to 96 targets starting with 5-10ng DNA with high reliability and is currently under investigation for methylation testing of cell free DNA in serum.

P11.023

Placental lipoprotein lipase DNA methylation levels are associated with impaired glucose tolerance and maternal and fetal blood lipid profiles

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Background: According to the fetal programming (or Barker's) hypothesis, newborns exposed to a detrimental fetal and perinatal environment are more susceptible to develop obesity, type 2 diabetes (T2D) and related chronic disorders but the underlying molecular mechanisms are poorly understood. Lipoprotein lipase gene *(LPL)* is an important regulator of lipid metabolism and transport and has been associated with obesity, T2D and dyslipidemia. The aim of this study was to determine the impact of impaired glucose tolerance (IGT) exposure on newborn *LPL* gene DNA methylation levels and lipid profile.

Methods/Results: Placental tissues and blood samples were obtained from 128 women (31 with IGT) and their offspring. Glucose tolerance was assessed using a 75-g oral glucose tolerance test (OGTT) between weeks 24 and 28 of pregnancy. *LPL* DNA methylation was determined by bisulfite pyrosequencing. There were up to 9% ($p<0.05$) differences in *LPL* DNA methylation levels between women with or without IGT. Placental *LPL* gene DNA methylation levels were associated with 2h-glucose post-OGTT levels (r=-0.204, $p=0.022$) and first trimester maternal triglycerides concentration (r>-0.201, $p<0.05$) and total cholesterol/HDL ratio (r=-0.207, $p=0.021$). Cord blood (n=93) cholesterol (r=-0.216, $p=0.041$), HDL (r>-0.208, $p<0.05$) levels and total cholesterol/HDL ratio (r>0.248, $p<0.05$) were also correlated with placental *LPL* DNA methylation.

Conclusion: These results suggest that fetal *LPL* DNA methylation levels are dysregulated by maternal glucose tolerance with a possible functional impact on cord blood lipid profile. They provide insights on the molecular mechanisms that may be involved in fetal programming and long-term development of obesity and dyslipidemia.

P11.024

DNA methylation patterns of mitotic and meiotic chromosomes from human spermatogenic cells

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We studied DNA methylation patterns of mitotic and meiotic chromosomes from human spermatogenic cells. Samples were obtained from 5 patients with fertility problems in IVF clinic by testis biopsy. Chromosomes were fixed on slides with ethanol:acetic acid (3:1) after colchicines treatment in hypotonic solution. Analysis of DNA methylation was performed by immunocytochemistry with monoclonal antibodies against 5-methylcytidine. Chromosomes were identified by QFH/AcD staining. Among 119 analyzed dividing cells several types were detected by their specific morphological features: mitotic diploid and polyploid spermatogonia and meiotic spermatocytes at the pachytene, diplotene and diakinese stages.

DNA methylation pattern (MeC-pattern) of mitotic chromosomes appeared to be a specific banding pattern, resembling R-banding. It differed by number of bands and DNA methylation intensity from that in lymphocytes and human mesenchymal stem cells, described in our previous study. DNA methylation level of heterochromatic blocks of chromosomes 1,9,16 and Y demonstrated the most feasible difference: in most mitotic spermatogonia these regions were hypomethylated.

Pachytene chromosomes showed less obvious MeC-banding pattern, probably due to the increased length of chromosomes. The most intensive DNA methylation was registered at the peritelomeric regions of pachitene chromosomes. Diplotene and diakinese cells demonstrated high, but fairly homogeneous DNA methylation along chromosomes with increased intensity of signal in chiasmas and heterochromatic regions.

Thus, chromosomes of spermatogenic cells demonstrate unique DNA methylation patterns, different from these in somatic cells, possibly explained by their crucial role in chromatin stability and faciliating recombination events.

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P11.025

A genomic approach for DNA methylation & hydroxymethylationanalysis

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DNA methylation and hydroxymethylation are some of the most important epigenetic modifications that can occur in the human genome. For instances, DNA methylation plays a vital role in the regulation of gene expression in normal cell development and aging, but also in the formation and progression of cancer and other diseases. Large scale identification of putative epigenetic biomarker candidates is achievable with the ability to profile DNA methylation and hydroxymethylation at the genomic level. Once validated, specific biomarkers could be applied to clinical and molecular diagnostic fields. Due to the availability of Next Gen sequencing technology, a number of new technologies have been developed for interrogating DNA methylation and hydroxymethylation at the genomic scale. Zymo Research has recently perfected sample prep and bioinformatic analysis as part of its new DNA Methylation and Hydroxymethylation Profiling Services. These epigenetic services combine next generation sequencing with Zymo's well-established epigenetic technologies and innovative bioinformatic algorithms for the most streamlined, comprehensive genome scale data generation to date. With these new services··· hundreds of epigenomic biomarker candidates can be discovered simultaneously.

P11.026

A genome-wide search for novel imprinted genes

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NLRP7 is a maternal effect gene as mutations in this gene cause recurrent hydatidiform moles, spontaneous abortions and stillbirths, whereas live births are very rare. We have studied a patient with multiple developmental defects born to a mother with a heterozygous *NLRP7* mutation. By genome-wide CpG methylation analysis of blood DNA from the patient, his parents and 18 normal controls on Illumina 27K arrays we found that the patient had methylation changes (delta ß>0.3) at almost all known imprinted loci as well as at 77 other loci. Using each control as a pseudoproband, we found methylation changes at only 11-26 (median 17) loci not known to be imprinted. In order to identify novel imprinted genes among the 77 conspicuous loci in the patient, we selected 22 genes (mainly hypomethylated genes) for deep bisulfite sequencing on the ROCHE/454 Genome Sequencer in the patient and at least two additional controls who were heterozygous at the test locus. Apart from FAM50B, which we proved to be imprinted, we did not observe allele-specific DNA methylation at these loci. We conclude that the patient has methylation defects at almost all imprinted loci as well as an excess of methylation changes at apparently non-imprinted loci. Our data also suggest that the number of genes that are imprinted in blood cells is rather low and that most of them have been discovered.

P11.027

Genome wide DNA methylation profiling of monozygotic twins discordant for trisomy 21

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DNA methylation is essential in mammalian development and has an effect in gene expression. We hypothesize that methylation differences induced by trisomy 21(T21) contribute to the phenotypic characteristics and variations in T21 patients. In order to determine the methylation differences in T21 without the noise of the genomic variation, we studied samples from monozygotic twins discordant for T21. We also collected samples from monozygotic twins concordant for T21, normal monozygotic twins without T21, and unrelated normal and T21 individuals to use as controls. We applied Reduced Representation Bisulfite Sequencing (RRBS) to generate nucleotide resolution of DNA methylation based on high throughput sequencing (HiSeq 2X 100bp) between each pair of twins. Methylation state of 4,278,488 CpGs with at least 8X coverage was obtained for the monozygotic twins discordant for T21. An initial analysis of methylation percentages differences between these twins identified 1000 differentially methylated regions (DMRs) (FDR<0.001 and at least 4 fold change in methylation differences). These DMRs were correlated with the deregulation of gene expression. The analyses of the control samples are ongoing. The study of methylation differences in monozygotic twins discordant for genetic abnormalities is a promising approach to understand the molecular mechanisms of aneuploidies.

P11.028

Comprehensive DNA methylation profiling with the SureSelect target enrichment system

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DNA cytosine methylation is a critical epigenetic modification involved in human diseases such as cancer and imprinting disorders. Various cellular processes including gene regulation, embryonic development, X chromosome inactivation, and chromatin remodeling are strongly associated with DNA methylation changes. Next-generation sequencing combined with sodium bisulfite treatment allows identification of methylation changes at single base resolution. However, whole genome bisulfite sequencing is prohibitively expensive. Additionally, many of the regions in whole genome bisulfite sequencing are in repetitive regions, and provide little information. In many cases, the researcher is only interested in profiling a subset of biologically relevant regions. To address these needs, we have developed SureSelect Methyl-Seq, which combines Agilent's SureSelect Target Enrichment platform with bisulfite sequencing to detect methylation changes. Our Methyl-Seq design targets human genomic CpG sites within CpG islands/shores, promoters, known differentially methylated regions (DMRs) and previously determined regulatory regions. This comprehensive design covers 84Mb making it well suited to study cancer- and tissue-specific DMRs. Here we describe the SureSelect Methyl-Seq workflow and demonstrate efficient target enrichment and precise methylation level detection. Further, we show high concordance with whole-genome bisulfite sequencing of known model systems and describe the detection of tissue and cancer specific DMRs.

P11.029

DNA-methylation in Dutch and Texan NTD children

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Neural tube defects (NTDs) are congenital malformations developing during the first weeks of pregnancy. Interactions between environmental and genetic factors are involved in the pathogenesis and prevention of NTDs. Maternal folate shortage during the sensitive period of neural tube development contributes to the occurrence of NTD. Folate is an important B vitamin and donor of methyl groups, essential for DNA methylation, a key epigenetic mechanism to explain gene-environment interactions. We hypothesize that folate-related NTD are due to derangements in DNA methylation in the child of (non)imprinted genes implicated in neural tube development.

Methylation levels of three imprinted genes (IGF2, H19, and KCNQ1OT1), and three non-imprinted genes (MTHFR, VANGL1, and LEKR) were measured using Sequenom MASSARRAY EpiTYPER in 34 cases and 78 controls from a Texan study and in 48 cases and 62 controls from the Dutch population. Linear mixed model analysis was used to calculate the association of DNA-methylation levels with NTD.

The Texan samples revealed no significant association of DNA-methylation levels of any of the genes with NTD. In the Dutch samples we found a borderline significant association of MTHFR methylation levels with NTD (-1.3% methylation in cases, P-value=0.072). In the combined analysis of the Texan and Dutch samples we observed a borderline significant association of MTHFR DNA-methylation with NTD (-0.6% methylation in cases, P-value=0.067).

In conclusion, slightly lower MTHFR DNA-methylation levels may be associated with NTD. Additional studies are warranted to confirm our results.

P11.030
Differential expression of microRNAs in peripheral blood mononuclear cells of Down syndrome children

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Down syndrome (DS) or Trisomy 21 is the most common human chromosomal disorder. DS phenotype includes several dysmorphic features, intellectual disability, immunological alteration, congenital heart disease, high risk for specific types of leukemia and neurological alterations. Recent studies show that DS results in the over-expression of microRNAs, which could result in low expression of specific proteins and contribute to DS phenotype. To identify differentially expressed microRNAs in peripheral blood mononuclear cells of six DS and six non-DS children and the biological processes relevant to DS pathogenesis associated with their predicted gene targets of microRNAs differentially expressed, we investigated the expression pattern of 754 mature microRNAs using TaqMan® Low Density Arrays (Applied Biosystems). Of the 490 mature microRNAs expressed in this cell type, 49 are low-expressed in DS group. The microRNAs located in chromosome 21 did not present differential expression between the groups. Target prediction was performed using TargetScanHuman v. 5.2. software and information about gene targets was obtained using the Bioprocess, a database that obtains data from NCBI. Bioinformatics analysis showed that genes involved in relevant biological process to DS, including apoptosis, reactive oxygen species metabolism, mitochondrial metabolism, immune system, cell aging, cycle and division and control of gene expression, are predicted targets of microRNAs differentially expressed in DS children. In conclusion, DS children present low expression of microRNAs not located on chromosome 21 in peripheral blood mononuclear cells and biological processes relevant to DS pathogenesis are associated with predicted gene targets of these microRNAs differentially expressed in DS chidren.

P11.032
Epimutations of imprinting genes and early pregnancy loss

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Genomic imprinting is one of the most significant epigenetic phenomena, which is involved in reproduction and fetal development. We have performed DNA methylation analysis of the cytotrophoblast (CT) and extraembryonic mesoderm (EM) from 5 first-trimester spontaneous abortions (6.8±1.1 weeks) with normal karyotype and 6 induced abortions (7.5±0.7 weeks) as a control group using HumanMethylation27 BeadChip (Illumina, USA) comprising 409 CpG sites oriented near promoter region of 60 imprinting genes. Sixteen (26.6%) imprinting genes have revealed abnormal methylation in most miscarriages. Most CpG sites of these genes (ATP10A, CDKN1C, DIRAS3, GNAS, GRB10, KCNQ1, MEST, MKRN3, SLC22A3, SNRPN, TP73, WIF1, WT1, ZIM2) were hypomethylated, whereas only in two genes (INS, OSBPL5) hypermethylation of CpG sites was detected. The presence of several epimutations affected from 1 to 9 imprinting genes in CT and from 1 to 6 ones in EM was observed in each embryo. All epimutations had a postzygotic origin since were confined by EM or CT, that indicates forming epimutations after differentiation of these tissues. Most imprinted genes with epimutations participate in cell cycle (CDKN1, DIRAS3, INS, TP73, GO:0007049), in cell differentiation (CDKN1C, GNAS, INS, TP73, WIF1, GO:0030154), in cell death (INS, TP73, WT1, GO:0008219), cell proliferation (CDKN1C, INS, WT1, GO:0008283) and growth (GNAS, INS, WT1, GO:0040007). This study was supported by Federal Program (contracts P303).

P11.033
Methylation analysis of three imprinted genes in ICSI versus IMSI sperms by limiting dilution bisulfite pyrosequencing

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Optimum selection of sperm cells may increase the success rate of intracytoplasmatic sperm injection (ICSI) for human infertility treatment. For standard ICSI the embryologist selects normal looking sperm under an inverted microscope. Recently, a new method, intracytoplasmatic morphologically selected sperm injection (IMSI), was introduced. For IMSI only the most normally shaped sperms without a vacuole in the nucleus are retrieved using a higher definition microscope. Several recent studies have shown that abnormal sperm parameters are associated with aberrant methylation imprints. To compare the epigenetic quality of different sperms from the same males, we have used limiting dilution bisulfite pyrosequencing, which allows one to study the methylation levels of multiple genes in pools of about 10 sperms each. First, we have analyzed the methylation patterns of two maternally imprinted genes, *hLIT1* and *hPEG3*, and one paternally imprinted gene, *hGTL2*, in ICSI versus IMSI sperm samples from 5 males. Secondly, we have compared IMSI sperms versus sperms with a clearly visible vacuole from 10 males. Thirdly, we compared IMSI sperms versus abnormally shaped sperms with a vacuole from 5 males. Overall, we found a low rate (0-4%) of epimutations (abnormal allele methylation) in all groups and no between-group differences. Although we cannot exclude epigenetic differences in genes other than those studied, our results do not support the hypothesis that IMSI selects better quality sperms, improving fertilization and pregnancy outcome.

P11.034
Utilizing next generation sequencing for exome analysis

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Next generation sequencing has changed the possibilities for analyzing human exomes. Several commercial kits are available allowing exome enrichment with subsequent library preparation in a cost efficient way. These libraries can be directly analyzed on e.g. an Illumina HiSeq. Enrichment is performed by pull down of coding regions with baits, which differ in overall design depending on the manufacturer. The depth of analysis can be enhanced by increasing the number of reads by using higher sequencing coverage mapped on the genome or the targeted region.

We used commercially available exome enrichment kits and designed a pipeline for data quality analysis, mapping and especially SNP detection. The mapping of reads was analyzed in detail to determine the efficiency of enrichment for the targeted exons. Variants were filtered according to several criteria like base quality, read quality, thresholds for coverage, and the overall mapping quality.

In order to improve the overall quality of reads for data processing, a quality score which represents the probability of a particular base mismatch in the reference genome was established. This highly increased the probabilities of validating certain mismatches.

In addition, FFPE samples were used for exome enrichment and the mapping and SNP detection compared to other starting material.

Utilizing exome sequencing in clinical and genetic diagnosis and personalized disease risk profiling is expected in the near future. Optimizing the sensitivity and data analysis pipelines will help to integrate exome analysis into a common clinical setup.

P11.035
Accurate detection of *de novo* mutations in rare and common neurodevelopmental disorders

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Many dominant Mendelian disorders occur sporadically because the severity and early onset of the disorder preclude transmission to subsequent generations. Exome sequencing represents the first systematic approach to identify many genes that cause sporadic diseases. *De novo* mutations causing rare syndromic forms of dominant Mendelian disease, such as Schinzel-Giedion syndrome and Kabuki syndrome were first identified by applying overlap strategies, *i.e.* identifying mutations in the same gene in multiple independent affected individuals. Subsequent studies focused on the role of *de novo* mutations in common neurodevelopmental disorders, such as intellectual disability, autism and schizophrenia by applying a trio design, i.e. exome sequencing of patient-parent trios.

In order to establish a robust approach optimized for *de novo* analysis, we used latest SOLiD 5500XL sequencing technologies in combination with the recently announced wildfire product (Life Technologies, Foster City, CA). The latter allowed the highest number of analyzed DNA molecules (>1 billion raw reads per lane), a fast process, long reads, high throughput and importantly allowed reliable calling of disease causing *de novo* mutations by applying exome sequencing to patient-parent trios. As a proof-of-concept for this novel technology we studied a patient with Baraitser-Winter syndrome, a well-defined disorder characterized by distinct craniofacial features, ocular colobomata and a neuronal migration defect. Using whole-exome sequencing of a patient-parent trio, we identified a *de novo* missense mutation in the cytoplasmic actin-encoding gene *ACTB*.

P11.036

Detection of Interstrand and Intrastrand DNA Crosslinks with Two-Dimensional Strandness-Dependent Electrophoresis

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Two-Dimensional Strandness-Dependent Electrophoresis (2D-SDE) is a novel technique for nucleic acid analysis to assess quality of samples, efficiency of molecular procedures and DNA damage. In the first dimension nucleic acid fragments are separated based on length and strandness i.e. double-stranded DNA, single-stranded DNA and RNA•DNA hybrids. The nucleic acids are heat denatured before the second dimension electrophoresis and thereafter fragments separate only based on length. After 2D separation, different arcs can be seen representing different strandness and lengths of the nucleic acids in the original sample.

We tested if 2D-SDE could be used to detect DNA crosslinks. Patients with Fanconi anemia (FA), a group of rare recessive disorders with heterogenic clinical features, are extremely susceptible to DNA crosslinking agents. Human genomic DNA in solution and fibroblast cell cultures with mutations in *FANCA* and *FANCD1* genes were treated with the different crosslinking agents diepoxybutane, mitomycin C and cisplatin and analysed with 2D-SDE. Increased amount of DNA migrating behind normal dsDNA was observed as expected for molecules with interstrand crosslinks since they prevent full denaturation of dsDNA. Intrastrand crosslinks causing bending of nucleic acids and ssDNA migrating in front of arc dsDNA were also observed. Lesions were dosage-dependent and correlated with cytogenetic abnormalities. Repair efficiency, as measured with 2D-SDE, was lower in the FA cell types compared to the wild-type cells.

2D-SDE has potential use in research, for Fanconi anemia diagnosis, and in chemosensitivity testing as the cytotoxicity of many cancer medications depends on their DNA crosslinking ability.

P11.037

Application of next generation sequencing to identify causative variant at the BCL11A locus

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In the past few years, genome-wide association studies have improved our understanding of the molecular basis of fetal hemoglobin (HbF) levels, which is one well known ameliorating factor of both beta-thalassemia and sickle cell anemia disorders. This approach led to the identification of the BCL11A transcription factor as one of the main genetic modifier of the two disease phenotypes.

Although several studies have shown that BCL11A functions as a developmental stage-specific repressor of HbF expression controlling globin switching both in human and mouse, the specific contribution of associated variants in gene action is still unclear. Here we report the integration of data from genotyping and targeted and whole-genome sequencing using next generation technology to identify causative variants at the BCL11A locus.

We performed at two steps imputation using BCL11A targeted sequencing of 33 patients with Thalassemia Intermedia (300x coverage, on average) and low pass whole-genome sequencing of 347 unrelated individuals from the SardiNIA project (4x coverage, on average) to impute the discovered variants in 2343 individuals, from the same cohort, genotyped with Affymetrix 500K and 6.0 arrays. Accounting for all known HbF levels modifiers, the association at the BCL11A locus can be explained by two independent variants, mapping 304 bp apart. They represent the strongest haplotypic signal within the gene and are likely to be the causative polymorphisms. Experimental assays are ongoing to assess their specific impact in BCL11A function.

P11.038

The contribution of histone modifications to fetal programming of adult disease - studies in offspring of mothers with gestational diabetes

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Several human epidemiological studies and experimental animal studies link in utero conditions very early in development with adverse effects on the risk of developing obesity, diabetes and other metabolic diseases later in life. Children born to mothers with obesity and/or diabetes have been shown to have a greater prevalence of metabolic disorders later in childhood, adolescence and adulthood. Epigenetic modifications of gene expression by DNA methylation and histone modifications are very attractive candidates for modulating these fetal programming effects. To investigate the impact of maternal overnutrition on the epigenome of the offspring, we aim at analyzing the histone modification profiles of candidate genes in chorionic villi of newborns from mothers with insulin-dependent gestational diabetes (GDM), dietary-treated GDM, and healthy controls (without GDM and overweight) using Chromatin immunoprecipitation (ChIP) real-time PCR. We started our analysis with the maternally imprinted MEST gene since our previous studies detected a significantly decreased DNA methylation at this gene in offspring of mothers with GDM. First experiments revealed a marked increase of the activating histone H3-lysine 4 trimethylation and histone H3-lysine 9 acetylation marks that is associated with maternal GDM. No significant changes in the repressive histone H3-lysine 27 trimethylation mark were observed. Histone modification analysis of several other candidate genes is also in progress. These preliminary results support the view that early exposure to overnutrition involves not only altered DNA methylation but also changes in histone modifications that possibly lead to increased MEST gene expression and effects on fetal programming of adult disease.

P11.039

Elucidating the role of *FOXP1* in striatal development

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De novo heterozygous *FOXP1* gene deletions were recently identified in 3 patients with intellectual disability and language impairment. These findings, together with support from independent studies, show that *FOXP1* may play a role in both cognitive as well as language development. This is supported by the fact that Foxp1 is strongly expressed in specific brain structures, particularly in the striatum. To date, very little is known about the role of FOXP1 in brain development. Examination of different developing brain structures in the absence of FOXP1 would shed light on the neurodevelopmental pathways in which it is playing a role. We have used a knockout mouse model to investigate the role of Foxp1 in early striatal development. The striatum is composed mainly of medium spiny neurons (MSNs) which can be divided into patch and matrix MSNs and form axonal connections with other components of the basal ganglia. Analysis of the Foxp1 knockout brain using various immunohistochemical and histological stains, which identify patch and matrix MSNs, have revealed that these specific neuronal populations are seemingly unaffected up to E14.5. We have additionally used axon tracing techniques to trace the striatal projections to the substantia nigra and these experiments revealed no misprojection of these axons. Taken together our results show that Foxp1, which is strongly expressed in the striatum is not essential for early striatal development and that its function is likely to be rather more central in later developmental stages.

P11.040

Role of CTCF protein in regulating FMR1 gene transcription.

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Fragile X syndrome (FXS) is mostly caused by expansion and subsequent methylation of the CGG repeat at the 5' UTR of the FMR1 gene (full mutation). Rare individuals of normal intelligence, carrying an unmethylated full mutation (UFM), have been reported and the epigenetic modifications in their FMR1 gene were previously characterized. CTCF (CCCCTC-binding factor), a zin-finger protein, is an important regulator of the transcription of genes harbouring trinucleotide repeats, acting as chromatin insulator. We investigated the role of CTCF in regulating FMR1 gene expression and observed that the amount of CTCF bound to FMR1 was higher in the UFM cell lines and in normal controls, compared to FXS cells. Knock-down of CTCF with anti-CTCF siRNA resulted in a reduction of FMR1 transcripts (both sense and antisense) in normal and UFM fibroblasts. After CTCF knock-down, the epigenetic analysis of the FMR1 promoter demonstrated a reduction of H3-K4 methylation and an increase of H3-K9 methylation, while the DNA methylation of the FMR1 promoter region and of the upstream methylation boundary remained unmodified. CTCF knock-down affected its binding to the 5' UTR of the FMR1 gene. These results suggest that CTCF is a modulator of the FMR1 transcription, given that its depletion causes FMR1 transcript reduction and the transition to a heterochromatic configuration. The elucidation of the mechanism sparing UFM males from inactivating their full mutation is important for planning therapeutic attempts at converting methylated into unmethylated full mutations, restoring FMR1 gene expression. Supported by FRAXA Foundation and Telethon Onlus.

P11.041

The prediction of Pathogenesis of Mitochondrial 12397 A>G substitution in Friedreich's ataxia with Bioinformatic Procedures

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Friedreich's ataxia (FRDA) is the most common ataxia that has an autosomal recessive inheritance. The disorder caused by mitochondrial defects. The prediction of pathogenesis of nucleotide changes by bioinformatics is useful method for Geneticians. In this study, Mitochondrial NADH dehydrogenase IV (ND5) gene was investigated by PCR-SSCP in 25 patients. The samples with shift bands sent for sequencing. Sequencing results were determined 12397 A>G substitution in 2 patients. This substitution causes to change of Thr to Ala (T21A). The determination of pathogenesis of this mutation were performed by SIFT database (Sorts Intolerant from Tolerant amino acid substitutions). The Substitution at position 21 from T to A is predicted to affect protein function with a score of 0.00 and median sequence conservation is 4.32. Although the prediction of this database is sequence homology-based tool, but that is useful method for the determination of effect of mutation on phenotype.

P11.042

Correlation analysis of clinical parameters with epigenetic modifications in the DUX4 promoter in FSHD

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Aim of our study was to identify relationships between epigenetic parameters correlating with a relaxed chromatin state of the DUX4 promoter region and clinical severity as measured by a clinical severity score or muscle pathologic changes in D4Z4 contraction-dependent (FSHD1) and -independent (FSHD2) facioscapulohumeral muscular dystrophy patients.

21 primary fibroblast and 26 primary myoblast cultures originating from patients with FSHD and controls were analyzed. Histone modification levels were determined by chromatin immunoprecipitation. We examined correlations between the chromatin relaxation score (CRS) defined by the H3K9me3:H3K4me2 ratio and an age corrected clinical severity score (CSS) or muscle pathology score (MPS). Possible relationships were investigated using linear regression analysis and significance was tested by Pearson's product-moment coefficient.

We found a significant difference of the CRS between controls and patients with FSHD1 and between controls and patients with FSHD2. Tissue specific differences in CRS were also observed. We also found a near-significant relationship between CRS and the age corrected CSS in fibroblasts but not in myoblasts. Surprisingly, we found a strong correlation between the MPS of the vastus lateralis and the CSS.

Our results confirm the D4Z4 chromatin relaxation previously shown to be associated with FSHD in a small number of samples. A possible relationship between clinical and epigenetic parameters could be established in patient fibroblasts, but not in myoblasts. The strong correlation between the MPS of the vastus lateralis and the CSS suggests that this muscle can be used to study for surrogate markers of overall disease severity.

P11.043

Gene expression analysis using functional genomics techniques

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Background: This work presents an overview of evolutionary models and some functional genomics methodologies in the specific context of analyzing the gene expression.

Functional genomics techniques are intended to aid in rapidly identifying gene function by correlating gene expression with cell or tissue phenotype. Despite many complex studies performed, the clinical utility of genotype-phenotype associations remains unclear.

Profiling transcriptomes and examining the coordinate expression of genes in diverse pathobiologic pathways is now possible with techniques such as gene array analysis.

Functional genomics techniques compare mRNA transcript pools (transcriptomes), they can also be used to compare genomes, and to study the proteome. We use those methods in which no prior gene sequence is required (open architecture systems), as well as those in which prior sequence data are required (closed architecture systems).

The aim of this work is the most widely used functional genomics methodology, gene array analysis applied to gynecological fields for diagnose the future diseases of the new born and to prevent the surgical complications of the malformative interventions in the first days of life.

Key words: gene expression, microarray technology, evolutionary models.

Abbreviations: mRNA - messenger ribonucleic acid

P11.044

Systematic Characterization of PRR-activated Monocyte Transcriptional Program

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Dynamic cellular processes, such as the response to an invading pathogen, are governed by complex transcriptional regulatory networks. These networks typically involve a large number of transcription factors (TFs) that are activated in different combinations and in a certain temporal order to produce a particular cellular response.

Here, we pursued a gene expression profiling strategy to systematically explore regulators of transcription after stimulation with prototypical microbial ligands. To this effect, we isolated monocytes from four healthy donors and exposed the cells to pathogenic compounds such as viral nucleic acids. We measured mRNA expression at six time points after stimulation. Clustering differentially expressed genes via smoothing spline clustering (SSC) method revealed a broad range of activation kinetics. We hypothesize that clustered genes are to some extent co-regulated by common TFs. To find overrepresentation of transcription factor binding sites (TFBS) we conducted promoter analysis using TFs whose expression was validated in our microarray data set. These results revealed a systematic overview of novel transcriptional regulators that may play a role in the response to pathogens. Elucidating the transcriptional network and identifying key regulators and their functions will greatly enhance our understanding of the innate immune response and its role in infection.

P11.045

Generation and comprehensive phenotyping of mouse models at the ICS

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The Institut Clinique de la Souris (ICS) is a research infrastructure of excellence for translational research and functional genomics. Founded in 2002 by Prof. Pierre Chambon, it provides a comprehensive set of highly specialized mouse services to scientists from academia and industry. The ICS combines the capacity of generating mutant mice on a large scale with a high-throughput and comprehensive phenotypic analysis of mice, but also customized protocols to better answer the scientist's needs. The ICS phenotyping platforms are adapted for the study of genetically engineered mouse models, as well as for pharmacological and toxicological studies, allowing better understanding of human diseases and their underlying physiological and pathological basis. More than 250 assays are available for evaluation of various functions including behavior, cognition, sensory systems, nutrition, metabolism, clinical chemistry, cardiovascular, respiratory function and anatomopathology. Beside its services, the ICS is a member of several European programs: EUCOMM (www.eucomm.org) to generate the mutant strains, EMMA (www.emmanet.org) to archive and distribute them, and EUMODIC (www.eumodic.org) to phenotypically characterize 500 knock-out models. The EUMODIC is extended to the 20 000 potential genes identified in the mouse genome and continued by the International Mouse Phenotyping Consortium (www.mousephenotype.org). ICS is also a partner of the Fondation Maladies Rares. Up to now, about 80 mouse mutant lines were phenotyped and around 80% of the analyzed lines show at least one phenotypic trait. The data are available to the scientific community (www.europhenome.org), as well as the mouse models through EMMA.

P11.046

Integrating genomic technologies in clinical practice: A novel approach

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Medical genetics is shifting from the present "phenotype-first" medical model to a "data-first" model which leads to multiple complexities. This abstract discusses a "phenotype-first" approach, Individualized Mutation-weighed Phenotype On-line Search (I-MPOS), which could render widespread use of Exome Sequencing (ES) and Whole Genome Sequencing (WGS) in the immediate future practical, ethical and clinically useful. In brief, patients have their exome/genome sequenced and their encrypted data stored on a password-protected platform which remains at the disposal of the individual patient. A patient presents to clinic with a specific medical concern. The physician performs a clinical evaluation and identifies some important features. After obtaining authorization, the clinician temporarily and anonymously uploads the patient's encrypted genomic data to a search engine (I-MPOSE) which simultaneously operates on the patient's encrypted data and on a regularly updated database containing all well characterized genetic diagnoses. I-MPOSE identifies the genetic changes present in the patient's sequenced encrypted genome relative to the reference genome. Using preset criteria, I-MPOSE automatically assigns a weight score to each variant based on the level of certainty for its pathogenicity. The physician performs a database search using keywords related to the clinically assessed phenotype thereby providing an initial ranking of possible genetic diseases. This initial ranking is then adjusted by I-MPOSE based on the weight scores automaticaly assigned to the variants identified by ES/WGS. The proposed approach allows for a more efficient prioritization of the genes to be tested in a clinical lab and an incremental integration of genomic technologies into clinical practice.

P11.047

Impact of common regulatory single nucleotide variants on gene expression profiles in whole blood

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Genome-wide association studies (GWAS) have uncovered susceptibility loci for a large number of complex traits. Functional interpretation of candidate genes identified by GWAS and confident assignment of the causal variant still remains a major challenge. Expression quantitative trait (eQTL) mapping has facilitated identification of risk loci for quantitative traits and might allow prioritization of GWAS candidate genes. One major challenge of eQTL studies is the need for larger sample numbers and for replication. The aim of this study was to evaluate the robustness and reproducibility of whole blood eQTLs in humans and test their value in identification of putative functional variants involved in the etiology of complex traits. In the current study, we performed comphrehensive eQTL mapping from whole blood. The discovery sample included 322 Caucasians from a general population sample (KORA F3). We identified 363 cis eQTLs and 14 trans eQTLs after stringent Bonferroni correction for multiple testing. Of these, 98.6% and 75% of cis and trans eQTLs respectively could be replicated in two independent populations (KORA F4 (n = 740) and SHIP-TREND (n = 653)). Furthermore, we identified evidence of regulatory variation for SNPs previously reported to be associated with disease loci (n = 59) or quantitative trait loci (n = 20), indicating a possible functional mechanism for these eSNPs. Our data demonstrate that eQTLs in whole blood are highly robust and reproducible across studies and highlight the relevance of whole blood eQTL mapping in prioritization of GWAS candidate genes in humans.

P11.048

The novel BTB-kelch Protein, KBTBD8, is ubiquitously expressed but specifically localized in the Cis-Golgi-Apparatus

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Proteins of the BTB/Kelch family are known to be involved in multiple biological processes such as migration, cytoskeletal arrangement, regulation of cell morphology, protein ubiquitination and gene expression. Kbtbd8 is a new undescribed member of this family. The gene was found to be highly expressed in mouse pluripotent stem cells by analyzing their transcriptome and was therefore suggested to be a putative pluripotency regulating gene. Comparative analysis of the gene and protein sequences revealed a high conservation throughout evolution especially in the characteristic domains of BTB, BACK and Kelch.

Starting with expression analysis on RNA level, Kbtbd8 was found to be ubiquitously expressed in mouse and human cell lines and tissues. In mice two transcripts can be detected - one full length and a shorter one lacking a part of exon 1 - which encode for two isoforms of the protein. Next we performed Western Blot and indeed both suggested isoforms were detectable at the predicted size. By immunocytochemistry on mouse and human cell lines we found a striking staining pattern next to the nucleus which we later identified as cis- Golgi apparatus by staining with GM130 antibody. Specificity of the KBTBD8 antibody was confirmed by tagging the KBTBD8 protein with an E2 tag. Detection of E2 and endogeneous KBTBD8 in transfected NIH-3T3 cells showed a perfect overlap suggesting the specificity of the antibody.

In conclusion, Kbtdb8 is a new member of the BTB/Kelch superfamily that is specifically expressed in the cis-Golgi apparatus in mouse and human.

P11.049

Epigenome-wide association study for HDL-cholesterol levels in familial hypercholesterolemia

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Background: A low high-density lipoprotein cholesterol (HDL-C) level is a well-known cardiovascular disease (CVD) risk factor. Although its heritability estimate is high, a few associated genetic variations accounting for a small percentage of its heritability has been reported. The aim of this study was to assess whether epigenetic changes, a non-traditional heritable mechanism, may account for HDL C variability. The study was conducted in familial hypercholesterolemia (FH), a recognized human model to study CVD risk modulators.

Methods/Results: A genome-wide DNA methylation analysis (Infinium HumanMethylation27 BeadChip, Illumina) was performed on blood DNA samples obtained from FH men subjects with low (L-HDLC; n=11) or high (H-HDLC; n=11) HDL-C concentrations. A total of 619 loci (β-value between 0.10 and 0.90; $p<0.05$) were found differentially methylated between groups. Among these loci, 232 were hypomethylated in the L-HDLC group compared to the H-HDLC group, whereas 387 were hypermethylated. According to gene ontology analyses (GeneCodis 2.0 software), hypomethylated regions revealed a pathway related to lipid metabolism ($p<0.02$), whereas hypermethylated regions were more likely to be associated with inflammatory ($p<0.0003$) and oxidative stress pathways ($p<0.003$). Furthermore, the initial association with one of the top differentially methylated locus located in the promoter of Troponin T type 1 gene was replicated in a cohort of 276 FH subjects using bisulfite pyrosequencing.

Conclusion: These results suggest that epigenome-wide changes contribute to the interindividual variations in plasma HDL-C levels in FH patients. If replicated, these findings could influence our understanding of the molecular mechanisms involved in the pathophysiological processes of CVD.

P11.050

Massive targeted resequencing for the diagnosis of Hearing Impairment: a 69-gene panel

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Hearing impairment (HI) is the most frequent sensory disorder, with a big impact in the quality of life of affected individuals. About 1 of 500 children present with prelingual HI. Genetic causes underlie over 60% of cases. Early detection is essential for the success of special education and treatments. To date, conventional techniques have been insufficient to provide a comprehensive molecular diagnosis, given the high number of genes that are implicated in HI.

We designed an NGS targeted resequencing panel for 69 genes, including: i) all genes currently known to be involved in non-syndromic HI (NSHI), with autosomal dominant, recessive, X-linked and maternal-mitochondrial inheritance patterns; ii) genes involved in some syndromic conditions, in which HI is the clinical sign that is earliest observed. The panel includes a total of 0.49 Mb comprising coding exons, splice sites and 5' and 3' UTR regions of the 69 genes. These regions were fully sequenced in 12 control patients with known mutations and in two HapMap cell lines (NA12144 and NA12892). Enrichment of the exonic regions was carried out using SureSelect Enrichment System (Agilent) and sequencing was performed with a SOLiDv4 Genetic Analyzer (Life Technologies). Sequencing reads were mapped and aligned against a reference sequence (GRCh37/hg19); variants were identified and classified. We present the results obtained during the validation of our panel, showing a high level of efficiency. Our targeted re-sequencing system offers massive analysis of 69 genes involved in HI, making the comprehensive molecular diagnosis of this disorder feasible.

P11.051

Generating complex descriptions of sequence variants using HGVS nomenclature based on sequence comparison.

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Descriptions of sequence variants can be checked and corrected with the Mutalyzer sequence variation nomenclature checker to prevent mistakes and uncertainties which might contribute to undesired errors in clinical diagnosis. Construction of variant descriptions accepted by Mutalyzer requires comparison of the reference sequence and the variant sequence and basic knowledge of the Human Genome Varia- tion Society sequence variant nomenclature recommendations. With the advert of sophisticated variant callers (e.g., Pindel) and the rise of long read sequencers (e.g., PacBio), the chance of finding a complex variant increases and so does the need to describe these variants. An algorithm performing the sequence comparison would help users to describe complex variants. The algorithm closely follows the human approach to describe a variant. It will first find the „area of change", and then finds the largest overlap between the original area and the area in the observed sequence. This process is repeated until the smallest description is found. This algorithm ensures that the same description will be generated every time researchers observe this variant. Furthermore, no knowledge of the HGVS nomenclature is required to generate this description. This not only helps clinicians to generate the correct description, but its implementation also allows automation of the description process. We have incorporated this algorithm in the Mutalyzer suite under the name Description Extractor.

P11.052

Minigene study of cryptic exons inclusion controlled by competition of hnRNP C with the core splicing machinery

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It was previously shown that heterogenous nuclear ribonucleoprotein C1/C2 (hnRNP C) silences inclusion of alternative exons by binding to long uridine tracts at the 3' splice sites of pre-mRNAs. With genome-wide approach using iCLIP and RNAseq methods, we identified its binding to the polypyrimidine tracts is mediated by direct competition with the core splicing factor U2AF65. Observed binding at deep-intronic sequence positions was shown to prevent inclusion of cryptic exons, of which majority is represented by ALU elements. Further, we performed more detailed study with use of reporter minigene assays, so far the most effectual choice to study regulation of alternative splicing by RNA-binding proteins in vivo. The competition of hnRNP C and U2AF65 was confirmed with minigenes containing mutations in polypyrimidine tracts, with aim of disrupting long uridine tracts to prevent hnRNP C binding and maintain binding of U2AF65. Cryptic exonization under hnRNP C regulation was verified in control and hnRNP C knock-down conditions. Introduced mutations lead to constitutive inclusion of ALU elements even in the presence of hnRNP C, what confirmed that hnRNP C regulates exonization of ALU elements through competition with U2AF65. Moreover, exonization of Alu elements in hnRNP C knock-down conditions was shown to regulate inclusion of neighbouring alternative exons, as well as effecting normal polyadenylation process. By constructing examples of minigenes with naturally occurring sequence polymorphisms, we demonstrated important role of Alu exonization in primate evolution and in disease.

P11.053

Loss of heat shock protein HSPA4 aggravates pressure overload-induced myocardial damage

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Failure of molecular chaperones to direct the correct folding of newly synthesized proteins leads to the accumulation of misfolded proteins in cells. HSPA4 is a member of the heat shock protein 110 family (HSP110) that acts as a nucleotide exchange factor of HSP70 chaperones. We found that the expression of HSPA4 is upregulated in murine hearts subjected to pressure overload and in failing human hearts. To investigate the cardiac function of HSPA4, *Hspa4* knockout (KO) mice were generated and exhibited cardiac hypertrophy and fibrosis. *Hspa4* KO hearts were characterized by a significant increase in heart weight/body weight ratio, elevated expression of hypertrophic and fibrotic gene markers, and concentric hypertrophy with preserved contractile functions. Cardiac hypertrophy in *Hspa4* KO hearts was associated with enhanced activation of gp130-STAT3, CaMKII, and calcineurin-NFAT signaling. Further analyses revealed a significant increase in cross sectional area of cardiomyocytes, and in expression levels of hypertrophic markers in cultured neonatal *Hspa4* KO cardiomyocytes suggesting that the hypertrophy of mutant mice was a result of primary defects in cardiomyocytes. Gene expression profile in hearts of 3.5-week-old mice revealed a differentially expressed gene sets related to ion channels and stress response. Taken together, these results reveal that HSPA4 is implicated in protection against pressure overload-induced heart failure.

P11.054

Development of a single comprehensive gene screening test for Familial Hypercholesterolaemia using Next Generation Sequencing

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Bristol Genetics Laboratory provides a comprehensive three level genetic testing service for Familial Hypercholesterolaemia (FH) using; level 1 FH20 ARMS for 20 common mutations; level 2 MLPA and Sanger sequencing of *LDLR*; level 3 Sanger sequencing of *PCSK9* and the *APOB* exon 26 mutation hotspot. Over a three year period of service provision a variant was identified in 93/232 index cases (40%). The ARMS methodology detected 48% of positive cases, *LDLR* sequencing 46% and the MLPA assay 6%.To reduce test costs, reduce turn around times and increase throughput we are developing a single assay for FH to detect all point mutations and copy number variation in *LDLR*, *APOB* and *PCSK9*. The coverage of *APOB* has increased to include all coding exons and an additional gene, *LDLRAP1*, which is associated with autosomal recessive hypercholesterolaemia, is included in the panel. The assay uses a targeted capture next generation sequencing approach (Haloplex PCR, Illumina MiSeq) and a bioinformatic analysis pipeline is under development using the Galaxy platform. The assay is currently being validated using 32 known positive control samples comprising: 25 samples with point mutations and indels, and 7 samples with exon duplications/deletions. To explore the benefits of extended screening we are analysing a cohort of previous test-negative patients with a high scoring clinical/biochemical index for FH. A comprehensive high throughput assay at reduced cost should facilitate extended uptake and commissioning of FH testing in the UK and be generally applicable to all European populations.

P11.055
Genomics and transcriptomics in Hypertrophic Cardiomyopathy: from the bench to clinics

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Hypertrophic cardiomyopathy (HCM), the most common hereditary cardiovascular disease, affecting 1:500 individuals in the general population, is typically characterized by genetic and allelic heterogeneity (more than 1000 mutations in 30 genes). The understanding of the genetic basis of HCM, namely the identification of new pathological biomarkers of heterogeneity that could contribute to genotype -phenotype correlations is of extreme importance. In this regard, the correlation between genomic and transcriptomic profiling and their integration into the clinical data was performed for 120 Portuguese Caucasian HCM-patients. DNA was extracted from peripheral blood samples and genotyping was performed by iPLEX Mass Array and High Resolution Melting to detect known and novel DNA variants in sarcomeric/non-sarcomeric genes, respectively. RNA extracted from cardiac and skeletal biopsies from HCM-patients were used for both sarcomere and non-sarcomere transcript levels and microRNAs profiling. Unsupervised machine learning methods were used to distinguish differences between groups of patients, tissues and genes. 85 of the 120 genotyped patients presented genetic alterations, 14 of them are novel ones and not presented in 200 chromossomes from healthy control individuals. All the novel mutations affected highly conserved residues. The most frequently mutated genes were MYH7 and TNNT2. Statistical analysis revealed a strong correlation between MYH7 and TNNI3 expression pattern in cardiac and skeletal muscles. Transcriptional profile also revealed an upregulation of CSRP3 gene in cardiac tissue. microRNAs expression profile changes observed during HCM remodeling, seems very promising to identify novel pathological biomarkers.

P11.056
Heterodisomy at 20q as a cause of Pseudohypoparathyroidism-Ib

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Introducction: Pseudohypoparathyroidism-Ib results from epigenetic GNAS defects. Familial and sporadic forms of PHP-Ib have been reported with distinct GNAS imprinting patterns: familial PHP-Ib patients have an exon A/B-only imprinting defect whereas sporadic cases have abnormal imprinting of the four differentially methylated regions (DMRs) in GNAS. In addition, they present a different underlying genetic alteration.

Objective: to analyze the methylation pattern at GNAS locus in a patient diagnosed with Pseudohypoparathyroidism-Ib and to identify underlying molecular genetic defect(s).

Design: We have studied dosage and methylation pattern at GNAS locus in the patient and her family by MS-MLPA. We also analyzed microsatellite and SNP markers along chromosome 20 looking for causative molecular alteration.

Results: We found that the index case and one of her brothers presented an altered methylation pattern at GNAS locus. It seems that the genetic alteration causative of this epigenetic defect was a paternal heterodisomy at least at 20q13.13.

Conclusion: Our work underlines the importance of analyzing apparently healthy family members of affected patients because of the subtle clinical features. Additionally, we emphasize that obtaining parental samples is essential to exclude/confirm uniparental disomy (isodisomy or heterodisomy) as a molecular underlying defect of Pseudohypoparathyroidism-Ib.

P11.057
In silico approach to Identification of novel cancer/testis-antigen genes by Expressed Sequence Tag(EST)

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Cancer testis antigens (CTAs) expressions are restricted to normal testis tissue. CTAs have been also recognized to be found in some tumor tissues .It has been reported by many researchers that the expression of CTAs could be served as biomarkers to diagnosis, prognostic and immunotherapy in some cancers. Computational approaches have been used for the identification CTAs mRNA expression pattern and led to subsequent identification of many novel genes with similar characteristics to known CTAs.

In this work we have used the UniGene database for gene clusters composed of expressed sequence tags (ESTs) generated from normal testis and tumor-derived cDNA libraries. Using this data base, we analyzed the entire human genome in order to identify new CTAs gene by some selective ESTs.

These candidate genes subsequently can be assayed with experimental approaches to reconfirm their roles as CTAs genes.

In this presentation we will introduce some of the possible novel CTAs genes.

P11.058
Gene-Specific DNA Methylation Analysis in Inflammatory Bowel Disease

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The two main types of Inflammatory bowel diseases (IBD) are Crohn's disease (CD) and Ulcerative colitis (UC): CD can affect the whole bowel wall of each part of the gastrointestinal tract, while UC is restricted to the mucosa of the colon and the rectum. The etiology of IBD is unknown but thought to involve environmental, immunological, microbiological and genetic factors. A contribution of epigenetic factors to IBD pathogenesis has been assumed but not been studied in detail up to now. We used using bisulfite pyrosequencing to investigate the methylation levels at the promotor regions of nine IBD candidate genes in inflammatory and non-inflammatory terminal ileal and colonic mucosal biopsies from IBD patients as well as terminal ileal and colonic mucosal biopsies from non-IBD patients (controls). Our analysis revealed no significant methylation differences between terminal ileal biopsies of CD patients and controls. MUC6 and IL17REL, however, displayed significantly increased methylation levels in inflammatory colon samples of CD patients compared to non-inflammatory patient samples and control samples. Methylation levels of MUC2, MUC6 and IL17REL were even more significantly increased in non-inflammatory and inflammatory colon samples of UC patients compared to controls. MUC15 was the only gene with a significantly decreased methylation detected in both non-inflammatory and inflammatory colon samples of UC patients. Overall, the detected DNA methylation changes appeared to correlate with the progression of IBD. A comprehensive understanding of the epigenetic mechanisms contributing to IBD will likely enable development of new therapeutic agents and strategies targeting epigenetically dysregulated genes.

P11.059
CHERISH - Improving Diagnoses of Mental Retardation in Children in Eastern Europe and Central Asia through Genetic Characterisation and Bioinformatics/Statistics - a GENOMIC PROJECT

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The CHERISH consortium was funded by EU FP7 under grant agreement #223692 and its first aim was the creation of a large collection of patients with developmental delay (syndromic and non-syndromic), mainly from Eastern Europe. General information and updates can be retrieved through http://www.cherishproject.eu/.

During the first 2 years of the project, 233 patients underwent analysis through a 44K (135 patients) or 105K (the remaining 98) array platform, to search for cryptic chromosome rearrangements. Of these, 166 patients were carriers of benign CNVs only, while 34 patients had "pathogenic" anomalies and 33 patients were carriers of a total of 39 variants of uncertain pathogenicity. Analysis of these last variants is underway.

SNP-array analysis was performed on another group of patients (~450). Analysis of variants is more complex due to high resolution of this assay and is being carried out by each partner. For instance, out of 95 patients analyzed in the Armenian cohort, 7 had known micro-del/dup syndromes, 11 had variants interpreted as pathogenic, while 7 variants are still under investigation.

A subset of patients with X-linked intellectual disability underwent analysis through a highly specific array, covering most exons from known X-chromosome genes. Out of 46 cases, 41 turned out normal while 5 cases need further investigation.

Global analysis of the identified variants will be presented.

As a last part of the project, selected familial cases (1 autosomal dominant, 3 X-linked and 8 autosomal recessive), where chromosome rearrangements had been excluded, will undergo exome analysis through next generation sequencing.

P11.060

Seeking for novel genes for autosomal recessive intellectual disability in overlapping runs of homozygosity in a large sample of consanguineous families

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A significant part of the unsolved cases of intellectual disability is probably of autosomal recessive inheritance pattern and shows an extreme heterogeneity. To discover novel genes causing autosomal recessive intellectual disability (ARID), 93 simplex, consanguineous families of Syrian descent with affected members were clinically examined and 316 individuals were genome-wide genotyped using SNP chips (Illumina 610K and CytoSNP as well as Affymetrix 6.0). We conducted autozygosity mapping and identified 552 runs of homozygosity (ROH). Fourteen families showed one ROH, 44 families showed two, three or four ROHs, and 35 families showed five or more ROHs. We considered highly interesting candidate regions, i.e. with five or more overlapping ROHs, and selected 31 candidate genes based on expression, function, and results of association studies of neurological phenotypes. We Sanger sequenced the genes in all candidate families. Subsequently, we identified 14 not annotated (dbSNP 132) candidate mutations and *in silico* analyzed them with help of the programs *MutationTaster*, *PolyPhen*, and *SIFT*. In 4 cases, the analysis predicted a pathogenic effect of the candidate mutations and those were examined by genotyping in a Syrian healthy control sample of 95 individuals. All candidate mutations were excluded because of relatively high frequencies in controls. Our analysis shows an extreme heterogeneity of ARID and suggests that massive parallel sequencing is a better strategy to elucidate its causes.

P11.061

Screening of a cohort of patients with intellectual disabilities from Cyprus using a high-resolution 400K microarray

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Intellectual Disabilities (ID) are very heterogeneous conditions with an estimated prevalence of ~1-3%. While it is estimated that genetic factors contribute to ID in ~50% of affected individuals, the identification of autosomal loci and chromosomal regions associated with ID has been challenging. One approach for the identification of copy number changes unique to individuals with ID is by array-CGH. The availability of custom-designed high-resolution oligonucleotide arrays enables interrogation at an extraordinary resolution and coverage not previously experienced with older platforms. The purpose of our study was to screen a cohort of patients from Cyprus using a new whole-genome 400K microarray which we designed, optimized and validated. Our new high-resolution array consists of >400,000 probes, and includes the entire 4X180K ISCA design as well as comprehensive coverage of >9,000 CNV regions identified by the WTCCC. In addition, ~200,000 probes were used to generate a high-resolution backbone spanning the entire genome. Here we report the preliminary results obtained from the initial screening of 12 patients and family members from 9 Cypriot families. CNCs were identified in 7 patients and included 5 duplications and 3 deletions that ranged in size from ~99kb to ~7Mb. All of the duplications and two of the deletions harbored several genes while one deletion resided in a single gene. Family studies are in progress to determine segregation of the aberrations with the disease and association with the phenotype. In addition, the performance of this new platform for the detection of CNCs associated with ID will be assessed.

P11.062

Large-scale validation and genotyping of inversions in the human genome by inverse PCR

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In the last years, different types of structural variants (SVs), have been discovered in the human genome and their importance to human health has become increasingly clear. Typically arrays have been used to characterize unbalanced changes. Inversions, however, are more difficult to study and less known. In this study we investigate the general applicability of inverse PCR (iPCR) for the analysis of inversions. We have tested different reagents and conditions to optimize the iPCR method and designed a high-throughput iPCR protocol to genotype inversions in a large number of individuals in just one day and with a small amount of DNA (10 ng for each inversion). As an example of the potential use of this method, we have analyzed 19 inversions predicted in humans with a size between 8 kb to 200 kb and mediated by inverted repeat sequences of 1.5-25 kb. First, we validated 17 of the 19 inversions in a panel of 9 Hapmap individuals (Yoruba, European, and Asian). Then, we genotyped these inversions in >60 additional European individuals and found total frequencies for the inverted allele between 1.5% and 62%. For these inversions we also checked the genetic transmission in ~10 mother-father-child european trios. Finally, we have determined the possible gene effects of the validated inversions, with around half of them changing the orientation of genes or exchanging the 3´or 5´ regions. In conclusion, the iPCR is a powerful, simple and fast method for high-throughput validation and genotyping of a wide range of inversions.

P11.063

Producing iPS cells: a biobank service for translational genetic research

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The goal of this project is to use the expertise of the Laboratory of Human Genetics and the Galliera Genetic Bank (GGB) to provide researcher with the possibility to obtain induced Pluripotent Stem Cells (iPSCs) from samples of patients involved in a specific research project. The iPS cell lines are validated using classical cytogenetic karyotype, immunofluorescence assay and RT-PCR, in order to be ready to be used by the researcher requesting the service. The iPSCs are a powerful tools to perform in vitro functional analysis of mutation causing diseases. The application of this tool will be to investigate the effects of mutations causing epilepsy and to assess the effect on neuronal membrane excitability of different neuron subtypes, to shed light into molecular pathways regulating brain functions and biological events leading to epileptic seizures. We have generated iPS cells using STEMCCA vector, that allows the most efficient generation of iPS cells, combined with the high availability of the cell lines stored in GGB/TGB Network. STEMCCA vector is a single lentiviral „stem cell cassette“ encoding all four reprogramming factors, OSKM in a single polycistronic vector. By combining all reprogramming transgenes in a single cassette, STEMCCA accomplish reprogramming of fibroblasts with high efficiency and allow the derivation of iPSC containing a single viral integration. It is also available a excisable version of gene cassette vector flanked by loxP sites to achieve highly efficient reprogramming of normal or diseased fibroblasts to allow the derivation of human iPSC free of exogenous transgenes.

P11.064

Assessment of promoter DNA methylation for an entire family of laminin-encoding genes in normal and tumorous breast tissues

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Laminins are key components of the extracellular matrix playing important roles in morphogenesis of breast tissues and involved in normal development and pathological processes. We evaluated methylation status for 12 promoter regions of laminin-encoding genes in 106 samples of breast cancer, 106 paired adjacent nonmalignant samples, 4 samples of normal mammary gland from autopsy, 30 samples of peripheral blood and 7 samples of buccal epithelium of healthy donors.

Promoters of 6 genes, *LAMA1, LAMA2, LAMA3B, LAMA4, LAMB1*and *LAMC3* have demonstrated abnormal methylation in 2% to 42% samples of breast cancer and/or adjacent tissues.

Promoters of the *LAMA3A, LAMB2, LAMB3* genes were constitutively methylated in breast tissues as well as in normal lymphocytes and buccal swabs, and of the *LAMC2* in breast tissues only. Our findings contradict previously reported abnormal methylation of laminin-5-encoding genes *LAMA3A, LAMB3* and *LAMC2* in 44%, 4%, and 20% of breast tumors respectively against the background of next to never methylated nonmalignant breast tissues, normal lymphocytes and buccal swabs (Sathyanarayana et al., 2003). Our results are supported by recently published Reduced-Representation-Bisulfite-Sequencing and our own bisulfite sequencing data. Constitutive methylation of laminin-5-encoding genes abandons its use as a marker of the pathological process and requires revision of the mechanisms of disregulation of these genes in cancer.

This study is among the first to evaluate promoter methylation for the whole family of cancer-related genes.

	Methylation in breast cancer and/or adj. nonmalignant samples (%)	Methylation in normal mammar gland from autopsy (%)	Methylation In buccal epithelium (%)	Methylation In peripheral Blood (%)
Genes constitutively methyl. in breast tissue				
LAMA3A	100	100	100	100
LAMB3	100	100	100	100
LAMB2	100	100	100	100
LAMC2	100	100	0	0
Genes abnormally methyl. in breast cancer				
LAMA1	35	0	0	0
LAMA2	42	0	0	0
LAMA3B	6	0	0	0
LAMA4	2	0	0	0
LAMB1	16	0	0	0
LAMC3	8	0	0	0
Genes unmethyl. in breast cancer				
LAMA5	0	0	0	0
LAMC1	0	0	0	0

P11.065

New non-invasive test for Limb Girdle Muscle Dystrophies type 2

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One over 8000 persons worldwide are effected by inherited or acquired neuromuscular diseases (NMD) presenting with muscle weakness, skeletal deformities, early loss of functions and mortality. The most common type of NMD is muscular dystrophies, which also have the most unfavourable outcome. Diagnostic pathway requires invasive muscle biopsy, later molecular confirmation.

Aim of the study was to develop non-invasive DNA based test for the common Limb Girdle muscle dystrophies (LGMD) mutations.

Materials and methods. Twenty patients with symptoms of LGMD without muscle biopsy data were recruited in various NMD centres. Illumina GoldenGate technology was applied for the 82 selected mutations in DYSF (35 sequence variations), CAPN3 (28), SGCA (8), SGCB (4), SGCD (3) and SGCG (4) genes.

Results. LGMD diagnosis was confirmed in six persons (30%) with applied DNA diagnostics technique. CAPN3 gene mutation 550delA were identified in 59 % of identified mutations, 2184G>A in 6 %, 664G>A in 5%, DYSF 2372C>G in 12 %, 1566C>G in 12 %, 1368C>A in 6%.

Conclusions. 550delA mutation in the CAPN3 gene is considered as a Slavic founder mutation, and it is frequently met in LGMD2A patients from Eastern Europe.

Non invasive DNA test is advisable for patients with LGMD prior muscle biopsy.

P11.066

Limb Girdle muscle dystrophies mutation analysis using Illumina's VeraCode GoldenGate Genotyping Assay

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Limb-girdle muscular dystrophies (LGMDs) are a group of muscular dystrophies characterized by a predominant involvement of the scapula, pelvic girdle and trunk muscles without affecting the facial muscles. Different autosomal recessive LGMDs (>10) have been identified as distinct entities with a similar phenotype and clear clinical overlap that makes their differential diagnosis difficult. To date, several hundreds different mutations have been described with their biological relevance remain unclear.

We have developed genotyping analysis of 96 mutations - insertions/deletions and SNP, within different genes related to LGMDs (SGCA, SGCB, SGCD, CAPN3, DYSF, several more mutations, and 4 control SNP within X/Y chromosomes) using Illumina's VeraCode GoldenGate Genotyping Assay.

Here we report study analysing a group of 107 unrelated Latvian controls with no sign of neuromuscular diseases matching general Latvian population by gender and nationality with our developed assay. 31 mutations had minor allele frequency (MAF) higher that 0.01 (0.014 - 0.294) suggesting no confirmation of their pathological effect.

Our data suggest that miscellaneous mutations found in LGMD patients and described as pathological need to be studied more intensively in terms of general population to clarify their effect.

P11.067

VarioML micro-framework for comprehensive variation data representation and exchange

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The VarioML micro-framework is a set of tools and practices improving the availability, quality, and comprehensivity of human variant information. It enables researchers and clinics to share that information with ease, clarity, and without ambiguity. Sharing of variation data is a critical need, yet attempts to meet this need have come up against difficulties at each level of design and implementation. Variant information can be arbitrarily complex, making a single standard vocabulary elusive and re-formatting difficult. Complex standards have proven too time-consuming for clinicians to implement.

The GEN2PHEN project addressed these difficulties by developing a comprehensive data model for capturing biomedical observations, Observ-OM, and building the VarioML micro-framework around it. VarioML pairs a simplified open specification for describing variants, with a toolkit for adapting the specification into one's own research or clinical workflow. Straightforward variant data can be captured, federated, and exchanged with no overhead; more complex data can be described, without loss of compatibility. The open specification enables push-button submission to gene variant databases (LSDB's) e.g., LOVD (http://lovd.org), using the free Cafe Variome data publishing service (http://cafevariome.org/), while the micro-framework bidirectionally transforms data between semantic and web-application code formats, opening up new possibilities for open source web applications building on shared data. Semantic vocabularies and translation schemas allow variant data to be part of the semantic web. A JAVA implementation toolkit makes VarioML easily integrated into biomedical applications.

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P11.068

Genomic organization of macrosatellite repeats

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Macrosatellite repeats (MSRs) comprise a significant proportion of the human genome, usually spanning hundreds of kilobases of genomic DNA. Because of their highly polymorphic nature, MSRs represent an extreme example of copy number variation, but their structure and function is largely understudied. This study comprises the genetic analysis of autosomal and X chromosomal MSRs of HapMap individuals representing Caucasian, Asian and African populations. Copy number variation, repeat stability and genetic heterogeneity of the autosomal macrosatellite repeats RS447 (chromosome 4p), MSR5p (5p), FLJ40296 (13q), RNU2 (17q) and D4Z4 (4q and 10q) and X chromosomal DXZ4 and CT47 were investigated. Repeat array size distributions show that these MSRs are indeed highly polymorphic with the highest genetic variation among Africans and the least among Asians. A mitotic mutation rate of 0.4-1.5% was observed, exceeding meiotic mutation rates and explaining the high size variability found for these MSRs. Rather than a uniform size distribution we observed a multimodal size distribution in seven MSRs using a Bayesian approach for the estimation, where for five of these MSRs the posterior probability was larger than 99.33%. In three of these five MSRs equidistant intervals between the modes were very likely as well, suggesting that MSR sizes are restricted and that MSRs are possibly organized into higher order chromatin structures. This study represents the first comprehensive study of MSRs in world populations identifying novel commonalities and differences in the organization and function of the human genome.

P11.069
Intellectually disabled children with normal molecular karyotypes: Genome-wide screening for altered methylation

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The aim of this study was to evaluate the impact of epigenetic alteration for intellectual disability (ID). We evaluated 81 clinically well characterised patients with ID. Most of them showed in addition muscular hypotonia, short stature, obesity or epilepsy. In these patients a monogenetic disorder could clinically not be diagnosed. All showed normal results on ArrayCGH (105K or 244K Agilent Array). Epigenetic alterations were analysed using the Illumina HumanMethylation450K Bead Chip, covering more than 485.000 CpG sites. The control group consisted of age and sex matched patients mainly affected by recurrent upper airway infections.

The analysis for differences in methylated loci between the patient and control group revealed 266 differentially methylated loci which were enriched for HLA, the interferon gamma-pathway, and antigen presentation. As this global comparison of both groups is not suited to detect private DNA methylation aberrations we are currently investigating local enrichment of DNA methylation changes aiming to identify methylation changes over several consecutive CpGs being typical e.g. for epimutations at imprinted loci. This strategy is driven by the observation that we identified one proband in the patient group with hypomethylation in MEG3. This finding was corroborated by bisulfite pyrosequencing.

Our results show that imprinting disorders are still underdiagnosed and may have a possible impact on ID. This is in agreement with the results of (1) who analysed a comparable cohort of 90 patients for known imprinting disorders and found two patients with Silver-Russell- respectively Beckwith-Wiedemann syndrome.

(1)Poole et al., Am J Med Genet A. 2010;152A:1990-3.

P11.070
Molecular and cytogenetic screening of children with idiopathic mental retardation in Ukrainian population

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Mental retardation (MR) is a generalized term, which includes disorders of adaptive behavior associated with a lack of cognitive development. MR occurs in 2-3% of the general population. In the majority of MR individuals a specific cause can not be identified.

We represent the data of 113 patients from 96 Ukrainian families with well clinically characterized MR included in the CHERISH project (grant agreement n°223692). Preliminary analysis (metabolic investigation, karyotype, molecular tests of known syndromes, MLPA for subtelomeric rearrangements) have been done. We found 1 patient with FRAXE expansion and 2 patients with Prader-Willi Syndrome. In 57 patients with normal results or with complex chromosomal rearrangements search for cryptic chromosome rearrangements was carried out through 44K, 105K or 400K array-CGH analysis in partner laboratories in Bologna (Italy) and Nicosia (Cyprus). Quantitative Real Time PCR were used to confirm the array-CGH findings. In total, copy number variations (CNVs) in patients from 26 Ukrainian MR families were detected (size: from 11Kb to 24378 Kb). CNVs with potential clinical significance were found in 9 cases. These CNVs were characterized as pathogenic or probably pathogenic, based on their size, position and genes involved in.

The obtained results show the strong genetic heterogeneity of hereditary forms of MR in our group of patients. These results generally agree with those of previous array-CGH cohort studies, showing, however, previously unreported genomic rearrangements in MR and a higher incidence of clinically relevant CNVs.

P11.071
Mutation screening of new candidate genes for mental retardation by next generation sequencing

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Next-generation sequencing (NGS) technologies generate high throughput sequence data very rapidly at a lower cost by massively parallel sequencing of clonally amplified DNA molecules. NGS is anticipated to transition into clinical-diagnostics use by avoiding massive PCR preparation and sequencing of multiple large regions of interest simultaneously. For the successful transition streamlining of processes is required, especially sample preparation, coupled with improvements in technology robustness and characterization of accuracy through validation studies. The purpose of this study was to establish the amplicon-based NGS technology for screening novel mutations in mental retardation (MR) patients. In this pilot project, 'Universal Tailed' Amplicon sequencing method was used. The amplicon library preparation was optimized by using multiplex PCR. We performed mutation detection of complete coding regions of two genes (total 32 amplicons) implicated in MR in 40 individuals on a bench-top 454 GS Junior platform (Roche). The 10-hr run was able to generate approximately 72,000 reads and about 22 million high-quality bases at an average read length of 308 bp. Altogether, 12 sequence variants were found in this study with seven known SNPs and five novel changes. All the variants were confirmed by traditional Sanger sequencing providing evidence of NGS accuracy. In conclusion, next-generation amplicon sequencing with enhanced efficiency and accuracy can be used as an efficient tool for high sensitive mutation detection in large patient cohorts and complex phenotypes. We identified one mutation in these two genes that may potentially be involved in MR and need to be studied in larger patient samples.

P11.072
Methylation analysis of breast cancer in Cyprus and Slovenia

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The best-studied epigenetic alteration in cancer is DNA methylation. It has been demonstrated that during tumorigenesis methylation is usually decreased at a genome-wide level, with selective hypermethylation of CpG islands occurring within the promoter regions of a number of tumor-suppressor genes. This leads to transcriptional silencing of these genes and subsequent tumor progression. Analysis of abnormally methylated genes has received a lot of attention lately since it is a feature of most cancers and is speculated to play a role in cancer etiology. Within the scope a bilateral project between Cyprus and Slovenia we investigated quantitative methylation changes in twenty four tumor suppressor genes, with the ultimate goal of identifying novel biomarkers applicable to the management of breast cancer patients. Methylation study was performed on 100 matched normal and tumor paraffin-embedded breast tissues from Cyprus and Slovenia, using methylation-specific MLPA. The cumulative methylation index (CMI) was calculated as the sum of the percentage methylation for all genes. Mann-Whitney and Kruskal-Wallis tests were used for comparing medians between groups. The χ2-test was used for comparing proportions. Hierarchical clustering was applied using R and SPSS statistical packages. Our results showed promoter methylation of in a number of tumor suppressor genes. The prognostic value of promoter hypermethylation is currently being further evaluated by studying additional samples.

P11.073
Rapid and efficient mutation detection in the mitochondrial DNA using a bench-top next-generation DNA sequencer

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Mitochondria play an important role in essential cellular functions. Each eukaryotic cell contains hundreds of mitochondria with hundreds of mitochondrial genomes (mtDNA). Human mtDNA is a 16,569-kb circular, double-stranded molecule, which contains 37 genes: 2 rRNA genes, 22 tRNA genes, and 13 structural genes encoding subunits of the mitochondrial respiratory chain, where ATP is generated. Mutant and wild-type mtDNA may co-exist as heteroplasmy, and cause human disease with diverse and variable clinical features and a loose genotype-phenotype relationship. Next-generation sequencing (NGS) technologies can be a boon to human mutation detection given their high throughput: consequently, many genes and samples may be simultaneously studied with high coverage for accurate detection of heteroplasmy. In circumstances requiring the intensive study of a few genes, particularly in clinical applications, a rapid turn around is another desirable goal. To this end, we assessed the performance of the bench-top 454 GS Junior platform as an optimized solution for mutation detection by amplicon sequencing. The purpose of this protocol is to simultaneously determine mtDNA sequence and quantify the heteroplasmic level. This protocol includes two independent PCR amplifications of the entire mitochondrial genome. Resulting PCR products are then mixed at an equimolar ratio. Subsequently, samples of twelve individuals are then barcoded and sequenced with high-throughput, next-generation sequencing technology. A 10-hr run was able to generate ~72,000 reads and ~25 million high-quality bases at an average read length of 348 bp. This technology is highly sensitive, specific, and accurate in determining mtDNA mutations and the level of heteroplasmy.

P11.074

Mitochondrial DNA analysis in the Genome of the Netherlands

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The Genome of the Netherlands (GoNL) is a national collaboration aimed at establishing a map of Dutch genetic variation by whole genome sequencing of 250 trio families consisting of unselected individuals of Caucasian origin. The unprecedented trio-based setup of this scale and the abundance of mtDNA in each sample give us the unique opportunity to study both population-wide and intra-human variation on the mitochondrial genome.

We developed a number of techniques in which mtDNA can assist in quality control of whole genome sequencing experiments. The high coverage (averaging ~1100x) enables us to easily detect

sample contamination with a low percentage of foreign DNA. One such case is present in our data set and its contamination has been confirmed by autosome analysis. By looking for violations of the inheritance pattern we readily identified sample swaps. Indeed, in two of the trios there had been a swap of the parents which was independently confirmed by immunochip data.

One of the goals of our mtDNA study is to refine the Dutch mtDNA phylogenetic tree. Preliminary results show that the data set contains more than 165 different haplogroups, where H and its subclades are most abundant, representing ~40% of individuals. This is in concordance with previous studies on the distribution of haplogroup H in Europe. 127 haplogroups are supported by at least 2 individuals, while 68 are supported by at least 4. Our samples disagree on defining polymorphisms for some haplogroups, indicating opportunities for refinement of the phylogenetic tree.

P11.075

A simplified Sanger sequencing workflow for mitochondrial variant detection provides high data quality

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Variations in the human mitochondrial genome are frequently used in forensic analyses, disease association research and human evolution. Because of the abundance of variations in mitochondrial DNA, either the complete mitochondrial genome or the critical region is sequenced. We present here a new PCR/sequencing workflow for mitochondrial DNA that uses capillary electrophoresis and that is integrated with data analysis and variant detection. This simplified re-sequencing workflow utilizes novel universal M13 sequencing primers for improved 5' sequence resolution, increased throughput, and reduced hands-on time. This workflow generates high quality bases from base 1 when using POP-7™ polymer comparable to data typically seen when using the considerably slower POP-6™ polymer and standard Sanger sequencing chemistry. In addition to improved 5' data quality the new workflow eliminates the need for a separate PCR clean-up step. Taken together these improvements reduce the entire workflow from PCR to finished sequence data to under 5 hours, compared to approximately 8 hours for the standard workflow. The sequencing output is analyzed with Variant Reporter® Software that applies quality control metrics, including the use of Quality Values for DNA trace values and confidence values for variant validity. We will present examples to demonstrate this mitochondrial re-sequencing workflow for variant detection.
Research Use Only

P11.076

Changes of DNA mismatch repair MLH1 promoter methylation and expression in DMT2 patients after a dietary intervention

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The genotoxic effects caused by reactive oxygen species (ROS) are among the most common DNA damage causes. Oxidative stress may lead to an increased level of unrepaired cellular DNA damages, discussed for tumor initiation. Mismatch repair (MMR) enzymes act as proofreading complexes that maintain the genomic integrity. MMR-deficient cells show an increased mutation rate. A diet containing a high amount of antioxidants lowers the potential harmful effects of ROS. The MutL homolog 1 (MLH1) gene belongs to the MMR complex.

Objectives: The influence of an antioxidant and vitamin rich diet on the epigenetic pattern of MLH1 was analyzed in non-insulin depended diabetes Mellitus type 2 (NIDDM2), impaired fasting glucose (IFG) patients and a lean control (LC) group.

Methods: CpG methylation of the MLH1 and MGMT promoter region was analyzed by pyrosequencing. Gene expression of MLH1 was measured by quantitative RT-RCR cDNA. DNA integrity was evaluated by COMET Assay.

Results and Discussion: The region on the forward strand of the MLH1 had higher methylation level in both intervention groups ($p<0.05$) after the intervention. The expression of the MLH1 gene decreased ($p<0.15$) in both groups. IFG and NIDDM2 groups showed makeable differences. The COMET Assay data suggests that the DNA stability was increased and a correlation between the expression of MLH1 and the DNA damage was found in the IFG group ($p<0.05$). The down-regulation of the MLH1 expression correlating with the observed increased CpG promoter methylation might reflect a lower MMR requirement by the higher DNA stability following the dietary intervention.

P11.077

A DNA resequencing array for genes involved in MODY/Type 2 Diabetes: a new era in clinical and molecular diagnosis

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Genetic testing for MODY has become a routine procedure allowing to set up proper treatment and discriminate from type 2 diabetes (T2D) whose symptoms are often overlapping. Common variants implicated in risk of diabetes explain only a minor proportion of the familial clustering observed in sporadic cases. We apply targeted resequencing technology to detect both known and novel mutations on a single high-throughput platform. We until now resequenced 12 cases with clinical diagnosis of MODY/T2D for 46 genes involved in this disorder. All the detected variations were confirmed by direct sequencing and potential pathogenicity was assessed by functional predictions and frequency in controls. We identified modest associations with common variants and a trend suggestive of an overrepresentation of rare variants in cases compared to controls for several genes, although it is plausible that combinations of rare and/or common variants in genes already implicated might explain the genetic basis of the disease. Between detected variants, we focus on 11, of which 2 are double heterozygous and plausibly pathogenic. In addition, our data show that, at least in the Italian population, mutations in the *PDX1* are likely the second cause of MODY/T2D, and that many patients resistant to traditional therapies are double heterozygous for some genes individually associated with diabetes/hyperglycemia.Next-generation sequencing technologies are becoming the first tier for an immediate understanding of the molecular basis of most diseases. Altogether, these efforts should allow to better understand the disease pathogenesis, find new targets for clinical therapy, and allow prediction of disease.

P11.078

Mosaic Homozygosity Reporter: A tool to detect low levels of revertant mosaicism in SNP array data.

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Revertant mosaicism is a phenomenon that occurs when the replacement of a pathogenic allele by the wild type allele favors cell-survival. Especially within a tissue with high turnaround time, such as blood, this can lead to near complete replacement of the pathogenic allele by the wild type allele. The presence of two copies of one allele can be detected by genome-wide single nucleotide polymorphism (SNP) arrays. However, when stretches of homozygosity are present in a small percentage of cells, like in the early stage of revertant mosaicism, these are often missed by allelic algorithms.

Here we present Mosaic Homozygosity Reporter, a method that facilitates the detection of low levels of allelic imbalance using the genotyping data from SNP arrays. The method detects mosaic stretches of homozygosity with high sensitivity by comparing the signal distribution of heterozygous calls between telomeric regions.

We have applied this method on Affymetrix SNP6.0 array data from 19 individuals with an autosomal dominant form of Dyskeratosis Congenita (DC), a multisystem disorder. The Mosaic Homozygosity Reporter detected mosaic reversion in blood cells of six patients with high significance (P<7.9•10-25), two of which could not be detected by visual inspection of the array's B-allele frequency plots. These data show that revertant mosaicism may be a common event in autosomal dominant DC and that Mosaic Homozygosity Reporter is able to detect low level mosaic stretches making it a valuable tool for the reanalysis of previously unsolved clinical cases where mosaic homozygosity may play a role.

P11.079

Searching for Multiple sclerosis genomic candidate regions by genome-wide synthesis of heterogeneous data sources

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Multiple sclerosis (MS) is a debilitating autoimmune condition characterized by demyelination in central nervous system, leading to symptoms of severe motosensory neurological disturbances. Development of high -throughput technologies opened the possibility of scrutinizing complete profile of molecular alterations in MS. Inherent statistical issues of multiple testing and high false-positive rates have, however, hampered attempts to entirely elucidate molecular background.

To propagate discovery and increase detection specificity of these studies, we performed an integrative synthesis of data originating from heterogeneous sources of global molecular alterations in MS. Data for inclusion was collected from 39 studies or bioinformatic sources. Altogether, 158.520 distinct significant signals discovered on 16 different biological levels were included. Custom rank product prioritization approach based on genomic position of included results was utilized for data synthesis. Nested permutational cycling was employed to determine significant accumulation of most significant results discovered on most diverse biological levels.

In total, 381 genomic regions were characterized with significant accumulation of results, reaching local permutation p-value minima below 0.001. Follow-up characterization of selected regions revealed them to contain 409 genes of which 87 (21.3%) overlapped with those tracked by HuGENet disease-gene associations database, while a notable proportion have not been investigated in MS. This suggests that there exists a substantial body of genes, whose involvement in MS is suggested by evidence in a complex body of data from 'omic' studies, but focused studies of their direct role in MS have yet to be performed and they thus present plausible targets in further validation studies.

P11.080

Molecular characterization of human sialidase Neu4 gene promoter region

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Sialidase Neu4 is one the four mammalian sialidases. We characterized previously that human Neu4 sialidase has activity against sialic acid containing substrates including ganglioside GM2. Biological role of human sialidase Neu4 enzyme has been shown by the transfection of neuroglia cells from a Tay-Sachs patient that Neu4 clears accumulated GM2. It has been also shown that sialidase Neu4 enzyme is responsible for degradation of ganglioside GD1a in brains of Neu4 knock-out mice. To expore human sialidase Neu4 gene regulation, we aimed to determine minimal promoter region and identify several transcription factors. We used TESS (Transcription Element Seach System) tool to predict the sequence motifs. We amplified seven different DNA fragments from human Neu4 promoter region, cloned into luciferase expression vector and performed reporter assays. We used electrophoretic mobility shift assay to demonstrate binding of transcription factors to candidate promoter region. Human sialidase Neu4 gene has TATA-less promoter but contains CCAAT elements. Luciferase reporter assay demonstrated that 187 bp upstream of Neu4 gene is minimal promoter region which regulate transcription. Electrophoretic mobility shift assay showed that 187 bp upstream region recruits several transcription factors such as c-myc. Overall, our results show some of cis- and trans- acting factors involved in the human sialidase Neu4 gene regulation. The data we obtained might be useful to discover small molecules which control Neu4 gene expression. Selective high expression of sialidase Neu4 gene might be controlled using drugs or small molecules and the accumulated ganglioside GM2 in lysosomes of Tay-Sachs patients can be reduced.

P11.081

Development of a custom designed aCGH chip (Neuromuscular Chip) for investigation of Neuromuscular Disorders

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Neuromuscular disorders (NMDs) are a group of genetically determined diseases encompassing many conditions that impair muscle function (DMD/BMD, LGMD2A, Sarcoglycanopathies, etc). Similar phenotypes may be caused by mutations in many different genes and consequently molecular diagnosis can sometimes be inefficient. Array-based comparative genomic hybridization (aCGH) is a high-throughput technology for detecting copy-number variations (CNVs) in the human genome.

In order to address the NMDs diagnostic problem, a custom Agilent a-CGH oligonucleotide chip (SurePrint G3, 8x60k platform) was designed using the e-Array application. The chip covers a selection of genes most commonly involved in NMDs: *DMD* (>25,513 probes, every 90bp for exons and approximately150bp for introns), *LARGE* Congenital muscular dystrophy type 1D (MDC1D), (5963 probes) Sarcoglycanopathies & Limb Girdle Muscular Dystrophies: *SGCA* (17q21.33), *SGCB* (4q11), *SGCG*(13q12), *SGCD* (5q33.3) (4609 probes), *POMT1/ POMT2/ POMGNT1/ FKTN/ FKRP* (2681 probes), *UTRN, LMNA, EMD, TRIM32, MYL7, ACTA1* (7089 probes), *CAPN3* (1020 probes), *LGMD2A* and Caveolinopathies: CAV3 (141 probes).

The neuromuscular aCGH chip was evaluated by a retrospective analysis, using DNA samples from NMD patients with known deletions or duplications. The expected CNVs, in all previously characterized patients, were successfully confirmed. Additionally, new exonic and intronic CNVs were detected, which possibly contribute/ modify to the patient's phenotype. Clinical application of the custom-designed

for general diagnosis of NMD patients, can therefore, provide significant additional information on the molecular pathogenesis for NMDs. We are planning to add it to our diagnostic investigation for neuromuscular diseases.

P11.083

Accurate determination of the length of homonucleotide stretches by highly parallel sequencing

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The number of nucleotides in long homonucleotidestretches, especially when 7 or more nucleotides long, cannot be accurately determined by highly parallel sequencing based on pyrosequencing or Ion Semiconductor Sequencing. Most genes, however, harbor homonucleotidestretches in this size range. This pitfall prohibits implementation of these sequencing formats in routine genetic diagnostics.

We present a method in which the length of a long homonucleotidestretch is reduced to a series of shorter nucleotide stretches in which each of them can be accurately determined. The combined accurate analysis of the smaller homonucleotidestretches then allows accurate determination of the length of the original longer homonucleotidestretch. The reduction in size of the homonucleotidestretch can be achieved by PCR, which we call homonucleotide-stretch-reduction-PCR (hnr-PCR). An hnr-primer is used, which is complementary against the region of the homonucleotidestretch, which extends in the homonucleotidestretch but not until its end, and which contains at least one non-complementary nucleotide. Since 100% complementary of a primer is not needed to allow DNA synthesis, as long as its 3' end is complementary against its target, non-complementary nucleotides can indeed be incorporated at certain positions of a primer. When a 5'adapter sequence is added to the primers used for hnr-PCR, and a different 5'adapter sequence is added to the primers used for standard PCR, the combined analysis of the standard amplicon and the hnr-amplicon against a given region then concludes the actual sequence. In this way, a 'one-stop'genetic test is obtained for these sequencing formats.

P11.084

Storing and sharing NGS variant data and phenotype information in web-based LOVD3 gene variant databases

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Exome and genome sequencing studies currently flood us with sequence variants. The free, open-source, platform-independent Leiden Open-source Variation Database software (LOVD, http://www.LOVD.nl) was developed to build standardized databases for curating and sharing gene variants. To cope with the new demands posed by exome/genome studies we have developed a new version of the LOVD platform. LOVD3 is compatible with the Gen2Phen data model, implemented with additional tables for phenotype, screening and transcript information. Genome-wide sequence variant data can be stored in a single LOVD installation using chromosomal nucleotide positions as reference. Web services retrieve gene and transcript information on the fly. Data from exome/genome studies can be stored and displayed in several ways: variant-by-variant or all connected to one patient. To promote early release of exome data, both phenotypes screened but unresolved and variants not-excluded from being causative can be stored (and identified) individually. Data can be public and non-public for both with the option to query. This leaves submitters in control of the data, ensuring that they will be contacted to obtain essential information. Finally LOVD3 supports a new access level, "collaborator", allowing collaborating groups to share otherwise non-public data. Other new features include: display of disease-specific phenotype information, storage of temporal phenotype information, improved interface and speed, and queries in and across data columns. Web services will be developed to communicate between LOVD installations as well as to allow database queries. Funded by the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement nº 200754 - the GEN2PHEN project.

P11.085

Quality control of DNA from formalin-fixed paraffin-embedded and fresh-frozen tissues prior to target-enrichment and next generation sequencing

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There are over 400 million formalin-fixed paraffin-embedded (FFPE) tissue samples archived in biobanks worldwide. These diseased and normal tissue collections are valuable resources for molecular genetic studies. However, the challenges of DNA extraction from FFPE tissues, including formaldehyde cross-linking, degradation, and mixtures of single-stranded and double-stranded DNA, result in low amounts of usable high quality material for downstream assays. Hence, assessing the quality of samples to be processed for highly sensitive and costly applications, such as next generation sequencing, becomes a critical consideration. On-chip and automated electrophoretic devices were evaluated for the characterization of FFPE and fresh-frozen DNA samples prior to and during target-enrichment and next generation sequencing workflows.

P11.086

A new horizon - assessment of the GnuBIO next gen clinical sequencing platform

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Next generation sequencing is poised to revolutionize medicine. Current platforms have been optimized for the research market; however, many challenges remain that prevent the technology from being widely used in clinical settings. Target enrichment and sample preparation are laborious and require highly technical personnel. In addition, data analysis is costly and time consuming. These challenges are compounded by high per sample cost, and a need for sample batching to minimize these costs.

GnuBIO's first generation sequencer is a fully integrated platform requiring only raw genomic DNA as starting material. Enrichment for hundreds to thousands of PCR targets occurs on board followed by data analysis and variant calling. Run times are short, e.g. a 500 PCR target run is estimated to take 3 hours. This is the first attempt to highly automate the next gen sequencing process from gDNA to data analysis with a fast turn-around-time while offering high accuracy and long reads in a single product.

The City of Hope and GnuBIO will present results that for the first time demonstrate the detection of unknown mutations in a blinded cohort of clinical patient samples in p53 cancer gene sequenced on the GnuBio platform. The depth of coverage realized from these analyses, combined with the accuracy, turn-around-time, and ease of sample handling, provides the potential framework to make routine clinical diagnostics a reality with the GnuBIO instrument.

P11.087

Sample Quality Control within various Next Generation Sequencing workflows using the new Agilent 2200 TapeStation System

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This study evaluates the performance of the 2200 TapeStation System in various Next Generation Sequencing (NGS) workflows. Numerous sample types were checked for quality at different stages of various NGS protocols including pre- and post-shearing, post adaptor ligation as well as pre-hybridisation and post-hybridisation within the SureSelect target enrichment workflow. The data shows that this new automated electrophoresis system provides qualitative information that enables informed decision making in all downstream steps. By providing a range of ScreenTape consumables with standard and high sensitivities along with a tailored analysis package, the system is able to QC gDNA, fragmented DNA, whole genome libraries and target enriched libraries, presenting descriptive analyses at each stage for multiple sequencer protocols. The data described here demonstrates that the 2200 TapeStation System has the range, sensitivity, precision and accuracy to meticulously QC samples within the NGS workflow.

P11.088

Direct in-flowchip isothermal amplification of sequencing libraries at ultra-high density for next-generation sequencing

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We have developed a new nucleic-acid template-preparation methodology, called "WildFire", where sequencing libraries are *in-situ* amplified directly in-the-flowchip; no cycling-steps, no sequencing beads, no material exchanges. Sequencing libraries are added directly to the 5500-series Genetic Analyzer flowchip, whose surfaces have been coated with a special library-adaptor capture oligonucleotide. A DNA polymerase reaction mix is added, and in a single isothermal step lasting ~ 30 minutes, individual nucleic acid fragments are amplified *in-situ* on the flowchip. The net density of sequencing-colonies created in this manner far exceeds anything currently utilized in next-generation sequencing, reaching ~ 1 million colonies per mm^2 per flowchip surface (~ 2 million colonies / mm^2 optical viewing surface). During *in-situ* amplification, the capture oligonucleotide is "consumed", and each individual nucleic-acid fragment "spreads" inside the flowchip until reaching an adjacent library fragment(s). When the individually-growing fragments "meet", the amplification step terminates, because all of the surface-bound primer was consumed. These "self-assembled", spatially resolved, monoclonal colonies, are then sequenced-by-ligation. The resulting colony-sequencing reads maintain the same high accuracy as our bead-based method. Ligase-reactions also appear to be more efficiently completed on sequencing colonies (vs. sequencing beads), with the overall chemistry cycle-time decreasing (~ 1.5X) and the read-lengths increasing (towards 100 bps). Full genomes (bacterial to human), exomes (human), and transcriptomes (human) have now been sequenced using WildFire technology (detailed statistics will be presented). WildFire technology greatly improves NGS workflow (*in-situ* amp), increases throughput (via ultra-high density colonies), and significantly decreases net cost-per-genome (elimination of costly template preparation steps).

P11.089

Similar nucleosome occupancy pattern of NF-Y histone substitute family proteins, in human embryonic stem cells and induced pluripotent cells

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Introduction

Pluripotent ES and iPS cells are indebted their unique properties like their wonderful developmental plasticity to unique structure of their chromatin. The dynamics of this structure is regulated by a variety of complex pro-

cesses such as use of histone substitutes. Transcription factor NF (Nuclear Factor)-Y, is a histone substitute family protein that specifically recognizes the CCAAT box present in the promoter region of many constitutive, inducible, and cell-cycle-dependent eukaryotic genes and plays a key role during eukaryotic development. As induced pluripotent cells show many similarities to embryonic stem (ES) cells also because of their excellent potential to study developmental mechanisms and human disease in addition to new hopes to use of them for regenerative medicine, vast and stringent comparison of them to replacement of ESC by iPSC may resolve ethics limitations.
Material and Methods
In this experiment total level of NFY-A, B and C of a few human cell lines including ESC and iPSC as pluripotent cells, and differentiated ESC and fibroblast cells as differentiated lines was evaluated by the method of nucleosome ELISA (Nu-ELISA) to compare their epiproteome signature.
Results
Results showed remarkable similarities in nucleosome occupancy pattern of NFY-A, B and C in ESC and iPS cells and lower levels in comparison to differentiated ESC and fibroblast.
Conclusion
Current finding implies the dynamic epigenetic role of NF-Y family members in gene regulation involved in development and differentiation.

P11.090

Establishment of a core unit laboratory for ultra deep sequencing

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With continued advances in sequencing technologies, next-generation sequencing has been successfully used in a variety of targeted and genome-wide analysis techniques to uncover the genetic basis of a number of Mendelian diseases. The analysis of the genome-wide coding region (exome) is now economically feasible and quickly accomplishable.
We established a core unit for ultra-deep sequencing equipped with two systems, a SOLiD-4 and its descendant SOLiD-5500XL (Life Technologies). We are using both with appropriate automation for library and templated bead preparation. The main application is the whole exome sequencing approach with single samples as well as index-parents trios using the Agilent-SureSelect 50Mb-Kit for the exome enrichment. The data analysis pipeline for read mapping and variant calling starts with LifeScope 2.5 (Life Technologies) running on a cluster in the university high performance-computing group (HPC). Subsequently the usage of publicly available annotation tools like ANNOVAR are simplified for end user with own scripting solutions. A commercial software NextGENe (Softgenetics) may also be used by end users for variant calling and annotation. Up to now, we sequenced 135 whole exomes, (86 SOLiD-4 and 49 SOLiD-5500XL). The transition to the newer system resulted in a significant higher coverage at same sequencing parameters (SOLiD-4 54 - 61, SOLiD-5500XL 111 - 114). In particular the detection of homozygous variants in single samples with a recessive model and *de novo* variants in trios with a dominant model was very successful in different projects. More than half of the samples of both models recovered straightaway functionally meaningful candidate variants.

P11.091

The $1000 exome dilemma: expectations and facts

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The rapid evolution of Next-Generation Sequencing platforms is transforming today's medical research and diagnostics. Currently, the combination of exome capture enrichment systems and high-throughput sequencing techniques is the method of choice for genomic research in Mendelian disorders and is becoming more popular for genetic diagnostics purposes as the cost per base decreases.
At present, many institutions, both public and private, offer exome analysis services at a very attractive price which can vary in price tremendously from one site to another. Frequently, users with not much expertise in either NGS sample preparation issues or bioinformatics analysis approaches use price as the key selection criteria overlooking other important issues that are critical for the achievement of reliable results.
Here we report the results of the exome sequencing and analysis of a Human controlled cell line, previously sequenced by the HapMap project, that was sent to three different anonymous sequencing sites that offered the "$1000 exome sequencing service" in order to provide an overview of what one can actually expect from such deal and how differences on sequencing data quality, exome capture enrichment systems and bioinformatics analyses influence on the final results.

P11.093

Rapidly profiling thousands of large non-coding RNAs from nanogram amounts of total RNA using a single microarray design

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Large intergenic non-coding RNAs (lincRNAs) are emerging as key regulators of diverse cellular processes. As researchers face the challenge of investigating the function of lincRNAs, there is a need for tools that can accurately and rapidly measure the expression of both lincRNAs and mRNAs simultaneously. A catalog of more than 8,000 human lincRNAs was recently annotated from more than 4 billion RNA-Seq reads across 24 tissues and cell types by scientists at the Broad Institute of MIT and Harvard. Using this new catalog of lincRNAs we have updated the content of the Human SurePrint G3 microarrays to enable systematic profiling and simultaneous detection of coding and non-coding gene expression from a single sample. We used low nanogram amounts of total RNA from matched tumor and adjacent normal tissues to detect both large and subtle differences in gene expression profiles that were consistent with the current literature. GeneSpring GX software rapidly identified differentially expressed lincRNAs and protein-coding RNAs resulting in expression measurements from total RNA in less than two days. Microarray data demonstrated good reproducibility, wide dynamic ranges and high sensitivity, and the tumor versus normal ratios generated from the microarrays showed high correlation with ratios generated from whole transcriptome sequencing of the same matched RNA samples. In this study we demonstrate that microarrays can provide accurate differential expression measurements of both protein coding and non-coding RNA from very low amounts of total RNA rapidly providing expression data that is equivalent to whole transcriptome sequencing measurements.

P11.094

MicroRNA expression profiling meta-analysis identifies pathways associated with lung cancer development and reveals potential therapeutic drug targets

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The development of non-small cell lung cancer (NSCLC) involves a strong enviromental component and alteration of many genes and pathways. MicroRNAs, short non-coding RNAs are important gene regulators on post-transcriptional level and aberrations in their expression are often directly linked to tumorigenesis and disease outcome. During recent years, a number of studies have reported microRNA expression changes in NSCLC. Several platforms with different number of detectable microRNAs have been used for these profiling studies, causing the variance in the results due to the differences in methods of detection and data analysis.
To identify microRNA drivers in lung cancer, we performed comprehensive meta-analysis of 16 available miRNA expression profiling studies using a novel Robust Rank Aggregation method. As a result, we identified a signature of four significantly over- and seven under-expressed microRNAs. To assess the biological function of identified miRNAs, we used several target prediction algorithms and gene enrichment tests to identify possibly perturbed biological processes and pathways.
In conclusion, we have shown that our approach is suitable and effective for meta-analysis of gene expression studies in case when different technological platforms have been used or raw data is unavailable. Our pathway analysis further confirms the link between aberrant regulation of miRNA expression and lung cancer.

P11.095

The importance of alignments in evaluating missense variants

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Many missense sequence variants have been implicated in human disease phenotypes. However, in the absence of functional assays, the related pathogenicity of many variants remains unclassified. A number of in silico tools have been developed to predict the effect of missense variants. In combination with other evidence, these tools are used routinely by diagnostic labs to advise clinicians of disease likelihood. However, errors may arise through uninformed choice of tools and use of inappropriate parameters which may compromise their accuracy.

We have benchmarked the predictions from a range of algorithms on a set of genes with well characterised missense variants. We find that prediction success is highly gene-specific and that different algorithms perform optimally with different genes. Prediction algorithms based on multiple sequence alignments (MSAs) can be sensitive to the quality of the alignments used. In order to satisfy statistical considerations, certain levels of sequence divergence are required and carefully curated alignment use is recommended. In this respect, we also assessed the impact of alignment quality on these algorithms and find that the majority of possible orthologue alignments do not affect prediction success greatly when assessing a set of variants. However, when assessing individual variants, prediction success can vary with alignment quality and diversity in MSAs is an important consideration to ensure optimum algorithm performance.

We suggest that provision of benchmark data for different algorithms, and of standard MSAs, will allow the optimal use of missense prediction tools.

P11.096

RNA analysis, a diagnostic tool

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DNA sequence variants of uncertain significance (VUSs) is a common challenge in all types of genetic testing. Sequence variants located all over the exons and introns have the potential to disrupt proper splicing of mRNA, either by disrupting a normal splice site, by creating a *de novo* splice site or by disturbing the interaction of splice regulatory factors.

In order to reveal the effect of VUSs identified by DNA sequencing, we have offered RNA analysis for genes expressed in blood and with identified sequence variants which might cause aberrant splicing. These are sequence variants located within the normal splice sites, synonymous variants and intron variants which are predicted to create *de novo* splice sites. In the poster we will present the method of choice and some examples of results.

RNA analysis is a simple and straightforward tool to increase the knowledge about the effect of sequence variants identified by DNA sequencing.

P11.097

Enabling next-generation knowledge integration for human genetics

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It is widely accepted that information overload, in terms both of the growing volumes of biomedical data and of the associated literature, is making it increasingly difficult for researchers to keep pace. We present 3DM, a suite providing information systems for protein families, relieving many of the burdens that researchers face in dealing with the growing amounts and complexity of biomedical data. For each protein family a large amount of information that is extracted from protein structures, alignments and scientific literature, among others, is available. All this information is integrated, validated, and can be analysed and interacted via a number of methods. As a response to the amount of scientific literature outgrowing researchers' ability to keep pace, we have developed software that can place this integrated data and information from 3DM systems in the context of full-text PDF articles. Users are able to jump seamlessly from a particular paper both to its underlying data and to other related information and literature at the click of a button, thereby allowing readers to extract more knowledge more swiftly from the literature.

Having many heterogeneous data types readily available in an integrated and validated fashion can greatly speed up research, and a wide variety of questions can be answered when such protein-related data from many different origins can be flexibly combined. As an example of particular interest to the human genetics community, by intelligently combining all this heterogeneous information the system is able to provide state-of-the-art predictions about the effects of genetic variations.

P11.098

Comparative Analysis of Strand-Specific RNA Sequencing Approaches

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Standard RNA-sequencing approaches generally require double-stranded cDNA Synthesis, which erases RNA strand information. Synthesis of a randomly primed double-stranded cDNA followed by addition of adaptors for next-generation sequencing leads to the loss of information about which strand was present in the original mRNA template. The polarity of the transcript is important for correct annotation of novel genes, identification of antisense transcripts with potential regulatory roles, and for correct determination of gene expression levels in the presence of antisense transcripts. This work investigates the performance of different strategies for directional RNA-Seq using multiple next generation sequencing platforms. Here, we examine the effect of different RNA fragmentation methods (divalent cations plus heat versus enzymatic fragmentation). We provide a comparative analysis (library complexity, continuity of gene coverage, strand specificity and 3'and 5'-end bias analysis) of strand-specific RNA methods.

P11.099

GeneTalk: an expert exchange platform for assessing rare sequence variants in personal genomes

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Next-generation sequencing (NGS) has become a powerful tool in personalized medicine. Exomes or even whole genomes of patients suffering from rare diseases are screened for sequence variants. After filtering out common polymorphisms, the assessment and interpretation of detected personal variants in the clinical context is an often time consuming effort. We have developed GeneTalk, a web-based platform that serves as an expert exchange network for the assessment of personal and potentially disease relevant sequence variants. GeneTalk enables a locus centered knowledge management for genetic variants and may assist a clinical geneticist who is searching for information about specific mutations: Our platform gets a geneticist directly connected to other users with expertise for the sequence variant of interest. GeneTalk is available at www.gene-talk.de.

P11.100

The sensitivity of detecting heterozygous variants in next-generation sequencing data is increased by applying an allele frequency distribution derived from a stochastic branching process

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Next-generation sequencing (NGS) technology allows us to detect genetic variants on a genome-wide scale. A deeper knowledge about the allele distribution at heterozygous loci is essential for sensitive variant detection. We describe the pivotal steps of the library preparation before sequencing as a stochastic branching process and derive a mathematical framework for the expected distribution of alleles at heterozygous loci in a short read alignment. Based on technical replicates of human exomes we demonstrate that the variance of allele frequencies is higher than expected from a simple binomial distribution. As a consequence algorithms for mutation detection that apply this wrong prior distribution are less sensitive for variants that deviate strongly from the expected mean frequency. Due to the stochasticity inherent to the sample preparation we show that there is an upper bound for heterozygous variant detection even with increasing sequencing depth. Our results therefore indicate that technical replicates are an effective means in the reduction of error rates.

P11.101

Assessing the quality and the population background of high-dimensional human variant data sets from next-generation sequencing platforms using similarity metrics

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In reference based resequencing projects a list of all detected genetic variants represents an important intermediate step in bioinformatics data processing. Although scores are used to estimate the error probability of single variant calls, the heterogeneity of analysis pipelines as well as the high dimensionality of the data make it difficult to compare the quality of entire data sets. Here we describe a similarity metric that allows a distance-based quality assessment of human variant data on a genome wide scale irrespective of the platform it was generated on. Using the individuals of the 1000 genomes project as a high-quality reference set we are able to robustly identify the population background, answer questions about relatedness and estimate error rates in a data set. Our distance-based scoring system is accessible at www.gene-talk.de and may be applied in high-throughput sequencing laboratories for an easy means of quality control and routine consistency checks in sample workflow.

P11.102

Molecular diagnosis and genotype-phenotype studies in patients with osteogenesis imperfecta by next generation sequencing

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Background: Osteogenesis imperfecta (OI) is a heritable disorder of bone formation, which is characterized by bone fragility and low bone mass. As reported, autosomal dominant OI is associated with the mutations in *COL1A1* and *COL1A2* genes and autosomal recessive OI is caused by mutations in *CRTAP, LEPRE1* and *PPIB* genes.
Methods: In this study, exon-wide mutation analyses of *COL1A1, COL1A2, CRTAP, LEPRE1* and *PPIB* genes were performed by PCR and next-generation sequencing (NGS). One hundred unrelated patients and their family members diagnosed with OI clinically from Taiwan population were enrolled in this study.
Results: 46 patients had mutation in *COL1A1* gene, and 25 in *COL1A2* gene. The mutation detection rate of *COL1A1* and *COL1A2* genes was 71%. Furthermore, one patient was identified heterozygous mutations in *PPIB* gene. No mutation in *CRTAP* or *LEPRE1* gene was found. In total, there was an overall mutation identification rate of 72%.
Conclusion:To gain more insight into the mutational spectrum in Taiwanese patients with OI, we conducted this study to perform extensive exon-wide mutational analysis of *COL1A1, COL1A2, CRTAP, LEPRE1*and *PPIB* genes based on the high- throughput mutation scanning system with NGS. There are several hot mutation regions in *COL1A1* and *COL1A2* gene had been proposed. PCR followed by NGS is an alternative strategy for molecular diagnosis.

P11.103

A fish-specific transposable element shapes the repertoire of p53 target genes in zebrafish

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Transposable elements, as major components of most eukaryotic organisms' genomes, define their structural organization and are known to establish new cellular functions during evolution. Recent discoveries support the hypothesis that TEs can directly mediate gene regulatory novelties at the network level by contributing to transcriptional and post-transcriptional modulation of nearby genes. For instance, TEs participate to the origin of new functional elements, as promoter sequences, transcription factors binding sites and enhancer, silencer and insulator elements. For example, binding sites of the pleiotropic master transcription factor p53 reside in LINE1, Alu and LTR repeats in the human genome. Similarly, here we describe the first case of a mobile element shaping the repertoire of the p53 target genes in zebrafish (Danio rerio). Through their embedded p53 responsive elements, the multiple copies of the non-autonomous DNA transposon EnSpmN6_DR drive in several instances p53-dependent transcriptional modulation of the adjacent genes, whose human orthologs were often previously annotated as p53 targets. These transposons define a set of target genes that contribute to axonogenesis, synaptic transmission and the regulation of programmed cell death. Consistent with these biological functions, the EnSpmN6_DR-colonized loci are enriched for genes expressed in both adult and fetal brain. Our data corroborate the recent findings concerning the role of p53 in the regulation of neural stem cell development and pinpoint a remarkable example of convergent evolution: the exaptation of lineage-specific transposons to establish networks of p53-regulated genes crucially involved in neuronal morphogenesis in both a hominid and a teleost fish.

P11.104

Rare variants in TMEM132D contribute to the risk for panic disorder in a German sample

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Background: Genome-wide association studies have identified a large number of common variants associated with common diseases. Most variants, however, explain only a small proportion of the estimated heritability, suggesting that less common and rare variants might contribute to a larger extent to common diseases than assumed to date. Here we use next-generation sequencing to test whether such variants also contribute to the risk for anxiety disorders by re-sequencing 40 kb including all exons of the TMEM132D locus which we have previously shown to be associated with panic disorder and anxiety severity measures.
Methods: DNA from 300 patients suffering from anxiety disorders, mostly panic disorder (84.7 %), and 300 healthy controls was screened for the presence of genetic polymorphisms using the SOLiD 3+ next-generation sequencing platform (Life Technologies) in a pooled approach. Results were verified by individual re-genotyping.
Results: We identified a total 371 variants of which 247 had not been reported before, including 15 novel non-synonymous SNPs. The majority, 76 % of these variants had a minor allele frequency less than 5 %. While we did not identify additional common variants in TMEM132D associated with panic disorders, we observed an overrepresentation of rare functional variants in healthy controls as compared to cases.
Conclusions: Our data suggest that not only common but also rare variants within TMEM132D might contribute to the risk to develop anxiety disorders. In addition, the overrepresentation of rare functional variants in controls supports previous results associating an increased function of this gene with anxiety measures.

P11.105

Isolation and characterization of the RNA content of exosomes derived from blood and cell culture media using the Ion Torrent Personal Genome Machine (PGM™)

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Exosomes are small (30 - 120 nm) vesicles containing nucleic acid and protein cargo secreted by all cell types in culture. They are found in abundance in body fluids including blood, saliva, urine, breast milk. There is an exponentially growing interest to studying the function exosomes and utilizing them for diagnostics development. At the moment, the mechanism of exosome formation, the make up of the "cargo", biological pathways and resulting functions are poorly understood. There is an urgent need to further our understanding of microvesicles, and critical to this, is the development of reagents and tools for their isolation, characterization and manipulation. We will present data on (1) isolation of exosomes from blood and cell cultures, using modified ultracentrifugation and other protocols; (2) initial characterization of the RNA content from these exosome fractions using next generation sequencing.

P11.106

Enabling Whole Transcriptome RNA-Seq on the Ion Torrent PGM Sequencer

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As processes for the PGM continually improve, library construction has quikkly become one of the most apparent bottlenecks for the RNA-Seq pipeline. Addressing this obstacle is extremely important to enable the developing clinical sequencing market by improving speed of sample to sequence. Also, with the already vast number of stored FFPE samples in tissue banks and a growing impetus to study samples that may be rare or precious, a library method that can start with total, limited, and/or sheared RNA is necessary. Historically, RNA-seq libraries have started with microgram quantities of total RNA in order to perform a polyA selection or ribo minus depletion and make the sample useful for downstream applications. These processes remove up to 95% of the sample, the majority of which being unwanted rRNA and mtRNA. At Ambion, we have developed a library preparation method that can begin with a minimal amount of total RNA and selectively enrich for mRNA using a not-so-random (NSR) priming approach. We will detail the NSR priming strategies to build an RNA library kit for sequencing whole transcriptomes using the Ion Torrent PGM. We will show results from experiments optimizing hybridization parameters and workflows for library using less than 100ng total RNA, significantly reduce the representation of rRNA and mtRNA, and be performed in less than 4 hours. This will permit a clinician to build a library in less than half the time, at a reduced cost, using 1/100th the amount of starting RNA as compared to a ligation-based approach.

P11.107

Expert system PharmakoGen intended to increase efficacy of drug therapy

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Nowadays pharmacogenetics is a very rapidly developing field of knowledge which allows to predict therapeutical effect of different drugs and occurrence of pathologic medical reactions depending on genotype of the patient.

Every day more and more scientific articles are being published worldwide regarding this matter. So extensive data which include information on different genes, mutations, polymorphisms and drug-to-drug interactions cannot be stored in one's mind and that is why cannot be used by the doctors without applying for special literature and databases.

Our aim was to develop expert system for increasing efficacy of drug therapy depending on genotype of the patient. As a result of the project expert system PharmakoGen was created.

Expert system PharmakoGen is developed on the platform xGen IDS, successfully used for creation of diagnostic systems on hereditary neuromuscular diseases, eye illnesses, and chromosomal syndromes.

Our expert system includes following elements: the module of input of patient information, the knowledge base module, the module of results explanation, and finally the module of expert conclusion with special individual recommendations.

The system can be used by doctors of various specialities and will help to prescribe correct dosage of medicine and to prevent undesirable drug effects while treating patients.

Our system also can be helpful in forming the list of genes and polymorphisms which should be tested in patient before beginning of specific treatment.

Thus developed software will be interesting to the practicing doctors and will allow to optimize treatment of diseases depending on specific genetic features of the patient.

P11.108

The distribution of risk alleles for clopidogrel response (CYP2, PON1, ABCB1): pilot study in Czech patients with coronary stenting

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Clopidogrel - an inhibitor of platelet activation - is widely used in the prevention of atherothrombotic events in patients undergoing percutaneous coronary interventions (PCI) with stent implantation. Genetic variations in genes involved in the absorption (P-glycoprotein/ABCB1) and metabolism (CYP2C19, CYP2C9, PON1) of clopidogrel are thought to influence the response to the drug. The purpose of our study, therefore, was to characterize the distribution of alleles which are associated with altered response to clopidogrel in Czech patients after PCI.

A spectrum of risk genetic variants in genes CYP2C19, CYP2C9, PON1, ABCB1 were assessed in a cohort of 294 patients after PCI employing multiplex PCR analysis by MassArray technology (Sequenom).

Carriage of loss-of-function alleles, which may cause reduced conversion to active metabolite of clopidogrel was as follows: CYP2C19*2 27.9 % (2.4 % homozygotes), CYP2C9*3 16.0 % (0.7 %), PON1 192R 46.9 % (4.4 %). Carriage of CYP2C19*17 - the allele associated with ultra-rapid metabolism - was 42.5 % (4.5 %). Genetic variation of P-glycoprotein influencing drug absorption - ABCB1 3435TT genotype and ABCB1*2 allele (haplotype ABCB1 1236TT-2677TT-3435TT) - was found in 33.3 % and 21.8 % patients, respectively.

In conclusion, more than 40% of our PCI patients possessed alleles and/or their combinations, which have been reported to be associated with impaired efficacy of antiplatelet treatment and/or increased risk of cardiovascular complications. The evaluation of risk allele(s) genotyping on the rate of subsequent cardiovascular events in our PCI patients is under progress.

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P11.109

The human platelet transcriptome: RNA-seq reveals a greater complexity of protein-coding and non-coding transcripts than previously appreciated

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Platelets are essential to hemostasis and thrombosis. Having a complete understanding of the platelet transcriptome will give insight into the genetic basis of these disease traits ultimately leading to new methods for treatment. Genome-wide RNA expression studies using microarrays has provided novel insights to the platelet transcriptome. However, limitations of microarrays including the use of probes only to known transcripts and a limited dynamic range for quantifying very low and high levels of transcripts has not yet revealed the complete picture of the platelets transcriptome. To capture the complexity of all the transcripts in human platelets, we performed RNA sequencing (RNAseq) in leukocyte-depleted platelets derived from four healthy donors. The platelet whole transcriptomes for long and short RNA from total and ribosomal RNA-depleted samples were analyzed on the AB SOLiD platform. We estimate that there are ~9500 protein-coding genes expressed in platelets, 85 of which were validated by qRT-PCR. Many classes of non-coding RNAs were identified, including a larger number of miRNAs than previously appreciated, as well as pseudogenes and retroelements. Comparison of microarray results to the RNAseq revealed a significant correlation of well-expressed mRNAs, but RNAseq identified many more transcripts of lower abundance and permitted the discovery of novel transcripts. The RNAseq performed here revealed the complexity of the platelet transcriptome that may permit a better understanding of the molecular mechanisms that regulate platelet physiology and contribute to disorders of thrombosis and hemostasis.

P11.110

Guidelines for splicing analysis in molecular diagnosis derived from a set of 327 combined *in silico/in vitro* studies on *BRCA1* and *BRCA2* variants

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Assessing the impact of variants of unknown significance (VUS) on splicing is a key issue in molecular diagnosis. This impact can be predicted by *in silico* tools, but proper evaluation and user guidelines are lacking. To fill this gap, we embarked upon the largest *BRCA1* and *BRCA2* splice study to date by testing 272 VUSs (327 analyses) within the *BRCA* splice network of Unicancer. All these VUSs were analyzed by using six tools (Splice Site Prediction by Neural Network, Splice Site Finder, MaxEntScan, ESE Finder, Relative Enhancer and Silencer Classification by Unanimous Enrichment, Human Splicing Finder) and the predictions obtained were compared to transcript analysis results. Combining MaxEntScan and Splice Site Finder gave a 96% sensitivity and a 83% specificity for VUSs occurring in the vicinity of consensus splice sites, i.e. the surrounding 11 and 14 bases for the 5' and 3' sites, respectively. This study was also an opportunity to define guidelines for transcript analysis along with a tentative classification of splice variants. The guidelines drawn from this large series should be useful for the whole community, particularly in the context of growing sequencing capacities that require robust pipelines for variant interpretation.

P11.111

Targeting human *KIAA0649P* into mouse *Rb1*: Pseudogene integration is causative for genomic imprinting of *RB1*

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The human retinoblastoma gene, *RB1*, is imprinted. Gene expression is skewed in favour of the maternal allele. This is due to parent-of-origin specific DNA methylation on the truncated and inverted pseudogene *KIAA0649P*. This pseudogene evolved after integration into intron 2 of the *RB1* and in its present form harbors a new CpG island, CpG85, which serves as promoter for an alternative *RB1* transcript, transcript 2B. CpG85 is methylated on the maternal allele only and, as expected, expression of transcript 2B is restricted to the paternal allele. Transcription of the paternal transcript 2B interferes with transcription of the regular *RB1* transcript on the same allele.

Mouse *Rb1* does not contain *KIAA0649P* and is not imprinted. To determine if *KIAA0649P* is sufficient to result in skewed expression of *Rb1* we generated a knock-in of human *KIAA0649P* in intron 2 of the murine *Rb1* gene. To be able to distinguish expression of the two *Rb1* alleles, mouse embryonic stem (ES) cells were first modified such that they harbor a single nucleotide variant in exon 3 of *Rb1*.

In the generated ES cell lines we could show that integration of a second active promoter leads to transcriptional suppression of the targeted *Rb1* allele. In addition, we determined that the *Rb1* promoter and CpG85 are free of DNA methylation. Currently we determine if an alternative transcript 2B is expressed of CpG85.

At the present stage our analyses of genetically modified mouse ES cells provides evidence that the integration of a pseudogene can suppress gene expression.

P11.112

Amplicon based targeted resequencing analysis of PDE6A and PDE6B in a cohort of patients with a clinical diagnosis of Retinitis Pigmentosa using the Fluidigm 48.48 Access Array™ system and the Roche 454 GS FLX Next Generation Sequencing technology

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The genetic heterogeneity of Retinitis Pigmentosa hampers the identification of the underlying mutation in many cases. Current genetic diagnostics frequently takes several months or even years and, due to the need for investigation of many large genes, is very expensive. Moreover, there is a lack of data about mutations in low-prevalence disease genes due to the technological limits that have hampered comprehensive studies.

We aimed to find all possible disease-associated variants in coding sequences of the *PDE6A* and *PDE6B* genes in a large cohort of patients diagnosed with autosomal recessive Retinitis Pigmentosa (arRP) using a time- and cost-efficient next generation sequencing (NGS) approach.

Ninety-six patients with a clinical diagnosis of arRP were screened for mutations in the *PDE6A* and *PDE6B* genes using an amplicon based targeted resequencing approach. Target enrichment was performed using the Fluidigm 48.48 Access Array™. NGS was performed using the Roche 454 GS FLX technology. Identified variants were confirmed or rejected by Sanger sequencing.

We verified a total of 16 sequence variants in *PDE6A* and 14 variants in *PDE6B*, respectively. Five of these were clearly pathogenic because they fulfilled the criterion of homozygosity or compound heterozygosity and, moreover, have been previously described. The pathogenicity of the remaining variants remains unclear since they were only found in heterozygous state.

Due to the heterogeneity of Retinitis Pigmentosa, genetic diagnostics based on traditional Sanger sequencing is laborious and expensive. We found that the NGS approach is a time- and cost-efficient tool for screening low-prevalence disease genes in large cohorts.

P11.113

Time course RNA-seq: A potential avenue with somewhat different approach in tandem of differential analysis

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RNA-seq is exponentially becoming the de facto standard approach to compel considerable advantages over conventional technologies such as microarray by directly sequencing transcripts in gene expression profile. Thanks to the reduced cost to sequencing, studies to dynamic change of gene expression in a given biological system over time have shown steady growth over the past few years as microarray, however, statistical approaches to characterize dynamic temporal complexities are currently elusive. In differential gene expression analysis, as somehow limited but intuitive solutions, static differential expression methods without respect to time can be applied, which do not take into account the inherent dependencies in time series explicitly that the expression patterns at later stages are dependent on patterns at earlier stages. We present a statistical framework to define dynamic gene expression patterns over time using empirical trajectory index and Hidden Markov Model (HMM) approach in time series RNA-seq data, and our methods are validated through Markov Chain Monte Carlo (MCMC) simulation study in time dependent data. The utility of the proposed dynamic methods for temporal RNA-seq is demonstrated by application to the analyses of gene expression patterns in RNA-seq real data sets and MCMC simulation study in details.

P11.114

Long term investigation of gene expression variations in venous blood from healthy individuals

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Gene-expression profiles from human venous blood have been applied in diagnostic tests and laboratory settings. A new method to obtain these profiles is RNA-Seq, a high-throughput sequencing technique. This method outperforms hybridization-based array technologies in sensitivity and dynamic range and provides a digital readout of transcript levels. Thus, it creates a high resolution snapshot of the transcriptome. While reproducibility of replicates and technical biases of high-throughput sequencing has been extensively studied, within individual variability has not. By applying RNA-Seq, we will categorize intra-individual variations over a time span of 2 years and analyze the impact on diagnostics. We would like to confirm that sample extraction on a "bad-day" does not sicken a patient.

Human venous blood samples will be collected from 3 healthy volunteers on a day to day, week to week and month to month basis over 2 years. Multiplexed samples will be analyzed on the Illumina HiSeq system and prepared by 3 approaches. The first is designed to analyze only polyadenylated RNAs, the second to investigate rRNA depleted RNA and the third focuses on small non-coding RNAs. The sequence reads will be mapped with Tophat and analyzed at the (gene) transcript isoform level with tools as Cufflinks, edgeR and(or) DESeq.

Defining these limitations is a crucial step in applying RNA-Seq in diagnostics. Characterizing intra-individual transcriptome variations will enable diagnostics to determine the limitations of RNA-Seq's predictive power. This study will provide practical recommendations that will significantly improve the accuracy of RNA-Seq studies.

P11.115

A simple method for improving the limit of detection for capillary electrophoresis DNA sequencing - a comparison of methodologies for KRAS variant detection

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Fluorescent dye terminator Sanger sequencing (FTSS), with detection by automated capillary electrophoresis (CE), has long been regarded as the gold standard for variant detection. However, software analysis and base-calling algorithms used to detect mutations were largely optimized for resequencing applications in which different alleles were expected at heterozygous mixtures of 50%. Increasingly, the requirements for variant detection are an analytic sensitivity for minor alleles of <20%, in particular, when assessing the mutational status of heterogeneous tumor samples. Here we describe a simple modification to the FTSS workflow that improves the limit of detection of cell-line gDNA mixtures from 50-20% to 5% for G>A transitions and from 50-5% to 5% for G>C and G>T transversions. In addition, we use two different sample types to compare the limit of detection of sequence variants in codons 12 and 13 of the KRAS gene between Sanger sequencing and other methodologies including shifted termination assay (STA) detection, single-base extension (SBE), pyrosequencing (PS), high resolution melt (HRM), and real-time PCR (qPCR).

P11.116

Investigating levels of precision in gene expression measurement by digital PCR

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Gene expression studies profiling mRNA are central to biomolecular research offering considerable potential for research, diagnostics and prognostics. Gene expression measured by reverse transcriptase (RT) linked PCR, is commonly used to interpret the effects of specific signals both *in vitro* and *in vivo*, often on low copy transcripts extracted from tissues. The RT step, necessary to convert mRNA to cDNA, is widely accepted as both inefficient and imprecise, with studies reporting variabilities up to 17-fold. This study tested RT measurement variability of synthetic RNA transcripts (ERCC developed targets; European RNA Control Consortium) using RT digital (d) PCR evaluated through one-step reactions; where RT-dPCR is performed in a one-step (one reaction vessel) process rather than the standard two-step approach (independent reactions in different tubes for the RT and then PCR stages). dPCR applies single molecule amplification achieved by sample partition for absolute quantification. The measurement capability and reproducibility of RT to aid quantification of targets spiked into human cell-line derived total RNA was assessed. Furthermore, to compare measurement accuracy of RT-dPCR with more established DNA dPCR, six well-characterised ERCC targets of known concentration were evaluated, enabling assay bias assessment. Our results demonstrate RT reaction sensitivity is assay dependent, with different quantification determined for each target despite evaluation of equal copy numbers. This study is one of the first to demonstrate application of RT-dPCR for absolute quantification of low copy RNA targets. This approach allows RT precision, sensitivity and linearity to be monitored alongside reaction efficiency, facilitating accurate interpretation of gene expression data.

P11.117

Excess of novel nonsense mutations identified in putative susceptibility genes for schizophrenia and autism spectrum disorders

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Schizophrenia (SZ) and autism spectrum disorders (ASD) are complex genetic neurodevelopmental disorders that share certain phenotypes (e.g. cognitive deficits), and may share an underlying pathology due to shared genetic risk variants (e.g. already-identified NRXN1). This study involves next-generation sequencing of the exonic regions of 215 putative susceptibility genes in an Irish sample of 151 cases of ASD, 274 cases of SZ and 287 controls, to identify rare mutations contributing to one or both disorders. A multiplex target enrichment method combined DNA samples using indexes/barcodes followed by enrichment of exonic regions using Agilent SureSelect and paired-end sequencing on an Illumina GAII. Selected genes were categorised as: 1) NRXN1 and interactors, 2) Postsynaptic Glutamate Receptor Complexes (NMDA, mGluR5 and AMPA), 3) Neural cell adhesion molecules, 4) DISC1 and interactors, and 5) Functional and Positional Candidates. Analysis of 2,170 rare variants revealed an excess of nonsense mutations in cases (n=12) compared to controls (n=1; p=0.019). All nonsense mutations were novel and 3 were in DST (2xSZ, 1xControl). The other SZ mutations were in DLG5, FAT1, FYN, INADL, MACF1 and MYO16. Five of 7 SZ nonsense genes have NRXN1-related function. ASD mutations were in CNTNAP1, GRIP1, GRIN2B and NRG1. Rare ASD mutations have previously been reported for GRIP1 and GRIN2B. Analysis of all rare variants in the 11 nonsense genes identified an excess of cases carrying one or more mutations compared to controls (p=0.019). These results supply new supportive data for known ASD risk genes and identify putative new susceptibility genes for both disorders.

P11.118

Determination of methylation profile in patients with schizophrenia

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Schizophrenia is a severe chronic mental disorder that affects most of the higher brain functions. Its prevalence is estimated on about 1% worldwide. The classical symptoms occur in several other psychiatric disorders, which makes difficult the exact diagnosis. Schizophrenia is a serious social and economic healthcare problem. Currently, there is no etiological treatment.

DNA methylation is a major epigenetic modification. It is a biochemical process that is important for normal organismal development and cellular differentiation. DNA methylation stably alters the gene expression pattern in cells. This modification can be inherited through cell division.

Materials and methods: We analyzed age matched pools of 110 female schizophrenia patients and 110 female healthy controls. We've performed high-resolution genome-wide methylation array analysis (Agilent 1x244K). We've analyzed the methylation status of 27 800 CpG islands of both groups to identify methylation profile differences.

Results: Our experiments show significant difference in the methylation profile between patients and controls. In patients we observed significantly higher number of hypermethylated genes compared to healthy controls. In controls we established that hypomethylated genes predominate in comparison to schizophrenia patients.

Conclusions: Our data suggest that there is a major differences is methylation profile between patients and controls. This dysregulation can play a critical role in schizophrenia pathogenesis.

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P11.119

QCTool: an efficient toolkit to automatically generate quality metrics of next-generation sequencing data

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With the wide use of next-generation sequencing platforms and the increase in sample throughput, it is necessary to automate the determination of data quality metrics, not only for sanity check purposes but also to monitor the complex workflow of the sequencing process to locate, and eventually correct, possible errors. To facilitate this process in a large ongoing effort to study the whole genomes, exome and transcriptome of about 1500 Sardinians, we have developed „QCTool".

The software takes as input SAM/BAM files and produces, in addition to standard statistics as base and mapping qualities, reads-to-reference mismatch rate, and genome coverage, also several parameters useful for wet-lab quality validation such as PCR duplicates count, nucleotide relative content, and insert size distribution statistics. It is enriched with contig/chromosome specific breakdowns as well as per-cycle quality plots. Furthermore for exome sequencing, a simple command line option allows the parsing of targeted regions and the determination of quality statistics limited to such regions.

The package produces both PDF reports and an easily parsable file that can be integrated into LIMS systems or analysis tracking tools. For a direct use on presentations or papers, all plots are also generated in JPG format. The toolkit can be run stand-alone, just supplying the input file on a single core machine, or in a multicore fashion. Around 8Gb of memory are required for the complete analysis of a human genome aligned file, but deactivating some analysis options enables execution on desktop machines using only a few kilobytes of memory.

P11.120

Frequency of altered DNA methylation at imprinted loci in children born SGA

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Patients with imprinting disorders show a broad range of phenotypic variability. This leads to the hypothesis that imprinting defects might frequently be undetected in patients that share some, but not all typical symptoms being present in imprinting disorders, e.g. children born small for gestational age (SGA). Furthermore recent studies propose hypermethylation in IGF2R DMR2 to be enriched among patients with growth restriction. Within the BMBF funded German Network "Imprinting Disorders" we here performed quantitative DNA methylation analysis of 10 imprinted loci (PLAGL1, H19DMR, IGF2, GRB10, NDN, SNRPN, NESP, NESPAS, MEG3, IGF2R DMR2) by bisulfite pyrosequencing of 97 patients born SGA and 50 controls. For IGF2R DMR2 we additionally screened 95 parents of patients born SGA (47 parent pairs and one single mother). We established in one child the diagnosis of Temple syndrome most likely due to an epimutation. Furthermore five patients and six individuals from the SGA parent cohort displayed IGF2R DMR2 hypermethylation. Of these in two families IGF2R DMR2 hypermethylation was detected in the child and one parent. Five individuals in the control cohort displayed IGF2R DMR2 hypermethylation. We conclude that imprinting disorders may still be underdiagnosed and are a relevant differential diagnosis in children born SGA. Hypermethylation in IGF2R DMR2 is not enriched in our patient cohort. Lack of genotype-phenotype correlation in our and previous studies leads to the assumption that IGF2R DMR2 hypermethylation most likely represents an epigenetic polymorphism, however a Mendelian inheritance cannot be excluded from our observations.

P11.121

Determining the role of SIRT6 as an epigenetic regulator of gene expression in hepatocytes

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The mamalian sirtuin family consists of seven members (SIRT1-7) that target a wide range of cellular proteins in nucleus, cytoplasm, and mitochondria for post-translational modification by deacetylation or ADP-ribosylation. SIRT6 is located in the nucleus and promotes a number of important key functions like DNA repair, genome stabilization and telomere maintenance. In accordance with these functions, Sirt6 knock-out mice show several dramatic symptoms of premature ageing and die within four weeks.

We performed microarray gene expression profiling of hepatocytes from Sirt6-deficient and wildtype mice. This analysis detected a significant upregulation of the imprinted H19, Igf2 and Peg3 genes which was subsequently confirmed by quantitative PCR. Bisulphite pyrosequencing of the imprinting control regions of these three and other imprinted genes did not reveal significant methylation differences between Sirt6-deficient and wildtype hepatocytes. Similarly, DNA methylation analysis of these cells at subtelomeric regions known to exhibit DNA methylation changes associated with increased telomeric recombination in DNA methyltransferase-deficient cells gave normal results. However, using quantification of global DNA methylation with a specific antibody in an ELISA-like reaction, we observed significantly decreased levels of 5-methylcytosine in Sirt6-deficient cells. Bisulphite pyrosequencing excluded that repetitive elements as frequently used indicators of global DNA methylation changes are affected by this demethylation process.

Our results provide further evidence that the epigenetic regulatory role of SIRT6 is more likely associated with higher order chromatin structures and specific histone modifications. Nevertheless, there is an obvious decline in global DNA methylation in Sirt6-deficient cells that needs to be studied in more depth.

P11.122

Detecting SNP interactions associated with HDL using GPUs

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In recent years many genome-wide association (GWA) studies have been performed. Many of these have been successful in identifying loci associated with complex diseases. Until now, these results failed to fully explain the heritability of many of the diseases.

Searching for interactions between Single Nucleotide Polymorphisms (SNPs) is one of the many possible explanations to the missing heritability. However, the computational time needed for testing all pairs of SNPs is proportional to the square of the number of SNPs, translating into months of CPU time. We therefore evaluated GLIDE [1] which makes use of the computational power of consumer-grade graphics cards (GPUs) to detect interactions via linear regression.

We present our first experiments with GLIDE for which we analysed the HDL levels in 3,000 individuals of the Rotterdam Study. The first results show that GPUs can indeed be used for fast genome-wide analysis of SNP-SNP interactions. We found multiple regions showing genome-wide significant interactions which are currently being investigated. However, occasionally problems occur using imputed data hampering meta-analysis and replication. Further developments are currently ongoing to overcome this issue and will also be presented.

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[1] Kam-Thong et al, submitted

P11.123

Genetic and functional investigation of the *SOX9* regulatory region in development and disease

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Mutations in the coding sequence of *SOX9* cause campomelic dysplasia (CD), a disorder of skeletal development, in association with 46,XY sex reversal, reflecting the essential roles of *SOX9* in chondrogenesis and testis development. Non-coding genomic lesions, including translocations and deletions, within a ~1.4 Mb region upstream of *SOX9* can recapitulate the CD phenotype fully or partially, suggesting the existence of an unusually large cis-regulatory control region. Indeed, studies in transgenic mice have demonstrated that this interval contains several highly conserved non-coding elements that can function as enhancers of tissue-specific transcription, partly recapitulating the *SOX9* expression pattern. Pierre Robin sequence (PRS) is a craniofacial disorder that is typically a component of the CD phenotype, and we have previously reported a locus for isolated PRS at ~1.1-1.4 Mb upstream of *SOX9*. We now report two novel deletions in PRS patients; one partly overlapping the previously identified locus, and the other falling much closer to *SOX9*. In parallel, we performed ChIP-Seq analysis to identify genome-wide binding sites for p300, a marker of active enhancers, in mouse craniofacial tissue. Several binding sites were identified upstream of *SOX9*, and were validated as craniofacial enhancers in transgenic mouse reporter assays. Notably, some of the p300 binding sites fall within the novel PRS deletions, and sequencing of these regions in a large cohort of PRS patients revealed several rare variants. These studies add a further level of complexity to our knowledge of the mechanisms governing transcriptional regulation of *SOX9*.

P11.124

Sex determining region Y (SRY) contributes to normal development by regulation of genes involved in pluripotency and differentiation

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Introduction: Members of the SOX (SRY box) family proteins play critical roles in multiple aspects of development.SRY, as a founder member of SOX family, has long been believed to be involved in development of sexual gonads by triggering signaling cascades that lead to formation of testis or ovary from bipotential gonads. However, less is known about whether SRY has role in other developmental procedures. In order to gain further insight into the possible roles of SRY during development, we looked into possible SRY-regulated genes and their levels of expression in a human embryonic carcinoma cell line, named NTera2, before and after induction of differentiation.

Material and methods: For this respect two groups of genes including *OCT4*, *NANOG* and *SOX2* as pluripotency marker genes, and *NESTIN* and *PAX6* as differentiation marker genes were evaluated.Chromatin Immunopercipitation (ChIP) was performed using SRY antibody on chromatin extracted from NTera2 cells before and after neural differentiation and SRY incorporation on the regulatory regions of the aforementioned genes were evaluated quantitatively, using real time-PCR technique.

Results and conclusion: Our results showed that incorporation of SRY in both groups of marker genes was increased after induction of differentiation. Besides, the low expression level of *OCT4*, *SOX2* and *NANOG* and the high expression of *PAX6* and *NESTIN* in the differentiated cells suggest that SRY may act as a transcription repressor for pluripotency-associated genes and as a transcription activator for differentiation -related genes.

P11.125

Targeted sequencing experiments for rare disease alleles: implications in clinical practice and diagnosis of steroid-resistant nephrotic syndrome

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During recent years, several podocyte genes have been implicated in severe forms of steroid-resistant nephrotic syndrome (SRNS) progressing to renal failure. To date, at least 15 genes highly expressed in the podocyte have been associated with the syndrome and different mutations in these genes have been identified; it is now known that the phenotypes associated with mutations in these genes display significant variability, rendering genetic testing and counselling a more complex task. Traditional methods for sequencing genes are often laborious and not easily available and a screening technique that enables the rapid detection of the pathogenetic variants would be very helpful in the clinical practice. The scope of our work is to apply next-generation sequencing (NGS) technology to study patients affected by SRNS, in which the previous analysis of *NPHS2* and *WT1* genes had not shown any mutations. We perform in 8 affected subjects a targeted resequencing of 46 genes including those already known as causative of the disorder and several other genes highly expressed in the podocyte.

We found new heterozygous missense mutations in different genes that occasionally are associated with the disease in childhood (*PLCE1, ACTN4, MYO1E, PODXL)*. We also detected a variation in the *ZHX2* gene, to date never associated with SRNS. This gene encode for a transcriptional factor which regulates the expression of several genes in the podocyte, including *WT1*. This results and further clinical investigations provides an exciting opportunity to reveal more insight into the pathogenic mechanisms that underlie this debilitating disorder.

P11.126

A systems biology approach to linking genotype to phenotype, using celiac disease as an example

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Genome-wide association studies (GWAS) have been successful in identifying genes involved in complex diseases. However, the critical challenge is to translate GWAS hits into a biological hypothesis. We have previously identified 57 independent, non-HLA loci to be strongly associated with celiac disease (CeD). Many of these loci harbor multiple genes and for many of these it is difficult to link genotype to phenotype. We therefore undertook a systems biology approach to fill this gap, integrating results from eQTL analysis, network/pathway-based analyses, imputation results, and SNP function annotation. The eQTL mapping, using whole genome gene-expression data and genotyping data from 1,240 samples and replication in an independent set of 229 blood samples, suggested significant eQTLs at 16 CeD SNPs (FDR P < 1.12*10-7 to 1.51*10-137). Pathway analyses using seed genes identified by GWAS and eQTL analysis suggested causative genes at 55 out of 57 CeD loci. Surprisingly, along with enrichment for T-cell, B-cell and neutrophil genes, co-expression network analysis also suggested that 11% of CeD genes are highly expressed in epithelial tissues and are involved in epithelial cell-cell adhesion. We imputed CeD-associated regions using 1000 Genome project and annotated the function of all the susceptibility variants. SNPs with strong eQTLs lay within binding sites of either microRNAs (e.g. for IL18RAP, UBE2L3) or transcription factors (e.g. for MMEL1, CSK). Thus, our study provides a biological hypothesis for almost every CeD susceptibility locus to connect the genotype and CeD phenotype and it implicates the involvement of a novel pathway in CeD pathogenesis.

P11.127

Analisys of MMP-3 gene promoter methylation status in epithelial cells from oral mucosa and gingival tissue cells and genetic expression in smokers and nonsmokers subjects with chronic periodontitis

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The objective was to investigate the methylation status of CpG site at position -686 of MMP-3 gene promoter and the levels of mRNA expression of MMP-3. Subjects were divided into: nonsmokers healthy, non-smokers and smokers with chronic periodontitis. DNA was purified from buccal epithelial cells, that were obtained after oral rinse and DNA and RNA were purified from biopsies gingival cells. The methylation status at -686 of MMP-3 gene promoter was investigated by restriction enzyme sensitive to methylation *HpaII*, the PCR and electrophoresis. Methylated samples showed positive bands after PCR and non-methylated samples showed no bands. The statistical analysis between the methylation status found in this samples in each groups was performed by X^2 test at 5% level. The relative expression of MMP-3 was examined by real time PCR and statistical analysis was performed by Kruskal-Wallis test at 5 % level. There was not difference in the methylation status found in each group. The frequency of the unmethylated status found in the epithelial oral cells was 18,3% in healthy non-smokers, 12,5% in chronic periodontitis smokers and 9,7% non-smokers. And the unmethylated status of gingival cells was higher in all groups: 28% in healthy non-smokers, 38% in smokers with chronic periodontitis and 27% in non-smokers with chronic periodontitis. Also, there was not difference in genetic expression of MMP-3 among these groups. We conclude that there is not association between the methylation status observed at position -686 of MMP3 gene promoter and chronic periodontitis in smokers and non-smokers.

P11.128

Agilent Technologies SureSelect™ Human All Exon Designs: High Performance Target Enrichment System for Human Exome Sequencing

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Next generation sequencing technologies have reduced high-throughput sequencing costs by several orders of magnitude and enabled numerous whole-genome analyses. Nevertheless, the expense and operational capacity necessary for large scale whole genome studies is still prohibitive for many laboratories. Since large portions of the genome consist of repeat elements and regions of unknown phenotypic value, targeted exome capture combined with massively parallel sequencing has become one of the most viable options to gain novel insights into the genetic causes of inherited disorders. By focusing on the protein-coding regions of the human genome, scientists are now able to more efficiently identify both common and rare polymorphisms that are more likely to result in cellular dysfunction and exhibit significant penetrance in disease association studies. This includes Mendelian disorders and complex diseases like cancer or neurological pathologies. Herein we describe our latest SureSelect Human All Exon designs, which incorporate updated exome coordinates from the major databases. Performance and workflow improvements including automation and multiplexed samples are demonstrated with respect to capture efficiency, uniformity, reproducibility of enrichment, and ability to detect SNPs and indels on multiple sequencing platforms. The specificity and accuracy of the human All Exon design ensures coverage of biologically relevant coding regions for small and large scale sequencing operations.

P11.129

Multiplex enrichment of genomic regions using HaloPlex PCR

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Targeted sequencing methods, in combination with bench top sequencers, enable studies of select regions of large genomes in multiple samples with sufficient coverage.

For a target sequencing to be useful for genetic research as well as for clinical applications it is important that the regions of interest are enriched with high specificity and uniformity to maximize the amount of useful data retrieved from each run. Furthermore it is also important that the protocol is fast to make use of the short turnaround times on bench top sequencers.

The HaloPlex PCR method combines target enrichment and library preparation in a single protocol without the requirement of any dedicated instruments. The protocol associates all targeted fragments with common primer motifs which can then be used to run multiplex PCR using only one primer pair. To make use of the capacity in the sequencing run, up to 96 samples can be enriched, barcoded and pooled on the same flowcell.

We have developed a faster version of the protocol that can be completed in six hours and with improved enrichment uniformity. The key improvements are shortened restriction digestion and hybridization times and consolidation of several of the reaction steps.

The performance of the new protocol was demonstrated by the enrichment of a 400 kb region in ten samples that were subsequently pooled and sequenced on one MiSeq flowcell. All samples were enriched with >95% specificity and 90% of the targeted bases were covered at >10% of average depth.

P11.130

Development of a gene panel for targeted next generation sequencing of twelve thoracic aortic aneurysmal genes

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Dissection/rupture of aortic aneurysms represent important causes of death in the Western world. The genetic contribution to thoracic aortic aneurysm (TAA) is significant and a dozen causative genes are known. The molecular confirmation of the clinical diagnosis is important as the identification of the underlying mutation has implications for further patient management and therapy. Because of overlapping phenotypes, the high allelic/locus heterogeneity and large size of the involved genes, the molecular diagnosis for TAA is not always straightforward. Moreover, the consecutive molecular screening of genes using conventional mutation screening methods is expensive and labor intensive. Next generation sequencing (NGS) after hybridization or amplification based enrichment offers an attractive alternative.

Here, we propose a cost- and time-efficient method for simultaneous screening of twelve TAA genes (ACTA2, COL3A1, FBLN4, FBN1, FLNA, MYH11, MYLK, NOTCH1, TGFBR1, TGFBR2 and SLC2A10) based on the Haloplex technology. The latter is an innovative, simple method for specific enrichment of target regions. The protocol simultaneously incorporates the sample identification barcodes (up to 96 different) and the primers for the subsequent NGS run and does not require the acquisition of expensive dedicated equipment. It requires 2 x 150 bp paired-end runs, which we have performed on a Miseq (Illumina). We have obtained an overall coverage "by design" of 99.7% and a "real-life" sequencing coverage of 96.6% of the targeted regions. We are currently optimizing the assay, which includes design improvements and validation in large sample groups.

P11.131

Sample preparation of animal and plant tissues prior to automated nucleic acid purification with Thermo Scientific KingFisher Duo and KingFisher Kits

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Efficient homogenization of animal and plant tissues is an essential step to ensure good DNA and RNA yield and quality. Certain sample types, e.g. harder tissue samples, require strong, either chemical or mechanical treatment for destroying tissue and cell structures before nucleic acid purification process.

Magnetic particle technology enables fast and effective purification of high quality nucleic acids. Thermo Scientific KingFisher Duo uses magnetic particle technology based on the use of magnetic rods with specially designed plastic consumables, BindIt software for protocol development and optimized KingFisher Kits for nucleic acid purification. KingFisher Duo enables purification of 12 samples during one run in working volumes of 30-1000 µl. Running two protocols sequentially without interruption raises throughput up to 24 samples per load. In addition, the instrument gives an option to choose working volumes up to 5 ml.

We tested several different mechanical homogenization methods in addition to chemical lysis to optimize sample preparation before nucleic acid purification in the KingFisher Duo. We analysed performance of homogenization and purification using several different sample materials, including tobacco leaves as well as animal liver and heart samples. The purification process in the KingFisher Duo was performed using three different KingFisher Kits, suitable for cell and tissue DNA or RNA purification. The results indicate high sensitivity of the purification process.

The KingFisher Duo in combination with the KingFisher Kits and new BindIt software 3.2 constitute an exceptional purification system for obtaining excellent yield and purity of nucleic acids.

P11.132

Different Next Generation Sequencing approaches detect mosaic mutations and deep intronic mutations in tuberous sclerosis complex

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Tuberous sclerosis complex (TSC) is caused by TSC1 or TSC2 mutations. Current molecular genetic testing combining Sanger sequencing of all 62 and MLPA of 58 coding exons, respectively, identifies a mutation in approximately 80% of the individuals with a definite clinical diagnosis. Possible reasons for this detection gap are mosaicism for a mutation underrepresented in lymphocyte DNA or mutations in regulatory regions.

We reanalyzed patients with the definite clinical diagnosis TSC by resequencing the entire genomic regions of TSC1 and TSC2 applying two Next Generation Sequencing approaches. Firstly, target enrichment with the Agilent SureSelect technology was combined with the Life Technologies SOLiD4 sequencing system. Probe design for 63.885 bp TSC1 and 44.255 bp TSC2 resulted in 43 and 27 genomic regions, respectively. Secondly, 108.140 bp genomic region was amplified by 20 overlapping long-range PCR fragments of 5.000-5.400 bp and subjected to the 454 pyrosequencing technology using the GS FLX Titanium system.

The SureSelect/SOLiD4 technology revealed a bias with an average of 46.68% reads (coverage 612-1332) mapped to TSC1 but only 5.8% (coverage 170-390) to TSC2. Coverage gaps in 17 of the 27 TSC2 regions are obviously due to its high GC content. The long-range PCR/454 GS FLX Titanium approach achieved a more uniform coverage ranging from 697-1719 for TSC1 and 687-3905 for TSC2.

Preliminary results following the two approaches for 10 TSC patient samples uncovered four mosaic mutations composed of two missense and two splice site mutations as well as one true heterozygous deep intronic mutation leading to aberrant splicing.

P11.133

Expression, Purification and Molecular Evolution Studies of Human Tyrosinase

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Tyrosinase is a copper- containing enzyme that has been isolated and studied from a wide variety of plant, animal and fungal species.Tyrosinases found in different sources such as prokaryotic or eukaryotic microorganism, mammals and plant, differ from each other with respect to the primary structure, size, glycosylation pattern and activation characteristics, but, all tyrosinases, have in common within their active site. In tyrosinase active site, two copper atoms are each coordinated with three histidine residues. Histidine residues are located in two regions named cuA and cuB. A loop containing three residues including M374 connects two copper atoms centers. Therefore this loop is essential for stability and activity of enzyme. M374 is conserved in the phenol oxidase superfamily. A set of mutants were generated previously by replacement of this residue with glycine. In this study, M374 was replaced with asp, Lys, arg, and thr. To carry out mutational characterization of human tyrosinase, an expression plasmid, pHis-tyrosinases, which contain the entire coding sequence of tyrosinase with site directed mutations that mentioned above, were constructed and expressed in p.lysis. The expressed enzymes were purified by an affinity chromatography. Oxidative activity and km values for native and recombinants enzymes were detected respectively. These newly recombinant tyrosinases may have therapeutic potential due to their activity and stability.

P11.134

GenomeNL variant database; towards a deep genetic encyclopedia of Dutch variation

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Background

The Dutch biobank collaboration BBMRI-NL has initiated the „Genome of the Netherlands" (GoNL) project to produce ~12x whole DNA genetic profiles of 769 Dutch people: 231 trios of a child with its parents, 11 quatros of monozygotic twins and 8 quatros of dizygotic twins. This deep genetic resource will offer unique opportunities for science, which is currently being expedited by an international team of researchers on optimal variant discovery, population genetics and evolution.

Results

Here we present the GoNL variant database, an online encyclopedia of Dutch variation. At submission of this abstract, researchers can already query the GoNL variant database to verify whether variants in their own sample are unique against 25 million SNPs observed in the panel of 500 parents. By April we expect a high quality SNP set based on the full panel and a collection of structural variations using the unique trio structure to filter false positives. We will enrich the database with reference annotations on each variant to disclose a wealth of new information and possible applications the diagnostics.

Methods

The SNP data was produced using Illumina sequencing, BWA alignment, Immunochip for QC, and GATK SNP calling. The data is represented in the database using the Observ-OM standard, developed in collaboration with EU-GEN2PHEN. The software is implemented using the open source MOLGENIS biosoftware toolkit and is freely available for groups to setup their own repositories. The database is accessible via http://www.nlgenome.nl

P11.135

Adhesion Protein VSIG1 Is Required for the Proper Differentiation of Glandular Gastric Epithelia

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VSIG1, a cell adhesion protein of the immunoglobulin superfamily, is preferentially expressed in stomach, testis, and certain gastric, esophageal and ovarian cancers. Here, we describe the expression patterns of three alternatively spliced isoforms of mouse Vsig1 during pre- and postnatal development of stomach and potential function of Vsig1 in differentiation of gastric epithelia. We show that isoforms Vsig1A and Vsig1B, which differ in the 3' untranslated region, are expressed in the early stages of stomach development. Immunohistochemical analysis revealed that VSIG1 is restricted to the adherens junction of the glandular epithelium. The shorter transcript Vsig1C is restricted to the testis, encodes an N-terminal truncated protein and is presumably regulated by an internal promoter, which is located upstream of exon 1b. To determine the role of VSIG1 during the development of stomach epithelia, an X-linked Vsig1 was inactivated in embryonic stem cells (ESCs). Although Vsig12/Y ESCs were only able to generate low coat color chimeric mice, no male chimeras transmitted the targeted allele to their progeny suggesting that the high contribution of Vsig12/Y cells leads to the lethality of chimeric embryos. Analysis of chimeric stomachs revealed the differentiation of VSIG1-null cells into squamous epithelia inside the glandular region. These results suggest that VSIG1 is required for the establishment of glandular versus squamous epithelia in the stomach. To perform more functional analysis of *Vsig1*, generation of conditional knockout mice is underway.

P11.136

Genotyping Fanconi Anemia Patients by Whole Exome Sequencing

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The rare genomic instability disorder Fanconi Anemia (FA) is characterized by bone marrow failure, variable malformations and increased predisposition to leukemia and solid tumors. Biallelic mutations in at least 15 genes involved in the FA/BRCA network of DNA interstrand crosslink repair (ICL), are known to be disease causing. FA can be diagnosed in different ways: At cellular level functional assessments like chromosomal breakage analysis or cell cycle testing are state of the art. However, at the molecular level it becomes more difficult, time consuming and expensive to detect mutations the more FA genes are identified. Therefore we performed whole exome sequencing (WES) in four FA patients with previously unknown mutations in order to evaluate the benefit of this method for molecular diagnosis and FA genotyping. To find the best combination between enrichment and sequencing technologies we tested two different pairings: *NimbleGen* enrichment from Roche along with the *Illumina* Next Generation Sequencing (NGS) platform and Agilent *SureSelect* enrichment with the *SOLiD* platform from *Applied Biosystems*. Irrespective of the enrichment method or sequencing platform we were able to detect and confirm the pathogenic mutations in each of our four patients. We found homozygous and heterozygous single base pair substitutions in *FANCJ, -D1, -D2* and *-P* but also an insertion of two base pairs and a splice site mutation. Therefore WES is proposed as a valuable tool for molecular diagnosis of FA which may replace classical genotyping approaches.

P11.137

Alternative Splicing Governs miRNA Biogenesis

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MicroRNAs (miRNAs) are noncoding RNAs of 22 nucleotides that induce post-transcriptional gene silencing through base-pairing with their target mRNAs. miRNA primary transcripts contain a local hairpin structure that is cleaved by the Microprocessor complex, consisting of RNAse III Drosha and DGCR8. The processing reaction releases the hairpin-shaped intermediates (pre-miRNAs). The majority of mammalian miRNAs are located in introns. Splicing of the introns in which such miRNAs reside was recently suggested to derive independently from the miRNA processing. We have uncovered a novel regulatory mechanism in which splicing determines the processing of pre-miRNAs. Based on a bioinformatic analysis of predicted transcription units of all miRNAs genes in 18 species, we identified a group of 52 miRNA precursors that share an intriguing genomic location - positioned on exon-intron junctions, these miRNAs are exposed to a regulatory mechanism in which splicing or alternative splicing might determine their biogenesis.

We experimentally demonstrated that in these cases the splicing machinery and the Microprocessor complex compete for processing of the same region on the RNA transcript and therefore, either the spliced mRNA or the pre-miRNA can be produced separately from the shared transcript. We also showed that tissue-specific and embryonic-specific alternative splicing negatively regulates the levels of mature miRNA produced from splice-site overlapping miRNA precursor, and reciprocally, that Drosha and DGCR8 modulate inclusion of alternatively spliced exon that overlaps with the miRNA stem-loop. Our data reveal a potential role for alternative splicing in the regulation of miRNA biogenesis.

P11.138

Pseudogenes: an unsolvable problem in Whole Exome Sequencing?

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Over the past years Whole Exome Sequencing (WES) became an established tool for the detection of mutations underlying rare human genetic disorders. We performed WES in nine patients and in all cases the same genes consistently caught our attention, because they were overrepresented by heterozygous single nucleotide variants (SNV). One of our patients carried a large number of heterozygous base substitutions in CDC27, even though one allel of this gene was known to be partially deleted. We found three listed pseudogenes of CDC27 which could be responsible for wrong mutation calls by misalignment of short sequence reads. Via selective primer design we were able to specifically resequence the functional gene by Sanger technique. As expected, all variants were identified as false positive calls due to overlap with the pseudogenes. In consequence of this finding, we became more alert concerning alignment artifacts due to paralogous sequences in the other projects as well. But contrary to the general expectation that these misalignments should be detectable because of their increased SNV counts, we also found several isolated SNVs which should have mapped to a related pseudogene. Analysis of the same data with different bioinformatic tools could only partially decrease the error rate. We therefore conclude that pseudogenes cause an enormous amount of false positive SNVs and require careful attention because they cannot be withdrawn as easily as sequencing errors. Mutation validation by Sanger sequencing still seems to be indispensable and the possibility of false negative mutation calls should be regarded as well.

P11.139

Functional characterization of Williams-Beuren syndrome chromosome region 22 protein

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Williams-Beuren syndrome (WBS) is a multisystem disorder associated with the hemizygous deletion of 26-28 genes on chromosome 7q11.23. Typical features of WBS comprise a recognizable pattern of facial dysmorphism, supravalvular aortic stenosis, connective tissue abnormalities, hypercalcemia and distinctive neurobehavioral phenotype. Hemizygosity of elastin gene is associated with supravalvular aortic stenosis; however it is unknown how the deletion of other genes in 7q11.23 contribute to the phenotype. Altered expression level of dosage-sensitive genes within aneuploid segments is the main cause of WBS.

WBSCR22 (protein expressed from Williams-Beuren chromosomal region 22) is a nuclear protein which has a predicted S-adenosylmethionine (SAM) binding motif and it is assumed that WBSCR22 has methyltransferase activity. SAM-dependent MTases represent a diverse class of enzymes which act on protein, small molecule, lipid or nucleic acid methylation and therefore mediate numerous cellular processes. The yeast homolog of WBSCR22, Bud23, sharing 47% similarity on amino acid level, is a ribosomal 18S rRNA methyltransferase required for ribosome biogenesis.

In our laboratory, we investigate the cellular function of WBSCR22. In sucrose gradient ultracentrifugation WBSCR22 cosediments with free ribosome small subunit (40S). WBSCR22 knock-down reduces the level of free 40S and alters 40S/60S ratio. WBSCR22 is involved in processing of 18S rRNA since WBSCR22 knock-down causes the accumulation of 18S-E pre-rRNA in cell nucleus. Our data suggest that WBSCR22 protein is involved in ribosome biogenesis.

P11.140

Screening of a European cohort of 150 male XLID patients using a custom-designed chromosome X exon-specific microarray

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X-linked Intellectual Disability (XLID) is a common cause of intellectual impairment in males with an estimated prevalence of ~1:1000. Many studies have been performed for the identification of genes associated with XLID. One approach for the identification of regions that may harbour novel XLID genes is through the detection of copy number changes (CNCs) by array-CGH. The purpose of our study was to screen a European cohort of male XLID patients using a high-resolution oligonucleotide 105K microarray specific for the X chromosome. This custom-designed chromosome X exon-specific microarray provides full coverage not only of the X chromosome itself but complete exome coverage with at least 6 probes for each exon thereby ensuring a robust screen for the identification of CNCs down to the individual exon level. To date, we have screened 153 patients and found CNCs in 11 patients (7.2%). These aberrations consisted of seven deletions and six duplications that ranged in size from 165bp to 7.26Mb. All of the deletions and two of the duplications resided in single genes while the remaining duplications spanned regions that harbored several genes. Follow-up studies including confirmation and segregation analysis with family members are in progress. In addition, the clinical significance of these aberrations and the possible role of these genes as novel XLID loci will be assessed.

P11.141

Expanding and enhancing access to the Sequence Read Archive (SRA) through a complementary new web-based mirror

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Public institutions such as the National Center for Biotechnology Information (NCBI) have made tremendous investments in generating and archiving a wide array of valuable genomic data for use by the research community. Expanding access to these valuable public data and streamlining the ability to integrate them into data management tools and powerful analyses, will further expedite their use and value in medical research, discovery and applications.

Teaming up with Google, DNAnexus has developed a complementary hosted mirror of the NCBI's Sequence Read Archive (SRA) that provides researchers an additional way to access these important datasets. This freely accessible resource provides a new web-based user interface built using the latest "cloud" technologies and genomic data standards. As the most comprehensive archive of publicly available next-generation sequencing data, the SRA is an important resource to researchers around the world. The SRA remains the single best source of useful sequence data from research initiatives such as the 1,000 Genomes Project and institutions like the Broad Institute, Washington University, and the Wellcome Trust Sanger Institute.

Here we discuss our work with the NCBI and Google to create a complementary mirror of the SRA available at sra.dnanexus.com. Through a typical user scenario, we will discuss the underlying data processing pipeline, key features of the new web-based interface that enables researchers to quickly identify and browse datasets of interest, link-outs to PubMed references, and integration of those data into an analysis workflow for hypothesis generation.

P11.142

A nonsense mutation in ATR-X gene responsible for nonsyndromic XLID in MRX77 family

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In 2003 we reported a linkage analysis performed on a 3-generation Greek family (MRX77) with seven affected males. Clinical evaluation showed apparently nonsyndromic X-Linked Intellectual Disability (XLID). The affected males have moderate to severe intellectual disability, severe speech problems and aggressive behavior. Two point linkage analysis using 26 polymorphic markers spanning the X chromosome had localized the disease gene to a large interval Xq12- Xq21.33 (flanking markers DXS983 in Xq12 and DXS6799 in Xq21.33) with a maximum lod score of 2.36. In 2010, the affected MRX77 patients were screened using the new full coverage chromosome X exon-specific array designed by our group which showed no copy number changes. In 2011, a proband was screened for mutations in 92 XLID genes using next generation sequencing prior to undertaking a whole X exome sequencing. A nonsense mutation, p.R37X (c,109C>T), was identified in exon 2 of the *ATR-X* gene. Although, the *ATR-X* gene had been sequenced in 2003, this mutation was not detected since this exon was not annotated at that time. Segregation and the skewed X-inactivation pattern in carrier females were consistent with the clinical phenotype of the MRX77 family. This nonsense mutation had previously been observed in the Chudley-Lowry syndrome and another XLID family, both of which had a phenotype less severe than ATRX syndrome. Since our family has nonsyndromic XLID, the finding further confirms that the variability of the phenotype associated with this mutation is likely due to the presence of alternative transcripts which do not contain exon 2.

P11.143

Genome-wide association in 1198 Dutch individuals for 163 serum metabolites

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Metabolites, or the small molecules involved in cellular metabolism, are presumed to be directly linked to (patho)physiology. Hence, it is important to gain a deeper understanding of the genetic and environmental contributions to interindividual variation in metabolite levels. As part of our ongoing contributions to the European Network of Genomic and Genetic Epidemiology (ENGAGE) consortium, we carried out a genome-wide association (GWA) study for serum metabolite levels in 1198 unrelated individuals who participated in the Netherlands Twin Register Biobank project. From 2004 until 2008, blood samples were obtained from these individuals (67.2% male, 32.8% female; mean age 52 years [SD, 13]) after overnight fasting. Metabolomics analysis was performed on the serum fractions of these samples, using the Biocrates AbsoluteIDQ p150 kit and electrospray ionization-MS/MS enabling detection of 163 metabolites belonging to five different classes: acylcarnitines (N=41), amino acids (AA, N=14), glycerophospholipids (N=92), sphingolipids (N=15), and a compound measure for hexoses (N=1). Genotyping was performed using various GWA chips. Imputation against HapMap 2 Build 36 Release 24 resulted in data for 3.8M unfiltered single nucleotide polymorphisms (SNPs) for each participant. SNPtest v2.2.0 was used for association analysis, including age, sex and principal component scores for population stratification as covariates. Genome-wide (conservative Bonferroni-corrected p <3.1E-10) significant hits were observed for several metabolites. The associations for 22 glycerophospholipids with SNPs in the fatty acid desaturase (FADS) gene cluster were among the most prominent. In conclusion, we found several clusters of genome-wide significantly associated SNPs for metabolites detected by MS in human serum. Future meta-analyses will combine these data with the results of six ENGAGE partners, to determine whether the results found in our Dutch sample are replicated in other cohorts.

P12. Molecular basis of Mendelian disorders

P12.001

Mutational analysis of RPE65, ABCA4 and RHO genes on Greek patients with Retinitis Pigmentosa, Leber congenital amaurosis and Stargardt's disease

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Retinal dystrophies are a clinically and genetically heterogeneous group of disorders which affect more than two million people worldwide. This heterogeneity raises difficulties on genetic testing and counseling for the affected families. Although our knowledge about genetic factors underlying these diseases has recently increased dramatically, a lot of disease causing mutations still remain unknown. Our study deals with identifying the disease-associated variants in Greek families with Stargardt disease, Cone-Rod dystrophy , Retinitis Pigmentosa and Leber Congenital Amaurosis.

We focused our research on the possible role of three genes (ABCA4, RHO and RPE65) in the pathogenesis of hereditary retinal dystrophies in Greek patients. All exons of ABCA4 were sequenced in families with Stargardt Disease, Cone-Rod Dystrophy and Retinitis Pigmentosa. RHO was sequenced in families with Retinitis Pigmentosa and RPE65 in families with Leber Congenital Amaurosis and Retinitis Pigmentosa.

A great number of genetic variants in coding and non-coding regions were found in this study. Most of them were known variations and include disease-causing mutations and known polymorphic variants. Mutations in ABCA4 were found in patients with Stargardt Disease, autosomal recessive Retinitis Pigmentosa and Cone-Rod Dystrophy. Mutation c.272G>A (p.R91Q) in RPE65 was found in a patient with Retinitis Pigmentosa and in his healthy mother. This mutation has been associated with autosomal recessive Retinitis Pigmentosa. We also found novel variants in non- coding sequences which may affect the splicing process. These variants were analyzed with the use of bioinformatic tools. We also attempted possible phenotype/genotype correlations.

P12.002

Two ABCB4 mutations involving two strategic NBD-motifs do not prevent the targeting to the plasma membrana but promote MDR3 dysfunction

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MDR3 protein translocates phosphatidylcoline (PC) from the inner to the outer leaflet of the hepatocanalicular membrane; its deficiency, related to ABCB4 mutations, favours the formation of "toxic bile". A continuum of hepatobiliary diseases have been associated with ABCB4 mutations but, for most of them, the detrimental effect on the protein is speculative only. The functional relevance of two strategic mutations within the N-terminal Nucleotide-Binding-Domain was examined with stably transfected HUH28-cell-lines expressing wild type and mutant MDR3 proteins by western-blotting, immunocytochemistry and chromatographic quantification of lipids, collected from culture medium after sodium-taurocholate (NaTC) stimulation. As suggested by our three-dimensional model of MDR3 (Degiorgio D et al., 2007), the p.Y403H mutation involves the A-loop while the p.L481R mutation is contained into the Q-loop. Our results show that both MDR3-mutant proteins were expressed in a comparable way to the MDR3-wild-type protein: a molecular mass of 160KDa associated with a green fluorescent signal, intenser and sharper in the plasma membranes, was constantly identified. However, compared to the stably transfected HUH28-cell-line expressing wild-type-MDR3 protein in the presence of NaTC 3mM, the lipid dosage into culture medium has shown (with five indipendent experiments) that
i) the efflux of PC is reduced ($p<0.01$) from cell lines expressing p.Y403H and p.L481R mutant proteins;
ii) the efflux of cholesterol is increased ($p<0.01$) from cell line expressing the p.Y403H mutant protein.
In conclusion, these mutations could promote in vivo formation of toxic bile with reduced amounts of PC (p.L481R) or with reduced amounts of PC and increased level of cholesterol (p.Y403H).

P12.003

New clues on the differential diagnosis between acrodysostosis and pseudohypoparathyroidism type Ia

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Acrodysostosis is a skeletal dysplasia characterized by short stature, nasal hypoplasia and brachydactyly. When multihormonal resistance is also present (mainly, PTH and TSH), the disorder might be clinically misdiagnosed as pseudohypoparathyroidism type Ia (PHP-Ia). Acrodysostosis with multihormonal resistance is caused by mutations in PRKAR1A, which participates in the Gsα protein signaling pathway.
Patients and methods: PRKAR1A was sequenced in five PHP-Ia patients with negative genetic and epigenetic analysis in GNAS locus.
Results: A heterozygous mutation in PRKAR1A was identified in three of the five patients. Parental testing showed that the mutation arose de novo in the three cases. A detailed analysis of clinical and radiological data revealed the characteristic bone abnormalities of acrodysostosis.

Conclusion: Acrodysostosis is an infradiagnosed disorder that might be confounded with PHP-Ia. An exhaustive radiologic study of patients presenting with short stature and PTH resistance could help clinical diagnosis and genetic testing.

Clinical and genetic information

	Patient 1	Patient 2	Patient 3
Sex	F	M	M
Age	3yr4m	12yr6m	8yr
Height (cm)	91.3 (-1.6SD)	138.6 (-2.1SD)	113.4 (-2.8SD)
BMI (kg/m2)	17.4 (+0.9SD)	22.3 (+0.5SD)	16.4 (-0.4SD)
PRKAR1A mutation	p.Q285R	p.R368X	p.R368X
At birth:			
- Gestational age	40w	39w	38w
- Weight	2400g (-2.4SD)	2840g (-1.2SD)	2270g (-1.9SD)
- Length	49cm (0SD)	47cm (-1.6SD)	45cm (-2.2SD)
Alterations:			
Bone:			
- Peripheral dysostosis	Yes	Yes	Yes
- Generalized brachydactyly	Yes	Yes	Yes
- Nasal hypoplasia	Yes	Yes	Yes
Biochemical and hormonal			
- PTH	Increased	Increased	Increased
- Serum calcium	Normal	Normal	Normal
- Serum phosphate	Normal	Normal	Normal
- TSH	Increased	Increased	Increased
- Serum T4	Normal	Normal	Decreased

P12.004

A new form of autosomal recessive syndromal acroosteolysis with recurrent infections, sensory neuropathy and mental retardation.

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We describe a syndromic form of acroosteolysis characterized by mental retardation, sensory neuropathy and recurrent infections with autosomal recessive inheritance. SNP haplotyping mapped the disease locus to the short arm of chromosome 11. Whole-genome sequencing using unchained base reads on self-assembling DNA nanoarrays allowed identification of a homozygous missense mutation in a gene localized in 11p15 that encodes a SNARE-associated Golgi protein. We show that the gene's mRNA is markedly upregulated in osteoclast cell cultures and patients' osteoclasts (induced by blood mononuclear cells) exhibit a significantly enhanced bone resorption activity. Mutations in the NOTCH2 gene (associated to Hajdu-Cheney syndrome), mapping in 1p12-p11.2, and in the WNK1 gene (associated to hereditary sensory and autonomic neuropathy type IIA), mapping in 12p13.33, have been excluded by both haplotype analysis and whole-genome sequencing.

P12.005

Autosomal Dominant Polycystic Kidney Disease: A comprehensive mutation analysis of the PKD1 and PKD2 genes in Spanish patients

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Autosomal Dominant Polycystic Kidney Disease (ADPKD) is one of the most prevalent inherited disorders, with an incidence of 1:1000. ADPKD is caused by mutations in the *PKD1* (85%) and *PKD2* genes (15%). The genetic diagnosis of this disease has so far been a very complex task because of the presence of 5 pseudogenes with a high identity (98%) with the *PKD1* gene. We have developed a PCR+sequencing based technology that allows a specific and accurate analysis of the *PKD1* sequences, excluding the pseudogenes ones.
In this work we have used this methodology to obtain a genetic diagnosis in a cohort of ADPKD from different Spanish hospitals. Results do not show the presence of prevalent mutations in the Spanish population. We have identified a high percentage of non-previously mutations making difficult their clinical interpretation. In these cases, familial analyses have been performed and results have been supported by *in silico* analyses. The identification of the disease-causing mutations in several Spanish families has allowed to provide a genetic counselling and an accurate genetic diagnosis to the patients and opened the possibility to offer familial testing and prenatal genetic diagnosis.

P12.006

Recurrent agnathia caused by DNA replication slippage in PRRX1 gene

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Agnathia-otocephaly is a rare craniofacial malformation which was recently found to be caused by de novo dominant as well as recessive mutations in the PRRX1 gene in two unrelated babies. We studied the PRRX1 gene in a non-consanguineous Indonesian family with a 4 month old daughter who was diagnosed prenatally with severe micrognathia (bilateral Pruzansky III). Her older affected brother died shortly after birth and clinically had agnathia-otocephaly. A frame shift mutation in a poly lysine (poly A) tract in the PRRX1 gene was identified in the proband while her father just had an insertion of one lysine residue . Expression of both mutations in COS7 cells showed loss of function of the frame shift mutation only. SNP analysis coupled with the recurrence of this mutation in this family are consistent with paternally derived germline mosaicism rather than autosomal recessive inheritance. Also, severe micrognathia (bilateral Pruzansky III) and agnathia-otocephaly represent a spectrum of craniofacial malformations in this family.

P12.007

COL4A5 mutational analysis of 51 unrelated Portuguese patients with Alport syndrome - preliminary report

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Introduction: Collagen type IV glomerulopathies include Alport syndrome (AS) and thin basement membrane nephropathy (TBMN). X-linked AS (XLAS) is caused by *COL4A5* mutations and the autosomal recessive and dominant forms of AS and TBMN are due to *COL4A3* and/or *COL4A4* mutations. Approximately 80% of AS is X-linked.
Aim: Describe the molecular pathology of XLAS in Portuguese families.
Patients and methods: In the setting of an ongoing national multicenter study, 51 unrelated patients with the clinical diagnosis of AS referred by nephrologists have already been studied. Mutational analysis of *COL4A5* gene was performed by direct PCR sequencing and multiplex ligation-dependent probe amplification (MLPA). Direct PCR sequencing of the *COL4A3* and *COL4A4* genes will be subsequently performed in *COL4A5*-negative cases.
Results: *COL4A5* direct sequencing identified 5 missense mutations [c.4342G>C (p.Gly1448Arg); c.715G>A (p.Gly239Arg); c.1009G>A (p.Gly337Ser); c.1844G>A (p.Gly615Glu); c.2633G>T (p.Gly878Val)], 4 splice site mutations [c.1339+6C>G; c.4297+1G>A; c.4803+1G>A; c.891+83_84insACTT], 3 deletions [c.2423del (p.Gly808fsX818); c.590del (p.Gln197fsX202); c.2510_2554del(45bp)], 2 nonsense mutations [c.4444C>T (p.Gln1482X); c.2815G>T (p.Glu939X)] and one previously reported mutation of unknown significance [c.1992G>T (p.Lys664Asn)]. Four large deletions were detected by MLPA [del ex.1_13 + (del ex.1_2 COL4A6); del ex.2_29; del ex.2_51; del ex.43_45].
Discussion: *COL4A5* mutational analysis confirmed the diagnosis of XLAS in only 19 families (≈37%), allowing the identification of 15 novel mutations, comprising ≈90% of all genetically confirmed AS cases. Two index patients carried the same missense mutation with a similar microsatellite haplotype, suggesting that they may share a common ancestor. The clinical criteria used by nephrologists appear to overestimate the diagnosis of AS.

P12.008

Alport syndrome epidemiology in Greek-Cypriots

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Alport syndrome (AS) is a hereditary hematuric nephritis, associated with sensorineural deafness, eye defects and progression to end stage kidney disease (ESKD) around 20-25 yo. *COL4A5* gene on chromosome Xq22-23 accounts for ~80% of all AS cases (XLAS). *COL4A3/COL4A4* genes on chromosome 2q36-q37 account for the remaining cases (ARAS).
We are studying *COL4A3/COL4A4/COL4A5* genes (in AS and in familial hematuria) for the last 9 years. Depending on inheritance pattern, we screen either *COL4A5* or either *COL4A3/COL4A4* or all three genes (totally ~150 exons). *COL4A5* is analyzed by genomic PCRs and direct re-sequencing. *COL4A3/COL4A4* screening is accomplished by genomic PCR and SURVEYOR endonuclease, followed by targeted DNA re-sequencing. PCR-RFLP is used for detecting more mutation carriers in the AS families.We have DNA samples from all Greek-Cypriot AS families (totally nine) refered to Cyprus' hospitals. Two were studied and published by a French laboratory in 2001 (one homozygous for the COL4A3-c.3533delC mutation and one with the de-novo COL4A5-G618R mutation). In two of them, we found a novel mild mutation, COL4A5-P628L. Two others were found to be compound heterozygous cases [COL4A3-G1334E with COL4A3-G871C (novel); COL4A3-c.2621-2622-delGAinsT with COL4A3-G1077D (novel)]. The remaining three were not studied yet, but the inheritance pattern is obvious (two ARAS, one XLAS). Totally: four XLAS families (45%) with ten living patients (71% of all cases) and five ARAS families (55%) with four living patients (29% of all cases). Although we found more XLAS families than ARAS, the total percentage of XLAS and ARAS cases resembles that in other countries.

P12.009

The p.Gly624Asp mutation in the *COL4A5* gene is the prevalent mutation in the Czech families with X-linked Alport syndrome

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Aim of the study: Alport syndrome is characterized by progressive hereditary nephritis, hearing loss, and ocular anomalies may also be present. The disease is genetically heterogenous, 85% of cases being X-linked caused by *COL4A5* gene mutations. The aim of the study was to detect *COL4A5* gene mutations in patients with hereditary nephritis or hematuria.
Patients and methods: Molecular genetic analysis of the whole coding sequence, i.e. exons 1-51 of the *COL4A5* gene was performed in 61 unrelated patients. Denaturating gradient gel electrophoresis, high resolution melting analysis, direct sequencing, and the MLPA method were used. Besides that, 48 family members of the patients with disclosed mutation were tested for carriership of the familial *COL4A5* mutation. Computer modelling was performed to simulate the impact of some mutations at the protein level.
Results: A pathogenic mutation has been found in twenty-nine of 61 patients (i.e. in 47%). The c.1871G to A / p.Gly624Asp mutation in exon 25 was the prevalent one being found in 12 families (i.e. 41% of pathogenic mutations). This is the only mutation that has been published in the HGMD and ARUP databases. All the other mutations are novel. Thirty-eight of the 48 tested family members were found to carry the familial mutation.
Conclusion: In nearly half of the Czech families with *COL4A5* gene mutations the c.1871G to A / p.Gly624Asp was found suggesting common ancestry.

P12.011

Determination of the exact GGGGCC-hexanucleotide repeat length of C9ORF72 in German and Swedish families with amyotrophic lateral sclerosis and frontotemporal dementia

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Amyotrophic lateral sclerosis (ALS) is a progressive, adult-onset neurodegenerative disorder affecting the upper and lower motor neurons. Mutations in 15 genes have been identified in familial cases and mutations in SOD1 have been reported to account for 12-23%% of familial cases. Recently, an intronic GGGGCC-hexanucleotide repeat expansion in C9ORF72 was found to be associated not only with ALS but also with frontotemporal dementia (FTD) and an overlapping phenotype combining ALS and FTD. Indeed, a repeat expansion in C9ORF72 was identified in up to 30% of familial and even 5% of sporadic ALS cases making C9ORF72 the most commonly mutated ALS gene. In most of the studies published so far, molecular testing solely relies on an indirect PCR-based methodology for repeat detection without determining the exact repeat size. Therefore, little is known about the exact size and size range of causative C9ORF72 alleles. Here, we report on a Southern-blot based analysis of the exact repeat length in a cohort of German and Swedish ALS families as well as on possible repeat expansions in seemingly healthy controls. Our study thus broadens the knowledge about the size range of normal and pathological C9ORF72 alleles, which is important e.g. for the identification of genotype-phenotype correlations as well as for improved individualized risk predictions and genetic counseling.

P12.012

The world largest ALS-FTD pedigree is linked to a massive GGGGCC-repeat expansion in the C9ORF72 on 9p21: The Mörtsell Disease.

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We present a 2200-individual pedigree in 6 countries where individuals either with amyotrophic lateral sclerosis (ALS) or frontotemporal dementia (FTD) in 1999 was found to be linked to 9p21. We now demonstrate that the patients with ALS or FTD share a massive GGGGCC-hexanucleotide expansion in C9ORF72 as a cause of neurodegeneration. The patients have variable degrees of expansion in leucocytes from peripheral blood. Autopsies performed on 6 patients (4 ALS, 2 FTD) show very similar morphological cellular findings but in different cell populations depending on the phenotype of the individual. Remarkably, while no mutations could be detected in the SOD1 in any of the patients, all FTD and ALS patients showed neuronal inclusions that stained positive for highly specific antibodies for misfolded wild-type SOD1. While the disease penetrance for either ALS or FTD is complete in some parts of this huge family, in other parts it is incomplete. Whether this is due to variable size of the hexanucleotide expansion in different cell populations or have other causes is being studied.

P12.013

PAX6 mutations in 93 aniridia Italian cases

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Aniridia is a rare human congenital malformation of the eye characterized by almost complete absence of the iris and other eye malformations. This disease is a dominantly inherited condition and sequence analysis had established that causative mutations involve *PAX6* gene located in chromosomal region 11p13.

We have investigated the presence of *PAX6* mutations in 93 cases of aniridia come from different Italian regions. We have used molecular techniques such as sequence analysis and MLPA for every case.

Among the 93 cases, 44 are familiar (47%). The causative mutation was identified in 50 subjects (54%). In 19 cases (38%), the mutations was a deletion not identified through MLPA, in 31 remaining cases (62%) by sequence analysis. In two cases with deletion, both *PAX6* and *WT*1 genes are involved. In 10 cases, the deletion regard only *ELP4* gene (located about 35 Kb downstream *PAX6* 3' region), suggesting that the disease is caused by a mutation that affect element controlling gene expression.

Among the identified mutations by sequence analysis, 21 consist in nonsense mutations, 5 in missense mutations, and 5 in site splicing mutations. Several of these mutations are novel, not present in the PAX6 mutation database (http://www.hgu.mrc.ac.uk/Softdata/PAX6/).

These data provide important indications: i) Mutational screening of *PAX6* gene must include techniques such as MLPA, which is able to identified mutations not observed by analysis sequence. ii) In a relevant fraction of patients with aniridia (about 10%), the alteration consist in a deletion of element that play a role in the transcriptional control.

P12.015

PKHD1 mutations in autosomal recessive polycystic kidney disease in Hungary

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Autosomal recessive polycystic kidney disease (ARPKD) is a severe inherited monogenic disease, which is characterized by enlarged polycystic kidneys and congenital hepatic fibrosis. Prognosis of the disease is very poor, 30% of the affected children die within the first year of life. Incidence of ARPKD is 1 in 10.000-40.000. ARPKD is caused by mutations in polycystic kidney and hepatic disease gene 1 (PKHD1) that encodes a large protein called fibrocystin/polyductin. PKHD1 gene consists of 67 exons.

The goal of our study was to establish the mutational spectrum of ARPKD families in Hungary. All exons of the longest open reading frame of PKHD1 gene and their intronic boundaries were amplified in 77 amplicons and sequenced. We have analyzed 18 families with ARPKD. Of the 15 different detected mutations, 8 missense mutations, 5 nonsense mutations and 2 small deletions could be identified. Four novel mutations were found, of which 1 missense, 2 nonsense and 1 small deletion. In 15 families both causative mutations could be identified, in one patient one mutation was found while no genetic alteration could be detected in two patients. Mutation detection rate was 86%. Five mutations, namely c.107C>T (p.T36M), c.3407A>G (p.Y1136C), c.5513 A>G (p.Y1838C), c.6992T>A (p.I2331K) and c.7916C>A (p.S2639X) were detected in more than one patient and are responsible for 58% of PKHD1 disease-causing alleles among Hungarian patients. One of them, c.7916C>A, was found to be surprisingly frequent, being responsible for 22% of all PKHD1 null alleles.

P12.016

Islet1 is a direct transcriptional target of the homeodomain transcription factor Shox2 in the sinoatrial node of the developing heart

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The heart's rhythm is initiated and regulated by a group of specialized cells in the sinoatrial node (SAN), the primary pacemaker of the heart. Abnormalities in the development of the SAN can result in irregular heart rates (arrhythmias). Although several of the critical genes important for SAN formation have been identified, our understanding of the transcriptional network controlling SAN development remains at a relatively early stage. The LIM homeodomain transcription factor Islet1 (Isl1) represents a prominent marker for cardiac progenitor cells of the second heart field and has been proposed, very similar to Shox2, to play an essential early role in the specification and patterning of the SAN.

Here, we compared gene expression levels in the right atria of wildtype and *Shox2*$^{-/-}$ hearts using microarray experiments and identified *Isl1* as one of its putative target genes. The downregulation of *Isl1* expression in *Shox2*$^{-/-}$ hearts was confirmed and the affected region narrowed down to the SAN by whole mount *in situ* hybridization. Using luciferase reporter assays and EMSA studies, we identified two specific SHOX2 binding sites within intron 2 of the *ISL1* locus. We also provide functional evidence for *Isl1* as a transcriptional target of Shox2 by rescuing the *Shox2*-mediated bradycardia phenotype with *Isl1* using zebrafish as a model system.

Our findings demonstrate a novel epistatic relationship between *Shox2* and *Isl1* in the heart with important developmental consequences for SAN formation and heart beat.

P12.017

Genetic screening of plakophillin-2 (PKP2) gene in Russian patients with arrhythmogenic right ventricular dysplasia

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Background: Arrhythmogenic right ventricular dysplasia (ARVD) is an inherited cardiomyopathy characterized by progressive fibro-fatty replacement of the myocardium, specific ECG-pattern, and high risk of life-threatening ventricular arrhythmias. Disease mainly

affects the right ventricle, but left ventricle, atriums and septum might be involved. Mutations in the desmosome armadillo repeat protein plakophilin-2 are common in patients with ARVD. Methods: A group of 12 unrelated Russian ARVD patients were examined. Clinical and instrumental examination included collecting of personal and family history, physical examination, standard and 24 h-ECG, Echo-CG and cardiac MRI. Genetic analysis of the PKP2 gene was performed by direct Sanger sequencing. Results: Genetic screening of mutations in PKP2 gene in DNA samples of 12 Russian ARVD patients was performed. Two mutations p.S140F and P.W538X were found in two unrelated families. Female patient (35 y.o.) carried heterozygous p.S140F variant had thinning of anterior wall infarction of the right ventricle and the presence of epicardial fat. Male patient (71 y.o.) carried heterozygous nonsense PW538X had sustained ventricular tachycardia since 41 y.o., ventricular fibrillation, AVB(I) and hypertrophy and dilation of RV. ICD was implanted, patient had repeated appropriate shocks. Those two variants were previously described as disease-causing mutations. Three additional variants without apparent clinical significance were detected in 3 patients. Conclusion: We identified two mutations in PKP2 gene in 2 of 12 Russian unrelated ARVD patients (16%). This prevalence matches with the prevalence of ARVD9 (MIM*609040). Genetic analysis of family members and genotype-phenotype correlation is in progress now.

P12.018

Asphyxiating Thoracic Dysplasia: clinical and molecular review of 42 families

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Asphyxiating Thoracic Dysplasia (ATD) belongs to the short rib polydactyly group and is characterized by a long and narrow thorax, short long bones and trident acetabular roof. Other features have been reported including polydactyly, retinal, renal and liver involvement. Today, mutations in IFT80, DYNC2H1, TCC21B and WDR19 genes have been reported in ATD. The clinical and molecular heterogeneity lead to difficulties in the evaluation of the long term prognosis.

Through a national grant (PHRC AOM 06031), we investigated 55 ATD cases from 42 families, and including 29 fetuses. They benefited from a combined approach of deep phenotyping, and IFT80 and DYNC2H1 molecular screening. The series included 26 postnatal cases, ranging in age from 6 months to 36 years. Significant pulmonary insufficiency was noted in 46% of cases, with tracheotomy requirement in 4 cases. Renal and liver diseases occurred in 16% of cases; whereas retinal alteration was present in 40 % cases aged more than 2 years (6/15). The molecular screening allowed the identification of DYNC2H1 mutations in 63% and IFT80 mutations in 6%. In 6 cases, only one heterozygote mutation in either IFT80 or DYNC2H1 was identified. Finally, the two genes were excluded in 31% cases.

These results emphasize that DYNC2H1 is the major gene responsible for ATD. The presence of only one mutation in 38% of mutated cases may suggest a digenic diallelic inheritance. The pulmonary prognosis is probably less pejorative and retinal involvement more frequent than previously thought. Follow up guidelines are proposed.

P12.019

Novel G2 micronucleus test allows detection of defects in homologous recombination and G2/M cell cycle control in a patient with adult ataxia telangiectasia (A-T) and parents

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We report here a patient (aged 23 years) with dystonic dyskinetic cerebral palsy and elevated serum alpha fetoprotein levels. The clinical diagnosis of adult A-T was confirmed by the identification of two heterozygous ATM mutations. The patient inherited a previously reported missense mutation, c.8122G>A (p.Asp2708Asn), from her father and a novel splice site mutation, c.8851-1G>T, from her mother.

AT patients are known to display enhanced chromosomal radiosensitivity. With the G0 micronucleus test, whereby the cells are irradiated in G0 phase of the cell cycle, 3-5X enhanced radiosensitivity is observed in typical AT patients compared to control persons. However, in this adult A-T patient, we only found a 1.7X increased radiosensitivity and no enhanced radiosensitivity in the parents. As ATM is involved in homologous recombination and G2/M cell cycle checkpoint, we developed a novel test, evaluating the number of micronuclei in lymphocytes irradiated in G2 phase of the cell cycle. This test showed about 3.5X enhanced radiosensitivity for the A-T patient compared to controls. For the heterozygous parents values were lower than for the proband, but significantly higher than for controls.

This novel G2 micronucleus test showed the potential to distinguish bi-allelic and mono-allelic ATM mutation carriers from controls. We will evaluate if this test allows determination of radiosensitivity in carriers of germline mutations in other genes involved in G2/M checkpoint and associated with an increased risk for breast cancer (BRCA1&2, Chek2, etc.). This could be an interesting approach to identify patients who would benefit from mutation testing of these genes.

P12.020

Atrichia with papular lesions: a recurrent missense mutation with novel phenotype

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Atrichia with papular lesions (APL) is a rare autosomal recessive disorder, characterized clinically by occurrence of universal congenital alopecia and the development of papular lesions of keratin-filled cysts over extensive areas of the body. Hair loss in APL is irreversible and the histology is consistent with an absence of mature hair follicles. In this study we ascertained a family AP7 from northern part of Pakistan. We used candidate gene approach by selecting four genes like *HR*, *P2RY5*, *LIPH*, and *DSG4*. Linkage analysis and direct sequencing of the PCR products carried out. A recurrent missense mutation (c.1859G>A) in exon 6 of the hairless gene (*HR*) is identified. This mutation has already been reported in a large family of Irish Travellers. Phenotypic appearance of affected individuals in this family was marked by complete absence of hair on the eyebrows, eyelashes and scalp. Cystic lesions were present on the elbows of affected individuals with no other abnormalities. This mutation found in the Pakistani population that segregates with APL in homozygous form in affected individuals. Phenotypes of the affected members of family AP7 are different from the already reported phenotypic descriptions. Hypotrichosis and nail dysplasia are observed in this family which is not reported before with this mutation in *HR* gene. Papular lesions are present in affected individuals. This research will help in the establishment of carrier screening test for prevention of above disease and to understand the molecular basis involved in the gene function.

P12.021

Inherited and *de novo* SHANK2 variants associated with autism spectrum disorder impair neuronal morphogenesis and physiology

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Mutations in the postsynaptic scaffolding gene *SHANK2* have recently been identified in individuals with autism spectrum disorder (ASD) and intellectual disability (ID). However, the cellular and physiological consequences of these mutations in neurons remain unknown. We have analyzed the functional impact caused by two inherited and one *de novo SHANK2* mutations from ASD individuals (L1008_P1009dup, T1127M, R462X). Although all three variants affect spine volume and have smaller SHANK2 cluster sizes, T1127M additionally fails to rescue spine volume in Shank2 knock-down neurons. R462X is not able to rescue spine volume and dendritic branching and lacks postsynaptic clustering, indicating the most severe dysfunction. To demonstrate that R462X when expressed in mouse can be linked to physiological effects, we analyzed synaptic transmission and behavior. Principal neurons of mice expressing rAAV transduced SHANK2-R462X present a specific, long lasting reduction in miniature postsynaptic AMPA receptor currents. This dominant negative effect translates into dose-dependent altered cognitive behavior of SHANK2-R462X expressing mice, with an impact on the penetrance of ASD.

P12.022

Evaluation of adRP microarray (Asper Biotech) for the diagnosis of autosomal dominant macular dystrophies

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Purpose: autosomal dominant macular dystrophies (adMD) are a group of diseases clinically and genetically heterogeneous. Currently there is no specific tool for genetic diagnosis. AdRP Microarray (Asper Biotech), which includes genes responsible for adMD, has recently demonstrated to be a cost-efficient tool for autosomal dominant retinitis pigmentosa diagnosis (1). We aim to test the AdRP Microarray for its potential use in adMD diagnosis.

Methods: 54 unrelated Spanish adMD patients affected were tested with adRP microarray. All mutations found were confirmed by sequencing. Peripherin 2(PRPH2), the most frequently mutated gene responsible for adMD, was sequenced in all negative samples for the microarray. The rate of false negatives (real mutations in PRPH2 gene represented but not detected by the array) and false positives (microarray results not confirmed by sequencing) were established.

Results: adRP microarray detected the mutation in 10 of the studied patients (diagnostic accuracy: 18.5%). Nine patients presented a mutation in PRPH2 gene, one in RHO gene. All mutations were confirmed by sequencing. Sequencing of PRPH2 of non-characterized families allowed the identification of one false negative. These results show a high level of analytical both sensitivity (91%) and specificity (100%).

Conclusions: adRP microarray shows high levels of analytical specificity and

sensitivity but sequencing of PRPH2 gene seems to be a more cost-efficient tool for adMD diagnostic than the adRP microarray.
(1) Blanco-Kelly et al, 2012.

P12.023
Identification of genetic defects in Iranian GJB2-heterozygous deaf individuals

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Hereditary hearing loss (HHL) is one of the most common sensorineural problems, affecting approximately one in 500 children. Although significant genetic heterogeneity exists as the cause of sensorineural HL, one locus, DFNB1, comprising the GJB2 and GJB6 genes, is responsible for up to 20-50% of cases with congenital non-syndromic HL in many populations. Homozygous or compound heterozygous mutations in GJB2 are detected in most cases with DFNB1-relatedHL. Interestingly, in some studies10-42% of deaf subjects showed recessive pattern of inheritance and withGJB2 mutations, carried only one mutant allele.
In this study, using direct sequencing; second mutant allele of GJB2 which leads to deaf phenotype was screened for possible mutations. One hundred patients with autosomal recessive non-syndromic hearing loss(ARNSHL) through Iranian population bearing first mutation in their coding exon (exon-2) of GJB2, were assessed for any other mutations in non-coding exon of GJB2 (exon-1)as well as promoter region of the gene.
We have identified the second mutant allele in splice site of exon-1 of GJB2 which known as -3170G to A in 14 probands (14%). No mutation in promoter region of GJB2 was found. Furthermore, Real-time PCR has been set up in order to check four known deletions which encompass both GJB2 and GJB6, for remainder probands.

P12.024
A novel ILDR1 gene mutation in two Iranian families with autosomal recessive non-syndromic hearing loss

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Hearing loss (HL) is the most common sensory deficit in human. It affects approximately 10 percent of the world population. Genetic causes of HL are estimated to account about 68% of newborns and 55% of cases by the age of four. Autosomal recessive non-syndromic hearing loss (ARNSHL) is the most common type of inherited hearing impairment. Due to the wealthy gene pool, Iran is a valuable source to identify the genes involved in different conditions.To date, several genes have been studied in Iranian deaf population.
DFNB42, one of the related loci in ARNSHL, was first identified in a Pakistani family. Another study on more Pakistani and Iranian families, led to the identification of 10 different mutations in the related gene, *ILDR1*.
To estimate the contribution of this gene in Iranian deaf population, we have set out to perform homozygosity mapping with flanking STR markers on 140 Iranian deaf families.
Mutation detection, using conventional sequencing, for three out of 140 families showing linkage to DFNB42 locus, led to identification of one splice site mutation (c.379+1G>A) in two of the families.
Our data shows that *ILDR1* gene has the prevalence of about 2.14% in Iranian deaf population, that comparing to other loci seems to have a small proportion of the ARNSHL causes in Iran.
Keywords:Autosomal recessive non-syndromic hearing loss, *ILDR1*, sequencing, Iran.

P12.025
A novel PJVK gene mutation in Iranian family with autosomal recessive non-syndromic hearing loss

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Hearing impairment is one of the common sensory disorders in world and affects about 1 of every 1000 newborns. In developed countries at least 50% of the cases are due to genetic defects resulting in non-syndromic deafness (70%), of which autosomal recessive inheritance predominates (80%). Hereditary hearing loss is very heterogeneous, so that nearly 95 loci and 40 genes have been identified as the causes of autosomal recessive non-syndromic hearing loss (ARNSHL) to date.
DFNB59 is one of the contributing loci in ARNSHL. Several studies showed mutations in PJVK as the cause of deafness in this locus. PJVK is considered as the first human gene implicated in non-syndromic deafness due to a neuronal defect. To date, several mutations have been reported in Iranian families from different studies and also some reports in Moroccan and Turkish populations as well.
In order to have more comprehensive look into PJVK gene in Iranian population, 144 ARNSHL families with two or more affected individuals from different Iranian ethnic groups were selected for this study.
Homozygosity mapping with flanking STR markers following conventional sequencing of the gene, revealed 2 mutations in 2 families, in which one of them is a novel nonsense mutation(c.274C > T or p.Arg92X) in exon 3. This data shows a prevalence of less than 2% in Iranian population for PJVK mutations. Although, mutations in this gene had been reported in different studies in Iranian deaf families but our data shows that this gene is not as prevalent as it seems.

P12.026
Molecular approach in Spanish families affected by Bardet-Biedl Syndrome

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Bardet-Biedl Syndrome (BBS, OMIM 209900) is a rare autosomal recessive ciliopathy associated with several features including obesity, retinopathy, polydactyly, mental retardation, hypogonadism, and renal defects. Until now 16 genes (*BBS1-BBS16)* have been involved in ~70% of the families.
We selected 36 individuals from 30 families from a Spanish population previously screened for mutations on *BBS* gene using a genotyping microarray (Asper Biotech Ltd) and with negative results. We performed direct sequencing of *BBS10* gene in these 36 patients. This gene accounts for more than 20% of the mutational load in our population. Without taking into consideration the recurrent mutations (*BBS1*-M390R, *BBS10*-C91LfsX95), *BBS10* gen carried the largest mutational load in our population.
Sequencing of *BBS10* gene revealed compound heterozygous mutations p.L533fsX554/ p.V331A (novel mutation) in one affected individual and a single heterozygous novel mutation p.Y197del in another patient. Our work led to the identification of mutations in two families (4.2%)/3 alleles.
In conclusion, two novel heterozygous mutations in *BBS10* gene were found. Our study shows the important of testing preferably *BBS10* gene when *BBS* genotyping microarray is negative in the diagnosis of Bardet-Biedl syndrome. Since we could not detect any mutations in 28 families, sequencing of next gene carrying the largest mutational load (*BBS1*) is convenient.

P12.027
Mutations in *RIPK4* that encodes Receptor-Interacting Serine/Threonine Kinase Protein 4 cause the Autosomal Recessive Form of Popliteal Pterygium syndrome

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The autosomal recessive form of popliteal pterygium syndrome, also known as Bartsocas-Papas syndrome (BPS), is a rare, but frequently lethal disorder characterized by marked popliteal pterygium associated with multiple congenital malformations. Using a genome-wide SNP homozygosity mapping strategy for this malformation syndrome in a Turkish family we identified a homozygous segment co-segregating with disease on chromosomal region 21q22.3. Since the phenotype of the deficiency of mouse ortholog of *RIPK4* consistent with anomalies seen in BPS, *RIPK4* was selected as candidate gene from the critical interval. Sequencing of the *RIPK4* showed a homozygous missense mutation p.Ile121Asn (c.362T>A) in the kinase domain of the protein. Screening of additional two BPS families showed a homozygous missense mutation p.Thr184Ile (c.551C>T) and a homozygous one base-pair insertion c.777_778insA (p.Arg260Thr*fs*X14) within the kinase domain of the protein. Molecular modeling and Luciferase reporter assays showed that both Ile121 and Thr184 positions are critical for the stability and catalytic activity of RIPK4. RIPK4 mediates activation of the nuclear factor-kB (NF-kB) signaling pathway that is required for keratinocyte differentiation and craniofacial and limb development. The abnormalities observed in presented individuals were similar, but less severe than those seen in Cocoon syndrome as a result of CHUK (IKKα) deficiency which is another component of NF-κB signaling pathway. In conclusion, our results showed that recessive mutations in *RIPK4* cause autosomal recessive form of multiple pterygium syndrome and RIPK4 and CHUK may function in closely related pathways to promote keratinocyte differentiation and epithelial growth.

P12.028

An identical *PRRT2* mutation underlies benign familial infantile epilepsy with and without paroxysmal dyskinesia

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Benign familial infantile epilepsy (BFIE) and paroxysmal dyskinesias are clinically and genetically heterogeneous paroxysmal neurological disorders. The ICCA syndrome is a phenotype combining both BFIE and paroxysmal kinesigenic dyskinesia (PKD). BFIE, PKD, and the ICCA syndrome have been linked to the pericentromeric region of chromosome 16. Recently, heterozygous mutations in the *PRRT2* gene on 16p11.2 were described as the cause of BFIE and paroxysmal dyskinesias. *PRRT2* encodes the proline-rich transmembrane protein 2 implicated to have a role in synaptic function. In this study we clinically and genetically characterized patients in three large Finnish families, which presented with either BFIE alone or in combination with a movement disorder with different types of triggers. Linkage analysis in two large ICCA families showed evidence for linkage to chromosome 16p12-p11.2 and a significant two-point LOD score of 5.55 was obtained at marker *D16S3022* (θ = 0.000). In the third family sharing a haplotype over the region on 16p12-p11.2 the clinical presentation was BFIE. After sequencing several positional candidate genes, the *PRRT2* gene was sequenced. Patients in all three families were heterozygous for the c.649dupC duplication mutation, the most frequently encountered *PRRT2* mutation. These data give further support for the association of *PRRT2* mutations to both epilepsy and movement disorders of both kinesigenic and non-kinesigenic type. The association of a single mutation with a variety of phenotypes highlights the contribution of modifying genetic and/or environmental factors to the clinical presentation.

P12.029

Two novel putative beta-globin gene mutations leading beta thalassemia intermedia phenotype

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There have been described approximately 800 different genomic alterations in the beta globin gene reported in the HbVar database and novel mutations are still rarely being reported. In this study, we aimed to identify two novel putative mutations in 3' un-translated region (3'-UTR) of the beta globin gene and to describe their clinical reflections. Four patients from two unrelated families referred with diverse set of hematological and clinical findings associated to beta thalassemia were included in this study. Molecular diagnosis of the beta globin gene mutations was performed by direct sequencing of the beta globin gene. A novel mutation HBB:c.*+108 A>G was found in combination with IVS-I-110 G>A mutation caused intermedia phenotype in two brothers and one sister with mild splenomegaly and occasional transfusion history in the first family. The second novel mutation named as HBB:c.*+132 C>T was found in combination with IVS-I-1 G>A in a 7 years old male diagnosed as beta thalassemia intermedia with irregular transfusion history in family 2. Based on beta thalassemia intermedia phenotypes despite of clinical diversity observed in our patients and taking into account the accompanying mutations, it would be concluded that these novel beta globin gene 3' UTR mutations are associated with mild phenotype of beta thalassemia.

P12.030

Novel indel mutation in CDMP1 gene is associated with brachydactyly type C in a four generation Turkish family

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The cartilage derived morphogenetic protein-1 (CDMP1), also referred as the growth/differentiation factor 5 (GDF5) gene, has been shown to be a key regulator in the bone morphogenic protein pathway (BMP) during skeletal and joint development. Heterozygous loss-of-function mutations reported to cause hypoplasia/aplasia of certain skeletal elements (brachydactyly), heterozygous gain-of-function mutations, occurring either on the gene itself or through the loss of its inhibitor noggin, result in joint fusion (symphalangism). Furthermore, homozygous mutations, predominantly affecting the limbs have been described; Grebe type dysplasia, Du Pan Syndrome, Acromesomelic Dysplasia-Hunter Thompson type. Also reported is homozygous missense mutation presenting with brachydactyly, formulating phenotype-genotype correlations by type and domain inconceivable, likely due to the influence of other factors impacting the developmental pathway. Presently, 34 mutations dispersed throughout propeptide and chain domains of CDMP1, associated with eight different OMIM entries, have been described.
We ascertain here two affecteds, one female and one male, with brachdactyly type C (MIM# 113100) presenting with disproportionate shortness of the 2nd and 3rd fingers and hypersegmentation of the proximal and middle 2nd and 3rd phalanges. These cases are from a family that reports an additional 8 affected members spanning across four generations. CDMP1 analysis revealed a novel heterozygous in frame indel mutation (c.803_827del25ins25) in the propeptide domain (p.cys268_ser276delCPSGRQPASinsLLSALLDVN). This is the second indel mutation ascribed to the CDMP1. The previously published indel mutation was of the out-of-frame type in homozygous state, in the chain motif, associated with Du Pan Syndrome. Our novel mutation further emphasizes the allelic heterogeneity of CDMP1.

P12.031

Altered Genomic DNA Binding Profile of a HOXD13 Mutant in a Novel Type of Brachydactyly

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Mutations in HOXD13 have previously been associated with synpolydactlyly. Here we describe a patient with brachydactyly and a novel mutation (Q317K) in the DNA binding homeodomain of HOXD13. Anomalies of hands and feet are characterized by shortness of fingers and toes, oligodactyly, and aplasia of some terminal phalanges. Functional analysis was performed by retroviral overexpression of FLAG-tagged HOXD13 wt and mutant in chicken micromass cultures, a well established model of chondrocyte differentiation. Targets of HOXD13 were identified by chromatin immunoprecipitation followed by next-generation sequencing (ChIP-seq). Bioinformatic analysis of precipitated sequences showed an altered DNA binding motif of the mutant protein. The inferred motif of HOXD13^{Q317K} showed similarity to that of PITX1. The mutant lysine at position 317 corresponds also to a conserved lysine at the analogous position of bicoid type homeodomain proteins such as PITX1. Thus we compared the binding pattern of HOXD13^{wt}, HOXD13^{Q317K}, and PITX1 using ChIP-seq and found a shift of HOXD13^{Q317K} towards a PITX1-like binding pattern. Furthermore the expression profile in HOXD13^{Q317K} overexpressing micromass cultures also shifted towards a PITX1 overexpression profile. Injection of HOXD13^{Q317K} into developing wing buds of chicken embryos showed a PITX1-like phenotype as well. Our results demonstrate how ChIP-seq can be used to characterize genome-wide binding profiles of mutant transcription factors. The mutation results in a partial conversion of HOXD13 into PITX1 and thus ectopic activation of PITX1 targets.

P12.032

New genes involved in metabolic disorders: Diseased states due to errors in the enzymatic regulation of the mammalian branched-chain α-keto acid dehydrogenase complex

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Mammalian branched-chain α-keto acid dehydrogenase complex (BCKDc) is a mitochondrial macromolecular multienzymatic complex consisting of three catalytic components that catalyzes the rate-limiting step in the oxidation of branched-chain α-keto acids. BCKDc deficiency leads to the autosomal inborn error of metabolism -maple syrup urine disease (MSUD)-. Activity of the complex is regulated by a specific kinase (BCKDK) which causes inactivation, and the protein phosphatase 2Cm (PP2Cm) which causes activation. Up to now, the 150 described MSUD-causing mutations have been only found on the genes encoding for the E1α, E1β and E2 catalytic subunits, but not much is known regarding diseased states that could arise due to errors in the enzymatic regulation of BCKDc. We present here the first two patients with a defective regulation of BCKDc. The first one corresponds to a MSUD patient with a mild variant phenotype. The second one is a patient with neurological abnormalities, showing a decreased amount of branched-chain amino acids levels in physiological fluids. The Sanger sequencing of BCKD related genes in both patients evidenced: a homozygous null mutation change c.417_418delTA (p.His139fs) in the *PPM1K* gene, which encodes for PP2Cm for the MSUD patient, and that was proved to be pathogenic by genetic complementation in patient's fibroblasts using the pT-REX-DEST30/*PPM1K*wt vector, and a homozygous c.1166T>C (p.Leu389Pro) nucleotide variation in the *BCKDK* gene, undetected in 300 control alleles, for the second one. The disease phenotype of these two patients demonstrates the importance of tight regulation of oxidative disposal of BCAA for normal growth and neurological functions.

P12.033

Application of RNA analysis for evaluation of unclassified variants in routine genetic diagnosis of breast cancer and neurofibromatosis 1

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A frequent problem in molecular genetic diagnosis is the assessment of unclassified variants (UCVs). Exonic missense or silent variants and intronic variants outside the invariably exon-flanking dinucleotides are ambiguous and *in silico* analysis has its limits in being theoretical. Expression analysis via RNA from blood lymphocytes can help to discriminate between variants that influence correct splicing and those that don`t. A precondition for this approach is the expression of the corresponding gene in blood cells as is the case for e.g. BRCA1, BRCA2, RAD51C and NF1. Using reverse transcription, RT-PCR and sequence analysis of the resulting product(s) we were able to show a splice effect for the BRCA1 variants p.G1366S, IVS19-12A>G, the RAD51C variant p.C135F and the NF1-variants IVS16del-6_3, IVS3+6T>G and p.L1569V. Conversely, a splice effect could be excluded for the BRCA1 variants p.D120N, p.T1548T and p.Q1604Q, the BRCA2 variants IVS9-11T>C and IVS22-7_4del, the RAD51C variant p.G3R and the NF1 variants p.K724K, L549Q, p.F1289F, p.R1375H and p.L1957L.

Preparing RNA from blood and performing cDNA analysis is usually not part of the routine molecular diagnosis. In our laboratory practice - if a possible splice mutation was identified - we recommend to take a second blood sample in the context of the subsequent genetic counselling in order to perform RNA analysis. In view of the potential benefit by solving the question of pathogenicity, this turns out to be a feasible effort.

P12.034

New mutation in SNTA1 gene in Russian Brugada Syndrome patient - a new causative gene?

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Brugada syndrome (BrS) is inherited cardiac arrhythmic disorder characterized by ST-segment elevation in right precordial leads, pseudo right bundle branch block, T-wave inversion and high risk of cardiac sudden death due to polymorphic ventricular tachycardia (PVT). The SCN5A gene was identified as causative in 1998 and has been only one known for BrS for many years. Mutations in this gene account 15%-30% of all cases. Starting from 2007 the list of genetic variants was increased up to eight. But all those genes seem to be much less prevalent and does not explain vast majority of BrS cases.

Clinical, instrumental and familial data, informed consent and blood samples were obtained from 20 unrelated SCN5A-negative Russian BrS patients. The PCR-based Senger sequencing of full coding and adjacent intronic areas of SCN5A, and few additional known and new candidate genes including SNT1A gene was performed.

We did find a new rare missense variant c.R106Q in SNT1A gene in patient with spontaneous BrS1-type ECG, registered episodes of PVT, and numerous appropriate shocks after ICD implantation. Quinidine was ineffective in decreasing VT frequency. This genetic variant had not been found in a control group.

New rare genetic variant in SNT1A gene had been found in Russian SCN5A-negative BrS patient. Syntrophin 1A is a protein interacting with Nav1.5 channel and can be considered as a candidate gene for BrS. It might influence on cardiomyocyte repolarization via altering sodium channel function. To elucidate possible causative role of c.R106Q in SNTA1 gene further functional investigations are required.

P12.035

Reliable and sensitive SCN5A high resolution melting curve analysis as a primary gene scanning assay for genetic diagnosis of Brugada syndrome

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Brugada syndrome (BrS) is an autosomal dominant inherited primary cardiac arrhythmia in a structurally normal heart, with a propensity to sudden cardiac death. Genetic defects have mainly been attributed to mutations in the alpha-subunit of the sodium channel gene (SCN5A), which account for approximately 20% of cases. The clinical diagnosis is based on an anamnesis of the patient and a baseline and/or infusion electrocardiogram, which should be confirmed genetically by the mutation analysis of the SCN5A gene. To allow a convenient and cost-effective diagnostic genetic testing for BrS a primary indirect gene scanning assay by high resolution melting curve analysis (HRMCA) of the SCN5A gene was developed.To assess the sensitivity and specificity of the HRMCA assay over 100 clinically diagnosed BrS patients were analyzed in parallel by bidirectional cycle sequencing of the 28 SCN5A exons and by HRMCA analysis of 24 exons. Sensitivity of the HRMCA could be increased by using spike-DNA completely homozygous in the amplified regions and by discriminatory analysis of melting patterns with dual melting domains. All of the Sanger sequencing confirmed mutations and SNPs could be detected through HRMCA, with the exception of a deep intronic mutation lying 8 nucleotides downstream of the 3'end of the forward primer. Specificity of the assay met expectations. This study demonstrates that SCN5A HRMCA analysis can be implemented as a cost-effective, high-throughput, user-friendly primary gene scanning method within the framework of the molecular diagnosis of BrS.

P12.036

The imprinted *C15orf2* gene in the Prader-Willi syndrome region encodes a nuclear pore complex associated protein

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The Prader-Willi syndrome (PWS) region in 15q11q13 harbours a cluster of imprinted genes expressed from the paternal chromosome only. Whereas loss of function of the *SNORD116* genes appears to be responsible for the major features of PWS, the role of the other genes is less clear. One of these genes is *C15orf2*, which has no orthologs in rodents but appears to be under strong positive selection in primates.

C15orf2 encodes an 1156 aa protein with six nuclear localisation sequences. To find out more about the function of C15orf2, we used the Phyre software for sequence threading against a structural database and InterProScan for a pattern and profile search. We found a highly significant similarity of the C-terminal part of C15orf2 to the nuclear pore complex (NPC) protein Pom121. Using the Invitrogen Flp-In system we generated a stable cell line that expresses flag-tagged C15orf2 upon doxycycline induction. By immunocytochemistry in expression-induced cells we found C15orf2 located at the nuclear periphery, where it co-localized with NPC and nuclear lamina proteins. Extending these observations to three-dimensional structured illumination fluorescence microscopy (3D-SIM), which achieves an eight-fold improved volumetric resolution over conventional imaging and can resolve single NPCs, we saw that C15orf2 is located at the inner nuclear membrane where it strongly associates with the NPC. Additionally, in nuclear envelope isolation and fractionation experiments C15orf2 co-purified with NPC and nuclear lamina proteins.

These experiments for the first time demonstrate that C15orf2 is part of the NPC or its associated molecular networks.

P12.037

Whole CYP21A2 gene analysis of congenital adrenal hyperplasia patients due to 21-hydroxylase deficiency

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Introduction: Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder, caused by mainly 21-hydroxylase deficiency. Females with severe 21 hydroxylase deficiency are exposed to excess androgens prenatally and are born with ambiguous genitalia. Also most patients can not synthesize sufficient aldosterone to maintain sodium balance and may develop potentially fatal "salt wasting" crises if not treated.
Methods: Whole CYP21A2 gene were analyzed in 100 patients and 80 controls. Distinctive primers were selected for the amplification of the functional gene (CYP21A2) as there is a pseudogene (CYP21A1P) with 98% homology. After the isolation of DNA from peripheric blood, functional gene was amplified by PCR. Mutations were detected with direct sequencing. Results were evaluated statistically.
Results: Totally 66 different mutations/polymorphisms were found in which 20 of them cause amino acid changes and 24 of them are novel. Also 12 of them were found statistically significant in patients when compared to controls. Additionally G→A mutation at position 711 (13 %) (novel mutation), A/C→G mutation at position 777 (38 %) and C→G mutation at position 1709 (20 %) were only detected in the patient group and were found statistically significant when compared to controls.
Conclusion: Prenatal diagnosis of CAH has been utilized for over 20 years especially by screening 9 known mutations. In case of obtaining the similar results of G→A mutation at position 711 in different populations, it may be used as a marker for prenatal diagnosis. Therefore, genital ambiguity and salt wasting can be reduced in CAH patients by using prenatal approaches.

P12.038

Targeted next-generation sequencing for the molecular genetic diagnostics of arrhythmogenic cardiac disorders in sudden cardiac death

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Sudden cardiac death (SCD) is the most common cause of death in the young. Ion channel disorders (channelopathies) such as the long QT syndrome (LQTS), catecholaminergic polymorphic ventricular tachycardia (CPVT) and Brugada syndrome (BrS) may account for more than 30% of unclear SCD cases lacking cardiac structural abnormalities. Other frequent causes of SCD are hypertrophic cardiomyopathy (HCM) affecting 1 of 500 people and arrhythmogenic right ventricular dysplasia (ARVD). As HCM and ARVD may present with only little structural symptoms, the differential diagnosis of these arrhythmogenic disorders may be difficult. Stepwise analysis of various genes with traditional Sanger sequencing is elaborate, time-consuming and expensive. Although clearly superior to Sanger sequencing, NGS has little impact on clinical testing to date. Sequence capture target enrichment followed by re-sequencing on the Roche Genome Sequencer FLX (GS FLX) system could be useful for novel mutation detection. A Roche-NimbleGen and an Agilent SureSelect in solution target enrichment assay was designed for the capture of coding regions including splice sites of 40 SCD genes known to be associated with SCD. Both enrichment assays were validated with two samples with known mutations in duplicates. Additional clinical SCD samples with unknown genotype were analyzed with a minimum coverage of 30-fold. Data analysis was performed with the CLC Genomic Workbench software. Potentially disease causing variants and regions with an insufficient coverage were re-analyzed with Sanger sequencing.

P12.039

The mutation makes the difference - Analysis of *RMRP* transcript levels in Cartilage-hair hypoplasia

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Cartilage-hair hypoplasia (CHH) is an autosomal recessive disorder characterized by short stature and metaphyseal dysplasia. Variable extraskeletal features include sparse hair, immunodeficiency, and predisposition to malignancy. CHH is caused by mutations in the *RMRP* gene, which encodes the RNA component of the RNase MRP complex. RNase MRP is involved in the endoribonucleolytic processing of specific RNA substrates (viperin and cyclin B2), RNA primers for mitochondrial DNA replication, and pre-5.8S rRNA. The *RMRP* RNA also forms complexes with TERT, the telomerase protein component.
When reanalyzing the *Rmrp* expression in heterozygous *Rmrp* 70A>G knock-in mice, we exclusively detected the wildtype allele in all tested tissues. We hypothesized that the instability of the mutated transcript had increased after neo-cassette removal and decided to analyse the expression of mutated *RMRP* transcripts in CHH patients. Our qPCR data show clearly reduced *RMRP* transcript levels in all patients and mild reductions in parents carrying one mutated allele. Then we compared the transcript ratios of both alleles by sequencing. Confirming our mouse data, the mutated transcript was nearly absent in heterozygous parents. Interestingly, we also observed shifted transcript ratios in compound heterozygous CHH patients.
Our results indicate that *RMRP* mutations lead to reduced *RMRP* transcript levels, probably due to instability of mutated transcripts. Furthermore, the transcript instability depends on the individual *RMRP* mutation. The different stabilities and resulting variable residual *RMRP* transcript levels might explain the diverse clinical presentation of CHH patients, as, e.g., the degree of telomerase dysfunction observed in CHH patients may be dosage-dependent.

P12.040

Identification of novel CAV3 gene alterations

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The human CAV3 gene, mapping to chromosome 3p25, encodes for caveolin-3 muscle-specific protein, which is the principal integral membrane component of caveolae. Caveolin-3 is localized to the muscle cell plasma membrane, where it forms a complex with dystrophin and associated-proteins. This suggests that the small vesicular invaginations of the plasma membrane (caveolae), involved in cell signaling and trafficking, might play an important role in muscle membrane physiology. In vitro and in vivo experimental models indicate that the maintenance of normal levels of caveolin-3 is essential for normal skeletal muscle development and postnatal function. Mutations in the coding sequence of CAV3 cause dominant limb-girdle muscular dystrophy (LGMD-1C). In this survey 121 Greek individuals, comprised of 119 normal controls and 2 patients presenting with increased serum creatine kinase (CK) levels, muscle weakness and a compatible with caveolinopathy muscle biopsy were screened for mutations along CAV3 gene. Two causative mutations in exons 1 and 2, respectively where disclosed in the 2 patients. Twenty three heterozygous polymorphic alterations were identified in the control group, including 3 missense mutations- one of which is novel, and two previously reported - a novel a microdeletion in 3'UTR and 19 silence mutations. As different alterations were detected between the control group and the patients, interpretation of gene defects will help towards the understanding of LGMD-1C pathogenesis.

P12.041

Genotype-phenotype correlations in cerebral cavernous malformations

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Cerebral cavernous malformations (CCM) are prevalent cerebrovascular lesions predisposing to chronic headaches, epilepsy, and hemorrhagic stroke. Individuals carrying an autosomal dominantly inherited mutation in *CCM3/PDCD10* have been reported to have a higher risk for cerebral hemorrhage during childhood when compared to *CCM1/KRIT1* and *CCM2/OSM* mutation carriers. Most recently, it has also been suggested that CCM3 function may be distinct from CCM1 and CCM2 and that CCM3 acts in different molecular pathways.

Genomic DNA sequencing and MLPA analyses allowed us to identify 38 additional CCM probands harbouring a loss-of-function mutation in one of the three CCM genes (21 in *CCM1*, 5 in *CCM2*, 12 in *CCM3*) over the past three years. Notably, the proportion of *CCM3* mutation carriers (32%) was significantly higher than previously reported. The mean age at referral was 17 years for index patients with a *CCM3* mutation (ranging from 1 to 51) while the mean age at referral was 36 years for *CCM1* probands (ranging from 6 to 79) and 43 years for *CCM2* probands (ranging from 17 to 72). We are currently inquiring for more details about disease manifestations and disease processes. However, our data already suggest a tendency towards earlier disease presentation in *CCM3* mutation carriers.

P12.042

The atypical Rett-syndrome protein CDKL5 promotes excitatory synapse formation by strengthening the interaction between NGL-1 and the postsynaptic scaffold protein PSD95

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Mutations in the X-linked gene cyclin-dependent kinase like 5 (CDKL5) cause a severe neurodevelopmental disorder with clinical features that are closely related to Rett syndrome (RTT), including intellectual disability, early onset intractable epilepsy and autism. However, very little is currently known about the biological role of CDKL5. We here show that Cdkl5 localizes at excitatory synapses and contributes to correct spine morphology and synaptic activity. Since we previously found that a balanced chromosome translocation in the Netrin-G1 gene (NTNG1) also caused atypical RTT with early onset seizures, we hypothesized that the two genes play a role in common pathogenetic processes. Immunofluorescence and immunoprecipitation experiments revealed that Cdkl5 interacts with the Netrin G1 ligand NGL-1, a postsynaptic neuronal adhesion protein. In addition, we could show that NGL-1 is phosphorylated and that this phosphorylation is mediated by CDKL5, in vitro. Using fibroblasts from a patient who carried a truncation of the CDKL5 gene, we obtained further evidence that CDKL5 phosphorylates NGL-1. Moreover, we have found that this phosphorylation is necessary for promoting a stable association between NGL-1 and PSD95, a scaffold protein of the post-synaptic density. Accordingly, phospho-mutant NGL-1 lacked the ability to induce synaptic contacts, while its phospho-mimetic form bound PSD95 more efficiently and partially rescued the CDKL5-specific spine defects. In conclusion, we provide novel mechanistic insights into how CDKL5 mutations can impact on neuronal function in atypical RTT.

P12.043

Further contributions towards the molecular analysis of NIPBL and SMC1A genes in a cohort of patients with Cornelia de Lange Syndrome

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Cornelia de Lange Syndrome [CdLS (MIM#122470)] is a rare multisystemic disorder, characterized by a typical phenotype that includes distinctive facial dysmorphism, hirsutism, growth and psychomotor developmental delay, abnormalities of the upper extremities, and relatively frequent gastrointestinal and congenital heart defects. CdLS is essentially caused by mutations in the *NIPBL* and *SMC1A* genes (~50% and 5% of cases, respectively), but mutations have been also described in other genes (*PDS5A*, *PDS5B*, *SMC3*) (http://www.lovd.nl/CDLS). This genetic heterogeneity in CdLS can however be explained by a close functional relationship at the cellular level, since all these proteins are involved in chromatid cohesion.

The molecular and clinical characterization of 42 Portuguese CdLS patients has been previously described (Oliveira *et al*., 2010). In this work we conducted the molecular analysis of *NIPBL* gene and more recently expanded this study to other 20 patients. Subsequently, all the molecularly unresolved patients were screened for large deletions and duplications in *NIPBL* by MLPA, and point mutations in *SMC1A* by high resolution melting curve analysis and sequencing.

Preliminary results allowed us to identify 4 previously known mutations (including a case with somatic mosaicism), 3 novel mutations (c.3316C>T, c.6983C>G and c.7307C>T) and 2 large deletions in the *NIPBL* gene. Mutation screening in *SMC1A* is still ongoing.

Our results, at least for *NIPBL* gene analysis, suggest that the use of several different techniques is essential for attaining a high mutation detection rate. CdLS cases with somatic mosaicism are probably underestimated in the literature and may explain some degree of phenotypical variability.

P12.045

CHD7 is involved in a multi-protein complex together with CHD8 and FAM124B

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Mutations in the *CHD7* gene are in approximately 2/3 of cases the underlying cause of CHARGE syndrome. To identify candidate genes involved in the pathogenesis of this disease, we searched for CHD7 interacting partners. We could previously demonstrate that a part of CHD7 interacts with a part of CHD8. Because CHD8 is a known component of the WDR5-ASH2L-RBBP5 (WAR) complex, we asking the question if CHD7 is also a member of this complex. Additionally, we search for new CHD7-CHD8 interacting partners by using the SILAC (***s***table ***i***sotope ***l***abelling with ***a***mino acids in ***c***ell culture) method in combination with mass spectrometry.

We identified FAM124B as a potential interacting partner of CHD7 and CHD8. We confirmed the result by co-immunoprecipitation. Furthermore, we studied the direct interaction between the CHD7 part, the CHD8 part and FAM124B by using the method of direct yeast two hybrid. We could demonstrate that the CHD8 part directly interacts with FAM124B, while the CHD7 part is not interacting. This result leads to the suggestion that CHD7 is together with CHD8 and FAM124B a component of the same complex, with direct contact to CHD8 and no direct interaction with FAM124B. Using the Duolink Pla II method we could demonstrate that CHD7 is also in near proximity with the WAR-complex members, leading to the suggestion that CHD7 is also involved in this complex.

The characterisation of CHD7 containing complexes and the identification of interaction partners will help to understand the pathogenesis of CHARGE syndrome.

P12.046

Targeted High-Throughput Sequencing for Diagnosis of Genetically Heterogeneous Diseases: Fast and Efficient Mutation Detection in Bardet-Biedl Syndrome, Alström Syndrome, and in clinically overlapping ciliopathies

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Bardet-Biedl syndrome (BBS) is a pleiotropic recessive disorder that belongs to the rapidly growing family of ciliopathies. It shares phenotypic traits with other ciliopathies such as Alström syndrome (ALMS), nephronophthisis (NPHP) or Joubert syndromes. BBS mutations have been detected in 16 genes and no clear genotype-to-phenotype correlation could be observed. This extensive genetic heterogeneity is a major problem for molecular diagnosis and genetic counseling. While various strategies have been proposed in order to optimize mutation detection they either fail to detect mutations in a majority of patients or are time-consuming and costly. We tested a targeted exon-capture strategy coupled with multiplexing and high-throughput sequencing on a total of 52 patients: 14 with known mutations as proof-of-principle, 38 with no previously detected mutations. Thirty genes were targeted in total, including the 16 BBS genes, the 12 known NPHP genes and the single ALMS gene. This strategy allowed the reliable detection of causative mutations (including homozygous/ heterozygous exon deletions) in 68% of BBS patients with no previous molecular diagnosis and in all proof-of-principle samples. Three probands were found to carry truncating mutations in ALMS1 confirming the phenotypic overlap between both disorders. The overall efficiency of detecting mutations in patients was positively correlated with their compliance to classical BBS phenotype suggesting that only a few true BBS genes remain to be identified. We will illustrate some interpretation problems one may encounter in diagnostic settings due to the multiplicity of variants detected. Targeted capture strategy appears highly efficient and cost-effective for genetically heterogeneous diseases.

P12.047

Whole exome sequencing in prenatal and postnatal investigations identifies the causative mutations associated with complex phenotypes

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By combining Next Generation Sequencing (NGS) with human whole exome capture, it is now feasible to identify, in a single step, inherited or somatic pathological mutations residing in coding regions of all known human genes. We applied whole exome capture and NGS in 2 complex clinical cases, involving: (1) an 18yr old male patient, exhibiting multiple abnormalities of the kidneys coupled to progressive visual impairment, and (2) a chorionic villus sample following termination of pregnancy, of a male fetus with skeletal and genital anomalies. Exome enrichment was achieved using Roche NimbleGen v2 capture, followed by NGS on an Illumina GAIIx at >50x coverage. Sequence data was analyzed and variants were filtered, identified and evaluated utilizing NextGENe v2.1 sequence analysis software (SoftGenetics) and various publicly available tools and databases. In the first case, a known or novel pathological heterozygous mutation was identified in each of 4 different genes (NPHP4, RPGRIP1L, CC2D2A, AVIL), consistent with a diagnosis of multi-allelic cilliopathy with retinal degeneration. In the second case, a predicted pathological hemizygous mutation c.194A>G (p.N65S) within the NSDHL gene on Xq28 was identified, and its presence was confirmed in the carrier mother. The presence of this novel mutation in the male fetus is most likely associated with malformation syndromes caused by dysfunction in cholesterol biosynthesis, in agreement with the clinical findings. In both cases, our findings provide new and important insights into the genetic causes of complex phenotypes and highlight the value of this new technology when applied in a clinical setting.

P12.048

Exhaustive molecular analysis and accurate estimation of type V collagen mutations in classic EDS

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Classic Ehlers-Danlos syndrome (EDS) is characterized by hyperextensible skin, atrophic scarring, easy bruising and generalised jointhypermobility. Mutations in *COL5A1* and *COL5A2*, encoding type V collagen, have been reported, but the proportion of classic EDS that result from defects in these genes remains unresolved. We studied 126 patients with a clinically established or suspected diagnosis of classic EDS. Of these, 102 patients fulfilled all major diagnostic criteria for classic EDS (Villefranche nosology) (group 1). We also included 24 patients in whom the diagnosis of classic EDS could not unequivocally be established, as they presented jointhypermobility and soft, mildly hyperextensible skin but no typical dystrophic scarring (group 2). Inclusion of this cohort allowed evaluating the most indicative clinical features for the presence of a type V collagen defect. In total, a type V collagen defect was identified in 93/102 patients in group 1, among which 49 novel mutations. Approximately half of these defects caused *COL5A1*-haploinsufficiency, whereas one-third were structural mutations in *COL5A1* or *COL5A2*. In 9/102 patients no type V collagen defect was detected. No obvious genotype-phenotype correlation was observed. In contrast, none of the patients of group 2 harboured a *COL5A1*/*COL5A2* mutation. Our data show that >90% of patients fulfilling all classic EDS Villefranche criteria harbour a type V collagen defect. Biochemical and molecular analysis in the mutation-negative patients from group 1 and 2 excluded the involvement of other fibrillar collagens and *TNXB*, which implies that type V collagen is the major, if not the only, protein involved in classic EDS.

P12.049

TRPV4 mutations in a selected CMT2 Norwegian patient cohort

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Mutations in the ankyrin domain of the TRPV4 (Transient receptor potential vanilloid) protein have been shown in patients with congenital distal spinal muscular atrophy (SMA), scapuloperoneal SMA and Charcot-Marie-Tooth (CMT) 2C. We aimed at identifying *TRPV4* mutations by sequencing exon 5 and 6 of the gene in a cohort of Norwegian patients with clinical and electrophysical findings suspicious of CMT type 2. We identified twenty two patients who were previously tested normal for the common CMT2 genes (*GJB1*, *MFN2*, *MPZ*, *GDAP1* and *NEFL*), and identified two patients heterozygous for the mutations p.Arg315Trp and p.Arg316Cys in the *TRPV4* gene. This suggests that *TRPV4* mutations might represent a relatively common cause of CMT2 in the Norwegian population and testing of the *TRPV4* gene should be considered where genetic testing of the common genes is inconclusive.

The patient harbouring the sequence variant c.946C>T (p.Arg316Cys) was referred at age 6 with clinical symptoms of muscular dystrophy. Investigation including FSHD genetic testing and muscle biopsy was inconclusive. On re-evaluation at age 13, electromyography, clinical and radiological investigations raised suspicion of scapuloperoneal SMA. Further genetic testing was negative regarding SMA and CMT2. He has a younger brother that also reports some overlapping clinical symptoms. A clinical re-examination and segregation analysis of the family will be presented and compared with patients previously reported with this *TRPV4* mutation.

P12.050

Cockayne Syndrome testing at BGL- a new service and case study

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Cockayne Syndrome (CS) is a recessive multisystem disorder characterised by early onset microcephaly, mental retardation, cachectic growth failure, photosensitivity and progressive neurological deterioration usually leading to death in childhood or early adulthood.

CS is associated with mutations in *ERCC6* (OMIM:609413) and *ERCC8* (OMIM:216400). 75% of CS mutations are in *ERCC6* and 25% in *ERCC8*. The proteins encoded by *ERCC6* and *ERCC8* both play important roles in transcription-coupled nucleotide excision repair.

We present an urgent prenatal case of a mother presenting at 10+ weeks, her 17 month old son having a definite clinical diagnosis of CS but no molecular or cellular confirmation. Time pressures precluded obtaining a molecular test result in European service/research laboratories, and cellular UV sensitivity studies requiring cultured skin biopsy typically taking 6-8 weeks. Sanger sequencing assays for *ERCC6* and *ERCC8* were designed and validated in ten working days and the proband identified as homozygous for an *ERCC6* pathogenic variant c.3052dupA. This facilitated prenatal testing by sequence analysis with results available in three days, confirmed by UV-test 5-6 weeks later.

Rapid reporting of sequencing assays recommends DNA testing as the first line approach in suspected CS cases meeting clinical criteria, complemented by the cellular UV sensitivity studies when variants of unknown significance (VUS) are detected. DNA testing avoids an invasive skin biopsy, and may reduce the number of additional tests undertaken upstream e.g. microarray CGH.

An accredited diagnostic genetics service for CS is now available for the UK and Europe, we present an audit of referrals to date.

P12.051

Mutations in Swi/SNF chromatin remodeling complex gene ARID1B cause Coffin-Siris syndrome

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Coffin-Siris syndrome (CSS) is characterized by intellectual disability, speech impairment, coarse facial features, hypoplasia of the fifth digits and/or fingernails and callosal agenesis. Since several sibships with CSS have been described autosomal recessive inheritance was considered a likely possibility. We selected three unrelated patients with CSS and the parents of one patient for exome sequencing. Filtering using public databases rendered recessive inheritance unlikely, as there was no gene with two heterozygous or one homozygous variant in all three patients. Focusing on autosomal dominant inheritance instead, only one gene with a variant in all three patients remained after filtering inherited and homozygous variants. Three truncating mutations in *ARID1B* were identified which were confirmed and shown to be *de novo* by Sanger sequencing. Array-based CNV analysis in 2000 patients with intellectual disability revealed deletions encompassing *ARID1B* in three patients with partially overlapping phenotypes. They share facial features, intellectual disability and severe speech delay with the CSS patients. Interestingly, they lack the typical CSS abnormalities and CSS was considered in only one if these patients as a diagnosis. Several patients with *ARID1B* haploinsufficiency have been described in literature with a very similar phenotype and often with agenesis of the corpus callosum. Therefore we conclude that haploinsufficiency of *ARID1B*, which encodes a (epigenetic) modifier of chromatin structure, emerges as an important cause of CSS and a potential common cause of intellectual disability and speech impairment. By screening additional groups of patients for *ARID1B* we have identified several new mutations.

P12.052

Genetic abnormalities in Coffin-Siris syndrome

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Coffin-Siris syndrome (CSS; MIM 135900) is a rare congenital anomaly syndrome characterized by growth deficiency, intellectual disability, microcephaly, coarse facial features and hypoplastic nail of the fifth finger and/or toe. The majority of patients are sporadic, being compatible with autosomal dominant inheritance. The genetic cause has not been elucidated.

To reveal the genetic basis of CSS, we performed whole exome sequencing of five typical subjects. Based on our scheme assuming that an abnormality of a particular gene would be shared in two or more patients, 51 variants remained as candidates. All the variants were checked by Sanger sequencing of PCR products amplified using genomic DNA from the five patients and their parents. Nine were false-positives (errors), 40 were inherited from either the father or mother, and two *de novo* heterozygous mutations were found in two patients.

Using this information, we carefully analyzed 23 CSS patients and found that at least 20 of them are genetically explained. Detailed information will be presented.

The following doctors are highly appreciated: Tsurusaki Y, Okamoto N, Ohashi H, Kosho T, Imai Y, Hibi-Ko Y, Kaname T, Naritomi K, Kawame H, Wakui K, Fukushima Y, Homma T, Kato M, Hiraki Y, Yamagata T, Yano S, Mizuno S, Sakazume S, Ishii T, Nagai T, Shiina M, Ogata T, Ohta T, Niikawa N, Miyatake S, Okada I, Mizuguchi T, Doi H, Saitsu H, Miyake N.

P12.053

A novel COL1A1 mutation in a family with infantile cortical hyperostosis (ICH, Caffey disease, OMIM#114000)

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Infantile cortical hyperostosis is a rare autosomal dominant disorder characterized by subperiosteal hyperosteogenesis manifesting in the prenatal period or in early infancy. It often affects the mandible, the clavicles, the ribs and the long bones. Typical clinical symptoms include painful swelling of the bones and fever. ICH is a self limiting disease which usually resolves spontaneously by 2 years of age.

A single mutation in the COL1A1 gene has been reported to date in ICH patients of different ethnic origins, namely the missense mutation p.Arg836Cys. The mutation was shown to lead to abnormal disulfide bonds and abnormal structures of the alpha 1 chain dimers.(1)

COL1A1 gene mutations, detected throughout the gene, are responsible for further connective tissue disorders such as: osteogenesis imperfecta and Ehlers-Danlos syndrome type III. Typical for these disorders is fragility of the bones and laxity of the skin/connective tissue, respectively.

We report here on the molecular findings obtained for a young girl with typical clinical manifestation of ICH. After having excluded the presence of the COL1A1 mutation p.Arg836Cys we sequenced all coding exons of the gene. The sequencing analysis revealed a heterozygous mutation, p.Arg918Cys, in the patient and her father, who also had typical features of the disorder. The mutation was not present in the patient's mother and 200 control alleles.

We suggest that ICH may not only be caused by the recurrent p.Arg836Cys mutation, but also by the novel p.Arg918Cys mutation.

Reference:

1.Gensure R.C. et. al (2005) J. Clin. Invest. 115:1250-1257

P12.054

A Loss of Function Mutation in the COL9A3 Gene Cause Autosomal Recessive Stickler Syndrome

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Stickler syndrome is a clinically variable and genetically heterogeneous syndrome characterized by ophthalmic, articular, orofacial, and auditory manifestations. Until now, it has been described with both autosomal dominant and recessive inheritance. The dominant form is caused by mutations in COL2A1 (STL 1, MIM 108300) , COLA11A1 (STL 2, MIM 604841), and COL11A2 (STL 3, MIM 184840) genes, while recessive forms have been associated with mutations of COL9A1 (MIM 120210) and COL9A2 (MIM 120260) genes. Here, we describe the first autosomal recessive Stickler family due to loss of function mutations (c.1176_1198del, p.P392fsX25) of COL9A3 gene. This findings further extend the role of collagen genes family in the pathogenesis of the disease.

P12.055

Consanguinity as a means to identify pathogenic recessive mutations

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Consanguinity and inbreeding increase the sharing of alleles among individuals. We have initiated a project to collect samples from families with recessive phenotypes in consanguineous families, in order to identify the functional genomic variation responsible for the disease. Any phenotype and family history compatible with autosomal recessive inheritance (and unknown molecular defect) is candidate for participation in the study. Forty two families of different ethnic background have already been collected. From each family, blood DNA from the patient(s), all unaffected siblings, and the parents is extracted. Samples from one or more of the affected individuals per family are first analyzed by array-CGH 400K for the detection of homozygous deletions. Then the samples of all family members are genotyped with a dense SNP array in order to identify Runs of Homozygosity (ROH), allowing the definition of chromosomal regions likely to contain the responsible genes. Finally exome sequencing is performed in one affected individual per family. Variants are called using publically available tools and filtered according to polymorphic SNVs deposited in public databases and predicted pathogenicity. We have so far analyzed twelve families using this approach. Causative variations of known disease genes have been identified in two families (VLDLR gene, causing disequilibrium syndrome and FKTN gene causing Fukuyama muscular dystrophy). In 5 additional families candidate genes have been identified. Consanguineous families provide an opportunity to identify pathogenic variants in known genes as well as candidate genes responsible for recessive phenotypes and rapidly fill in the space of genotype phenotype links.

P12.056

Molecular analysis of *TSC2/PKD1* contiguous gene deletion syndrome

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The *TSC2/PKD1* contiguous gene syndrome (PKDTS, MIM#600273) results in disruption of both the *TSC2* and *PKD1* genes. PKDTS is characterized by severe juvenile polycystic disease, combined with variable phenotypic expression of tuberous sclerosis (TSC2). This extensive renal damage by cysts usually results in end-stage renal disease (ESRD), often before the second decade of life. Currently, the mechanism for PKDTS is nearly unknown.

Previous findings suggest that PKDTS damage is associated exclusively with the severity of kidney symptoms, and not with the severity of other ones, such as TSC, liver cysts, and intracranial aneurysms. To our best knowledge, no whole studies including; clinical, renal imaging studies, nor histopathological and neither molecular biological analysis with new technologies (such as MLPA and aCGH), have been performed on the *TSC2/PKD1* contiguous gene syndrome patients.

The main aim of this work is to examine the genotype-phenotype correlations in this disease including previously reported patients with clarified breakpoints of the large deletions. To end this, we report herein 7 new patients with the *TSC2/PKD1* contiguous gene syndrome deletions in different extent, by means of MLPA and a custom design aCGH within 16p13.3 locus. The extent of the deletion concerning *TSC2* and *PKD1* genes, and the nature of the deleterious event are determined and discussed concerning clinical consequences and pathogenic molecular mechanisms.

P12.057
Familial recurrence and modifier genes in CDLS families with NIPBL mutations.

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Cornelia de Lange syndrome (CdLS; OMIM #122470) is a rare multisystem disease characterized by mental and growth retardation, typical facies, and limb reduction. Mutations in three genes encoding a regulator (NIPBL) and two structural subunits of the multifunctional cohesin complex (SMC1A and SMC3) , account for up to 60% of CdLS cases, with >50% due to NIPBL. Although germinal mosaicism has been reported, inherited NIPBL mutation have been rarely described.

We report on two familial cases out of a cohort of 90 NIPBL-mutated patients. In the first family, a mother with a very mild facial phenotype and her two CdLS sibs were carriers of c.5329-15A/G splicing mutation, leading to c.5328[TGTTTGCTTGGCAG]5329ins transcript that predicts the truncated p.Ile1777Cys fs22X protein. In the second family the p.Leu1238Phe change was transmitted by the mother, with a psychiatric phenotype, to the CdLS proband and to her brother affected with learning disability. The variant has not been found in either 800 control chromosomes or LOVD . Array-CGH has been performed to investigate whether CNVs acting as modifier factors could modulate the intrafamilial phenotypic variability.

A duplication containing the entire PIAS3 gene and a *de novo* duplication exiting in the whole duplication of RPAI1 gene were detected in the two sibs of the first family and the CdLS proband of the second family both involved in genomic stability.

Quantitative real-time-PCR is in progress These data, suggest a putative concurrent effect of rare CNVs and NIPBL mutation in the CdLS expression of clinical signs.

P12.058
Differential allelic expression of SMC1A gene in Cornelia de Lange female patients

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Cornelia de Lange syndrome (CdLS OMIM #122470, #300590, #610759) is a rare multisystem disorder characterized by facial dysmorphisms, upper limb abnormalities, growth and cognitive retardation. Mutations of NIPBL (5p13.2) and SMC1A (Xp11.22) genes, encoding a regulator and a structural subunit of the cohesin complex, account for about 60% and 6% of CdLS cases. The SMC1A gene escapes X chromosome inactivation, although uncompletely, with a variable expression of the allele located on the inactive X. This finding may contribute to the clinical variability of SMC1A-mutated female patients.

Four female patients carriers of different mutations of SMC1A gene and 8 controls have been tested by Pyrosequencing to assess the reciprocal level of allelic expression, discriminated by the heterozygous mutation in the patients and heterozygous coding SNP rs1264011 in the controls.

The analysis of the two alleles showed a 53/47 ratio in the controls group and a 67/33 ratio favouring the wild type allele in the patients. In particular the patient with the highest wild type expression (73/27=wt/mut) presents a borderline phenotype with mild dysmorphisms and cognitive deficits, at difference of the other patients (65/35=wt/mut) showing a moderate to severe clinical phenotype.

According to the proposed dominant negative mechanism of mutated SMC1A protein in females CdLS patients, the preferential, but variable expression of the wild type allele may influence the clinical phenotype. The overall findings, expanded to further cases and corroborated by quantitative expression data, enhance our understanding of the variable clinical spectrum of X-linked CdLS and highlight the occurrence of overlooked cases.

P12.060
Expanding the mutational spectrum of CRLF1 in Crisponi Syndrome.

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Crisponi syndrome (CS) and cold-induced sweating syndrome type 1 (CISS1) represent manifestations of the same autosomal recessive disorder with different degrees of severity, caused by mutations in CRLF1. The two syndromes share clinical characteristics, such as dysmorphic features, muscle contractions, scoliosis and cold-induced sweating, with CS patients showing a severe clinical course in infancy involving hyperthermia, associated with death in most cases in the first years of life. We suggested recently to rename the two genetic entities with the broader term of Sohar-Crisponi syndrome. We expanded the mutational spectrum of CRLF1 in the syndrome and carried out a meta-analysis of the literature for all the mutations described so far. In conclusion we found 9 new mutations in addition to the 29 already described. The higher prevalence is registered in Sardinia, Turkey and Spain. In Sardinia, where the syndrome seems to be more common than in the rest of Italy, due to 2 founder mutations, we performed a pilot screening to evaluate the carrier frequency. In details we found 16 carriers on 1,100 Sardinian control individuals from 4 different provinces, with an estimated prevalence of 1:20,000. A more detailed analysis (detection of heterozygous CRLF1 deletions by Quantitative Real Time PCR, CLCF1 and CNTFRα testing, exome sequencing) is ongoing to find the genetic cause in those patients with a clinical phenotype suggestive of Sohar-Crisponi syndrome, but with no evident mutations in CRLF1. This will help in better understanding the pathogenesis of the disease and the molecular pathways involved in the phenotype.

P12.061
Further molecular characterization of PYCR1-related cutis laxa

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Autosomal recessive cutis laxa (ARCL) sydromes are a group of overlapping disorders with progeroid features, but without lung and vascular involvement (ARCL type 2). In a cohort of patients initially as characterised as wrinkly skin syndrome, gerodermia osteodysplastica or De Barsy syndrome we identified mutations in the PYCR1 gene encoding pyrroline-5-carboxylate reductase 1. PYCR1-related ARCL (ARCL2B; OMIM #612940) is the second most frequent form of ARCL. We give an update on the clinical variability and report novel disease-causing mutations.

The PYCR1 protein is part of the proline-cycle, a conserved pathway described to generate NAD(P)+ in the cytoplasm via synthesis of proline. PYCR1-deficient fibroblasts from ARCL2B patients show decreased mitochondrial membrane potential, a disrupted mitochondrial network, and an increased apoptosis rate upon different kinds of stress. A detailed analysis of the subcellular distribution revealed an exclusive mitochondrial localization of PYCR1. After RNAi-induced loss of PYCR1 in HeLa cells we reproduced the fragmentation of the mitochondrial network and a decreased membrane potential. In addition, alterations of ATP and lactate levels imply mitochondrial dysfunction.

Thus, we conclude a role of PYCR1 in the regulation of the mitochondrial redox state, which influences mitochondrial fusion and possibly also metabolic activity. This combination of defects is likely to be a key event in the pathogenesis of ARCL2B and qualifies ARCL2B as a mitochondrial disorder.

P12.063
Clinical Genetic Analysis of Cystic Fibrosis in Republic of Moldova

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Determining the frequency of CFTR mutations which are specific for individual population having a scientific interest.

During the period from 1992 to present we investigated 147 Moldavian patients with CF aged from 1 month to 24 years (70 male and 77 female). We carried out comparison of phenotypic features in CF with type of CFTR mutation. The national distribution of patients was following: Moldavians - 66,2%, Russians - 14,6%, Ukrainians - 7 %, Gagauz - 6,2 %, Bulgarians - 2,3 %, and another nationalities - 3,7%.

To determine 9 mutations (F508del, G542X, N1303K, W1282X, R117H,

G551S, R347P, R334W, R553X) in CFTR gene we are used the method of PCR, in 15 patients was administered DNA diagnosis for 35 - 36 mutations jointly with professionals from Center of Cystic Fibrosis, Bordeaux, France, and Department of Genetics of University Clinic in Hannover, Germany.
CFTR-mutations were identified in 69,72 % investigated chromosomes, were determined 17 mutations. The mutation which have clinical importance in Moldova (> 1%) were: F508del- 58,84%, 2789+5G>A - 1,70 %, G542X - 1,36 % , N1303K- 1,36 %, 2184insA- 1,36 %. All of them are „severe" and are associated with pancreatic insufficiency. The frequencies of other mutations were < 1%. 16 mutations (excluding F508del) were determined in 10,88% studied chromosomes. For patients with non-identified mutations (30,28%) is characteristic high level of pulmonary pathologies (70,0%).
Therefore, the present study discovered fact what in Moldavian patients prevailed rare mutations, and severity of CF correlates with type of mutations in CFTR gene.

P12.064

Whole-exome sequencing identifies a novel nonsense mutation in the TTN gene in a large Dutch family with DCM

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Dilated cardiomyopathy (DCM) is an etiologically highly heterogeneous disorder, characterized by left ventricular dilation and dysfunction, leading to heart failure and sudden death. Approximately 20-50% of dilated cardiomyopathy cases are familial with significant genetic and phenotypic heterogeneity. In a multigenerational Dutch family with autosomal dominant transmission, we employed whole-exome sequencing in three affected family members. Using the GATK pipeline for whole exome data analysis, we identified between 1431 and1557 novel exonic/splice site variants per individual. Subsequent filtering steps, included filtering for dominant inheritance (heterozygous variants) and for coding regions, revealed a novel shared nonsense variant in the Titin gene (TTN) in all three affected patients. The respective substitution c.59845C>T predicts a premature stop codon (p.Arg19949X) at the protein level, leading to a truncated titin protein. The mutation was confirmed by Sanger sequencing and was shown to co-segregate with disease in all DCM patients in this family. Our results show that mutations in the gene encoding giant muscle filament titin (TTN) can cause DCM and may account for a significant portion of the genetic etiology in familial DCM in the Dutch population.

P12.066

Etiological study of hearing loss: clinical practice guidelines in Brazilian patients

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Deafness is considered the most prevalent sensory disorder in humans. In Brazil, there are no official data regarding the prevalence and etiology of hearing impairment, but it is known that environmental factors are among the major causes. Although a simultaneous testing approach, including clinical exams, audiological, laboratorial, imaging and genetic expands the etiological diagnostic, overloads the healthcare system due to high costs. Thus, the goal of the present study is to evaluate the effectiveness of imaging and genetics tests and their impact on public health, aiming to increase efficiency and reduce costs of the etiological diagnostic of hearing loss. It was conducted an analysis of 100 patients with sensorineural hearing loss. A detailed investigation was performed in patients, including imaging and genetics analysis. The number of individuals with unknown cause was reduced from 72 to 42 (42% of reduction). Radiologic abnormalities were identified in 29 of the patients, while molecular alterations were found in 31 individuals. The etiology remained unknown in 42% of the patients, was due to environmental factors in 25%, genetics in 19% and inner ear malformations or other defects in 14% of the cases. It was concluded that both imaging and genetic analysis were important to identify the etiology of hearing loss, however, molecular tests contributed mainly for diagnosis of patients with congenital deafness, while radiologic exams had greater contribution for diagnosis of cases with progressive or abrupt hearing loss. The sequential protocol enables an optimization of the etiological diagnosis and cost reduction.

P12.067

Novel GJB2 mutations in Greek patients with sensorineural, non-syndromic deafness

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Non-syndromic hearing loss is one of the most common hereditary diseases in human, and the disease is a genetically heterogeneous disorder. Connexins (Cxs) are membrane-spanning proteins that co-assemble into intercellular gap junction channels. Gap junction channels mediate electrical and biochemical communication between adjacent cells and play vital roles as mediators of intercellular molecular signaling. Cx-linked deafness highlights the key role of gap junctions in the physiological processes of hearing. Co-localization of Cxs with the gap junction system in the inner ear suggests a role in cochlear electrolyte homeostasis. During auditory transduction, they are proposed to maintain membrane potentials by regulating the flow of potassium ions between the sensory epithelia of the inner ear. Mutations in the GJB2 gene, encoding connexin 26 (Cx26), are a major cause of non-syndromic recessive hearing impairment in many countries and are largely dependent on ethnic groups. Here we describe the clinical and molecular genetic findings in two Greek families presenting with bilateral, sensorineural, non-syndromic deafness. In the first family with hearing parents we detected compound heterozygosity of the c.35delG and the novel c.292C>T (p.R98W) GJB2 mutations in a proband with postlingual hearing loss while in the second family where both parents and the proband suffered from prelingual deafness, we observed homozygosity of the c.35delG GJB2 mutation in the mother and compound heterozygosity of the c.35delG and the novel c.247-249delTTC (p.delF83) GJB2 mutations in the father and the proband.

P12.068

Mutations in the prostaglandin transporter SLCO2A1 cause primary hypertrophic osteoarthropathy with digital clubbing

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Digital clubbing with enlargement of the distal phalanges and overcurvature of the nails frequently occur secondarily as a feature of chronic hypoxia or neoplastic processes. We and others reported mutations in the 15-hydroxyprostaglandin dehydrogenase (*HPGD*) gene encoding the major prostaglandin PGE_2 catabolizing enzyme in patients with digital clubbing and primary hypertrophic osteoarthropathy (PHO). In this study, we hypothesized that PHO can also be caused by mutations in other components of prostaglandin signalling. Extracellular prostaglandins are taken up by a carrier-mediated process across the plasma membrane. For many prostanoids (e. g. PGE_2) this transport is mediated by SLCO2A1, a type IV transmembrane protein that belongs to the solute carrier organic anion transporter family. We performed sequencing of *SLCO2A1* in seven PHO patients previously found to be negative for mutations in *HPGD* and identified pathogenic mutations in all but one individual. All patients were of normal intelligence and height and did not suffer from pulmonary disease or any other interfering disease. All of our mutations in *SLCO2A1* represent truncating alleles or affect residues that are indispensable for prostaglandin transporter activity. To corroborate our data, we constructed molecular models and Ramachandran plots of wild-type and mutated SLCO2A1 proteins to visualize changes of protein folding and structure. We show that *SLCO2A1* is a frequent cause for autosomal recessive PHO confirming the data recently published by Zhang et al. (2012). Although most patients harbour mutations in *HPGD* and *SLCO2A1*, further genetic heterogeneity is likely and candidate approaches targeting other prostaglandin signalling components seem promising.

P12.069

The frequency of short pathogenic repeat expansions in DM2

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Myotonic Dystrophy type 2 (DM2), in Germany as frequent as DM1, is normally caused by huge CCTG repeat expansions in intron 1 of the zinc finger protein (ZNF9, CNBP) gene on chromosome 3q21. During the last few years we found some symptomatic patients with small CCTG expansions between the normal upper limit of about 26 CCTGs in the population and a putative old lower limit for pathogenic repeat expansions of 75 CCTGs during our routine diagnostic screening process for DM2. Pictures of a Southern blot and TP-PCR (repeat primed PCR) are given. There is good evidence that these „mini expansions“ are pathogenic: there were ribonucleic foci in the patients' muscle biopsies and alternative splicing events in muscle specific genes. From 2004 till now we detected 19 DM2-patients with such a short pathogenic repeat. This is a portion of roughly 2% of all patients with a long expansion (n=960) in this period; the distribution between male and female patients is nearly equal. The short pathogenic repeat expansions may arrise in most cases from a pool of long normal alleles (as suggested in DM1) by a slippage or unequal crosssing over mechanism; on the other side we could find at least one repeat contraction from an effected mother to an affected daughter (full expansion contracting to a mini expansion). The latter case supports the observation of „pathogenic mini expansions“.

P12.070

Clinical, pathological and genetic characterization of manifesting *DMD* carriers: the role of X-chromosome inactivation

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Mutations in *DMD* gene lead to Duchenne muscular dystrophy (DMD), milder Becker muscular dystrophy (BMD) and rare X-linked dilated cardiomyopathy (XL-DCM). Female carriers are mainly asymptomatic, however between 7,8% and 22% manifests some degree of the disease. Several mechanisms have been implicated to cause symptoms among manifesting carriers. These include X-autosomal translocations disrupting *DMD*, compound heterozygous mutations, co-occurrence of *DMD* mutations together with other genetic abnormalities (X-chromosome monosomy, X-chromosome uniparental disomy and male pseudohermaphroditism) and skewed X-chromosome inactivation. Clinical presentation among manifesting carriers are heterogenic and ranges from myalgia to disabling DMD-like forms.

We identified 22 manifesting carriers, ten of them with no family history of D/BMD affected males. We report findings concerning clinical presentation, muscle dystrophin expression, *DMD* mutation spectrum and X-chromosome inactivation (XCI) pattern in blood and muscle. Clinical pictures included: 1) isolated dilated cardiomyopahty (*n*=2, 9%), 2) isolated mental retardation or behavior issues (*n*=3, 14%), 3) myalgia and/or exercise intolerance (*n*=4, 18%) and, 4) mild to severe muscle weakness (*n*=13, 59%) that ranged from mild BMD-like (*n*=6, 27%) to severe BMD-like (*n*=4, 18%) and DMD-like (*n*=3, 14%).

Twenty-one different *DMD* mutations were identified in heterozygous state including: 12 exonic deletions (57%), 3 exonic duplications (14%) and 6 point mutations (29%). The aim of this study was to explore the potential correlation between the severity of the disease and X-chromosome inactivation. We have compared XCI ratios between manifesting carriers, non manifesting carriers and normal female controls.

P12.071

The LGMD2A prevalence among patients diagnosed as DMD in Russia.

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Duchenne muscular dystrophy (DMD) is the commonest and best-known of the muscular dystrophies, but other types of muscular dystrophy (MD) like limb-girdle muscular dystrophy type 2A (LGMD2A) may have a similar clinical presentation. LGMD2A is the most frequent autosomal recessive MD.

The molecular defect in *DMD* gene, accounting for approximately 60% of cases of DMD is deletion of one or more exons. The most common molecular defects in *CAPN3* gene in Russia are two missens mutations c.550delA and c.598_612del.

More than thousand DNA samples (1339) of patients diagnosed as DMD have been analyzed for deletions of exons 3, 4, 6, 8, 13, 17, 19, 32, 42-45, 47, 48, 50-53, 60 in *DMD* gene and in 539 of them (40.25%) have been revealed different deletions in *DMD* gene. 800 patients have no these deletions.

DNA samples of 698 patients without deletions in *DMD* gene have been screened for the most frequent mutations in *CAPN3* gene and these mutations were revealed in 34 of them (4.87%). Genotypes was: c.550delA/c.550delA - 14 patients, c.550delA/N - 15 patients, c.550delA/c.598_612del - 2 patients, c.598_612del/N - 3 patients.

From Hardy-Weinberg equilibrium summary the allelic frequency of this two mutations comprised 64%. Using this data we calculated that there have to be 45 patients with LGMD2A among 800 patients without deletions in *DMD* gene. So we found that the frequency of LGMD2A among patients diagnosed as DMD comprise 3.36%.

P12.072

An unusual form of autosomal recessive early onset nephropathy: mapping to chromosome 4.

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Early onset nephropathy (age of onset less than one year) with features of tubulo-interstitial nephritis, hypertension and tendency for hyperkalemia was diagnosed in 4 individuals of an Israeli Bedouin kindred. None of the affected individuals had rapid deterioration of renal function. Neurological manifestations commensurate with Cogan-type oculomotor apraxia were evident. Molar tooth sign was not seen on neuro-imaging. Association with any of the previously known 12 loci for nephronophthsis was ruled out. Genome wide linkage analysis identified a disease-associated locus on chromosome 4 between 37143147 and 57096602. No mutations were found in the coding sequence of WDR19 (NPHP13). Whole exome sequencing is underway.

P12.073

Identification of novel causal genes for early-onset Alzheimer disease through whole genome sequencing technology

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Alzheimer disease (AD) is the most common form of late-onset neurodegenerative dementia, that also occurs at young age in 10% of AD patients. In a small percentage of early-onset AD patients the disease is inherited in an autosomal dominant manner. Mutations in the 3 known causal genes (*APP*, *PSEN1* and *PSEN2*) are not explaining all early-onset AD patients.

A previous genome-wide linkage study on a well-documented early-onset AD family has implicated a novel genetic locus of 5.44 Mb at 7q36. Traditional Sanger sequencing of all coding exons of the genes present in the candidate region and array-based comparative genomic hybridization (aCGH) for CNV analysis both failed to reveal evident disease-linked mutations. Yet, the role of non-coding variations remains to be explored. To continue searching for the underlying genetic defect whole genome sequencing (WGS) was performed on 4 distantly related patients from the pedigree. The GenomeComb software tool has been used for filtering on quality and frequency and for variations prioritization. After this procedure, we obtained 93 variations of which 35 were segregating with the disease. These have been tested in the extended control cohort of Flanders-Belgian origin to exclude (common) polymorphisms. Leaving eventually 4 variations shared by the family patients and absent in controls. The screening of these variations in a larger AD population is ongoing to estimate the contribution of the newly identified variations to AD pathogenesis. Next, we will functionally characterize the newly identified variations to better understand their contribution to AD pathology.

P12.074

A RP1 common founder mutation is a major cause of Early-onset Autosomal Recessive Retinitis Pigmentosa in Spanish population

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Background. Retinitis pigmentosa (RP) is a clinically and genetically heterogeneous group of retinal degeneration disorders being one of the major causes of genetic blindness. While the RP1 gene is a major gene responsible for autosomal dominant RP (adRP), it also seems to be a rare cause for the autosomal recessive RP (arRP). However, very few studies for *RP1* have been performed in patients affected with arRP so far.

Methods. Homozygosity mapping or exome sequencing analysis were performed in three families segregating arRP. A mutational screening was performed in 241 additional unrelated families for the p.Ser452Stop mutation. Haplotype analysis was also conducted. Individuals who were homozygous, double heterozygotes or carriers of mutations in RP1, underwent an ophthalmic evaluation to establish a genotype-phenotype correlation.

Results. Four novel mutations in RP1 were identified. The new mutation p.Ser542Stop was present in 11/244 (4.5%) of the studied families. All chromosomes harboring this mutation shared the same haplotype. All

patients presented a common phenotype with an early age of onset and a prompt macular degeneration while the heterozygote carriers did not show any signs of RP.
Conclusions. p.Ser542Stop is a single founder mutation and the most prevalent described mutation in the Spanish population. It causes early-onset RP and is responsible for 4.5% of all cases. Our data suggest that the implication of RP1 in arRP could be underestimated.

P12.075
Molecular screening of KRT14 common mutations in Iranian patients affected with Epidermolysis bullosa

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Epidermolysis bullosa (EB) is a heterogenic skin disorder with three classic clinical forms includes Simplex, dystrophic and Junctional which characterized by blisters that follows minor physical trauma .The Simplex form is more common variation which has four subtypes, caused by mutation in KRT5 or KRT14.Although the Skin Biopsy is the first step in EB Testing strategy, it is needed to confirm and localize the mutation by direct molecular methods.We aimed to evaluate the KRT14 hot spot exons as a rapid, noninvasive and cost benefit panel test for first step screening of common mutations in EB affected patients.Methods: About 80 affected patients clinically diagnosed as EB, were collected and classified clinically so all DNA samples evaluated blind for selected exons of KRT14 by direct Sequencing method. Result: Molecular screening for KRT14 hot spots revealed that about 25% of affected probands show mutation in selected exons. It is confirmed that these exons beside the other selected hot spots include KRT5, COL7A1 as a screening panel could exclude about 40% of EB types.Key words: EB, KRT14, Sequencing, KRT5, COL7A1

P12.076
Identification of novel mutations in the amiloride-sensitive epithelial sodium channel in African patients with cystic fibrosis-like disease

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The defect in chloride and sodium transport in cystic fibrosis (CF) patients is a consequence of CFTR loss of function and/or an abnormal interaction between cystic fibrosis transmembrane conductance regulator (CFTR) and amiloride sensitive epithelial sodium channel (ENaC). Apart from the defective chloride secretion, loss of functional CFTR results in increased sodium absorption through the ENaC channel in CF patients.
We investigated whether mutations in the genes that code for the different subunits of ENaC gene might result in CF-like disease in patients in whom only one CFTR gene is mutated, or that carry no mutations at all in the CFTR coding region and its exon/intron junctions. We extensively performed ENaC genes sequencing in these CF-like patients and established the frequency of identified ENaC mutations in a cohort control.
In total, 66 sequence variants in ENaC genes were found in in 60 CF-like patients.
Several novel ENaC gene mutations were identified and some of them were located in highly conserved domains and consistent with a pathophysiological role. Only three novel mutations including p.V348M and p.W423R in SCNN1B subunit and p.R180W in SCNN1G were observed once in our patients, but not in controls. The preliminary functional studies using expression in Xenopus laevi oocytes showed that the p.V348M is a gain of function mutation with a high amounts (2-30%) functional ENaC activity.
Our data suggest that CF-like syndrome in Africa could be associated with ENaC mutations. The combination of ENaC and CFTR mutations may play a hitherto unrecognized role in lung diseases.

P12.078
Improving molecular diagnosis of epilepsy by applying multiple prediction algorithms

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Mutations in the voltage-gated sodium channel α1-subunit gene (*SCN1A*) have been associated with the spectrum of generalized epilepsy with febrile seizures plus, which includes severe phenotypes such as Dravet and Doose syndromes. However, the prognostic value of these mutations and a possible correlation with the different clinical subtypes remain unclear. Therefore, the aim of this study was to search for mutations in *SCN1A* in patients with Dravet and Doose syndromes, thus establishing genotype-phenotype correlations using predictive algorithms to determine potentially deleterious mutations.
We performed *SCN1A* mutation screening in 15 patients with Dravet syndrome and 13 with Doose syndrome. Eight algorithms were used to analyze the possible impact of the mutations in protein function. In addition, MLPA was used to detect copy number variations within *SCN1A*.
Twelve mutations were identified in patients with Dravet syndrome. All missense mutations were predicted to be deleterious and are mostly located in the pore region or the C-terminal of the protein. Patients with Doose syndrome showed no mutations. Moreover, no copy number variants were identified.
The high frequency of *SCN1A* mutations found in patients with Dravet syndrome (80%) suggests that molecular testing is particularly useful for individuals with this phenotype. In contrast, *SCN1A* testing does not seem to be clinically relevant in Doose syndrome. Our strategy for predicting deleterious effect of mutations using multiple prediction algorithms provided valuable information, helping clinicians with decision making. Furthermore, our results indicate that missense mutations can cause severe phenotypes depending on its location and the type of amino-acid substitution.

P12.079
Mutations in *PRRT2* cause familial and sporadic cases of Benign Infantile Epilepsy

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Objective: Benign familial infantile epilepsy (BFIE) is an autosomal dominant seizure disorder that occurs in infancy. Seizures begin around 6 months of age and cease by 2 years. In some cases a movement disorder, paroxysmal kinesigenic choreoathetosis (PKC), follows in childhood or adolescence: this combined disorder is known as infantile convulsions and paroxysmal choreoathetosis (ICCA) syndrome. We have recently shown that the familial disorders BFIE, PKC and ICCA are allelic, caused by mutations in *PRRT2*. We sought to determine if *de novo PRRT2* mutations cause sporadic benign infantile epilepsy and whether the phenotypic spectrum of *PRRT2* was broader than initially recognized.
Methods: 44 probands with infantile-onset seizures, infantile convulsions with mild gastroenteritis and benign neonatal seizures underwent phenotyping and *PRRT2* sequencing. The segregation of mutations identified in probands was studied.
Results: The recurrent *PRRT2* mutation c.649-650insC (p.R217fsX224) was identified in 11 probands. Nine probands had a family history consistent with BFIE or ICCA. Two probands without a family history of seizures or PKC had *de novo PRRT2* mutations. Febrile seizures with or without afebrile seizures were observed in two families with *PRRT2* mutations.
Conclusions: Mutations in *PRRT2* are an important cause of seizures seen in infancy. Mutations can be either familial or *de novo*. *PRRT2* mutations are present in >80% of BFIE and >90% ICCA families, but are not a common cause of other forms of infantile epilepsy. Seizures with fever may occur in BFIE such that it may be difficult to distinguish BFIE from febrile seizures in small families.

P12.080
NGS in molecular diagnostics of epilepsies.

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The molecular genetic diagnosis of epilepsy particularly in children is essential for precise diagnosis, prognosis and has significant implications for the therapeutic strategy. Until now mutation screening was performed by DGGE, Sanger sequencing and MLPA technique.
Here we report on a new mutation screening protocol for Dravet syndrome & GEFS+ associated genes based on Next-Generation Sequencing (NGS) using the Roche 454J platform. Our current testing panel includes mutation analysis of genes *SCN1A, SCN1B, GABRG2, GABRD* and *PCDH19*.
So far, 22% of the patients suspected for Dravet syndrome / GEFS+ harboured a mutation in the *SCN1A* gene, one patient had a mutation in the *GABRD* gene. The spectrum of identified mutations included small deletions, insertions, indel mutations, truncating and missense mutations, mostly located in the pore and voltage sensitive region of the *SCN1A* protein. We have also detected a deletion of the whole *SCN1A* gene as well as a large duplication by MLPA. Interestingly, in two patients with a severe clinical manifestation a homozygous and two compound heterozygous mutations were identified, respectively.

Genetic diagnostics based on the NGS platform enables the analysis of multiple disease-related genes in parallel, to reduce TAT and the cost of the test as well and therefore outperforms the conventional Sanger sequencing.

P12.081

Mutations of the PCDH19 Gene in 26 Female Patients with Epilepsy

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Dravet syndrome (DS), or severe myoclonic epilepsy of infancy (SMEI) is a genetically determined encephalopathy mainly caused by de novo mutations in the SCN1A gene. Recently, Depienne et al identified mutations in the gene encoding protocadherin-19 (PCDH19) in 11 females with epileptic encephalophathy of infancy who were negative for mutations in the SCN1A gene. The PCDH19 gene links to Xq22.1 and this gene contains 6 exons, of which the first is unusually large.

We studied 26 female patients from southern Italy with SMEI that were negative for mutations in SCN1A to investigate the frequency of PCDH19 mutations in our population.

The clinical features of patients are seizures onset before 12 months of age, mild to severe mental retardation with poor language development ,and ataxia. Genomic DNA from the patients was analysed by direct sequencing of the PCDH19 gene on an ABI 3130XL Avant automated sequencer.

In this study, we identified three different heterozygous novel mutations of PCDH19 gene in 26 patients with SMEI (11,5%). The c.1522_1528del/p.Ile508ProfsX59 novel mutation was identified in an isolated female patient. The second novel mutation identified is c.1649G>C/ p.Arg550Pro; the third novel mutation identified is c. 2568C>T/p.Ser856Cys.

The results of this study indicate that PCDH19 mutatinon is a relatively frequent cause of epilepsy in Southern Italy. This frequency (11,5%) is comparable to that reported in previous studies.

In conclusion molecular testing of PCDH19 should be considered in females with early-onset FS and/or epilepsy with or without cognitive impairment and family history.

P12.082

Targeted Next Generation Sequencing as Diagnostic Tool in Epileptic Disorders

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The epilepsies are a very heterogeneous group of common neurological disorders comprising many individually rare diseases. Thus, genetic diagnosis oft remains difficult. With our approach we aim to reveal the genetic basis of epileptic disorders in so far unresolved cases.

We enriched a panel of 323 epilepsy-associated genes using a custom designed Agilent SureSelect in solution kit and sequenced on a SOLiD 4 platform.

We screened >50 unknown cases with a broad spectrum of epilepsy phenotypes. We detected causative aberrations in commonly mutated ion channel genes (e.g. SCN1A, SCN2A) as well as in rarely affected genes (e.g. STXBP1, MFSD8). Surprisingly, we detected many mutations in extremely uncommon genes (e.g. KCTD7, ARHGEF9, KCNJ10, SMS). We also revealed SCN1A mutations in three patients where conventional testing (Sanger sequencing / HRM) failed to detect the mutations.

We have successfully established a fast and cost efficient genetic screening method for patients with seizure disorders. We were able to uncover the genetic basis of many so far unresolved cases with epilepsy. We detected mutations in patients with both clear and unspecific epilepsy phenotypes. We revealed false negatives in conventional genetic testing methods. Many mutations were detected in genes that only in very rare instances have been associated with epileptic disorders. Thus, many rare epilepsy disorders might perhaps be more common but simply underdiagnosed due to unspecific and diffuse phenotypes. Therefore, our approach may contribute in collecting information on both well-known and unacquainted epilepsy disorders and in revealing their true phenotypic spectrum.

P12.083

Mutation spectrum in the CACNA1A gene in 49 patients with episodic ataxia type 2

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Episodic ataxia is an autosomal dominant ion channel disorder characterized by episodes of imbalance and incoordination. Episodic ataxia type 2 (EA2) features recurrent episodes of vertigo and cerebellar ataxia, lasting from minutes to a few days, with often interictal nystagmus.

Many EA2 patients harbor mutations in the CACNA1A gene, encoding the α1A subunit of the P/Q-type voltage-gated calcium channel Cav2.1. The vast majority of them are loss-of-function missense or nonsense mutations leading to decreased channel currents. Recently, CACNA1A exonic deletions were reported in EA2 using quantitative approaches, such as multiplex ligation dependent probe amplification (MLPA) and quantitative multiplex PCR of short fluorescent fragments (QMPSF).

We performed a mutational screening of the CACNA1A gene, including the promoter and 3'-UTR regions, in 49 unrelated patients diagnosed with EA2. When point or small indel mutations were not found, we performed MLPA and QMPSF assays to screen for large duplications or deletions. Overall, a mutational screening by PCR amplification and sequencing allowed identification of 9 point mutations (4 nonsense and 5 missense changes) and a 2-bp deletion (predicting a truncated protein), covering 20% of the patients. Subsequently, quantitative analysis identified a deletion of exon 35 as a result of a homologous recombination event between flanking Alu sequences. Our data suggest that these variations are disease-causing, although functional studies are warranted.

P12.084

Does C-terminal deletion in the ALAS2 gene cause x-linked dominant or recessive protoporphyria?

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Erythropoietic protoporphyria (EPP) is an inherited disorder caused by over-production of protoporphyrin IX in the final step of heme synthesis. Most patients have autosomal-dominant EPP, which requires coinheritance of a null *FECH* mutation trans to a hypomorphic allele. Recently a new form of X-linked dominant protoporphyria associated to gain of function deletions in *ALAS2* has been described.

In this study we re-examined 7 Italian unrelated *FECH*-negative families for a total of 23 subjects by sequencing of exon 11 of *ALAS2*. In 4 families, 4 males and 5 females carried the deletion c.1706-1709 delAGTG, confirming the role of *ALAS2* in protoporphyria phenotype. Differently from what was previously described (Whatley et al., 2008) we found heterogeneity of phenotypes between females: out of five females with the *ALAS2* deletion, only two showed photosensitivity. Thus X-inactivation (X*i*) were evaluated by analysis of *ZMYM3* gene and HUMARA Assay. The symptomatic females showed extreme skewed X*i* pattern of the wild type allele (95% vs 5%), while the other three showed a balanced or a slightly skewed inactivation of the mutated allele. Our results demonstrate that the disease is transmitted as an X-linked recessive trait and the phenotype depends on the degree of X*i*.

In one family only the proband carried the deletion indicating a possible the novo onset of the mutation that was confirmed by segregation of microsatellites.

The mutations in 3 *FECH*-negative EPP families remain unknown, indicating that new gene targets can offer new opportunities for diagnosis.

P12.085

Whole-exome sequencing in patients with clinical diagnosis of Familial Hypercholesterolaemia

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Familial hypercholesterolemia (FH) is a monogenic condition caused, in most cases, by mutations in LDLR, APOB and PCSK9. From our previous studies only 40% of patients have an identifiable mutation so, other mutations in these genes or other gene defects must exist to explain the cause of hypercholesterolemia in the remaining families.

The main aim of this project was the whole-exome sequencing of 5 index

patients with clinical diagnosis of FH (4 without a detectable mutation and one patient (P1) with a mutation in LDLR but the genotype did not justified the phenotype), in order to identify the genetic cause of the hypercholesterolemia in these patients.
Multiple variants were identified for each patient, more than 15000 per sample. The present analysis only refers to variants occurring only in one sample. A total of 6139 were nonsynonymous single-nucleotide substitutions, 11740 were synonymous, 283 were frameshift alterations and 73 were stopgain or stoploss alterations. A total of 1566 of the nonsynonymous substitutions were not present in dbSNP. The 639 novel nonsynonymous substitutions were distributed among 501 genes. P3 has a nonsynonymous alteration described before in LDLR gene, that was missed before due to technical problem. P1 has a frameshift alteration and P4 has an insertion, both not described before, that are possible causes of hypercholesterolaemia. Family studies are being prepared but functional studies are required to prove pathogenicity.
Exome sequence produces a vast amount of data that needs careful analysis but it can lead to the discovery of new genes for FH.

P12.086
Evaluation of exome sequencing in genes associated with aortic diseases

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Exome sequencing is a combination of next generation sequencing (NGS) and the enrichment of all known human protein-coding exons and flanking splice sites. In addition to qualitative analyses, which can detect point mutations and small insertions/deletions, exome sequencing data can also be used for quantitative sequence analysis in order to detect large insertions/deletions. In this study, we have evaluated the qualitative and quantitative properties (i.e. mutation detection rate) of exome sequencing for different mutation types and genes. These genes are associated with syndromic forms of rare aortic diseases, such as Marfan syndrome *(FBN1)*, Loeys-Dietz syndrome *(TGFBR1, TGFBR2)*, and Ehlers-Danlos syndrome vascular type *(COL3A1)*, or with non-syndromic forms such as familial thoracic aortic aneurysms *(ACTA2, MYH11, MYLK)*. For this evaluation, DNA samples with known point mutations and small deletions/duplications detected by Sanger sequencing as well as large deletions/duplications detected by MLPA were used as template in exome sequencing. In a first step, we applied Agilent's in solution sequence capturing of all coding exons and flanking intronic sequences and performed NGS using a SOLiD4 platform. Exome sequencing data visualized by the Integrative Genomics Viewer revealed that the mutation detection rate of the used exome sequencing method was lower than that of Sanger sequencing and MLPA, varying between mutation types and genes. Whereas point mutations were successfully detected in enriched exons with sufficient read-coverage depth, the used exome sequencing protocol needs to be improved for the detection of small deletions and duplications/insertions as well as for the more balanced capturing of exons.

P12.087
Exome sequencing of a consanguineous family affected by a congenital muscular dystrophy with hyperlaxity (CMDH).

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Over the past two decades, there have been major advances in defining the genetic bases of congenital muscular dystrophies (CMD). Genetic research has allowed the identification of more than 14 genes responsible for various forms of CMDs. Despite the great progress in this field, there are still a significant percentage of cases for which the mutated gene is unknown. This is particularly the case for milder forms. Novel genomic techniques like Next-Generation DNA Sequencing (NGS) open new avenues in the elucidation of genetic defects causing monogenic disorders such as CMD. We recruited a consanguineous French-Canadian family from Southwestern Quebec affected by CMDH. A previous microsatellite genome wide-scan (GWS) has linked this family to 32cM region on chromosome 3p23-21. Most obvious candidate genes have been rule out in this region. A SNPs genome scan (Illumina OmniExpress) have identified six homozygous region shared by the two affected members. Among them, a 17Mb region on chromosome 3p24.3p21.3 was identified. The two affected cases as well as the mother were sent for exome sequencing at Perkin Elmer service lab. The Agilent SureSelect 50Mb kit was used for exons capture and the sequencing was performed on an Illumina HiSeq. A list of candidate genes with rare variants shared by the two cases in a homozygous state and heterozygous in the mother was produced. The uncovering of the genetic bases of muscular dystrophies serve as the essential original building blocks on which successful new therapeutic approaches can be designed.

P12.088
Next-generation sequencing in genome-wide investigations reveals novel candidates in genetic eye disease

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Many ocular disease including developmental eye conditions such as anterior segment dysgenesis and diseases affecting the retina show marked genetic heterogeneity. The underlying genetic causes are unknown in many cases. An understanding of genotype-phenotype correlations is required to facilitate improved treatment strategies in these conditions. Next-generation sequencing is a key strategy for assessing structural and sequencing variants on a genome-wide scale in personalized medicine. We identified two families with balanced chromosomal rearrangements and eye disease, one *de novo* and one segregating with the disease, indicating pointers to the underlying disease genes. For rapid breakpoint identification, we used a mate-paired-end-tag sequencing approach. Identification of discordant read pairs spanning the breakpoints showed the genomic rearrangements precisely. In the *de novo* case with retinitis pigmentosa, a strong novel candidate disease gene with a role in presynaptic neurotransmitter release was transected by the breakpoint. In the familial case with anterior segment dysgenesis, several candidates were in proximity to the breakpoint and expression results from the patient cells and mouse eye tissues have identified a strong candidate in eye anterior segment development. Exome target enrichment sequencing is being carried out for these and other candidates to screen for mutations in other patients with similar eye phenotypes. Our work emphasises the utility of combined genome-wide structural and exomic analyses in disease gene identification in genetically heterogenous eye disorders.

P12.089
Hemophagocytic lymphohistiocytosis developed in visceral leishmaniasis may have genetic etiology

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Hemophagocytic lymphohistiocytosis (HLH) is an immune dysfunction disorder with various etiologies. Familial HLH (FHL) has genetic basis, while acquired HLH develops secondary to infections, malignancies etc. BMT is the only curative therapy in FHL whereas HLH symptoms in secondary HLH subside by the treatment of main cause. HLH may rarely develop secondary to visceral leishmaniasis (VL). This first study demonstrates that HLH developed in VL may actually have genetic etiology. One-year-old only child of a consanguineous family had abdominal distention and high fever. Leishmania amastigots in BMA led to diagnosis of VL. Liposomal-amphotericin-B therapy resolved symptoms which reappeared subsequently. No amastigots but hemophagocytosis detected in second BMA. She received continuous HLH-2004 protocol therapy since remissions followed by HLH reactivations and/or five CNS relapses until she died at age four. HLA matched donor was unavailable. Haplotype analysis of the family for Perforin, UNC13D, Syntaxin 11 and STXBP2 genes of FHL revealed homozygosity for UNC13D gene. Sequencing identified homozygous 627delT frameshift mutation (Thr209fsX40) in exon 8, resulting in premature termination of translation 40 amino acids downstream. Parents were heterozygous for this pathologic mutation. In conclusion, HLH in this patient did not develop secondary to VL, but FHL, triggered by leishmania infection, most probably emerged at an earlier time. Clinicians must be aware that HLH developed in VL may have genetic etiology and leismaniasis may mask the diagnosis of primary disease, which may eventually lead to loss of the patient due to tardiness in proper therapy and BMT. Supported by TUBITAK (Project No:105S386-SBAG3193).

P12.090

Molecular analysis of *MEFV* gene mutations in Iranian patients with Familial Mediterranean Fever

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Familial Mediterranean Fever (FMF) is an autosomal recessive disorder characterized by recurrent attacks of fever and painful inflammatory manifestations in the abdomen, chest or joints. The marenostrin/pyrin-encoding gene (*MEFV*), mapped to 16p13.3, has been proposed as a candidate gene for FMF on the basis of the identification of mutations clustered in 10 exons.

In this study we used reverse-hybridization (FMF StripAssay, ViennaLab Diagnostics, Vienna, Austria) to analyze the following 12 *MEFV* mutations: E148Q, P369S, F479L, M680I (G/C), M680I (G/A), I692del, M694V, M694I, K695R, V726A, A744S and R761H in 331 Iranian patients, who were referred to us based on clinical criteria indicating FMF.

We identified *MEFV* mutations in 33.8% of our patients. Out of these, 66.5% were located in exon 10, while the remaining 33.5% were found in other exons. The most common mutations were p.M694V (20%), followed by p.E148Q (18%), p.V726A (9%), and p.M680I (c.2040G>C) (3%), which suggests a heterozygote advantage of these first three mutations in Iran. In 45.9% of patients we could not identify any mutation that could explain their clinical status; therefore, we plan the comprehensive sequencing of *MEFV* gene to elucidate the cause of their disease. Among these patients, for the first time we report on a novel disease causing compound heterozygote mutation (R202Q) in exon 2 and (c.1588-69 G>A) in IVS-5 of *MEFV* gene in one family which is followed by early onset FMF. As disease-causing mutations these two mutations should be further investigated in more patients in different populations.

P12.091

Homologous recombination - a pool for FA candidate genes

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Fanconi anemia (FA) is a rare autosomal or X-linked recessive genetic disorder, characterized by typical physical abnormalities, bone marrow failure and an increased risk for malignancies. Because of a DNA repair defect FA cells show elevated spontaneous and in particular MMC inducible chromosomal instability. To date, fifteen FA genes have been reported. The corresponding proteins are known to be members of the FA/BRCA network that promotes DNA interstrand crosslink repair by homologous recombination (HR).

A small subset of FA patients still cannot be assigned to established complementation groups. Therefore, we screened cell lines from those patients for defects in candidate genes involved in HR. These potential FA genes included MUS81, SFPQ and MMS22L. MUS81 is the structure-specific endonuclease of the MUS81/EME1 heterodimer that interacts directly with the FA protein SLX4 (FANCP). The splicing factor related protein SFPQ cooperates with RAD51D, a paralog of RAD51C (FANCO), and might play a role in homologous recombination. The third candidate gene is MMS22L. In complex with TONSL, it accumulates at stalled replication forks and is required for efficient formation of RAD51 foci after DNA damage.

The screening methods included Sanger sequencing and Western blotting and so far revealed no pathogenic mutations. More unassigned FA cell lines and additional FA candidate genes will be included in our screen, since candidate gene approaches have been successful in the past for the identification of the FA genes BRCA2/FANCD1, PALB2/FANCN and SLX4/FANCP.

P12.093

Evaluation of a Laboratory Developed High Resolution Melt Assay Design for Determination of Methylation Status of the FMR1 Promoter

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Fragile X Syndrome (FXS), a common cause of intellectual disability, is inherited via expansion of the trinucleotide (CGG) repeat in the *FMR1* gene. FXS is associated with methylation of the expansion and the neighboring *FMR1* promoter, preventing transcription. Fragile X diagnostic testing incorporating Southern analysis can detect expansion of the repeat region and evaluate methylation using a methylation-sensitive restriction digest, but the process is time-consuming and only queries a small number of CpG sites flanking the expansion. We recently developed and tested a PCR primer pair capable of amplifying both methylated and unmethylated bisulfite-converted DNA, for a region of the *FMR1* promoter incorporating 22 CpG sites. PCR amplification of bisulfite converted DNA with an intercalating dye and High-Resolution Melt analysis can be used in a laboratory developed (LD) protocol to determine the overall methylation status of the *FMR1* promoter. One possible High-Resolution Melt protocol was evaluated in experiments testing the methylation status of 20 clinical samples (4 premutation males, 5 premutation females, 4 full mutation males, and 7 full mutation females) for which the results were compared to methylation values estimated from Southern blot results. Additional clinical and Coriell DNA samples were tested that included normal, premutation and full mutation alleles from both genders. Our results showed qualitative methylation values were in line with those observed by other methods. A PCR-based LD assay for *FMR1* methylation would complement other PCR-based expansion sizing and screening LD tests to allow both expansion and methylation status of patient samples to be rapidly determined.

P12.094

Examination of FMR1 allele size in women with primary ovarian insufficiency from the Basque Country

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FMR1 premutation alleles (55-199 CGG repeats) have been associated with primary ovarian insufficiency (POI). More recently some studies have shown that alleles in the intermediate range (45-54 CGG repeats) and in the high end of the normal range (≥35 CGG repeats) are also related with the development of this condition. A group of 31 women with POI from the Basque Country has been analyzed to study the prevalence of alleles in the premutation, intermediate and the high end of the normal range. Considering the 35-54 CGG repeat range, the number of women carrying at least one allele with >35 CGG repeats was statistically higher in patients (16.3% vs. 6.67%). To make a more accurate analysis the patient group was classified into two categories, women with overt POI (19.36%) that presented with amenorrhea for at least four months and FSH levels >10 IU/L and women with occult POI (80.64%) that presented with decreased fecundity and regular menses. When comparing alleles with >35 CGG repeats, the prevalence of these alleles was statistically higher among women with overt POI, but not among women with occult POI. The data of this study suggests that carrying more than 35 CGG repeats in the FMR1 gene might be related with the development of overt POI, but not with occult POI.

P12.095

Mosaicism for a normal allele, a full mutation and a deletion involving the whole CGG repeat in the *FMR1* gene

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We report on a 29-year-old female who wants to start a family. She was referred for DNA analysis to see what risk she carries for having offspring with fragile X syndrome, because her mother carried a premutation of 80 CGG units in the *FMR1* gene.

DNA was isolated from lymphocytes. PCR and length analysis on an automated sequencer identified only a normal CGG repeat of 29 CGG units. Further Southern analysis revealed a mosaic pattern, that indicated presence of an *FMR1* allele with a deletion next to a normal allele and alleles with a full mutation. Sequence analysis indeed confirmed presence of a 2475 bp deletion that covered the whole CGG repeat and transcription initiation site until 45 bp proximal of the translation start codon.

Similar deletions have been reported and these result in inactivation of the *FMR1* gene and absence of the FMR1 protein. Our patient therefore has a 50% risk of transmitting either a full mutation allele or the deletion allele, either of which could cause fragile X syndrome in her offspring.

It is important to note that the *FMR1* deletion allele was not detectable with the Amplidex *FMR1* PCR kit (Asuragen, Austin, USA). Since recently, we use this new method as an alternative to Southern analysis in DNA testing for fragile X syndrome. We are now comparing various techniques to decide upon an optimal method that can be offered for prenatal testing.

P12.096

Fraser syndrome: Implementation and first results of FRAS1 molecular analysis at the University Hospital of Clermont-Ferrand, France

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Fraser syndrome is a rare disease, with autosomal recessive inheritance. It is a malformative syndrome, which most frequent signs are cryptophtalmos and syndactyly. They are associated with other anomalies like craniofacial, genital, urinary or lung malformations. The expression is variable. Clinical diagnosis criteria have been defined by Van Haelst in 2007. Currently, prenatal diagnosis uses ultrasonography during the second quarter of pregnancy and is difficult without family history orientation. Two genes, FRAS1 and FREM2, have been identified as responsible for Fraser syndrome. Only 2 different mutations have been described in FREM2 and 23 in FRAS1.

In this work, our initial goal was to identify, by PCR-sequencing, causative mutations of Fraser syndrome patients from eight families who were diagnosed on clinical criteria. We analyzed FRAS1 in priority.

We found in those patients 4 nonsense mutations, 2 intronic mutations affecting splicing, one 1-bp duplication and finally 4 new sequence variations (intronic and exonic) of unknown significance. We specified the value of these 4 sequence variations using bioinformatic algorithms and functional tests based on transfection of minigene constructs in HeLa cells. Using these tools, a missense mutation was shown to induce exon skipping.

Analysis of FRAS1 gene provides the clinician a molecular confirmation of Fraser syndrome diagnosis in about 40% of cases. This further allows the possibility to offer the parents an early antenatal molecular diagnosis for a future pregnancy. We are now considering the development of the analysis of coding exons of FREM2 gene to increase the coverage rate.

P12.097

Study of FRAXE-MR in intellectually disabled individuals referred for Fragile-X Syndrome testing in Portugal

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Among the genetic causes involved in X-linked intellectual disability (XLID), pathogenic variations in *FMR1* (Fragile Mental Retardation 1)*, AFF2 (*AF4/FMR2 family member 2) and *ARX* (Aristaless Related Homeobox) genes emerge as main causes. *FMR1* and *AFF2* genes contain (*polymorphic repetitive regions*) a repeat polymorphism which is susceptible to suffer dynamic mutation, a process that may induce pathogenic expansions. FRAXE-associated mental retardation (FRAXE-MR) is mainly a non-syndromic form of XLID and is due to *AFF2* gene silencing as a consequence of 5'UTR-CCG expansion or gene mutations. A CCG triplet number up to 30 repeats is consider normal, while full expansion (>200 repeats) and hypermethylation of CCG cluster results in FRAXE-MR.

AFF2 variants are not frequently sought. An implementation of a cost-effective strategy (co-amplification with other ID genes) represents an improvement in molecular diagnosis with consequent gains in clinical genetic diagnosis and counseling. Herein we present results of *AFF2* molecular analysis in a subpopulation of 5000 intellectually-disabled individuals with primary referral for FRAXA screening, by a novel multiplex-PCR strategy. This approach accurately detected normal to pre-mutated alleles. A pre-mutated allele with 68 CCG was identified and further characterized by Southern blot analysis in order to exclude methylation and/or repeat number mosaics, as well as PCR failure. Possible phenotype-genotype correlations based on the clinical data of one previously diagnosed family with *AFF2* full expansion, the newly characterized pre-mutation carrier and one case with a new variant of the *AFF2* gene will be investigated and presented.

P12.098

Molecular Combing for the Diagnosis of FSHD

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Facioscapulohumeral muscular dystrophy (FSHD) is the third most common neuromuscular disorder. It is associated with a contraction of D4Z4 macrosatellite repeats on chromosome 4q35. The copy number variation of the D4Z4 repeat varies between 11-100 copies in the normal population and 1-10 in FSHD patients.

Chromosome 10q26 contains a highly homologous repeat array. On both chromosomes, the array can be embedded in two major haplotypes - A and B, but only repeat contractions on the 4qA haplotype are associated with the disease.

The standard method for FSHD diagnostics is Southern blotting, but in about 20% of patients the FSHD genotype remains unclear. Therefore, *GenomicVision* has designed a diagnostic test for FSHD based on Molecular Combing and fluorescence *in situ* hybridisation.

Molecular Combing is a single-molecule analysis that enables direct visualization of multiple whole genomes and the detection of large genome rearrangements. Long DNA molecules are combed onto a solid surface in parallel alignment with uniform stretching allowing direct sizing of fluorescence signals. Probes have been developed to visualize the FSHD-locus as a three-coloured bar code in order to differentiate the repeats and the four haplotypes.

In our diagnostic lab this new technique has been ß-tested for diagnostic purposes. DNA of 30 suspected FSHD patients has been analysed by Molecular Combing, results have been compared to the corresponding Southern blots and show very good accordance.

Molecular Combing provides all molecular data required for the diagnosis of FSHD in a single experiment.

P12.099

Analysis of the Glucocerebrosidase Gene Mutations in 32 Turkish Gaucher Patients

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Gaucher disease (GD) is the most frequent autosomal recessive lysosomal glycolipid storage disease and is caused by acid b-glucosidase (E.C.3.2.1.45) enzyme deficiency. Mutations in the glucocerebrosidase gene (GBA; MIM# 606463; GenBank accession no. J03059.1) cause Gaucher's disease. The GBA (7.5 kb) gene contains 11 exons and 10 introns and is located at chromosome 1q21 locus which consist of several genes. A highly homologous GBA pseudogene is 16 kb downstream from the functional gene. More than 200 mutations such as subtitutions, splicing alterations, partial and total deletions insertions, including complex mutations due to genetic rearrangements between the functional gene and pseudogene have been defined in the GBA gene (2). In this study we report the molecular characterization of 32 unrelated Turkish GD patients having different types of GD. The allelic frequencies of GBA gene mutations in Turkish patients are reported. The most prevalent mutations are N370S and L444P accounting for 50 % and 35.48 % in our GD patient groups respectively. We identified one novel genetic alteration which was a missense change L385R that are associated with the severe phenotype of type II GD. Molecular genetic analysis of the GBA gene in GD is important for genotype phenotype correlation and also will provide reliable genetic counseling in families at high risk for GD.

P12.100

Prevalence of 35delG of the GJB2 gene in hereditary, prelingual, nonsyndromic hearing loss in Mexican population.

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Hearing impairment is the most common sensory disorder. About 1/1000 children is affected by pre-lingual deafness. Several gene mutations have been associated with recessive hearing impairment. The GJB2 gene mutations are the most frequent cause of hereditary, prelingual, non syndromic hearing loss; they present an autosomal recessive inheritance. More than 90 GJB2 mutations affecting Cx26 expression have been reported and linked to hearing loss. Up to 85% of abnormal Cx26 expression may be attributed to the 35delG mutation. This type of mutation showed a high prevalence among Caucasian populations. Prevalence of GJB2 mutations in the Chinese population seems to be different, it appears that 35delC is the most frequent cause of recessive hearing impairment among this population; similarly, Japanese families with bilateral sensorineural hearing loss, also present the 235delC defect, which seems to be the most prevalent mutation affecting Cx26 expression among this population. Previous investigations show ethnic association to other specific GJB2 mutations, per example R143W mutations among African populations or 167delT among Ashkenazi Jews. Objective: To identify the prevalence of 35delC GJB2 mutation in a sample of

Mexican patients. Methods The study included 96 patients from 37 non-related families with hereditary, prelingual-nonsyndromic-hearing-loss, all of them were analyzed through PCR and DNA sequencing from genomic DNA. Results and Discussion: We found in 7 families the presence of 35delC both in heterozygous and homozygous state. We discuss the prevalence of this mutations in the Mexican sample and compare it with the data previously reported in other populations.

P12.101

Identification of a *de novo* splice-site mutation in *SLC2A1* gene causing Glut1 deficiency syndrome in a Turkish patient

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Glucose transporter-1 deficiency syndrome (Glut1-DS) is a metabolic disorder characterized by early onset infantile seizures, movement disorders and a wide range of neurologic abnormalities. Heterozygous mutations in gene *SLC2A1* (*GLUT1*) have been implicated in Glut1-DS, which encodes Glut1-a major glucose transporter in the mammalian blood-brain barrier.

We analyzed *SLC2A1* in a Turkish patient with early onset infantile seizures, microcephaly, intellectual disability, ataxia, dyskinetic cerebral movement disorder and hypoglycorrhachia (cerebrospinal fluid (CSF) glucose concentration:31mg/dl; normal:>40mg/dl and CSF/blood glucose ratio:0.36; normal:>0.45). All exons and exon-intron boundaries of *SLC2A1* were PCR amplified in ten fragments from genomic DNA and screened for mutations by direct DNA sequencing. We detected a novel heterozygous splice site mutation (NM_006516.2:c.680-1G>T) at the last nucleotide of intron 5. Neither the parents nor the 5 healthy sisters of the patient carried this mutation indicating that mutation has arisen *de novo*. This novel mutation analyzed using high resolution melting assay was not detected in a control group of 92 samples selected from the general population. The theoretical consequences of mutation predicted with an *in silico* splice site prediction tool; Human Splicing Finder (HSF). HSF analysis resulted with broken acceptor splice site with 33.57% loss of scoring where scoring decrease >10% was considered predictive of splicing alterations.

We report a novel mutation that possibly creates an altered splice-site. Analysis of relative *SLC2A1* transcript levels by real-time quantitative RT-PCR shows a decreased level in the patient suggesting a pathogenic effect of this mutation on the transcript level, which will further be analyzed.

P12.102

Investigating the functional role of *GPM6A* as a possible intellectual disability gene

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In a patient with mild cognitive impairment/learning disabilities and behavioral anomalies, molecular karyotyping with an Affymetrix 6.0 SNP array revealed a de novo copy number gain of the *GPM6A* gene. Subsequent expression analysis in patient's blood lymphocytes showed increased *GPM6A* expression in the patient compared to healthy controls. Glycoprotein M6a (GPM6A) is a transmembrane protein of the PLP/DM20 protein family whose expression is restricted to neurons. In rats GPM6A has already been implicated in neurite outgrowth, neuronal differentiation and synapse formation. M6a has also been implicated in stress response in different animal models.

We employed Drosophila melanogaster as a model organism to further elucidate its role in stress response, synapse formation and behavior. Using the UAS-Gal4 system we analyzed the function of the Drosophila homologue m6 after tissue specific knockdown and overexpression with different m6 RNAi and m6 overexpressing lines. We found elevated expression levels of m6 in fly brains of L3 larvae and adult flies after heat shock stress treatment, which further increased with heat shock elongation. These findings indicate that m6 expression is also stress responsive in flies. Effects of different m6 levels on survival time upon starvation and oxidative stress are also being studied. Additionally we are currently investigating the role of m6 in synapse formation by dissecting L3 larvae to analyze various parameters of the synapses of neuromuscular junctions and a possible role in complex learning and memory processes by performing the Courtship conditioning assay.

P12.103

Mild haemophilia A in a female patient with an Xq27.3-q28 deletion and a missense mutation in the F8 gene

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Haemophilia A is an X-linked recessive bleeding disorder which is rarely reported in females. The main reason for the manifestation of haemophilia A in females is due to heterozygous mutations in the F8 gene combined with non-random inactivation of the X chromosome.

Here, we present a 7-year-old girl with mild haemophilia A (FVIII:c 20%) whose brother shows a hemizygous missense mutation (p.Met2164Val) in exon 23 of the F8 gene and suffers also from a mild haemophilia A (FVIII:c 18%).

Direct sequencing of exon 23 in the young girl revealed the same missense mutation as detected in her brother but strikingly it was present in homozygous form. Turner syndrome could be excluded by analyses of polymorphic markers on Xq13, Xq22 and Xq28. Multiplex ligation-dependent probe amplification (MLPA) revealed a large heterozygous deletion involving the whole F8 gene and adjacent control regions. In order to determine the extension of the deletion an analysis for copy number variation was performed on a 300 K Illumina Cyto SNP-12 array. Thereby, a large deletion of approx. 8.9 Mb comprising about 200 genes on Xq27.3-q28 was detected in this girl. Additionally, almost complete inactivation of the X chromosome containing the large deletion could be demonstrated by fragment analysis after methylation sensitive digestion.

Except for the manifestation of haemophilia A the contiguous deletion is supposed to have no or only mild phenotypic consequences for the girl herself in contrast to her potential male offspring: a male fetus carrying this deletion may not be viable.

P12.104

Genetic screening of MYH7 and MYBPC3 genes in Russian HCM patients

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Background: Primary hypertrophic cardiomyopathy (HCM) is inherited cardiac disorder characterized by clinical and genetic heterogeneity. Mutations in MYBPC3 and MYH7 genes, encoding myosin-binding protein C and myosin heavy chain beta, respectively, account of 40% of HCM cases. Some MYBPC3 and MYH7 mutation carriers have a high risk of sudden cardiac death (SCD).

Methods: By now panel of 30 patients with primary HCM have been formed. We have screened coding and adjacent intronic areas of MYBPC3and MYH7 by direct sequencing in 15 patients. Medical examination: personal and familial history collection, physical examination, standard ECG, 24-h HM and Echo-CG.

Results:We have found 3 mutations (S217G, Q1233ter, V896) in 3 probands inMYBPC3 gene (20% of cohort screened), and 2 HCM-associated polymorphisms (S236G, R326Q). Two mutations (R403W, R249Q) in 2 probands (13,3%) were found in MYH7 gene.

Patient carried heterozygous MYBPC3 variants R326Q and Q1233ter had early manifestation, fast progression and positive SCD familial history. Patient with mild form of HCM had heterozygous S217G mutation in MYBPC3 gene shown previously as leading to dilated cardiomyopathy (DCM) or HCM (with SCD) both. Carrier of heterozygous variants S236G in gene MYBPC3 and R249Q in gene MYH7, 4 y.o., male, had a hypertrophy of left ventricular and interventricular septum. His sister with HCM died suddenly at age of 14 y.o. Mutation carrier R249Q has a high risk of SCD.

Conclusion: Screening of mutations in MYBPC3 and MYH7 genes patients is reasonable and cost-effective in HCM patients.

P12.105

High throughput technologies aimed at the identification of new candidate genes in Italian and Qatari population

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Hereditary Hearing loss (HLL) is a common disorder accounting for at least 60% of prelingual deafness. Most cases (70%) are non-syndromic (NSHHL) with mutations in GJB2 and GJB6 genes playing a major role worldwide, and

almost no other common genes have been identified. Regarding the Qatari population, a molecular screening for these common genes/mutations clearly demonstrates that they accounts for a minor proportion of NSHHL cases in this population. Thus, these findings strongly suggest that many genes for NSHHL await identification. To increase our knowledge on the molecular bases of HHL in these populations, an extensive use of high throughput technologies such as High Density arrays (i.e for linkage data) and Next Generation Sequencing has been planned.
Six Italian families (dominant inheritance) and 5 Qatari families (recessive inheritance), all negative for the presence of mutations in the most common hearing genes, have been selected. High density SNPs arrays have been utilized to define a minimum number of candidate loci, to be applied in the filtering phase of NGS data. Whole exome sequencing data have been obtained and confirmed by Sanger sequencing. After filtering (dbSNP and in-house database), 2 new candidate genes have been identified in the Qatari population, while data on the Italian samples are still under the validation step. These results will definitely increase our knowledge of new deafness genes, and further confirm the importance of such new technologies for disease gene identification.

P12.106

Different contribution of DFNB loci in Hearing Impaired pedigrees in Iranian population

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Hearing loss is the most common sensory disorder. The autosomal recessive non-syndromic form (ARNSHL) accounts for 72% of monogenic HL. DFNB1 is the most common cause of ARNSHL in many populations (50%) including Iran (20%). The fact that many loci are involved together with the heterogeneity of the status, necessitate studying further loci in various Iranian ethnic groups. Fifteen large deaf pedigrees originating from the Southern Khorasan province of Iran were selected. The families analyzed for *GJB2*, exon I & II, mutations and *GJB6* large deletions (D13S1830, D13S1854). Pedigrees negative for GJB2 mutations were then subject to linkage analysis for loci DFNB2, DFNB3, DFNB4, DFNB7/11, DFNB9, DFNB21 & DFNB59. Individuals were genotyped for STR markers using touch-down PCR-PAGE. DFNB4, DFNB3, DFNB21 & DFNB59 have been analyzed and the project is proceeding for other loci. Three out of the 15 families showed *GJB2* mutations. One family carried homozygous c.35delG mutation. The other pedigree carried two different mutations; One patient was heterozygous for c.231G>A while another carried a heterozygous c.380 G>A mutation. The second alleles were not detected. The 3rd pedigree showed heterozygote mutation of p.V27I+E114G/wt . GJB6 deletions were not detected. One family showed linkage to DFNB3 and the remaining did not show linkage to the studied loci. Our results once again emphasize the heterogeneity of HL among different Iranian ethnic groups .These results could provide further insight into the etiology of HL and may lead to better genetic diagnostics & counseling. This study proceeds with more loci and more families.

P12.107

The genetic basis of non-syndromic hearing loss in Cyprus

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Mutations in the *GJB2* (Connexin 26) gene are responsible for more than half of all cases of pre-lingual recessive inherited non-syndromic deafness in Europe. This study presents a mutation analysis of the *GJB2* and *GJB6* genes in 104 Cypriot patients with sensorineural non-syndromic hearing loss compatible with recessive inheritance. Samples from patients were screened for the IVS1+1G splice mutation and the coding exon 2 of the *GJB2* gene including also the deletions del(GJB6-D13S1830) and del(GJB6-D13S1854). Twenty seven patients were verified with *GJB2* mutations in both alleles and with 35delG as the most dominating one, accounting for 76.3% (45 out of 59 mutated alleles), followed by p.R184P (6.9%), p.V37I (1.7%), p.E47stop (1.7%), p.L90P (1.7%), delE120 (1.7%), 167delT (1.7%) and p.V178A (1.7%). Additionally, five patients with severe sensorineural hearing loss were detected only in the heterozygote state. Three of these patients were heterozygous for p.V153I, a fourth was heterozygous for p.V37I and lastly a fifth for the spice site IVS1+1G>A.
Finally, no *GJB6* mutations or the known del*(GJB6-D13S1830)* and del(GJB6-D13S1854) were identified in any of the investigated Cypriot non-syndromic hearing loss patients. This work confirms that the *GJB2* 35delG mutation is an important pathogenic mutation for hearing loss in the Cypriot population and that the underlying molecular basis of autosomal recessive non-syndromic deafness in Cyprus is genetically relatively homogeneous . This finding will be used towards the effective diagnosis of non-syndromic hearing loss, improve genetic counseling and used as a potential therapeutic platform in the future for the affected patients in Cyprus.

P12.108

Nonsense Mutations In SMPX, Encoding A Protein Responsive To Physical Force, Result In X-Chromosomal Hearing Loss

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Hereditary hearing loss is the most common sensory disorder in humans and is characterized by an extraordinary allelic and nonallelic genetic heterogeneity. X-chromosomal hearing impairment represents only a minor fraction of all cases. In a study of a Spanish family the locus for one of the X-chromosomal forms was assigned to Xp22 (DFNX4). We mapped the disease locus in the same chromosomal region in a large German pedigree with X-chromosomal nonsyndromic hearing impairment by using genome-wide linkage analysis. Males presented with postlingual hearing loss and onset at ages 3-7, whereas onset in female carriers was in the second to third decades. Targeted DNA capture with next-generation sequencing detected a nonsense mutation in the small muscle protein, X-linked (SMPX) of affected individuals. We identified another nonsense mutation in SMPX in patients from the Spanish family who were previously analyzed to map DFNX4. SMPX encodes an 88 amino acid, cytoskeleton-associated protein that is responsive to mechanical stress. The presence of Smpx in hair cells and supporting cells of the murine cochlea indicates its role in the inner ear. The nonsense mutations detected in the two families suggest a loss-of-function mechanism underlying this form of hearing impairment. Results obtained after heterologous overexpression of SMPX proteins were compatible with this assumption. Because responsivity to physical force is a characteristic feature of the protein, we propose that long-term maintenance of mechanically stressed inner-ear cells critically depends on SMPX function.

P12.109

Transthyretin-related familial amyloid polyneuropathy in Bulgaria

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Transthyretin-related Familial Amyloid Polyneuropathy (TTR-FAP) is a hereditary amyloidosis, caused by amyloid formation and destabilization of the transthyretin tetramer.
The disease is autosomal dominant, caused by mutations in the TTR gene. The majority of the described cases concern small kindred or sporadic patients. The TTR mutation p.Val30Met is the most common one and only this mutation has been reported in large family cases, so far.
We report on 45 TTR-FAP patients from 26 Bulgarian families. The restrictive cardiomyopathy and the progressive polyneuropathy are the most typical clinical findings.
We found endemic region in the south-western part of the country, where 80.7% of the patients carry the mutation c.325G>C; p.Glu89Gln, while the worldwide most common mutation p.Val30Met was detected only once. Interestingly, one patient from this region was compound heterozygous for the mutations p.Val30Met and p.Glu89Gln. The clinical symptoms are typical, although the age of first symptoms is five years earlier in comparison to the average age. On the other hand, one patient was a 62 years old carrier of the mutation p.Glu89Gln without any clinical history.
In summary, FAP encompasses complex phenotype with marked variability within a single family. The screening for mutations in the TTR gene should not be restricted to common mutations, as a second mutation might exist. The detected mutations in pre-symptomatic cases, even well-known ones,

have to be interpreted with caution in respect to their pathological income.

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P12.110
Mutational analysis of *SERPING1* gene in Slovenian patients with hereditary angioedema: four novel mutations

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Hereditary angioedema (HAE) is a rare autosomal dominant disease characterised by the swellings of the face, lips, tongue, larynx, genitalia or extremities, with abdominal pain caused by intra-abdominal edema. HAE is caused by mutations affecting C1 inhibitor gene, also called *SERPING1*, resulting in low levels of C1 inhibitor (Type I HAE) or by normal levels of ineffective C1 inhibitor (Type II HAE).

We recruited 16 individuals with HAE from 7 unrelated Slovenian families. The diagnosis of HAE was established in the presence clinical and laboratory criteria (low C1 inhibitor antigenic levels and/or function), followed up with positive family history. Genetic studies were carried out by PCR and sequencing for the detection of *SERPING1* mutations, in promoter, noncoding exon 1 and in the 7 coding exons and exon-intron boundaries.

In all patients with HAE a mutation responsible for the disease has been identified. Four mutations were reported for the first time. In HAE type I families one already reported substitution (Gln67Stop, c.265C>T), together with four novel mutations have been identified. The new mutations included two missense substitutions, Ser128Phe (c.449C>T) and Glu429Lys (c.1351G>A), together with two frameshift mutations, indel (c.49G>TT; c.49delGinsTT) and deletion (c.593_594delCT). Both families with HAE type II harboured the two well-known substitutions affecting the arginyl residue at the reactive centre in exon 8, Arg444Cys, c.1396C>T and Arg444His, c.1397G>A, respectively.

Our study identified four novel mutations in the Slovenian HAE population, highlighting the heterogeneity of mutations in the *SERPING1* gene causing C1 inhibitor deficiency and HAE.

P12.111
The added value of targeted next generation sequencing in patients with cardiomyopathies. Substituting Sanger sequencing as a diagnostic test.

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Whole exome sequencing (ES) of a significant number of samples from a wide variety of diseases has become common practice for identifying putative disease linked variants. The advantage of this approach is the possibility of analyzing a large amount of genes in parallel. However, substituting Sanger sequencing with ES for mutation detection in daily diagnostics is yet not applicable due to dramatic differences in coverage within ES experiments, resulting in missing clinically relevant mutations. Targeted enrichment will circumvent this shortcoming by selecting only those genes involved in a particular disease. We developed two kits, based on Illumina's TruSeq™ Custom Enrichment and Agilent Sure Select Target Enrichment, for mutation detection in 48 genes that were proven to be involved in hereditary cardiomyopathies. Eighteen patients were analysed applying both kits and Sanger sequencing for up to six genes. An additional 18 patients were screened using the Agilent Target Enrichment kit only. Sample preparations were performed according to manufacturers protocols. Samples were multiplexed to an amount still permitting a theoretical coverage of 100 reads per targeted sequence/per patient. All samples were sequenced using 151bp-paired end reads on a Illumina MiSeq sequencer and analysed using the MiSeq Reporter pipeline. All (pathogenic) mutations previously detected with Sanger sequencing were identified. In addition, 103 novel putative pathogenic mutations (19 synonymous, 10 splice site and 74 missense) were found, on average three per patient. *In silico* analyses, confirmation by Sanger sequencing and co-segregation analyses are currently performed to identify the causal mutation in each patient/family.

P12.112
Novel mutation in the ENG gene in Russian patients with Hereditary Hemorrhagic Telangiectasia.

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Hereditary hemorrhagic telangiectasia type 1 (HHT 1) or Osler-Weber-Rendu disease (OMIM 187300) is an autosomal dominant disorder. HHT 1 characterized by recurrent epistaxis, telangiectasia, multi-systemic vascular dysplasia and clinical presentation of wide variation. Molecular-genetic analyses of HHT 1 have identified gene ENG on chromosome 9. This gene encoded protein endoglin, which is expressed predominantly on endothelial cells as a heavily glycosylated disulfide-linked dimer that binds TGF-β1 and TGF -β3.

Here, we report genetic analyses of one Russian family with HHT 1 diagnosed by clinical criteria. The proband is a boy 2,5 year old. He was diagnosed as HHT 1 with recurrent epistaxis and arteriovenous malformation (AVM) in the left lung. The history of HHT 1 was found also in his relatives: recurrent epistaxis, arteriovenous malformation in spleen, migraine headache in mother and spontaneous, recurrent epistaxis, telangiectases in maternal grandmother.

Amplicons of 1, 3, 7, 9, 12 exons and introns of ENG gene were directly sequenced. We found a heterozygous single nucleotide (G) deletion in the splice donor site of intron 7 (c.991+1delG or IVS 7+1 del G) that converted the 5' end of intron 7 from GT to TG. This deletion would lead to frame shift and produce unstable mRNA. Found mutation is a novel. Genetic testing of this family confirmed clinical diagnosis in individuals and provided for early detection of AVMs and helps us to prevent the complications of HHT 1 disease in our proband.

P12.113
The mutational spectrum of GDAP 1 gene in hereditary motor and sensory neuropathy patients from Bashkortostan Republic (Russia)

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Hereditary motor and sensory neuropathy (HMSN) comprises a group of clinically and genetically heterogeneous disorders of the peripheral nervous system. We examined HMSN patients from Bashkortostan Republic (BR) and detected spectrum of specific mutations in the GDAP1 gene using direct sequencing of its coding regions. The GDAP1 gene (8q21.11) codes ganglioside-induced differentiation-associated protein 1 - an important factor in the fission of mitochondria. The GDAP1 gene mutations cause autosomal - recessive disease type 4A. Molecular-genetic investigation of HMSN in 165 unrelated families showed 6 different nucleotide changes in the GDAP1 gene. Two of them haven't been described previously: c.685G>A (p.Glu229Lys), c.934G>A (p.Ala312Thr), and were not detected among healthy family members and controls (n=100), and one previously reported mutation c.715C>T (p. Leu239Phe) wide spread among patients with HMSN 4A type. Its nucleotide changes are supposed to be disease causing mutations. Three revealed nucleotide changes appeared to be gene polymorphic variants: c.102G>A, c.507T>G and c.933G>A. All mutations were heterozygous and revealed in different patients. Taking into consideration an autosomal-recessive type of HMSN, confirmed by genealogical analysis, every patient should carry a second undefined mutation. Perhaps, mutations, which we haven't found, may be located in the GDAP1 promoter region. Thus, it is established that HMSN are caused by the GDAP1 mutations in 2% cases in BR. The received data will contribute to optimization of medical and genetic consulting of HMSN families in our region.

P12.114
Candidate gene responsible for a new clinical form of hereditary recurrent neuropathy

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Inherited peripheral neuropathies that are recurrent and from which affected individuals make full or partial degrees of recovery are unusual. The most prominent disorders that fall into this category are: (i) hereditary neuropathy with liability to pressure palsies (HNPP; MIM 162500) caused by mutations in the *PMP22* gene; (ii) hereditary neuralgic amyotrophy (HNA; MIM 162100) due to mutations in the *SEPT9* gene; and (iii) primary erythromelalgia (MIM 133020) caused by mutations in the *SCN9A* gene. We recruited a large three generation family with eight affected members and the inferred diagnosis of recurrent neuropathy with an autosomal dominant pattern of inheritance. The main clinical manifestations are recurrent episodes of nerve paresis, lumbo-sacral plexopathy, erythromelalgia and migratory sensory neuropathy. In order to characterize the molecular bases which underlie this neuropathy, we first analyzed the candidate genes/loci (*PMP22*, *SEPT9* and *SCN9A*) by Sanger sequencing and/or by segregation analysis. Our findings showed that none of these three genes are involved in the disease. By combining exome sequencing with previous genome-wide linkage analysis, a novel heterozygous mutation was detected in a gene located on chromosome 17, which has not been associated with any neuropathy yet. This mutation co-segregates with the disease and was not observed in 132 unaffected individuals of matched geographical ancestry. We are currently investigating the pathogenicity of this mutation by cellular studies. *This work was supported by the Instituto de Salud Carlos III (Grants number CP08/00053 and PS09/00095) co-funded with FEDER funds.*

P12.115

Molecular testing for hereditary spastic paraplegia type 4 (SPG4) in a group of Polish patients - preliminary results

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BACKGROUND: Mutations in the SPAST gene are responsible for spastic paraplegia type 4 (SPG4), the most common among heterogeneous group termed hereditary spastic paraplegias (HSP).

MATERIAL AND METHODS: A group of 160 patients clinically diagnosed as HSP (90 familial, 70 sporadic cases) were screened for mutations in the SPAST gene. Molecular analysis was performed using multiplex ligation-dependent probe amplification (MLPA) and direct sequencing analysis.

RESULTS: Screening of 160 patients for mutations in the SPAST gene by MLPA enabled us identification of microrearrangements in 12 subjects (7.5%). Among those 11 deletions (9 multiexonic and 2 single exon deletion) and 1 duplication of two exons were found. Sequencing of the SPAST gene, which contains 17 exons, performed so far for 13 exons in 104 patients, revealed 11 different mutations in 12 individuals. Frameshift mutations (c.1215_1219delTATAA, c.1246_1247insG, c.1317delT, c.1418_1431delAGTCTGCTGGAGAT, c.1435_1436delAG, c.1779_1780insA), splice site change (c.1729-2A>G) and missense mutations (c.1079T>C, c.1100T>C, c1378C>T, c.1849T>G) were identified. Out of 11 point mutations 10 are localized in the AAA domain of spastin and one missense mutation in the last TAA STOP codon switched to coding for glutamic acid. Furthermore 5 intronic substitutions and 1 known synonymous variant in exon 6 were detected.

CONCLUSIONS: So far molecular analysis in 160 patients revealed 24 cases of SPG4 (15%). The study results confirm that the majority of point mutations as well as microrearrangements in the SPAST gene are localized in the AAA domain of the spastin protein (19/24 cases).

P12.116

Mutation analysis of SPG4 gene in Bulgarian patients with hereditary spastic paraplegia

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Background: Hereditary spastic paraplegias (HSP) are a group of clinically and genetically heterogeneous neurodegenerative disorders which main features are progressive spasticity and weakness of lower limbs. HSPs are characterized by degeneration of the longest axons in the central nervous system. Prevalence in European population is 3-10 cases per 100 000. All types of inheritance are described in this disorder - autosomal dominant (AD), autosomal recessive (AR) and X-linked. HSP is classified in two big clinical groups - pure and complicated. In about 35% of AD cases with pure form are detected mutations in SPG4 (spastin) gene, but can be found in sporadic cases as a result of de novo mutation.

Materials and methods: We performed a genetic screening for SPG4 mutations by direct sequencing in a cohort of 50 AD and sporadic HSP cases from three different ethnic groups (Bulgarian, Gypsy/Roma and Turkish).

Results and discussion: In this study we identified 9 mutations in SPG4 gene, 4 novel (1 splice site mutation, 2 missence and 1 deletion) and 5 already reported (3 missence, 1 nonsense and 1 deletion). In 6 cases the mutations are found in patients with AD type of inheritance. A novel finding for Bulgarian population are mutations in four sporadic cases confirming the need for SPG4 screening of this HSP group. No mutations were found in Turkish patients.

Conclusions: Our findings contribute to a better understanding the molecular basis of HSP and have implications for diagnostic testing and genetic counseling in Bulgarian population.

P12.117

Studying the molecular basis of hereditary spastic paraplegia in Gypsy/Roma patients from Bulgaria

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Hereditary spastic paraplegias (HSP) are a group of rare heterogeneous neurodegenerative disorders, characterized by progressive spasticity and weakness of lower limbs. All modes of inheritance have been described in HSP - autosomal dominant (AD), autosomal recessive (AR) and X-linked recessive. In order to better tackle the genetic heterogeneity of HSP, we are studying Bulgarian Gypsy/Roma families with this disorder. Gypsies share unique features as an isolated population, like high degree of inbreeding, decreased genetic heterogeneity and higher incidence of rare recessive disorders. So far we have collected 44 Gypsy patients belonging to 24 families (4 AD, 14 AR and 6 sporadic).

The dominant and sporadic families were screened for mutations in the spastin (SPG4) gene, the most common cause of AD-HSP. We identified a novel splice site mutation in one patient. The most frequent AR-HSP gene, paraplegin (SPG7), was screened for the recessive families. In only one of all the tested patients was detected a nonsense mutation.

Two consanguineous recessive families were subjected to genome-wide SNP genotyping followed by homozygosity mapping. In the first pedigree with four affected sibs we identified 4 autozygous regions which contain two known HSP genes, AP4E1 and spartin (SPG20), and their sequencing is ongoing. The homozygosity mapping in the other family with three affected sibs identified only four large homozygous regions that do not contain any known HSP gene, suggesting a novel genetic entity. Exome sequencing is currently underway to indentify the underlying genetic defect.

P12.118

Seven novel genetic mutations within the 5'utr and the housekeeping promoter of HMBS gene responsible for the non-erythroid form of acute intermittent porphyria.

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Acute intermittent porphyria (AIP) is an autosomal dominant disorder caused by molecular abnormalities in the *HMBS* gene. This gene is transcribed from two promoters to produce ubiquitous and erythroid specific isoforms of porphobilinogen deaminase (PBGD). In the classical form of AIP, both isoforms are deficient, but about 5% of families have the non-erythroid variant in which only the ubiquitous isoform is affected. Only one mutation sited in the housekeeping promoter have been previously reported as causative for this form of AIP. In this study we identified one small deletion and six nucleotide substitutions within the 5'UTR and the housekeeping promoter of *HMBS* gene: c.1-440_-427del14bp; c.1-421G>A; c.1-331C>T; c.1-270G>A; c.1-122T>A; c.1-103C>T; c.1-28A>C.

Using luciferase reporter assays and quantitative PCR experiments we characterized the functional role of these seven novel genetic variants demonstrating that all mutations cause a significant loss of transcriptional activity. Our investigations suggest that these nucleotide substitutions may alter critical binding sites for transcriptional factors and they confirm that these regions represent an important molecular target for pathogenesis of non-erythroid form of Acute Intermittent Porphyria.

P12.120

Abnormal Responses to Visual Cortex Activation in Early Stage Huntington Disease Patients using 31P-Nuclear Magnetic Resonance Spectroscopy

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Objective: To test the hypothesis that brain energy metabolism is abnormal

in patients at an early stage of Huntington disease (HD).
Background: Energy metabolism has been a major focus of HD research for many years due to several observations in both patients and models of the disease. However, there are currently no in vivo biomarkers of brain energy metabolism in HD.
Methods: We coupled noninvasive 31P-NMR spectroscopy with activation of the occipital cortex in order to measure the levels of ATP, phosphocreatine (PCr) and inorganic phosphate (Pi) before, during and after a visual stimulus. We studied 15 HD patients at an early stage of the disease (mean motor UHDRS= 18 ±9) and 15 age- and sex-matched controls.
Results: In controls, we observed an 11% increase in Pi/PCr ratio (p=0.024) and a 13% increase in Pi/ATP ratio (p=0.016) during brain activation, reflecting increased ATP synthesis and ADP levels. Subsequently, controls had a return to baseline levels during recovery (p=0.012 et 0.022 respectively). In HD patients, both Pi/PCr and Pi/ATP ratios were unchanged during and after visual stimulation, reflecting altered mitochondrial bioenergetics. In addition, in HD patients the ratio of Pi/ATP correlated with the UHDRS score during the activation (p=0.014) and recovery periods (p=0.009), while Pi/PCr ratio correlated with the UHDRS score during recovery (p=0.016), reflecting a correlation between brain energy metabolism and disease severity in HD.
Conclusions: 31Phosphorus nuclear magnetic resonance spectroscopy could provide functional biomarkers of brain energy deficit to monitor therapeutic efficacy in Huntington disease.

P12.121

Translation of HTT mRNA with pathogenic CAG repeats is regulated by the ubiquitin ligase MID1 and the translation modulators PP2A and mTOR

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Expansion of CAG repeats is a common feature of neurodegenerative disorders like Huntington's disease. We show here that expanded CAG repeats bind to a translation regulatory protein complex that contains MID1, PP2A, and the translation factor S6K. Binding of the MID1-PP2A protein complex increases with repeat size and leads to a stimulation of the translation of the CAG repeat expansion containing mRNA in a MID1, PP2A and mTOR dependent manner. Our data indicate that pathological CAG-repeat expansions upregulate translation leading to an overproduction of aberrant protein and suggest the MID1-complex as a promising therapeutic target for CAG repeat expansion disorders.

P12.122

Pelizaeus-Merzbacher-like disease caused by a homozygous mutation in AIMP1/P43

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Pelizaeus-Merzbacher-Like disease (PMLD) is a hypomyelinating leukodystrophy, a disorder involving aberrant myelin formation presenting with rotatory nystagmus, progressive spastic paraplegia, severe motor impairment and neurological deterioration within the first months of life. The known forms of the disease are caused by homozygous mutations in GJA12 and HSPD1.
Two remotely related Bedouin kindred in southern Israel presented with an autosomal recessive phenotype of PMLD. Homozygosity at the two known loci was ruled out in affected individuals. DNA samples of 5 affected individuals and 7 non-affected obligatory carrier first-degree relatives were analyzed using 250k SNP Affymetrix arrays, and fine mapping was done using microsatellite markers. The phenotype-associated locus was mapped to a 8.94 Mb region on chromosome 4q24 (maximum multipoint LOD score of 4.25). Sequence analysis of 14 candidate genes of the 39 genes in the region unraveled a two-nucleotide (CA) deletion mutation in AIMP1/P43 encoding ARS-Interacting Multifunctional Protein 1. AIMP1 functions as a non-catalytic component of the multi-synthetase complex, catalyzing the ligation of amino acids to their cognate tRNAs. The deletion causes a frameshift mutation resulting in a premature stop codon amputating the 312aa protein after 127 aa, abrogating AIMP1/P43's main catalytic domain .The mutation was not found in 200 control chromosomes.

P12.123

Identification of an Alu-mediated 12.2 kb deletion of the complete LPAR6 (P2RY5) gene in a Turkish family with hypotrichosis and woolly hair

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Hypotrichosis is a rare form of progressive hair loss characterized by sparse and occasionally woolly hair that is curly and breaks easily. Disease causing mutations in LIPH, LPAR6 and KRT74 have recently been identified. We describe a four generation pedigree from Turkey following an autosomal recessive pattern, in which the four affected members had hypotrichosis and woolly hair. By sequencing LPAR6 and the use of SNP-arrays we revealed a homozygous loss of the entire LPAR6 gene in the affecteds. We hypothesize that the 12 kb deletion resulted from illegitimate recombination secondary to slip-replication. The orientation of three Alu repeats around LPAR6 may have provoked the formation of a 'triple barrel' structure during replication, thereby allowing strand slipping. This first report of complete LPAR6 loss expands the spectrum of known LPAR6 mutations, and suggests a novel mechanism for this gene and for the formation of DNA rearrangements in general.

P12.124

A single amino acid residue deletion, p.Leu3230del, in the brain-specific isoform Dp71 of dystrophin results in intellectual disability without muscular dystrophy

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We have identified a single amino acid residue deletion, p.Leu3230del, in the brain-specific isoform Dp71 of dystrophin in a family with nonspecific X-linked intellectual disability by sequencing of all exons of the X chromosome. Linkage analysis supported causality as the mutation was present in the 7.6 cM linkage interval on Xp22.11-Xp21.1 with a maximum positive LOD score of 2.41. Molecular modeling predicts that the p.Leu3230del deletion results in the destabilization of the C-terminal domain of dystrophin and hence reduces the ability to interact with β-dystroglycan. Subsequent determination of the creatine kinase levels in blood of the index patient indeed showed a mild but significant elevation in serum creatine kinase, which points to impaired dystrophin function. Taken together, these data indicate that we have identified the first *DMD* mutation in Dp71 that results in intellectual disability without muscular dystrophy.

P12.125

Unravelling the genetic causes of syndromic Intellectual Disability in the era of exome sequencing.

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Intellectual Disability (ID) represents a large and heterogeneous group of disorders with variable phenotypes and severity, and impaired intellectual abilities as a common feature. Although the number of ID causing genes is increasing rapidly, at present the genetic aetiology remains unexplained for 60% of the ID forms, thus no molecular diagnosis can be made for the majority of the patients. With the aim to identify new genetic causes of ID, we used the exome sequencing approach and focused on syndromic autosomal recessive forms. So far we selected 4 families of Sardinian origin with at least 2 patients affected by Multiple Congenital Anomalies and Intellectual Disability (MCA/ID) associated into a clinically undefined syndrome, and 1 family with 1 patient affected by Filippi syndrome with unknown genetic aetiology. Up to date, 6 samples from 2 families were sequenced with the TruSeq Exome Enrichment Kit (Illumina). On average, 140,000 variants (SNVs and indels) were identified, of which 7% were novel (not yet included in dbSNP 132). Among the novel variants, on average 980 fall in exons and 1,452 in UTR regions, and we are currently searching the potential causative variants related to the diseases by applying a standard step-wise filtering approach. Our study will contribute to the molecular diagnosis of a larger number of subjects by improving the existing map and networks of ID causative genes, and likely to the detection of new syndromes through the so-called “reverse dysmorphology”, ie, using an approach from genotype to phenotype.

P12.126

Pitfalls of whole exome-sequencing: hidden *DYNC2H1* mutations in patients with Jeune Asphyxiating Thoracic Dystrophy

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In recent years whole-exome sequencing has been developed, a technique by which all exons of the genome (all the protein-coding DNA) can be sequenced at once. Here we show that whole-exome sequencing, using either 35 or 50 Mb Agilent kits for exome capture, was insufficient to detect pathogenic *DYNC2H1* variants in patients with Asphyxiating thoracic dystrophy (ATD; Jeune syndrome). Jeune syndrome is a rare inherited ciliopathy involving chondrodysplasia characterized by shortened ribs and long bones, and polydactyly, progressive kidney and liver disease as well as retinitis pigmentosa. Reduced thoracic capacity causes approximately 60% early lethality. *DYNC2H1* encodes a subunit of the dynein 1B motor that drives tip-to-base ciliary intraflagellar transport, and mutations have previously been associated both with embryonically lethal short rib-polydactyly and the milder, but overlapping Jeune aspyxiating thoracic dystrophy. Although the *DYNC2H1* gene was targeted in our whole-exome experiments many sequence reads were not properly aligned, resulting in 30-70% of the gene not being covered. Only a combination of whole-exome sequencing and a candidate gene approach (i.e. analysis of non-covered *DYNC2H1* exons using Sanger sequencing) enabled us to detect the missing *DYNC2H1* mutations. Whole-exome data analysis of the 90 exon *DYNC2H1* gene is therefore comparable to playing ‘hide and seek’, whereby certain mutations are easier to find than others according to their relative coverage. In conclusion, although whole-exome sequencing has revolutionized the field of human genetics, our findings emphasize that next-generation sequencing also presents significant challenges for gene identification and for implementation of this technique in DNA diagnostics.

P12.127

Molecular analysis of Polish patients with junctional epidermolysis bullosa

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Junctional epidermolysis bullosa (JEB) is a rare, autosomal recessive genodermatosis characterized by spontaneous or mechanically induced blisters formation at the level of lamina lucida of the epidermis-dermis junction. Clinical outcome of the disease varies from generalized, lethal Herlitz subtype, to milder, sometimes localized- non Herlitz JEB.

JEB is mostly caused by mutations in *LAMB3, LAMC2, LAMA3* encoding laminin332 and *COL17A1* encoding collagen type 17 - extrcellular proteins linking hemidesmosomes to extracellular matrix in epidermis-dermis junction.

According to literature data, mutations in *LAMB3* are most frequently found worldwide in JEB patients and some recurrent mutations were identified in this gene in different populations.

The aim of the study was to investigate the mutation spectrum in Polish JEB patients.

11 patients with clinical features of Herlitz-JEB (3 cases) or non-Herlitz JEB (7 patients), and a couple with history of 3 children demised of Herlitz-JEB were enrolled to the study. DNA analysis was performed using direct sequencing.

We established full genotype in 6 cases. The other patients are still under analysis. In 5/6 cases we detected mutations in *LAMB3* gene. 40% of mutated alleles accounted for R635X, in 30% of them we found c.1439_1443del and in 20% novel c.965_966+8del. Our preliminary results indicate that R635X is the commonest mutation in Polish JEB patients, as it is in general population, and that at least 2 other mutations - uncommon in other European countries- are recurrent in Poland. This finding has a practical impact while preparing an algorithm of molecular analysis for Polish JEB patients.

P12.128

Incomplete penetrance of a novel *KCNQ1* mutation in a large family with long QT syndrome

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Congenital long QT syndrome (LQTS) is an inherited potentially fatal arrhythmogenic disorder that is characterized by prolonged corrected QT (QTc) interval. Mutations in 3 genes (*KCNQ1*, *KCNH2*, *SCN5A*) account for the majority of the cases. However, 10 other genes are now known to be implicated in LQTS. In this work, we describe the clinical and molecular analysis in a large family with LQTS. Screening *KCNQ1*, *KCNH2*, *SCN5A* genes in the proband, who presented with episodes of syncope led to the identification of a novel heterozygous mutation (c.773 A> C; p.H258P) in *KCNQ1*. An extended clinical and genetic screening of the family identified 11 other members who were carriers for this mutation. All identified carriers had prolonged QTc intervals, yet, only two of them were clinically symptomatic. Nevertheless, the electrocardiographic and molecular analysis stratified seven carriers at high-risk of a cardiac event as they had a QTc of ≥ 500 ms and were carriers of a *KCNQ1* mutation. Our work illustrates the importance of extended family screening in LQTS to identify silent carriers and hence adopt the most appropriate therapeutic and preventive intervention.

P12.130

Inherited cystic kidney disease: a molecular screening in Italian patients

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Inherited kidney diseases are a heterogeneous cause of renal failure with great clinical variability. Once ultrasound and urinalysis have excluded polycystic kidney and glomerulonephritis, family history can help to distinguish nephronophthisis (recessive) from dominant nephropathies. Clinical features can also help to define the specific diagnosis, but this is still a demanding task due to phenotypic overlap.

Purpose of this study was to perform a molecular screening of some genes associated with these disorders, in order to validate a diagnostic algorithm related to clinical phenotypes, useful to drive the genetic screening. *NPHP1*, *NPHP5*, *UMOD*, *REN* and *HNF1B* genes were selected for the analysis. DNAs from 12 Italian patients with inherited nephropathies were submitted to direct sequencing. Furthermore, deletion analysis by multiplex PCR for *NPHP1* and MLPA for *HNF1B* were performed.

Three causative mutations were detected: 1) a novel heterozygous p.E48K variant in *REN* found in a patient with cystic nephropathy, hyperuricemia, hyperkalemia and anemia. The mutation lies in a conserved position, cosegregated with affected family members and was absent in 50 chromosomes; 2) a homozygous p.R489X mutation in *NPHP5* gene was detected in a patient with retinitis pigmentosa and recurrent cholangitis, confirming the diagnosis of Senior-Locken Syndrome. This mutation was previously reported in a Pakistani family; 3) a heterozygous pR295C variant in *HNF1B* was identified in a patient with renal failure and diabetes mellitus. This mutation is known to alter the DNA binding domain and was not previously reported in Italy. These preliminary results are promising for defining a diagnostic algorithm.

P12.131
A mutation detected by exome sequencing and phenotypic variability in a family with Lenz microphthalmia syndrome

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Lenz microphthalmia syndrome was first described as a type of X-linked microphthalmia in 1955. It is known to exhibit genetic heterogeneity and two loci, Xq27-q28 and Xp11.4, have been mapped to be associated with the syndrome.

We met a large family with syndromic microphthalmia. Bilateral but asymmetric microphthalmos and mental retardation were observed in all the patients and cardiovascular malformations or renal abnormalities were observed in some patients, showing phenotypic variability. All patients were male and the pedigree indicated X-linked recessive inheritance. According to their clinical findings and the form of inheritance, patients were diagnosed with Lenz microphthalmia syndrome.

Whole exome sequencing was performed by using a next-generation sequencer and TruSeq Exome Enrichment system (illumina) to identify a mutation in the family. Pooled DNA with four affected males in the family was used for one exome analysis to enrich hemizygous variations in the patients.

Of 552 called SNPs or indels on X chromosome, 51 were novel (not registered in the SNP131). Four hemizygous (not detected as heterozygous) variations were found in exons. After comparison of exome data between affected and unaffected males, one substitution, c.C254T, was identified and was confirmed by direct Sanger sequencing in all the patients. In addition, we confirmed heterozygous mutation in all female carriers as well.

We concluded that the mutation was responsible for the patients.

P12.132
Three novel mutations in Leptin and Leptin Receptor genes among 3 Egyptian families

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Background: Congenital leptin deficiency and congenital leptin receptor deficiency are a rare recessive genetic disorder resulting in severe hyperphagia and early onset obesity. It is caused by mutations in the LEP gene encoding leptin and mutations in the congenital leptin receptor gene respectively.

Objective: We report 6 patients from 3 Egyptian families presenting with severe hyperphagia and early onset obesity.

Method: Genomic DNA was extracted from peripheral blood leukocytes of all patients and their family members using a standard method. Direct sequencing of the whole coding region of the leptin gene was carried out in the two families with undetectable serum leptin levels while sequence analysis of the LEPR gene was performed in the third family with high serum leptin levels.

Results: We detect one novel missense mutation in the leptin LEP gene (N103K) and another novel nonsense mutation in the leptin LEP gene (W121X) as well as a missense mutation in the Leptin receptor gene LEPR (P316T).

Conclusion: Although genetic causes of obesity are rare autosomal recessive disorders, high consanguinity rate in our society will lead to the discovery of a high number of monogenic obesity.

P12.134
Transcriptional dysregulation in Hutchinson-Gilford progeria syndrome patients compared to age-matched controls

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Hutchinson-Gilford progeria syndrome (HGPS) is a rare genetic condition with symptoms of premature aging manifested at a very early age. Patients born with progeria typically live to their mid teens or early twenties and the most frequent cause of death is myocardial infarction or stroke. HGPS is usually caused by dominant mutations in the lamin A (LMNA) gene. There are 22 known LMNA mutations associated with the development of HGPS that have been published in the literature to date. These mutations can be detected by the classical or next generation sequencing, high resolution melting analysis (HRMA) and denaturing high-performance liquid chromatography. At the subcellular microscopic level, the disease is manifested by morphological abnormalities in nuclear envelope structure. Within the framework of our ongoing effort to integrate technologies (and their results) used to gain the information about genomic mutations (classical or next-gen sequencing, scanning or unlabelled-probe HRMA), gene expression (qPCR, microarray analysis, next-gen sequencing) and its regulation (ChIP-chip, ChIP-qPCR, ChIP-seq) in HGPS patients which could be further used to improve diagnosis, prognosis, treatment and the overall quality of life of the HGPS patients we conducted a pilot experiment on gene expression in fibroblast cell lines from four HGPS patients and four age-matched controls. Our reliminary data on this small group of target and control samples showed that the most affected biological processes are transcription, signal transduction, development and signal transduction. This work was supported by the grant FR-TI3-588 from The Ministry of Industry and Trade of the Czech Republic.

P12.135
Genome-wide SNP analysis to identify genetic modifiers for long QT syndrome in a consanguineous population

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Congenital long QT syndrome (LQTS) is an inherited potentially fatal arrhythmogenic disorder that is characterized by variable expressivity and incomplete penetrance. Common variants in *KCNQ1*, *KCNE1*, and *NOSA1P* genes have been recently implicated in modifying the risk of life-threatening arrhythmias in LQTS. In our highly consanguineous population, we conducted genome-wide SNP analysis in 15 families with LQTS to identify regions of homozygosity (ROH) that harbor loci known to be associated with either LQTS or risk of sudden cardiac death. In two families with previously known homozygous *KCNQ1* (LQT1) mutations, ROH encompassing *AKAP9* (LQT11) and *SNTA1* (LQT12) that were detected. In a third family, a ROH that harbors *ADRB2* gene, known to be associated with SCD without LQTS, was identified. In the context of consanguinity, our work illustrates the value of homozygosity analysis for detecting genetic modifiers in LQTS. In addition, our approach could also be adopted to detect the presence of digenic inheritance in consanguineous families with LQTS.

P12.136
Towards a better prediction of the age at onset in Spinocerebellar ataxia type 3

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Spinocerebellar ataxia type 3 (SCA3) or Machado-Joseph disease (MJD) is an autosomal-dominantly inherited, neurodegenerative disorder caused by the expansion of a CAG repeat in the *MJD1 gene*.

Statistically, a correlation between the number of CAG repeats and the age at onset of SCA3 patients exists and patients with more CAG repeats have an earlier onset of symptoms. However, this statistical correlation is not perfect and the number of CAG repeats contributes only about 55 % to the age at onset. Therefore, the remaining 45 % are influenced by other factors, which we aim to identify in this study. Aside from the CAG repeat itself, the *MJD1 gene* contains several polymorphisms within the coding region which lead to amino acid changes or even a premature stop in the encoded ataxin-3 protein.

Here, we assume that the amino acid changes within ataxin-3 resulting from these polymorphisms influence the function of normal and expanded ataxin-3 and/or its interaction with other proteins and therefore modify the age at onset, the pathogenesis and disease progression of SCA3 patients. We, therefore, genotyped more than 500 samples of SCA3 patients for these polymorphisms and generated haplotypes comprising the CAG repeat length and the polymorphisms located downstream. Two haplotypes turned out to be most common among SCA3 patients and additional haplotypes have a possible impact on the age at onset in SCA3. We hope that our results will improve the prediction of clinical symptoms and contribute to the understanding of pathogenic processes in SCA3.

P12.137
Applying Next Generation Sequencing to Molecular Diagnosis of Marfan and Loeys-Dietz Syndromes

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The Marfan syndrome (MFS) and the phenotypically related Loeys-Dietz syndrome (LDS) are caused by mutations in the genes coding for Fibrillin-1 (FBN1), Transforming Growth Factor Beta Receptor 1 and 2 (TGFBR1 and TGFBR2) and Sma- and Mad-related Protein 3 (SMAD3). Molecular diagnostics of thes two syndromes by PCR sequencing using the Sanger method is time-consuming and expensive, especially for the FBN1 gene with its 65 exons.

As an alternative, we started sequence analysis of these genes using a multiplex PCR approach followed by Next Generation Sequencing (NGS) on the Roche GS Junior System. Plexes were designed in silico considering amplicon length and avoiding potential primer dimer formations using the online tool http://biocompute.bmi.ac.cn/MPprimer/primer_dimer.html. Amplicons that comprise the up to now not extensively studied regulatory regions in the promoter and 5'-UTR areas of the four genes were also included. To distinguish and resolve correct amplification of all multiplex PCR products before NGS analysis, we performed PCR with Cy5-tagged M13 primers and subsequent detection by fragment analysis in a capillary sequencer.

Up to now, we studied genomic DNA samples from 11 patients with previously identified mutations and/or polymorphisms in FBN1, TGFBR1, TGFBR2 and SMAD3 and all known variants were correctly identified. Analyses of samples of 30 patients suspected of having MFS/LDS that have not yet been investigated for mutations in TGFBR1, TGFBR2 and SMAD3 are in progress. Mutation screening using this combined multiplex PCR-NGS approach is suitable to make diagnostics faster and less expensive.

P12.138

A recurrent ALU repeat-mediated deletion within the NFIX gene accounts for a missing part in Marshall-Smith syndrome

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Marshall-Smith syndrome (MSS) is a recognizable entity characterized by moderate to severe developmental delay, skeletal abnormalities, upper airway obstruction, and distinctive facial features. Mutations in the gene NFIX were recently discovered as the cause of MSS (Malan et al. AJHG 2010). In six patients exhibiting the typical phenotype, we identified four novel NFIX frameshift and splice-site mutations by direct sequencing, while two individuals turned out normal. For further evaluation, we set up an MLPA-based screening for exon deletions or duplications. Both patients were found to carry a heterozygous deletion of exons 6 and 7 of the NFIX gene. The same deletion was found in three additional cases of a cohort of 15 MSS patients, of which 9 were previously found to have NFIX point mutations. Breakpoint sequencing revealed the deletion to be mediated by a recombination event between ALU-Y repeats located in introns 5 and 7. Further studies on the mRNA level indicated that the transcript lacking exons 6 and 7 escapes nonsense-mediated mRNA decay, thus suggesting that the deletion leads to the expression of a mutant protein rather than haploinsufficiency of NFIX. We conclude that the recurrent NFIX deletion is specific for MSS, because it mimics the effects of other MSS-associated mutations that are thought to generate mutant proteins able to exert a dominant-negative effect over the wild-type allele. Intronic ALU repeats create predetermined breaking points facilitating *de novo* occurrence of this deletion, a mechanism that accounts for about one quarter of MSS cases in our joint cohort.

P12.139

Accumulation of nonsyndromic hearing loss associated with *Marveld2* gene mutation in Slovak and Hungarian Roma patients

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The wide group of nonsyndromic hearing loss disorders (NSHL) is one of the most common sensory impairment in humans. Approximately 50% of all cases are caused by genetic factors. Among all types of monogenic, Mendelian traits, autosomal recessive form stands for nearly 80% of all NSHL cases. The group of hearing disorders is characterized by tremendously high locus and allele heterogeneity with over hundred genes associated to these disorders. Most frequently analyzed genes in NSHL patients worldwide are *GJB2, GJB6, MYO7A, MYO15A, SLC26A4,* and *TMPRSS3*. In respect to the patient's population origin, mutations in otherwise scarce genes may occur in higher frequencies in some specific populations. Demographic history and population structure of Roma in Europe resulted in occurrence of such population specific mutations which are otherwise very rare or unseen in autochthonous populations in Europe. Studying a large Hungarian family of Roma origin with multiple NSHL patients we identified a founder mutation, IVS4+2T>C of the *MARVELD2* gene, previously identified in Pakistani patients. To test the prevalence of the identified mutation analyzed 167 Hungarian and 300 Slovak NSHL patients regardless the ethnic origin. Random population sample biobanked from unrelated healthy 502 Hungarian and 300 Slovak Roma individuals were also tested. Heterozygous presence of IVS4+2T-C mutation among healthy, control Roma individuals proved the population specific character of this mutation in Hungary and Slovakia. The common origin of the surveyed mutation identified in Hungarian and Slovak Gypsy patients was further analyzed using set of SNP markers located adjacent to the *MARVELD2* gene locus.

P12.140

Screening of a large cohort with UMOD associated kidney disease (UAKD) for mutations in UMOD, HNF1beta and Renin

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A spectrum of slowly progressive autosomal dominant kidney disease characterized by hyperurecaemia, reduced Uromodulin excretion, renal cysts, and endstage renal failure between the third and seventh decade is subsumized under the term UMOD associated kidney disease (UAKD).

The UMOD gene encoding for Uromodulin was the first MCKD gene identified. Mutations often affect a cysteine residue and result in endoplasmatic reticulum retention and subsequent reduced urinary excretion. However the precise pathopyhsiology of tubulointerstitial damage is ill understood.

Next to UMOD, mutations in HNF1beta can result in a similar clinical presentation.

Recently, dominant Renin mutations were shown to result in a UAKD phenotype. Initially recessive mutations in Renin were identified as one cause of renal tubular dysgenesis.

Here we report the results of a stepwise mutational analysis in 71 families compatible with a diagnosis of UAKD.

UMOD mutations were identified in 25, while HNF1beta mutations could be identified in 7 families. On the remaining 39 families complete Renin analysis identified one kindred with a mutation in the signal sequence (p.W10R) affecting four generations. We report the youngest patient showing renal impairment as early as 11 months of age and provide further functional data on signal sequence mutations. Only 4 heterozygous REN mutations all within the signal peptide have been published. For the three previously described mutations, a damaged targeting and cotranslational translocation of preprorenin into the endoplasmatic reticulum had been shown.

This study illustrates that the genetic of cause still remains to be identified in the majority of patients suffering from UAKD.

P12.141

Cellular localization of MCPH1 isoforms and effects of MCPH1 mutations on G2/M checkpoint release

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Biallelic mutations in the human MCPH1 gene are the cause of primary microcephaly associated with a unique cellular phenotype marked by premature chromosome condensation (PCC syndrome) in G2 phase and delayed decondensation post mitosis. MCPH1 encodes a multifunctional protein that was reported to be involved in brain development, DNA damage response and regulation of chromosome condensation.

In previous work of our group, Gavvovidis et al. found that MCPH1 encodes two major transcripts, full-length MCPH1 (MCPH1-FL) and another transcript lacking the six 3' exons (MCPH1Δ9-14). In addition, a splice variant lacking exon 8 (MCPH1Δ8) was detected. Here we re-examined the cellular localization of those isoforms after centrosomal localization of full-length MCPH1 had been reported by several groups. By transfection of human cell lines (U2OS, HeLa) with FLAG-tagged constructs, we were unable to satisfactorily show any of the MCPH1 isoforms colocalizing with centrosomes. We conclude that such localization must be a temporary or cell type-specific phenomenon.

Furthermore, experiments using knockdown of MCPH1 by RNAi or MCPH1-deficient MEFs had previously shown severe effects on the cellular response to DNA damage after irradiation. Here we compared the effect of patient-derived MCPH1 mutations on G2/M checkpoint control. We determined the mitotic index by phospho-histone-H3 flow cytometry after irradiation of cells with 1 Gy. There was no difference in terms of G2/M checkpoint control compared to normal control cells. However, delayed checkpoint release was observed in all of the analyzed MCPH1-deficient cell lines, featuring the way in which DNA damage response to IR is defective.

P12.142

Phenotypical consequences of mild Mecp2 overexpression in the mouse as an experimental approach to estimate gene-dose effects

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Overexpression of MeCP2 as found in the MECP2 duplication syndrome has a detrimental effect in both human and mouse. Thus, any future therapies directed at increasing the levels of MeCP2 in the patient must be considered carefully to avoid further neurological impairments. To estimate gene dose effects and predict the level at which potential non-tolerable side effects might occur, mouse models with mild overexpression are instrumental. We generated Mecp2_WT_EGFP transgenic mouse, in which the total amount of MeCP2 (endogenous plus transgenic) is mildly overexpressed (~1.5X). When Mecp2_WT_EGFP mice were crossed with Mecp2 knockout (KO) mice, our preliminary analysis suggest that major phenotypes of the KO mice were rescued, however further investigation will be necessary to exclude any late stage symptoms. To characterize Mecp2_WT_EGFP mouse model, we performed an extensive test battery which, apart from increased aggressiveness and seizure propensity, revealed essentially unaltered behavior. Evaluation of neuronal parameters both ex vivo and in vivo revealed no major abnormalities. Also, expression analysis of differentially regulated Mecp2 target genes, such as Bdnf, Ddc, Gdf11, Gpr26, Lrp1b, Pygm and Robo1 by quantitative RT-PCR analysis in Mecp2_WT_EGFP mice revealed only minor alteration in the expression of Mecp2 target genes. In contrast, a transgenic mice overexpressing 2X Mecp2 has been reported earlier to have considerable effects on target gene expression. We conclude that quantitative RT-PCR analysis of Mecp2 target genes may be a suitable approach to evaluate in future the success of MeCP2 supplementary therapy.

P12.143

MEFV mutations in patients with Familial Mediterranean Fever from Antalya province, Turkey

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Familial Mediterranean Fever (FMF) is mainly autosomal recessive and the most frequent hereditary inflammatory disease characterized by fever, arthritis, serosal inflammation and amyloidosis. FMF affects different ethnic groups and countries including Turkey, connected with the MEFV gene mutations. In this study, we reviewed the data of 2283 FMF suspected patients (1281 female and 1002 male) from May 2003 to December 2011. Automated DNA sequencing was used to identify the mutations in exon 2 and exon 10 of MEFV gene. Of 2283 suspected patients, 1065 (46.64%) had MEFV gene mutation and 52,84% of them were female. Seventeen different mutations (M694V: 67%, E148Q: %14.8, V726A: 11.4, M680I: 9.27%, R761H: 2.19%, K695R: 1.91%, A744S: 1.41%, M694I: %1.2, K695M: 0.14%, E230K: 0.63%, E167D: 0.42%, T267I: 0.014%, and E225D, E251K, L110P, V653I, M680V with 0.07% frequency) were found. Of these mutations, 16 different heterozygote and 10 different homozygote genotype were detected. The most five common mutations were M694V, E148Q, V726A, M680I, and R761H in order. Although the allelic frequency of V726A (9.21%) was found lower than E148Q (14.8%), the frequency of M694V/V726A compound heterozygote was found as higher than M694V/E148Q.

In conclusion, we first time report the mutations of MEFV gene from Antalya province in Southern of Turkey, and found different mutation types and frequencies than previous reported studies in Turkey.

P12.144

SHANK3 Microdeletion in a patient suspected to Rubinstein-taybi syndrome

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Mental retardation (MR) is a heterogeneous disorder with genetic and non-genetic etiology affecting 1 to 3% of the general population. Here we describe a copy number variation in a patient with MR and multiple congenital anomalies (MCA).

A 24 years old man with a history of infantile hypotonia and childhood developmental delay was referred to our clinic. His dysmorphic feature includes short stature, heavy eyebrows, ptosis, speech difficulty and high arched palate. He had convulsion episode due to epilepsy from 5 years old.

Conventional cytogenetic study revealed no abnormal finding. Further investigation was performed using Multiplex Ligation Probe Amplification (MLPA) technique with P245 kit, P070 and P036 kits (SALSA, MLPA Kit, MRC, Holland) to screen microdeletion syndromes and subtelomeric rearrangements. MLPA results were analyzed by Genemaker V1.95.

Micro-deletion was detected in CREBBP gene (16p13.3) and SHANK gene (22q13.33) in P245-kit. A subtelomeric micro-deletion was detected in DECR2 gene in P070 kit.

Patient's phenotype could be explained by this genetic finding as CREBBP gene is a known cause of Rubinstein-Taybi Syndrome (RTS) in most of cases. Nearly 3% of RTS are related to the deletion in EP300 gene (22q113.2) which was not investigated here. However we detected SHANK3 gene deletion that is characterized by severe expressive-language delay and mild mental retardation.

This is the first report of deletion in SHANK3 gene in Rubinstein-Taybi Syndrome that could be confirmed and further characterized by other methods like FISH, array CGH or confirmatory MLPA kit.

P12.145

Primary microcephaly (MCPH): Expanding the phenotype

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Primary MCPH (MCPH) defines congenital microcephaly with an occipito-frontal circumference (OFC) two standard deviations (SD) or more below the age- and sex-related mean. It is genetically heterogeneous with at least seven known loci and genes (MCPH1 to 7), for which autosomal recessive inheritance of mutations has been shown in families. Primary MCPH was initially defined as excluding gross structural brain malformations or severe neurological deficits, however additional malformations of cortical development (MCD) are increasingly being recognised. Few patients with primary microcephaly and mutations in the MCPH genes other than ASPM have been described. We have performed mutation analysis in the MCPH1, WDR62, CDK5RAP2, CEP152, ASPM, CENPJ, STIL and PNKP genes using haplotype analysis and direct sequencing in 14 families and 10 sporadic patients. Mutations were found in the ASPM (5 families, 8 patients), WDR62 (5 families, 5 patients), CDK5RAP2 (1 family, 2 patients), STIL (1 family, 1 patient) and PNKP (1 family, 1 patient) genes. Twelve of the 16 different mutations found have not been previously described. All patients had severe developmental delay, with speech development being most severely affected. All patients showed a MCD in addition to severe microcephaly. The mildest brain phenotype was associated with ASPM mutation and a characteristic combination of polymicrogyria and pachygyria was associated with WDR62 mutation. Patients with CDK5RAP2 and PNKP mutation had agenesis of the corpus callosum in addition to simplified gyration. The recognition of specific patterns associated with mutation of each of the MCPH genes will aid targeted diagnosis in the future.

P12.146

A novel locus for autosomal dominant microcephaly

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Autosomal dominant microcephaly with mild to moderate mental retardation with no dysmorphism or other anomalies was diagnosed in eleven individuals of an Arab Israeli family. Craniosynostosis and environmental factors were ruled out per history as possible contributors to the disease, thus verifying the diagnosis of primary microcephaly. Brain CT scan of affected individuals showed no architectural anomalies. Nine living affected individuals were available for clinical and genetic evaluation. Association with all known microcephaly-associated loci were ruled out using polymorphic markers and genome wide linkage analysis data. Genome-wide linkage analysis revealed association of the disease to a region on chromosome 4 with a maximal LOD score of Z = 3.44, at marker D4S1534 (θ=0). Whole exome sequencing is underway.

P12.147

Megalencephalic Leukoencephalopathy with subcortical Cysts type 1 (MLC1) due to a homozygous deep intronic splicing mutation (c.895-226T>G) abrogated by AMO treatment.

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Megalencephalic leukoencephalopathy with subcortical cysts is an autosomal recessive disease characterized by early-onset macrocephaly, developmental delay, motor disability in the form of progressive spasticity and ataxia, seizures, cognitive decline and characteristic MRI findings. Mutations in two genes, *MLC1*(22q13.33; 75% of patients) or *HEPACAM* (11q24; 5-10% of patients) are associated with the disease.

We describe an adult MLC patient with moderate clinical symptoms. *MLC1* cDNA analysis from lymphoblasts showed a strong transcript reduction, and identified a 246-bp pseudoexon containing a premature stop codon between exons 10 and 11, due to a homozygous c.895-226T>G deep-intronic mutation. The role of this mutation on splicing was confirmed using a minigene assay, and an antisense morpholinated oligonucleotide (AMO) targeted to the aberrant splice site partially abrogated the mutation *in vitro*.

The mutation c.895-226T>G has a leaky effect on splicing leaving part of the full length transcript and may explain the milder phenotype in our patient. This category of mutations is often overlooked, being outside of canonically sequenced genomic regions, and is particularly important because it can be corrected by antisense oligonucleotides therapy.

P12.148

New deletion in Currarino syndrome

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Currarino syndrome (CS, OMIM#176450) is an autosomally dominant inherited developmental disorder due to incomplete rostral separation of endo- and ectoderm. The condition is characterized by three main findings; a sacral bony abnormality, anal stenosis and a presacral mass. CS is associated with mutations in the *MNX1* gene (previously known as *HLXB9*). Penetrance is reduced and expression is variable within families. Molecular testing can facilitate diagnosis and accurate genetic counseling. *MNX1* mutations were identified by direct Sanger sequencing of the coding regions and intron/exon boundaries of *MNX1* and by multiplex ligation probe amplification (MLPA kit P277-B1 Human Telomere-10) and a specially designed kit with probes within the *MNX1* gene. A total of 23 individuals with CS from nine different families were tested. We found mutations in only three families. In one family four affected individuals had a deletion of a cytosine in exon 1 (c.340delC, p.His114ProfsX111), previously described by Ross et al. (1998) and referred to as DelC, nt 414-17. In the second family, the two affected individuals, harboured a duplication of a guanine in exon 1 (c.336dupG, p.Pro113ArgfsX110), previously described by Köchling et al. (2001) and referred to as 413delG. In the third family, four affected individuals had a microdeletion of *MNX1* detected by MLPA only. The extent of the deletion, which has not been reported previously, is defined. This case underscores the importance of dosage analysis as an adjunct to sequencing in determining the molecular cause of CS.

P12.150

Parallel approach in molecular diagnostics of progressive muscular dystrophies.

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There are two major disease groups to account for the phenotype of a progressive muscular dystrophy: X-linked dystrophinopathies (Duchenne, Bekker) and limb-girdle muscular dystrophies (autosomal dominant/recessive LGMD). Their common features are progressive muscle weakness and atrophy spreading form proximal to distal muscle areas. The particular type of the disease is clinically difficult to distinguish. Pedigree analysis, muscle biopsy and molecular-genetic analysis are used as main diagnostic tools.

In our laboratory, deletion/duplication analysis of the dystrophin gene and direct sequencing of relevant genes constitute the standard testing procedure. Until now our strategy involved a stepwise sequencing analysis starting with genes most frequently harbouring disease causing mutations.The stepwise strategy was selected due to

a) the large size of involved genes
b) high genetic heterogeneity of LGMD and
c) low throughput and high cost of Sanger sequencing method.

All these factors contributed to significantly prolonged testing time per patient. In many cases, the testing was stopped prematurely due to high cost/long duration.

Therefore, we have implemented a high-throughput sequencing platform (GS Junior, Roche), which has enabled us to switch from consecutive to parallel testing strategy. Our muscular dystrophy panel currently contains 14 genes (DMD, MYOT, LMNA, CAV3, CAPN3, DYSF, SGCG, SGCA, SGCB, SGCD, FKRP, POMT1, ANO5, POMT2) and further genes will be added as necessary. We aim to decrease the testing time and cost, increase the mutation detection rate and avoid muscle biopsy where possible.

P12.152

Molecular genetic diagnostics of myotonia congenita and structural analysis of mutations in the *CLCN1* gene

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Non-dystrophic myotonias are skeletal muscle disorders associated with mutations in the *CLCN1* and *SCN4A* genes. Mutations of *CLCN1* result in autosomal dominant (Thomsen) and/or autosomal recessive (Becker) myotonia congenita (MC). Mutations in *SCN4A* are typically inherited as an autosomal dominant trait. The ClC1 protein is a homodimer with a separate ion pore within each subunit. The varied inheritance pattern of myotonias appears to result from differential effects of a mutation on the channel dimer. Mutations causing recessive myotonia most likely affect properties of mutant monomer leaving the wild type monomer unaffected in the heterodimer. On the other hand, mutations causing dominant myotonia affect properties of both subunits in the wild type/mutant heterodimer. Our study addresses two points: 1) molecular genetic diagnostics of MC by tandem analysis of the *CLCN*1 and *SCN4A* genes, and 2) homology modelling of the dimeric ClC1 channel. In the first part, mutations associated with the disease were identified in 57 probands - 39 carried mutations in *CLCN1* and 18 in *SCN4A*. In the second part, we performed homology modelling of the dimeric ClC1 channel on the basis of known crystallographic structures. From this model, we predicted aminoacids (AA) forming the dimer interface and AA forming the Cl^- ion pathway. Further, we performed search for mutations of AA forming the dimer interface or/and the Cl^- ion pathway in our model which have been found in patients with MC to assess the correlation between the localisation of a mutation and the type of MC.

P12.153

FZD6 encoding the Wnt receptor frizzled 6 is mutated in autosomal-recessive nail dysplasia

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Isolated nail dysplasia is rare and has only been reported in a small number of families. In this report, we describe two Pakistani families with an autosomal-recessive inherited nail dysplasia. Thickening and hyperpigmentation of all finger and toe nails were present at birth, and the nails subsequently became claw-like around puberty. By genomewide linkage analysis, we mapped this genodermatosis to chromosome 8q22.3, and identified a homozygous nonsense mutation c.1750G>T (p.E584X) in the frizzled 6 (FZD6) gene in all affecteds. Expression analyses in nail sections from healthy individuals revealed strong expression of FZD6 in the ventral nail matrix and a less pronounced expression of FZD6 in the nail bed. FZD6 belongs to a family of proteins that serve as receptors in Wnt signaling pathways, and has been shown to act as a negative regulator of the canonical Wnt/β-catenin signaling cascade and a positive regulator of the non-canonical Wnt or planar cell polarity (PCP) pathway. The present results therefore suggest that FZD6 plays a pivotal role in the growth and guidance of the nail plate in humans by acting as a molecular switch between different Wnt pathways. Previous studies have identified mutations in the RSPO4 and LMX1B components of the Wnt pathway in patients with the hypoplastic nail disorders anonychia and nail-patella syndrome, respectively. Only recently, FZD6 mutations were identified in isolated nail dysplasia. The present results emphasize the important role of the Wnt pathways in nail development and increase understanding of Wnt-mediated developmental events in general.

P12.154

Identification and characterization of a rare gene variant in congenital interstitial lung fibrosis and nephrotic syndrome

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We saw a patient who presented with severe congenital interstitial lung fibrosis and nephrotic syndrome, characterized by kidney hypoplasia, hydronephrosis, glomerulosclerosis, pulmonary hypoplasia, and alveolar glycogenosis, leading to death 7 months after birth due to respiratory insufficiency. The purpose of this study was to describe the clinicopathologic findings and unravel the underlying genetic cause. Known nephrotic syndrome genes were screened and 250K SNP array analysis was performed. No novel variants were identified in the known nephrotic syndrome genes. A homozygous region of 20 Mb was identified in the patient's DNA. Sequencing of the strongest candidate gene revealed a novel homozygous missense variant that was inherited from the heterozygous unaffected parents and not found in 384 control chromosomes. Striking similarities were seen between the gene knockout mouse and the patient's phenotype. Further in vitro characterization studies demonstrated the effect of the variant on the protein function. Here, we report a novel human gene variant, causing congenital interstitial lung fibrosis and nephrotic syndrome for the first time. The variant leads to an amino acid substitution in a gene essential for basement membrane development, in a domain interacting with extracellular matrix components. This is the first clinical phenotype associated with a mutation in this gene in humans. Our findings have major implications for our understanding how basement membrane morphogenesis is regulated and facilitates the design of genetic screening tests for early diagnosis and genetic counselling for patients and their relatives.

P12.155

Next Generation Sequencing (NGS) in the diagnostics of Nephrotic Syndrome

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About 10% of children with idiopathic Nephrotic syndrome (NS) are steroid-resistant (SRNS), and develop end-stage renal disease. In the last couple of years, mutations in a number of genes highly expressed in podocytes have been identified causing SRNS.

The focus of this study was to establish a NGS approach for developing a fast and extensive method leading to increased genetic diagnostics of SRNS patients. As positive control 31 patients were analyzed, who had already been sequenced using the Sanger sequencing method following the mutational algorithm. These patients were examined in all thitherto known SRNS relevant genes (*ACTN4*, *CD2AP*, *COQ6*, *INF2*, *LAMB2*, *NPHS1*, *NPHS2*, *PLCE1*, *TRPC6*, *WT1*) using the Roche 454 technology. In 18/31 patients mutations were known, 13 patients did not show mutations in the already examined genes (Sanger detection rate 58%).

In 16/18 genetically diagnosed patients mutations were confirmed using NGS. Two mutations localized within homopolymer regions had not been identified. In 4/13 (31%) without mutations so far, mutations in one of the following genes have been identified: *CD2AP* (1 patient), *INF2* (1 patient), and *NPHS1* (2 patients). These genes were not sequenced using Sanger sequencing because they were excluded by the mutation algorithm. Altogether, in 20/31 patients causative mutations have been identified (NGS detection rate 65%).

NGS in the diagnostics of NS seems to be a good option for a rapid and reliable genetic analysis. However, mutations within homopolymer regions can be missed by the Roche 454 technology. These regions should still be analyzed by Sanger sequencing.

P12.156

Mutational analysis of the PLCE1 gene in Greek children with clinical presentation of nephrotic syndrome (NS) and diffuse mesangial sclerosis (DMS) or focal segmental glomerulosclerosis(FSGS)

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Mutations in *PLCE1* encoding phospholipase C epsilon 1(PLCe1) have recently been described in patients with early onset nephrotic syndrome (NS), diffuse mesangial sclerosis (DMS) and focal segmental glomeruloscerosis (FSGS). In 40 NS patients (35 sporadic, 5 familial) with histological findings of DMS or FSGS, 27/31 exons of *PLCE1* were analysed by direct sequencing. Pathological mutations in the *NPHS1, NPHS2* and *WT1* genes had been previously excluded in all patients. Sequencing analysis revealed *PLCE1* mutations in 4/35 patients. Three, homozygous for the previously described p.R1246X, had similar clinical findings (FSGS, age diagnosis 3.7, 2.5 and 3.5 years, respectively; age first dialysis 3.9, 2.6 and 4.5 years, respectively). Additionally, 1 case was found homozygous for a novel mutation, p.A945P. The patient (male) presented at 3months with DMD, initiating dialysis at 9months. *In silico* analysis (SIFT, Polyphen, Pmut and MutationTaster) were applied to evaluate causality of the p.A945P mutation. Pmut and SIFT found the nucleotide change "possibly damaging", while Polyphen and MutationTaster indicated p.A945P to be benign. Resequencing analysis in members of the patients' family (over 3 generations, all unaffected with NS) found only heterozygotes for p.A945P (according to expected phase). Further studies are required to conclude the pathogenicity of this novel *PLCe1* mutation, in the light of observations that pathogenicity of *PLCe1* gene mutations may be ambiguous (Boyer et al, J Med Genet, 2010). Overall our findings are consistent with previous conclusions that *PLCe1* mutations are not uncommon in childhood NS patients in whom mutations in other NS-gene have been excluded.

P12.157

Analysis of VANGL2 in 164 patients with neural tube defects reveals no mutations

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Van Gogh-like (Vangl2) is a protein of the non-canonical Wnt signalling pathway regulating polarized cell movements during embryonic development such as neurulation. Mutations of the Vangl2 gene have been shown to cause neural tube defects (NTDs) in loop-tail (Lp) mice and have become strong candidates for causing NTDs in humans. Mutations in the VANGL1 gene, which is homologous to VANGL2, were described to be associated with NTDs in 1-2% of cases. To examine the contribution of VANGL2 sequence variants to human NTDs, we studied lymphocyte DNA from peripheral blood of 164 patients with clinically relevant NTDs for VANGL2 gene mutations using PCR sequencing.

We identified no mutations, but 14 single nucleotide polymorphisms (SNPs) in the VANGL2 gene. Of these, seven were silent SNPs within the coding DNA (exons 3, 4, 6 and 7) and seven were intronic SNPs. Nine SNPs were already reported by the 1000 Genomes Project with similar frequencies, and five SNPs were novel and appeared with low frequencies. In silico testing of the five novels SNPs predicted no deleterious effects (no alteration of splice sites), but further studies remain to be done before final conclusions can be drawn. Furthermore, we performed a methylation analysis by bisulfite pyrosequencing of the potential VANGL2 promoter region to search for possible epimutations causing NTD and identified no methylation differences between patients with NTDs and controls. Other studies reported VANGL2 mutations in about 2% of NTD cases, suggesting that VANGL2 gene mutations are associated with NTDs in a small subset of cases.

P12.158

Neurofibromin (NF1) is required for skeletal muscle development

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Neurofibromatosis type I (NF1) is a multi-system disease caused by mutations in the *NF1* gene. *NF1* encodes a RAS-GAP protein, Neurofibromin, which negatively regulates RAS signaling. Besides neuroectodermal malformations and tumours, the skeletal system is often affected in NF1 patients, scoliosis and long bone dysplasias being a main cause of considerable morbidity. Interestingly, a reduction of muscle strength and size has been reported in NF1 patients. However it remained unclear whether the observed muscle weakness was a consequence of the skeletal ramifications developing during puberty and early adulthood or if there was a muscular phenotype before the onset of a bone phenotype. Nf1 gene inactivation in the early mouse limb bud mesenchyme using Prx1-cre ($Nf1^{Prx1}$) resulted in muscle dystrophy characterised by fibrosis, reduced number of muscle fibres, and reduced muscle force. This was caused by an early defect in myogenesis affecting the terminal differentiation of myoblasts between embryonic days (E)12.5 and E14.5. In parallel, the muscle connective tissue cells exhibited increased proliferation at E14.5 and an increase in the amount of connective tissue as early as E16.5. These changes were accompanied by excessive MAPK pathway activation. Satellite cells isolated from $Nf1^{Prx1}$ mice showed normal self-renewal, but their differentiation was impaired as indicated by diminished myotube formation. Our results demonstrate a requirement of Neurofibromin for muscle formation and maintenance. This previously unrecognized function of Neurofibromin may contribute to the musculoskeletal problems in NF1 patients.

P12.159

Mutation screening by Next generation sequencing technology in analysis of Neurofibromatosis type 1- our first experience.

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Neurofibromatosis type 1 (NF1) is autosomal dominant disorders and is caused by mutations in the *NF1* gene. Current approaches of NF1 analyses are complicated molecular diagnostics due to the large size of the *NF1* gene, the presence of pseudogenes, the great variety of possible lesions and high mutation detection rate.

We report our first experience of NF1 gene analysis using the next-generation sequencing (NGS) (Roche GS Junior). 20 patients with clinical symptoms of the disease were included to DNA mutational screening .The PCR of all exons of NF1 gene were performed by the use of primers composed by a gene-specific part and a universal tail. Each primer was labeled by MID tails (Multiplex Identifier Adaptors), which serve to identify specific patient´s DNA sample. Prepared amplicons of NF1gene were analysed using of two runs in the GS JUNIOR system. The generated NGS data were processed with Roche software Amplicon Variant Analyzer version 2.5p1 and acquired sequences were compared to reference genome.

We have detected point mutations within the coding region: deletion, nonsens, missense, splicing and frameshift mutations. All of these sequence variants were confirmed by conventional analysis method (Sanger sequencing). Since the data acquired within our first experiment by the Roche Junior system are in full accordance with results obtained by traditional approach and the process is both time and cost effective , we are involving NGS to our strategy of molecular genetics testing of NF1 patietns in combination with conventional analysis methods as MLPA, Sanger Sequencing of DNA and cDNA

P12.160

Next-Generation Sequencing in Neurofibromatosis 1

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Neurofibromatosis 1 is an autosomal-dominant disease characterized by multiple café-au-lait spots, axillary and inguinal freckling, multiple neurofibromas, and Lisch nodules. The disorder is caused by defects in the tumor-suppressor gene NF1. Mutation detection is complex and expensive due to the large size of the NF1 gene, the presence of pseudogenes, the lack of mutation hotspots as well as a large variety of minor and major deletions.

We have established a Next-Generation Sequencing (NGS)-based mutation detection method as a tool for routine analysis of the entire NF1 gene in patients with excluded deletion. So far, we have tested >45 patients with suspected NF1. 7% of the patients harbored a NF1 gene deletion, the remaining patients were subsequently examined using NGS: all protein-coding exons including the flanking splice consensus sites were amplified by PCR and afterwards sequenced using the Roche 454J platform. In >70% of the patients we have detected exonic and splice site mutations. All mutations identified by NGS were successfully validated by Sanger sequencing.

No causative mutation was found in over 20% of the patients, which might be explained either by the presence of deep intronic mutations or the fact that not all patients in our cohort met diagnostic criteria for NF1. To further increase the mutation detection rate we have recently included NGS-based testing of the SPRED1 gene, mutated in the clinically similar Legius syndrome.

We conclude that the use of NGS-based diagnostic procedures offers an affordable and highly sensitive alternative for NF1 diagnostic.

P12.161

Mutation analysis of NF1 and SPRED1 genes in Slovak patients

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Neurofibromatosis type 1 (NF1) is one of the most common autosomal dominant disorders with incidence of 1:3500. The main clinical features are “café au lait” macules, freckling, optical glioma, Lisch noduls, any types of neurofibromas, and dysplasia of bones. The disease is caused by inactivating mutations within the NF1 gene that maps to 17q11.2 and consists of 60 exons. Protein product of NF1 is a tumor suppressor neurofibromin that functions as a negative regulator of Ras proto onkogen.

Legius syndrome called NF1- like syndrome is disorder with similar clinical features like NF1. It is caused by mutation in the next negative regulator of MAPK signal pathway - SPRED1 (Sprouty-related EVH1 domain - containing protein 1). SPRED1 gene is localized on 15q13.2 chromosome and consist of 8 exons.

We identified 55 mutations in NF1 gene. 31 of them are new and 24 are recurrent. Using protocol base on sequencing of entire NF1 coding region developed by Messiaen and Wimmer (2008), we identified 25/55 (45%) frameshift, 8/55 (15%) splicing, 7/55 (13%) missense, 5/55 (9%) nonsence mutations and 1/55 (2%) small frame deletion. In all cases where no mutation was identified by sequencing we performed MLPA analysis we identified 5/55 (9%) deletions of entire gene type 1, 4/55 (7%) larger intragenic deletions of one or more exons.

If patients fulfil the main diagnostic criteria but they have no germinal mutation in NF1 gene we analysed SPRED1 gene. Analysis of SPRED1 gene was finished in 18 patients. No pathogenic mutation was found.

P12.162

Loss of neurofibromin increases micro- and macroporosity in cortical bone resulting in diminished mechanical resistance

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Skeletal manifestations such as osteoporosis, dystrophic scoliosis or tibial dysplasia are commonly in patients with neurofibromatosis type 1 (NF1). To further explore the origin of NF1 bone dysplasia we now performed detailed analysis of the cortical bone porosities in Nf1Prx1 mice and in NF1 patients employing high-resolution imaging techniques. One of our aims was to explore how NF1 loss of function affects osteocytes, the mechanosensory cells of the bone. The overall morphology of the humerus from Nf1Prx1 mice appeared severely disordered. Especially at the muscle to bone insertion sites we observed large amounts of fibrocartilaginous tissue. Within the diaphysis we detected large non-mineralized regions of bone tissue that are associated with blood vessels. Thus, the macroporosity was 5-fold increased in Nf1Prx1 mice as compared to controls. Microporosity, which is mainly determined by the size of osteocyte lacunae, was increased. While Nf1Prx1 cortical bone contained a normal number of osteocyte lacunae, the average lacuna volume was increased, yielding higher relative osteocyte volume per bone volume (3.4 % in the mutants vs. 2.0 % in controls). The osteocyte phenotype is likely cell autonomous as increased osteocyte lacuna volume was also detected in the Nf1Col1 mice. Similarly, a quantitative volumetric analysis of cortical bone samples from NF1 patients demonstrated increased osteocyte lacuna size. These findings suggest that neurofibromin is required for normal osteocyte function, and facilitates bone homeostasis. Thus, our collective data reveal a significant impact of neurofibromin on cortical porosity establishing a further aspect of NF1 bone dysplasia.

P12.163

In search of genetic defects in unrelated frontotemporal lobar degeneration patients using whole genome sequencing

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Frontotemporal lobar degeneration (FTLD) has a positive family history in up to 50% of patients and approximately 40% can be explained by causal mutations in one of the known genes. In spite of this, identifying and sampling informative families for genetic linkage studies becomes increasingly difficult due to a variable disease onset and penetrance, amongst other reasons. We use whole genome sequencing (WGS) as an alternative to identify Mendelian FTLD genes. We obtained the whole genome sequences of three affected sib pairs and ten unrelated at an average coverage of 70-fold capturing both alleles of 96.5% of the genome. Analysis of the genome sequences resulted in about four million variants per genome. Subsequent variant filtering procedures were based on sequence quality, genomic location, allele frequency, zygosity, predicted functional consequences and occurrence in sequenced patients. The number of variants was reduced to a manageable number of candidate variants, with 380 missense or splice variants segregating in each sib pair and 350 shared by at least three unrelated patients. We are now validating the selected variants and determining their frequency in neurologically healthy control individuals and extended patients cohorts. The identification of novel FLTD genes will provide valuable insights into the pathogenesis of the disease and will eventually lead to the development of e.g. disease models.

P12.164

Characterization of the delC.2970-2972 AAT mutation in exon 17 of the NF1 gene in Mexican patients with neurofibromatosis type 1 with no cutaneous neurofibromas

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Neurofibromatosis type 1 (NF1) is one of most common genodermatosis inherited as an autosomal dominant trait with complete penetrance and variable expressivity. With an incidence of 1:3000, it is caused by mutations in the NF1 gene, which encodes the protein neurofibromin. Recent reports identified a three nucleotide deletion in exon 17 of neurofibromin gene associated with absence of neurofibromas and benign course. The aim of the study is the molecular analysis of the gene NF1 in Mexican patients with NF1 and no neurofibroms. Methods: 10 patients were screened through PCR and DNA sequencing from genomic DNA. Results: 10 patients (all >20 years old) with a mild NF1 phenotype and no neurofibroms were screened for deletion in exon17 (c.2970-2972 AAT) of the NF1 gene. All patients included in the study were negative for the deletion reported in other populations. Conclusions: In this study we found no association between the absence of neurofibromas and delc.2970-2972 AAT in exon 17 in patients with NF1.. We consider that other genes or environment factors could be associated with the absence of neurofibromas in neurofibromatosis type 1.

P12.165

Identification and characterization of SMPD1 mutations causing Niemann-Pick types A and B in Turkish Patients

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Niemann-Pick disease Types A and B (NPD) are autosomal recessive sphingolipidoses caused by mutations in the sphingomyelin phosphodiesterase 1 (SMPD1) gene. These two types of the disease were described as early onset Type A disease and the milder Type B according to the presence (type A) or absence (type B) of neurological symptoms.
The 5 kb gene encoding the human SMPD1 gene (MIM# 607608,GenBank# M81780.1) is composed of six exons and is located at chromosome 11p15.1-11p15.4. Several mutations causing NPD have been described. We present a molecular analysis of six unrelated Turkish NPD patients in which mutant SMPD1 alleles were identified. One of the patients had type A and 5 had type B NPD. These mutations included two missense mutations: c.409T>C (p.L137P) and c.1262 A>G (p.H421R); the common frameshift mutation at codon 189, identified in 2 patient is caused by the deletion of the 567T introducing a stop codon 65 aminoacids downstream (p.P189fsX65) and a novel frameshift mutation c. 1755delC (p.P585PfsX24) which was not previosly reported. The known c.409T>C (p.L137P) and c.567delT (p.P189fsX65) were the most frequent mutations accounting for 50% and 25% the alleles, respectively. In this study genotype-phenotype correlations were established for the mutations.

P12.166

Frequent CLCN1 gene mutations in patients from Russian Federation (RF) with non-distrophic Thomsen and Becker myotonias

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Non-dystrophic Thomsen and Becker myotonias are muscle channelopathies caused by mutations in CLCN1 (7q35). The study group consisted of 79 unrelated patients with revealed myotonia by clinical examination. Patient's DNA samples were analyzed by direct sequensing of all 23 exons of the CLCN1 gene and 40 different mutations were detected in 118 chromosomes in 21 family cases (9-AD and 12-AR) and 45 sporadic cases (84% altogether). Eighteen mutations haven't been described earlier. There were detected several frequent mutations: p.Gly190Ser (5,9%), c.1437-1450del (9,3%), p.Ala493Glu (5,1%), p.Thr550Met (3,4%), p.Tyr686Stop (5,1%) and p.Arg894Stop (30,5%) composed 59,3% all mutant chromosomes. The most frequent p.Arg894Stop was revealed in homozygous and compound-heterozygous cases and allelic frequency of p.Arg894Stop among the control population is 1,2% (12/488). As the founder effect was expected to cause the high frequency of the p.Arg894Stop we analyze haplotypes of mutant chromosomes in 28 patients and 17 relatives using microsatellite markers D7S1824, D7S2513, D7S3068, D7S661 and D7S2511. Despite most linked markers flanking CLCN1 gene was in 1,7 Mb(D7S2513) and 0,6 Mb(D7S3068) away from the gene it wasn't any common haplotype in mutant chromosomes. Possibly the distance between markers and the mutation wasn't enough close. So we analyzed haplotype using introgenic SNP markers (rs940864, rs2272251 upstream and rs4489232, rs6464546 downstream the mutation Arg894Stop). Founder haplotype was detected in 13 out of 17 mutant chromosomes (rs940864-rs2272251-rs4489232-rs6464546: A-T-T-A) and it is statistically significant comparably with control group (p=0,05). Resume this data widespread occurrence of mutation p.Arg894Stop in Russia is result of founder effect.

P12.167

Clinical spectrum of intragenic CAMTA1 rearrangements: From non-progressive congenital ataxia to intellectual disability

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Non-progressive congenital ataxias (NPCA) with or without intellectual disability (ID) are clinically and genetically heterogeneous conditions. As a consequence, the identification of the genes responsible for these phenotypes remained limited. Using high resolution microarrays, we identified intragenic copy number variations in the CG-1 domain of the Calmodulin-binding Transcription Activator 1 (CAMTA1) gene, segregating with autosomal dominant ID with NPCA in two unrelated families, and a de novo deletion located in the same domain in a child presenting with NPCA. In the ID patients, the deletion led to a frameshift, producing a truncated protein, while this was not the case for the patient with isolated childhood ataxia. Brain MRI of the patients revealed a pattern of progressive atrophy of cerebellum medium lobes and superior vermis, parietal lobes and hippocampi. Although DNA sequencing of the CG-1 domain in 197 patients with sporadic or familial non-syndromic intellectual deficiency, extended to full DNA sequencing in 50 patients with ID and 47 additional patients with childhood ataxia, identified no pathogenic mutation, there is considerable evidence that CAMTA1 rearrangements are being disease causing. Indeed, CAMTA1 is a brain specific calcium responsive transcription factor expressed in the brain and cerebellum during development and later implicated in memory processes; intragenic rearrangements are concentrated in a highly conserved functional domain with transcription regulation ability and nuclear trafficking functions; CAMTA1 transcriptional studies have shown upregulation of genes already implicated in intellectual disability and autism spectrum disorder. Altogether, we show that CAMTA1 loss-of-function is responsible for NPCA with or without ID.

P12.168

The prevalence of *OTOF* mutations in Iranian deaf population

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Hearing impairment is the most common genetic sensory defect in humans worldwide. One in every 1,000 neonates is born with profound congenital deafness. About 70% of hereditary sensoryneural hearing loss (SNHL) is non-syndromic (DFN) and at least 80% of these cases have autosomal recessive (DFNB) inheritance. To date, genetic studies have shown that mutations in more than 90 loci and 40 genes are associated with DFNB. Mutations in *OTOF* gene are cause of neurosensory non-syndromic recessive deafness, DFNB9. *OTOF* gene contains 48 exons which encode a transmembrane protein, otoferlin. Several mutations in this gene have been found in Lebanese, Pakistani, Turkish, Colombian and Spanish families.

Recently, we observed an Iranian family with autosomal recessive non-syndromic hearing loss(ARNSHL),which showed linkage to *OTOF* gene. Mutation detection of this gene revealed a missense mutation. So we decided to screen our population for this gene.

One hundred and forty four ARNSHL families with two or more affected individuals originated from different ethnic groups of Iran were selected for this study. All the families were subjected to homozygosity mapping using flanking STR markers of *OTOF* gene. After screening all the families, one family showed linkageto this gene. Further analysis using direct sequencing of this gene revealed a splice site mutation (IVS28+2 T>C). So, in comparison with other countries such as our neighboring country, Pakistan, *OTOF* mutations are very rare in Iran.

P12.169

Exome sequencing identifies *PRPS1* as a major *locus* for X-linked nonsyndromic hearing loss in the Italian population

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An Italian family with two siblings affected by nonsyndromic sensorineural hearing loss (NSHL) and showing a recessive pattern of transmission was selected for whole-exome next-generation sequencing (NGS). Genomic capture and NGS on a HiSeq 2000 sequencer (Illumina) of the proband lead to the identification of a novel missense mutation within *PRPS1*. Mutations in this gene, which codes for the phosphoribosylpyrophosphate synthetase 1 (PRS-I) enzyme, were previously demonstrated to cause X-linked syndromic conditions associated with hearing impairment (e.g. Arts syndrome and Charcot-Marie-Tooth disease-5), and, most recently, NSHL in 4 families (DFNX1 *locus*). The identified mutation segregates with prelingual, bilateral, profound NSHL in the proband's family. A subsequent screening of the entire *PRPS1* gene by Sanger sequencing in 13 additional unrelated probands from NSHL families showing a likely X-linked inheritance pattern led to the discovery of a second missense mutation segregating with pre-lingual hearing impairment. The two novel variants were absent in a cohort of 126 Italian audiologically-tested, normal-hearing controls. Both amino-acid substitutions are predicted to cause a destabilization of the enzyme structure. The impact of both *PRPS1* mutations on the function of PRS-I is currently under analysis by measuring the enzyme activity in the patients' emolysates compared to controls. In conclusion, we provide evidence of the usefulness of whole-exome NGS for the genetic diagnosis of NSHL and we highlight the recurrence of *PRPS1* mutations, suggesting that it may represent a major *locus* for X-linked NSHL to be prioritised in genetic screenings.

P12.170

A custom multiplexing mutation panel for Noonan, Costello, LEOPARD and Cardiofaciocutaneous Syndromes

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Introduction: Noonan syndrome is a congenital genetic disease that affects both males and females equally. Often this syndrome is not diagnosed, but it is related to many complex problems such as coagulation defects and lymphatic dysplasias. Differential diagnosis includes major diseases in the same metabolic pathway - Costello, Cardiofaciocutaneous and LEOPARD Syndrome. Noonan Syndrome is often present in prenatal cases with increased nuchal translucency and normal karyotype. **Method:** We developed a custom multiplex mutation panel (CGC Mutation Panel - Pat. Pending) that contains a total of 81 point mutations on genes PTPN11, RAF1, SOS1 and KRAS, HRAS, BRAF, MAP2K1 and MAP2K2 involved in Noonan, LEOPARD, Costello and Cardiofaciocutaneous syndromes. With this panel we analysed 85 samples (35 prenatal and 50 postnatal). **Results**: From the 85 samples tested (35 prenatal and 50 postnatal), in 2 prenatal sample we detected mutations on PTPN11 gene allowing the diagnosis of Noonan and in 3 of the postnatal samples we found mutations on SOS1, BRAF and PTPN11 gene allowing the diagnosis of Noonan and Cardiofaciocutaneous syndromes. **Conclusion:** This approach is a valuable prenatal and postnatal diagnostics tool, since it detects the most common mutations associated with Noonan Syndrome and syndromes of the same metabolic pathway in a single test. This panel reduces time and costs to achieve a diagnosis improves its capability, independently of the sample type, allowing an earlier decision-making process in patient management, and is useful especially in prenatal diagnosis situations with increased nuchal translucency and normal karyotype.

P12.171

Novel mutations in PTPN11 gene in two girls with Noonan syndrome phenotype

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Noonan syndrome is an autosomal dominant disorder characterized by postnatally reduced growth, distinctive facial dysmorphic features, hypertrophic cardiomyopathy and/ or pulmonary valve stenosis , skeletal and hematological anomalies, and webbing of the neck. To date, mutations of nine different genes were identified as the molecular cause of this disorder. While mutations of these genes are responsible for the 75% of all the cases, mutations in PTPN11 is responsible for approximately 50% alone.

Here, we report two novel mutations c.A1690G and c.C155T in two girls with Noonan Syndrome phenotype, both with short stature, one with mild neuromotor developmental delay and the other with dysplastic pulmonary valve and pulmonary valve stenosis. We try to correlate genotype-phenotype relation.

P12.172

A spectrum of mutations in *PTPN11*, *SOS1* and *RAF1* genes in patients with Noonan syndrome clinical suspicion.

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Noonan syndrome (NS) is a relatively common developmental syndrome belonging to the group of RASopathies, that are characterized by increased activity of the Ras/mitogen activated protein kinase (RAS/MAPK) signaling pathway. NS is known to be a disorder with variable phenotypic expression including symptoms like short stature, dysmorphic features, congenital heart defects and many other.

Noonan syndrome is caused by a germline mutation in one of the genes that encode proteins involved in RAS/MAPK signaling pathway. In NS patients, heterozygous mutations have been identified in *PTPN11, SOS1, RAF1, BRAF, KRAS, NRAS, SHOC2, MEK1* and *CBL* genes.

Three hundred eleven individuals (237 patients with clinical suspicion of NS and 74 relatives) were referred to our laboratory for molecular examination. The *PTPN11, SOS1* and *RAF1* genes were examined in 237, 70 and 28 patients, respectively. The analysis of coding sequence was performed using direct sequencing method.

The known NS causing missense mutations were found in 89 (37.5%) cases: 67 had mutation in *PTPN11*, 10 in *SOS1* and 12 in *RAF1*. All the identified mutations, according to the literature, were gain-of-function type. Although most of the probands with NS have a *de novo* mutation, we have identified 17 (50%) familial cases (14 - *PTPN11*, 2 - *SOS1*, 1-*RAF1*). The low frequency of identified mutations among patients included in the study might suggest that more stringent criteria should be used for patients' qualification for molecular testing.

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P12.173

Interpretation of atypical *NOTCH3* mutations: lessons from patients with novel large NOTCH3 alterations but no CADASIL

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Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is characterized by early onset stroke and vascular dementia. It is caused by stereotyped missense mutations in *NOTCH3*, which invariably lead to an uneven number of cysteines in one of the 34 EGFL domains that constitute the NOTCH3 ectodomain. In addition to missense mutations, a few small pathogenic *NOTCH3* deletions and insertions have been described. All changed the number, or the spacing, of cysteine residues. A frame shift mutation leading to a stop was reported as pathogenic, but molecular and clinical testing was incomplete.

We describe three patients with large *NOTCH3* alterations, of which the pathogenicity was initially unclear. We combined extensive laboratory analysis (MLPA, DNA and RNA analysis, Western Blotting) with thorough clinical evaluation. The first patient has a deletion of exon 3-16, leading to a premature stop, the second patient has an exon 3 stop mutation. Clinically, they did not have a CADASIL phenotype. Molecularly, the mutations are predicted to result in small NOTCH3 fragments that lack transmembrane and intracellular domains and are highly unlikely to be expressed at the cell surface. The pathogenicity of the mutation in the third patient, a splice site mutation causing exon 7 skipping, remains uncertain but does not give classical CADASIL

We conclude that *i)* clinical and molecular investigation by an experienced team is indispensable for the correct interpretation of atypical *NOTCH3* mutations *ii) NOTCH3* stop mutations do not cause CADASIL, arguing against the theory that hypomorphic *NOTCH3* alleles also cause CADASIL.

P12.174

Recessive mutations in MCM4/PRKDC cause a novel syndrome characterised by a primary immunodeficiency and impairments in DNA repair

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We present a study on ten children from three families with a novel syndrome born to consanguineous parents from the Irish Traveller population. The syndrome consists of failure to thrive, a natural killer cell deficiency, an atypical Fanconi's type DNA breakage disorder and features of familial glucocorticoid deficiency. In addition the children had delayed bone age, clinodactyly and some presented with hypoglycaemia. Using SNP homozygosity mapping, we identified a locus for this syndrome on 8p11.21-q11.22. Targeted resequencing of the candidate region revealed a homozygous mutation in *MCM4/PRKDC* in all ten patients that segregated with the phenotype. Consistent with the observed DNA breakage disorder, *MCM4* and *PRKDC* are both involved in the ATM/ATR DNA repair pathway which is defective in patients with Fanconi's anaemia. Deficiency of *PRKDC* in mice has been shown to result in an abnormal NK cell physiology similar to that observed in the patients. Mutations in *MCM4/PRKDC* represent a novel cause of DNA breakage and NK cell deficiency. Our findings suggest that clinicians should consider this disorder in patients with failure to thrive who develop pigmentation or who have recurrent infections.

P12.175

Oncogenic NRAS G12S discovered as a germline mutation in a patient with Noonan/CFC syndrome

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Noonan syndrome (MIM 163950) and cardiofaciocutaneous syndrome (CFC syndrome; MIM 115150) are clinically overlapping syndromes caused by mutations in various genes encoding components of the RAS/MAPK signalling pathway. Here, we report on a 1-year-old girl with facial anomalies, heart defect, and developmental delay leading to the differential diagnosis of Noonan or CFC syndrome. She has not had any signs of a myeloproliferative disease or other malignancy, so far. Mutation analysis revealed the heterozygous *NRAS* mutation c.34G>A (p.G12S) in leukocyte DNA. The presence of the mutation was also confirmed in the patient's buccal cells and could be ruled out in the parents, thus indicating a *de novo* mutational event in the germline. G12S is one of the most frequent somatic *NRAS* mutations and predominantly observed in cancers of haematopoietic and lymphoid tissues, thyroid, and skin. Only very few germline *NRAS* mutations sparing the classical oncogenic mutation hotspots have been identified to date in patients with Noonan syndrome. It has been hypothesized that oncogenic mutations when occurring in the germline might lead to embryonic lethality. Two anectdotal reports, however, described the known oncogenic *NRAS* mutation G13D being apparently present in the germline of patients with a hematologic phenotype but no obvious signs of Noonan syndrome. Our observation documents a clear RASopathy phenotype and absence of myeloproliferative disorder in a patient with oncogenic *NRAS* G12S as a constitutional mutation.

P12.176

Cornelia de Lange Syndrome diagnostics in times of NGS

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Cornelia de Lange Syndrome (CDLS) is a rare autosomal dominant disease with an incidence of 1 in 10.000 to 60.000 newborns. It leads to severe developmental anomalies. This disorder is characterized by skeletal, craniofacial deformities, gastrointestinal and cardiac malformations. The physical as well as intellectual development of a child is affected. Clinical diagnosis is based on the phenotype.

This disorder is mainly caused by mutations in the Cohesin complex genes NIPBL, SMC1A, and SMC3. Mutations in the NIBL gene are responsible for about 50% of the cases. The two latter genes cause a slightly milder form of CDLS. In addition, patients with mutations in the UBE2A gene show a CDLS like phenotype. So far, molecular validation of CDLS has been done by Sanger sequencing or MLPA with focus on NIPBL gene analysis. All coding exons and their corresponding splice sites were analyzed.

Recently, the high throughput pyrosequencing technique using the Roche 454J platform was implemented in our laboratory and an open and flexibly upgradeable diagnostic panel for CDLS including the genes NIPBL, SMC1A, SMC3 and UBE2A was established.

Here, we report on our analyses of CDLS patients applying NGS technology into routine diagnostics and compare its performance with standard mutation detection. The preliminary data show that our diagnostic panel is a real alternative to classical sequencing in respect to sensitivity, TAT and cost. In addition, our approach is optimal for molecular analysis of genetically heterogeneous diseases with distinct clinical manifestation and caused by mutations in a limited number of genes.

P12.177
New insights in non-syndromic albinism

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Background: Albinism is a complex group of genetic disorders characterized by reduced or complete absence of melanin pigment in the skin, hair, and eyes associated with decreased visual acuity, nystagmus, and photophobia. Oculocutaneous albinism (OCA) is classified into several types based on clinical and molecular categories. The prevalence of all forms of albinism varies considerably worldwide and has been estimated at approximately 1/17,000.

Patients and Methods: After informed consent was obtained, blood samples were collected from patients with clinical signs of OCA. We screened 13 families (7/13 Spanish, 1/13 Rusian and the 2/13 Africans) for sequence variations in the coding region of the *TYR* gene avoiding the *TYRL* pseudogene and the 6, 7 and 13 exons of the *OCA2* gene. Also MLPAp325 was performed in order to detect deletions or duplicacions in the *TYR* and *OCA2* genes.

Results: At least one single mutation in either or both *TYR* and *OCA2* genes has been identified in 12 out of 13 families. Most of the identified mutations were detected in a heterozygous pattern, but with the exception of the mutation p.Pro81Leu and the 2,7kb including exon 7.

Discussion: The entire coding regions of 4 genes associated with non-syndromic OCA have been analysed in many cases, however one single mutation have been identified in many of the cases analysed what suggests that other genes yet unidentified probably exist. Epistasic phenomenon also has been described in mice assays. The new technologies such as Next Generation Sequencing would provide us new insights in this disease.

P12.178
Novel *OFD1* mutations in males extend the phenotypic spectrum to vermis hypoplasia, polydactyly, hypothalamic hamartoma and *situs inversus*, and basal bodies docking impairment

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OFD1 is classically responsible for dominant X-linked OFD I syndrome with male lethality, but mutations were recently reported in males, one syndromic mental retardation (MR) families (SGBS2, MIM300209) and two Joubert syndrome (JBS10, MIM300804) . We therefore sequenced the *OFD1* gene in a cohort of 92 males, including 32 JBS patients, 20 fetuses with Joubert/Meckel syndrome or ciliopathy spectrum, as well as 40 additional males with syndromic MR based on the association of at least 2/5 of the following criteriae: macrocephaly, obesity, polydactyly, recurrent respiratory infections, and retinopathy.

We identified 2 novel truncating *OFD1* mutations, both located in exon 21. The first *de novo* mutation was found in a fetus with polydactyly, vermis hypoplasia, but also hypothalamic hamartoma and *situs inversus* extending the phenotypic spectrum of *OFD1* mutations. The second maternally inherited mutation was found in a 10 year-old JBS male with polydactyly, obesity, recurrent bronchitis and abnormal nasal NO measurement. Electronic microscopy of airways epithelia in both cases showed cilia abnormalities and an abnormal centrosomal docking at the cell surface. *OFD1* expression analysis during early human development correlates with its phenotypic spectrum.

Our study further confirms that *OFD1* mutations in the C-terminal domain lead to syndromic recessive X-linked JBS in males. The *OFD1* gene should therefore be sequenced in JSRD males with molar tooth sign, in association with other suggestive features such as retinopathy, polydactyly, macrocephaly but also hypothalamic hamartoma and *situs inversus,* and recurrent bronchitis. Abnormal NO measurement might help orienting the molecular analysis towards *OFD1*.

P12.179
A compound heterozygous missense mutation and a large deletion in the *KCTD7* gene presenting as an opsoclonus-myoclonus ataxia-like syndrome

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Purpose: To describe a new presentation of KCTD7 mutations: an opsoclonus-myoclonus ataxia like syndrome with subsequent development of generalized continuous epileptic activity.

Methods: We recorded the clinical course of the disease, the evaluation and the response to steroid therapy. After excluding possible genetic causes, whole genome exome sequencing was performed in order to identify the causative gene. Sequence variants were filtered according to the phenotype. Sanger sequencing was performed to confirm the point mutation and MLPA was used for screening for a possible deletion in the second allele.

Key finding: Two pathological variants were found in the KCTD7 gene: R84W and a large deletion of exons 3 and 4. The father is heterozygous for the R84W mutation and the mother is heterozygous for the exon 3+4 deletion.

Significance: KCTD7 mutations were described in a single family with progressive myoclonus epilepsy. Our patient presented with non epileptic myoclonus, ataxia and opsoclonus responsive to corticosteroid treatment and only two years later developed an epileptic EEG witout overt seizures. The different phenotype broadens the spectrum of KCTD7 related diseases.

P12.180
Lack of mutations in COL1A2 gene in osteogenesis imperfecta patients from Russia

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Osteogenesis imperfecta (OI) is a heritable disorder of connective tissue mainly caused by dominant mutations in type I collagen genes COL1A1 and COL1A2.

The aim of our study was to identify mutations in COL1A1 and COL1A2 genes in Russian OI patients.

We examined 54 patients with OI from 43 families and 50 healthy controls corresponding by age, gender, ethnicity and place of residence. All 51 coding exons in COL1A1 gene and 52 exons and flanking intronic regions in COL1A2 gene were analyzed by SSCP-analysis and direct sequencing.

One frameshift mutation (c.579delT (p.Gly194ValfsX71), three nonsense mutations (c.967G>T (p.Gly323X), c.1081C>T (p.Arg361X), c.2869C>T (p.Gln957X)) and one splice mutation (c.4005+1G>T) were identified in patients with autosomal dominant inheritance of OI type I. Two mutations (c.2444delG (p.Gly815AlafsX293) and c.3540_3541insC (p.Gly1181AlafsX38)) occurred in sporadic cases of OI type I, whereas c.1243C>T (p.Arg415X) mutation - of OI type 3.

In conclusion, the present study revealed eight mutations in COL1A1 gene (two of them observed de novo: c.967G>T (p.Gly323X) and c.3540_3541insC (p.Gly1181AlafsX38)) and no mutations in COL1A2 gene in Russian patients with OI. However, no predominant mutations were detected. Despite the previous studies indicating the vast majority (70%) of COL1A1/COL1A2 mutations causing OI being glycine substitutions to amino acid with a bulky side chain, we detected no missense ones. Interestingly, all detected mutations were unique for each family except for the frameshift mutation c.579delT (p.Gly194ValfsX71) observed in two unrelated families. Future research should focus on other genes responsible for OI development in Russian patients.

P12.181
LEPRE-1 gene in Osteogenesis Impefecta: study of mutations in Brazilian patients

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Osteogenesis Imperfecta (OI) is a heterogeneous genetic disease characterized by bone fragility, recurrent fractures and clinical variability. The majority of OI cases has autosomal dominant inheritance and is caused by mutations in genes that codify type I collagen protein. The *LEPRE1* gene is one of the most frequent mutated gene described in OI patients with recessive pattern. In order to characterize mutations in *LEPRE1* gene a total of 33 unrelated Brazilian OI patients were studied using SSCP screening and sequencing analyses. This study was approved by the Research Ethics Committee and all patients agree in participate of the work. The previously described African mutation (c.1080+1C>T) was found in homozygous state in one isolated case with severe OI. Two mutations were identified in heterozygosis: the c.1087A>G/p.Lys363Glu missense mutation detected in a sporadic case with severe form and the c.2024G>T/p.Trp675Leu change detected in a moderated case with recessive inheritance. In these patients the other changes were not found probably because of the technique limitations. The c.1720+52C>T mutation and c.1812C>T silence change were found in one patient with mild OI and dominant heritance, but absent in his affected mother, suggesting that these were no-pathogenic changes. Our results showed that almost 10% of Brazilian OI patients carry mutations in *LEPRE-1* gene. Moreover most of the carry patients have severe phenotype and are sporadic cases. These dates showed the importance of the study of *LEPRE-1* gene in OI and corroborate dates observed in other populations. Supported by Brazilian Institutions: CAPES, FAPES, FACITEC, CNPQ, Arcelor-Mittal, Brazil.

P12.183

An overview of clinical, biochemical and molecular findings in series of 36 COL1A1/COL1A2 mutation-negative osteogenesis imperfecta patients

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Autosomal recessive osteogenesis imperfecta (OI) is associated with mutations in an expanding list of genes, encoding collagen-modifying enzymes and chaperones (CRTAP, LEPRE1, PPIB, FKBP10, SERPINH1 and PLOD2), and more recently SP7 and SERPINF1.

We studied the presence of mutations in these genes in a carefully selected cohort of 36 COL1A1 /COL1A2 mutation-negative patients with a clinical diagnosis of OI type II, III or IV, or with Bruck syndrome (BS), a related phenotype characterized by bone fragility and congenital contractures.

Homozygous/compound heterozygous mutations were found in 19/36 probands. The majority of mutations were present in LEPRE1 (n=6) and FKBP10 (n=5) and, to a lesser degree, in CRTAP (n=3), SERPINF1 (n=2), PPIB (n=1), SERPINH1(n=1) and PLOD2 (n=1). In agreement with previous reports, LEPRE1, CRTAP and PPIB defects cause a severe to lethal osteochondrodysplasia that overlaps with but is distinctive from OI type II/III, whereas FKBP10 and PLOD2 mutations are associated with Bruck syndrome. Two probands with progressive OI harboured homozygous SERPINF1 mutations, and a homozygous p.Arg222Ser was found in SERPINH1 in a patient with OI type III. Biochemical collagen studies provide a valuable contribution to the diagnostic work-up for recessive OI as important overmodification of the collagen type I α- chains is seen in the presence of CRTAP and LEPRE1 mutations. An abnormal electrophoretic pattern for type I collagen was also observed for the SERPINH1 mutation, but not for mutations in the other genes. Our findings also indicate that still other gene(s) are involved in the pathogenesis of OI.

P12.184

Identification of nucleotide substitutions and mutations in the *Otoferlin* (*OTOF*) gene in a cohort of patients with auditory neuropathy (AN)

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OTOF gene mutations were identified causative for an isolated autosomal recessive form of congenital auditory neuropathy, DFNB9. Since 1998, molecular investigation identified absence of hotspot mutations, numerous polymorphisms and variants of unknown significance. The first 10 local patients were reported at ESHG 2011 meeting. Additional patients were studied.

Material and methods: Patients were enrolled if they fulfill criteria for AN. Molecular investigation relied on complete ORF sequencing (NM_194248.2, long isoform, 1997aa). Our molecular lab is the only one in Belgium to sequence complete ORF of *OTOF* gene. Substitutions were noted as 'unclassified variants' when absent from Ensembl and Uniprot databases or from available publications. Family members were investigated when available.

Results: Among 32 patients referred for AN, 24 had isolated NA among whom 15 were congenital (prelingual).1/15 was identified with 1 homozygous mutation (c.3269C>A); 2/15 carried one mutation and a variant, probably pathogenic[1] (c.[2401G>T;2402A>T];[2464C>T] and c.[2401G>T ;2402A>T;c.4936C>T];[3608A>G]). These last two propositus originated from unrelated pedigrees from Congo and Burundi. 1 patient was carrier of 2 undetermined variants (c.[2123G>A];[206+9G>A]); 1 patient of 2 variants (c.[3751T>C(;)5063C>T]) and

6 patients had one variant only.

Conclusion: The present screening confirms substitutions considered as pathogenic reported once[1] and present in different genetic background population. Identified compound heterozygous (one mutation and one variant, this last reported so far as 'probably pathogenic') may account for a less severe phenotype. Precise genetic counseling remains delicate. Molecular strategy encompasses *GJB2* and *GJB6* genes before *OTOF* ORF sequence screening in AN.

Reference: [1]*Romano J. J Hum Genet 2009;**54:**382-385*

P12.186

Novel mutation in Cathepsin C Gene (CTSC) and the Modeling of Mutated Protein in Three Iranian Families with Papillon Lefèvre Syndrome

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Papillon-Lefèvre syndrome (PLS) is a rare autosomal recessive disorder characterized by hyperkeratosis followed later by periodontitis, destruction of alveolar bone and loss of primary and permanent teeth. Mutation of the lysosomal protease Cathepsin C (CTSC) gene is the genetic cause of PLS. The CTSC Gene was analyzed in three Iranian families with affected PLS showing premature tooth loss and palm plantar hyperkeratosis. Direct automated sequencing of genomic DNA was performed following amplification of exonic regions and associated splice intron site junctions of the CTSC gene. Mutation screening and sequence analysis of the CTSC gene revealed a novel mutation (P. 35delL) in exon 1 of one patient, and two previously reported mutations in other probands. The known mutations were a missense mutation in exon 4 which converts Arginine to Proline (CGT→ CCT, CM993131, codon 210) and a nonsense mutation in exon 6 that causes a stop codon (CGA→TGA, CM993134, codon 272) in the other two patients. RFLP for 100 normal alleles confirmed the new mutation. Protein modeling of the deduced novel mutation was performed by online server Swiss-Prot automated modeling and analyzed by special bioinformatic softwares including ZMM, ICM-browser and SPDB-viewer to better understand the structural defects. The structural defect caused by the mutation P. 35delL admits the polarity nature of the molecule. As this mutation occurred in the conserved domain of the CTSC, the structural analysis might reveal inconsistency of the special binding sites in the CTSC molecule which could be very important in the functionality of the protein.

P12.187

The guanine nucleotide exchange factor kalirin-7 is a novel synphilin-1 interacting protein and modifies synphilin-1 aggregate transport and formation

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Synphilin-1 has been identified as an interaction partner of alpha-synuclein, a key protein in the pathogenesis of Parkinson disease (PD). To further explore novel binding partners of synphilin-1, a yeast two hybrid screening was performed and kalirin-7 was identified as a novel interactor. Kalirin-7 is a brain-specific guanine nucleotide exchange factor (GEF) enriched in the postsynaptic density (PSD) of excitatory synapses. It contains a SEC14 domain, a spectrin-like domain, a RhoGEF domain and a Pleckstrin homology domain (PH) which control multiple functions of the protein. Kalirin-7 activates Rac1 and regulates dendritic spine morphogenesis, plasticity and development. An interaction of kalirin-7 with huntingtin-associated protein 1 (HAP1), nitric oxide synthase (iNOS), and disrupted in schizophrenia 1 (DISC1) further links the protein to Huntington disease (HD), Alzheimer disease (AD), and schizophrenia.

In order to evaluate the functional relevance of this newly discovered interaction, we first focused on the ability of synphilin-1 to promote inclusion formation, as this feature provides a functional overlap of kalirin-7 and synphilin-1. By means of Co-immunoprecipitation, Fluorescent immunostaining, and Live cell imaging, a novel role for kalirin-7 in aggresome dynamics was identified.

P12.188

Parkinson disease: whole genome sequencing for the identification of novel genes

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Gene identification studies can be instrumental in the elucidation of disease mechanisms underlying neurodegenerative brain diseases such as Parkinson disease (PD) and eventually support the development of earlier and more accurate diagnostic tools as well as formulation of preventive or curing therapies.

We therefore performed whole genome sequencing in a Flanders-Belgian PD patient with an onset age of 24 years and both unaffected parents. Successive filtering of the identified variations was based on sequence quality, genomic location and frequency in both the '1000 genomes project' and an extended collection of Flanders-Belgian individuals. Finally, variations were selected based on segregation in line with one of the four possible inheritance patterns. Focusing on high-confidence coding and splice site variations we identified 115 de novo, 40 homozygous recessive, 55 heterozygous compound recessive and 11 X-linked variations. Genetic validation in an extended cohort of geographically matched control individuals (N=1000), to exclude polymorphisms, resulted in 16 variations in 10 genes with a high potential to be linked to PD in this Flanders-Belgian nuclear family. We are currently estimating the contribution of these variations to PD pathogenesis in our cohort of Flanders-Belgian PD patients (N=600) using various genotyping platforms. Further we plan to sequence the selected candidate genes using a custom MASTR-NGS assay to identify other mutations in these genes to further support a role in PD pathogenesis. Eventually, functional characterization of the probable pathogenic variant(s) will be performed to gain insights into the mutation mechanism and disease processes.

P12.189

Caspase 8 and caspase 9 activation in peripheral blood lymphocytes of patients with LRRK2-associated Parkinson's disease

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Mutations in the LRRK2 are the most frequent cause of familial Parkinson's disease (PD). Although the precise physiological and pathological role of LRRK2 is unclear, direct link between mutant LRRK2 and apoptosis has been suggested. There are two main caspase activation pathways of apoptosis, receptor-mediated sequential activation of caspase-8, and cytochrome c-dependent mediated caspase-9 activation. Earlier we showed higher level of spontaneous apoptosis in patients with LRRK2-associated PD compared to controls (persons without neurological disorders).

The aim of our present work was to examine the level of active caspase 8 and caspase 9 in peripheral blood lymphocytes (PBLs) after incubation (37°C, 5% CO2). PBLs of patients were isolated from venous blood. Cells were resuspended in RPMI1640 with Fetal Calf Serum and were incubated for 48 hours. We estimate the level of active caspase 8 of PBLs in two patients with LRRK2-associated PD (the G2019S mutation) compared controls (n=4) after 1h, 24h, 48h and caspase 9 after 24h of incubation by western blot analysis. β-actin was tested as an internal standard. The active enzyme subunits are 18kDa for caspase 8 and 10kDa for caspase 9. We found the activation of caspase 8 in both patients with the G2019S mutation and in all controls after 1h, 24h, 48h of incubation. The active caspase 9 was detected in both carriers of the G2019S mutation and in one from four controls after 24h of incubation. Thus, predominant activation caspase 9 in patients with LRRK2-associated PD could be suggested.

P12.190

Novel PCDH19 mutations in Bulgarian epilepsy cases

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X-linked female-limited epilepsy is characterized by seizure onset in infancy or early childhood and cognitive impairment. The spectrum of phenotypes has been extended to include female patients with early infantile epileptic encephalopathy, type 9 (EIEE9). Heterozygous PCDH19 mutations were identified in EIEE9 patients. Interestingly, only heterozygous females are affected, while hemizygous males are devoid of seizures or cognitive impairment, although mosaic males can also be affected. This inheritance supposed cellular interference as possible pathogenic mechanism.

We report on two Bulgarian patients with typical clinical manifestation of EIEE9. Two novel mutations were detected in the PCDH19 gene: c.2705dupA; p(Asp902Lysfs*6) and c.1091delC; p.Pro364Argfs*4 (both in heterozygous state). Surprisingly, the mother of the first patient was heterozygous asymptomatic carrier of c.2705dupA. She has never suffered epileptic seizures and intellectual impairment. The second mutation c.1091delC was de novo.

To the best of our knowledge, this is the first case of a female heterozygous for a mutation in the PCDH19 gene who did not manifest any clinical features. Usually, the PCDH19 mutations arise de novo, are inherited by the unaffected carrier fathers or by affected mothers. The possibility for totally skewed X inactivation is very unlikely as it has never been detected in PCDH19 mutation-positive patients. Thus, one could speculate that, due to the complex interaction with other genetic, epigenetic factors, some PCDH19 mutation carriers could even fully "escape" any disease manifestation, and this could be the case in our patient's mother.

Acknowledgments
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P12.191

Identity-by-Descent Mapping Reveals a New Locus for Primary Congenital Glaucoma, GLC3E, on Chromosome 19p13.2

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Primary congenital glaucoma (PCG) is caused by developmental anomalies of the trabecular meshwork and the anterior chamber angle, resulting in an increased ocular pressure (IOP) and optic nerve damage from birth or early infancy. In general PCG displays an autosomal recessive inheritance and is genetically heterogeneous. To date, four PCG loci are known (GLC3A-D), in which two genes have been identified, *CYP1B1* and *LTBP2*. Here, we aimed to map the disease gene in a large, four-generation consanguineous family with PCG, originating from Jordan. Mutations in known PCG genes were excluded. Identity-by-descent (IBD) mapping was performed in six affected members using genome-wide SNP genotyping with 250K arrays. The common IBD regions did not overlap with any known PCG loci. Filtering on both size of the region and number of consecutive homozygous SNPs revealed a new candidate region on 19p13.2, named GLC3E. This region measures 2.67 Mb and contains 93 genes. Using prioritization tools, *BEST2* was selected as the best candidate gene. Indeed, Best2 is expressed in non-pigmented epithelial cells of ciliary body, which is responsible for formation of aqueous humour. Also, Best2-/- mice have significantly lower IOP than wild type littermates. Sanger sequencing of *BEST2* in affected individuals revealed no mutations however. To analyze the other genes in the IBD region, two affected individuals underwent exome sequencing for which data analysis is currently ongoing. We identified a potential new PCG locus, named GLC3E, confirming the genetic heterogeneity of PCG, and representing a unique opportunity to identify the third PCG gene.

P12.192

Molecular genetic background of PEHO syndrome

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The PEHO syndrome (Progressive encephalopathy with Edema, Hypsarrhytmia and Optic atrophy; MIM 260565) is a severe autosomal recessive inherited progressive infantile encephalopathy. The main features of PEHO syndrome are hypotonia, infantile spasms and/or hypsarrhytmia, psychomotor retardation, absence or early loss of visual fixation, edema of the face and limbs, and typical dysmorphic features. Brain atrophy is progressive and most prominent in the cerebellum, where the molecular layer is strongly reduced, Purkinje cells are abnormally small and misaligned, and the cells of the internal granular cell layer are almost absent. Based on imaging and neuropathological findings we have divided the PEHO syndrome into two types, the cortical atrophy and loss of myelin being pronounced in type 2.

Using a 318 k genome-wide SNP scan we identified a 435 kb region on chromosome 17 that was homozygous in Finnish type 1, but not type 2 patients. Sequencing of positional candidate genes revealed a missense mutation in a gene not previously associated with human disease in all but one patient with type 1 PEHO. Mutations were not present in type 2 patients. The

"PEHO1" gene encodes a protein possibly involved in transcriptional regulation. It is widely expressed in the brain, the expression pattern being compatible with the neuropathological findings. In developing mouse brain, the "PEHO1" protein expression is strong in neural progenitor and migrating premature granular cells. Transient overexpression studies revealed no difference in subcellular localization. This is the first step towards understanding the pathogenesis of PEHO, that is genetically heterogeneous.

P12.193
Perrault syndrome: Evidence for genetic heterogeneity and whole-exome sequencing to identify novel molecular mechanisms

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Autosomal recessively inherited Perrault syndrome (MIM233400) is a clinically and genetically heterogeneous disorder. It is typically characterized bilateral hearing loss, ovarian dysgenesis in female patients, and can be associated with neurological manifestations such as mental retardation and cerebellar ataxia. A proposed clinical classification suggests type I does not show neurological symptoms while type II patients do show variable affection of the nervous system. Recently, mutations in HSD17B4 and in HARS2 genes were described in some, but not all, patients with Perrault syndrome. Here we report a 16 years old girl with Perrault syndrome born to consanguineous parents, who presented with ovarian agenesis, microcephaly, mental retardation, neurologic abnormalities including ataxic gait, and no speech acquisition. Hearing impairment was not observed. Her karyotype was normal (46,XX). We excluded homozygosity of regions of described genes for Perrault syndrome by marker analysis. Subsequently, we performed whole-exome sequencing. Our strategy for the identification of the causative gene is based on an initial determination of homozygous stretches using all identified variants followed by a prioritization of likely damaging variants within these homozygous stretches. Using this innovative filter strategy we aim to identify a new gene underlying Perrault syndrome giving more insights into the pathogenic mechanism of the disease

P12.194
A novel splice site B3GALTL mutation confirms typical Peters plus syndrome in two Tunisian patients

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Peters plus syndrome is an autosomal recessive rare disorder comprising ocular anterior segment dysgenesis, short stature, hand abnormalities, distinctive facial features, and often other major/minor additional defects. Only six mutations in the B3GALTL gene were recently reported in patients with Peters plus syndrome, leading to the inactivation of the B1, 3 -glucosyltransferase involving in the synthesis of a rare disaccharide that occurs on thrombospondin type 1 repeats of many biologically important proteins. In our study, we screened the B3GALTL gene in two unrelated Tunisian patients with typical Peters plus Syndrome. A novel homozygous c.597-2 A->G mutation was identified in both patients. Bioinformatic analyses using the MFOLD and the EMBOSS programs showed that this mutation modulates the pre mRNA secondary structure of the gene, and decreases the score value related to the formation of splicing loops. Moreover, the c.597-2 A->G mutation is located in a CpG island of the B3GALTL coding region, eliciting a potential epigenetic role of this position including gene's methylation and regulation. These data confirm an important role of the B3GALTL gene test that provides diagnosis confirmation and improves dramatically genetic counselling for the families.

P12.195
Molecular genetic analysis of PAH gene in Belarus: two novel mutations

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Phenylketonuria (PKU) is one of the most common inherited monogenic diseases in Belarus, its frequency is 1:6000 newborns. Classical PKU (OMIM#261600) is caused by mutations in PAH gene.

We completed the molecular-genetic analysis of R158Q, R261Q, Y414C, IVS10-11G> A and IVS12+1G>A mutations and sequencing of six exons of PAH gene in patients with PKU in Belarus. Identified mutations are shown in table 1.

Table 1. Mutations of PAH gene in patients with PKU in Belarus.

Exon/Intron	Mutation	No. of Chr.	Frequency %
I2 E3	IVS2-13T>G	6	0,96
	R111X	3	0,48
	D84Y	1	0,16
	P89S	1	0,16
E5	R158Q*	40	6,4
E6	E221D222FSAGdel	1	0,16
E7	E280K	15	2,4
	R261Q*	9	1,44
	R252W	8	1,28
	P281L	4	0,64
	R241C	2	0,32
	R243X	2	0,32
	G272X	2	0,32
	R261X	1	0,16
E9	L311P	3	0,48
	I306V	2	0,32
I10 E11	IVS10-3C>T	5	0,8
	IVS10-11G>A*	3	0,48
	Y386C	2	0,32
	E390G	1	0,16
	c.1127_c1132dup**	1	0,16
I12 E12	IVS12+1G>A*	8	1,28
	R408W	429	68,75
	A403V	1	0,16
	R413P	1	0,16
	Y414C	3	0,48
	del427C**	1	0,16
Unidentified		69	11,06
Total		624	88,94

* identified by RFLP-analysis
** novel mutation

We identified two novel mutations, each on single chromosome: duplication c.1127_c.1132 in exon 11 and deletion 427C in exon 12. Both mutations were found in compound heterozygosity with R408W. In addition to mutation R408W, significant frequency in the Belarus population also have mutations R158Q (6,4%), E280K (2,4%), R261Q (1,4%), R252W (1,3%) and IVS12+1G>A (1,3%).

P12.196
Improvement of the PKHD1 mutation database for autosomal recessive polycystic kidney disease (ARPKD)

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Mutations in the PKHD1 gene on 6p12 are associated with autosomal recessive polycystic kidney disease (ARPKD). This primary ciliopathy is characterized by enlarged bilateral polycystic kidneys and congenital hepatic fibrosis. The PKHD1 gene (longest ORF 66 exons) is highly complex due to its genomic size (470 kb). Its gene product polyductin/fibrocystin contains 4074 aa. Mutation detection rate is up to 90% with at least one detected mutation. The mutations are scattered throughout the whole gene. The vast majority of PKHD1-mutations are "private" mutations. Pathogenic mutations divide into about 60% missense and 40% truncating/splice mutations. Our PKHD1 mutation database (http://www.humgen.rwth-aachen.de) has been established to catalogue all changes detected in the PKHD1 gene in a disease specific database e. g. for use in clinical practice. Here we report about the revision and extension of the database with the aim to alleviate the evaluation of PKHD1 variants regarding their possible clinical significance. For this purpose evolutionary conservation and a more detailed bioinformatic validation of the 329 included missense changes (PolyPhen2) and segregation analyses data were re-evaluated and included. Additionally all changes were synchronized to dbSNP (version 135) entries, the allele frequencies and rs-numbers of 116 changes were added. Furthermore about 50 new variants have been added. In total the database now includes 752 entries dividing into 396 clearly pathogenic changes, 202 likely neutral and 154 changes of unknown significance. Evaluation of these changes will be the topic of further studies.

P12.197
AMH gene mutations in two Egyptian families with persistent mullerian duct syndrome

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Introduction: The anti-mullerian hormone (AMH) is responsible for regression of müllerian ducts during male sexual differentiation. Mutations in AMH or its type II receptor lead to persistence of the uterus and fallopian tubes in male children i.e. persistent müllerian duct syndrome (PMDS). Both conditions are transmitted according to a recessive autosomal pattern and are symptomatic only in males.

Patients and Methods: We report two unrelated Egyptian consanguineous families with PMDS. The first family comprised 3 affected prepubertal sibs complaining of undescended testis, pelvic exploration and laparotomy revealed mullerian derivatives. The other family was presenting with an adolescent male with impalpable left testis and pelvic exploration showed remnant of fallopian tubes and rudimentary uterus. AMH levels were very low and almost undetectable in all affected patients in both families. Direct sequencing of the coding region of the AMH gene identified two homozygous mutations in exon 1, R95X in the first family and V12G in the second family.

Conclusion: These data confirmed the autosomal recessive type of the PMDS, which needs molecular investigations of this rare disorder on large number of cases with undescended testis in Egypt will be of great value for proper diagnosis and genetic counseling.

P12.198

Phenotype and genotype variability of the PMM2 gene mutation among the same Egyptian family

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Deficiency of phosphomannomutase (PMM2) is considered the most common congenital disorder of glycosylation (CDG) and designated as CDG1a. Phenotypic presentation is variable from severe disorder to milder phenotype. Here we describe an Egyptian family with 4 affecteds from 2 generations with mutation in PMM2 gene with different genotype and phenotype presentation in each generation. The older generation with 3 affected who was the paternal side sibship for our proband and were derived from first cousin parents. All of them had moderate to severe psychomotor retardation, the male patient never experienced walking and was non educable The older female affected was able to walk at the age of 6 year, and the younger female was able to walk with support and had a mild intellectual impairment. Interestingly, the female patients had no menstrual cycle. The hormonal and abdominopelvic sonar investigations revealed a premature ovarian failure in the affected females. Our proband was a six years old male patient from far relative parents with a normal cognitive function He had hypotonia, mild intesion tremors and ataxic gait although his MRI scans showed severe cerebellar atrophy. Strabismus was note in the second year of life and corrected by surgical intervention. The sequence analysis of the PMM2 gene revealed a compound heterozygous mutation in Exon 5 and in exon 8 in the proband and a homozygous mutation in Exon 8 in the other affected family members. This consanguine family demonstrated a phenotype and genotype variability of the PMM2 gene.

P12.199

Mutations in Rotatin link primary cilia function to organization of the human cerebral cortex

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Polymicrogyria is a post-migratory organization defect of the cerebral cortex characterized by many small gyri with abnormal cortical lamination. Here, we identified autosomal recessive mutations in the Rotatin gene, *RTTN*, in patients with polymicrogyria from separate families. Rotatin determines early embryonic axial rotation as well as anteroposterior and dorsoventral patterning in mouse. We show that Rotatin colocalizes with the basal bodies at the primary cilium. Cultured fibroblasts from patients have structural abnormalities of the cilia and show down-regulation of *BMP4*, *WNT5A* and *WNT2B*, key regulators of planar cell polarity, expressed at the cortical hem, the cortex organizing center giving birth to Cajal-Retzius (CR) neurons. Indeed, in mouse embryos Rotatin expression co-localizes with CR neurons. Knockdown experiments in human fibroblasts and neural stem cells confirm a role for Rotatin in cilia structure and function. Rotatin mutations thereby link aberrant ciliary function to abnormal development and organization of the cortex in human patients.

P12.200

Mutations in a novel dynein assembly factor PF22 (DNAAF3) cause cilia dysmotility and left-right-axis defects

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Primary ciliary dyskinesia (PCD) is an autosomal recessive disorder arising from dysmotility of cilia in the respiratory tract, brain ventricles, oviduct and the embryonic node leading to chronic obstructive pulmonary disease, reduced fertility and situs abnormalities. With a frequency of up to 1 in 10,000 PCD is a rather common "rare disease". To date, 12 genes causing approximately 40% of all cases have been identified, two encoding proteins (KTU, LRRC50) involved in cytosolic axonemal dynein co-assembly. Using homozygosity mapping and subsequent Sanger sequencing we have identified mutations in a new gene, C19ORF51 in PCD patients with absent dynein arms. We find that the Chlamydomonas ortholog of C19ORF51, PF22, is involved in the cytoplasmic assembly of the outer dynein arms preceeding their import into the axoneme. In PF22 mutants, axonemal dynein heavy chain stability as well as co-assembly of heavy with intermediate chains is significantly disturbed and PF22 appears to act downstream of KTU and LRRC50 in the dynein preassembly pathway. In zebrafish, PF22 knockdown results in a typical ciliopathy phenotype (axis curvature, pronephric cysts, hydrocephalus and situs inversus) due to loss of the axonemal dynein arms resulting in cilia dysmotility. We therefore propose a conserved multi-step pathway for formation of assembly-competent dynein complexes, and that PF22 (renamed DNAAF3, "dynein axonemal assembly factor 3") mutations causes PCD with situs inversus due deficient cytoplasmic dynein assembly resulting in absent dynein arms.

P12.201

Novel molecular findings in patients with primary hyperoxaluria and implications for advanced molecular testing strategies

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Primary hyperoxaluria (PH) constitutes a group of autosomal recessive disorders characterized by excessive endogenous oxalate synthesis resulting in nephrocalcinosis and/or urolithiasis. Currently three types of PH (PHI-III) can be accurately defined. In contrast to the well-characterized entities of PHI and PHII, the pathophysiology and prevalence of recently described PHIII, caused by *HOGA1* mutations, is largely unknown.

In this study, we analyzed a large patient cohort previously tested negative for PHI/II by complete *HOGA1* sequencing. Seven distinct mutations, among them four novel, were identified in 15 patients. In patients of non-consanguineous European descent the previously reported c.700+5G>T splice-site mutation was predominant and represents a potential founder mutation. *In vitro* analysis of the c.700+5G>T mutation using a minigene assay showed activation of a new splice site 52 bases downstream from the wild-type donor splice site leading to an in-frame insertion of 17 amino acids to the native protein. Interestingly, we furthermore identified a family where a homozygous mutation in *HOGA1* (p.P190L) segregated in two siblings with an additional *AGXT* mutation (p.D201E).

First functional analysis revealed that HOGA1 deficiency is causing PHIII, yet reduced HOGA1 expression or aberrant subcellular protein targeting are unlikely the responsible pathomechanisms.

Our results strongly suggest *HOGA1* as a major cause of PH, indicate a greater genetic heterogeneity of hyperoxaluria spectrum, and point to an exceptionally favourable outcome of PHIII despite incomplete or absent biochemical remission. Potential multiallelic inheritance in PH has implications for genetic testing strategies and might represent an unrecognized mechanism for phenotype variability.

P12.202

Etiopathogenesis of primary lymphedema

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Lymphedema is a soft tissue swelling resulting from abnormal accumulation of interstitial fluid containing high molecular weight proteins due to abnormal drainage of lymph by the lymphatic vasculature. Primary lymphedema is due to an abnormal development and/or function of the lymphatic system. Some of the cases are inherited with autosomal dominant or recessive mode of inheritance with incomplete penetrance and variable expression. Mutation screening of a large series (n=400) of sporadic and familial index patients led us to discover several mutations in syndromic and non-syndromic patients in the known lymphangiogenic, including FOXC2, VEGFR3, SOX18, CCBE1, PTPN14, GCJ2, GATA2 and KIF11. Yet, only 10% of the patients are explained by (an) identified inherited dominant or recessive mutation, or a de novo change. Samples without a mutation are extremely valuable for our ongoing genome-wide strategies to identify additional genes mutated in primary lymphedema. Among them, we have two consanguineous families with recurrence of a congenital lymphedema without a mutation in the known genes. Whole genome scan using Affymetrix SNP-Chip 250K was thus performed and no copy number change was identified in the affected members. Autozygosity mapping and parametric linkage analysis confirmed the exclusion of the 8 candidate lymphedema-genes. Interestingly, three overlapping autozygous regions were identified in the two families. These three identified loci are analyzed using massive parallel sequencing to identify novel lymphedema-causing mutations and genes. Our findings provide clear evidence for the existence of (a) new causative gene / genes associated with the autosomal recessive form of the disease. (miikka.vikkula@uclouvain.be).

P12.203

Clinical and genetic study of 46 Italian patients with primary lymphedema

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Primary lymphedema is characterised by altered morphological development of lymphatic vessels causing fluid accumulation in interstitial spaces. In familial forms, it is transmitted as a dominant Mendelian trait with heterozygous mutations in genes involved in lymphangiogenesis. We used PCR and direct sequencing to analyse only the region of the FLT4 gene encoding the „tyrosine-kinase domain" and the single exon of the FOXC2 gene, in 46 Italian probands with primary lymphedema, 43 of whom had familial forms. We identified 12 mutations in 12 patients (12/46, 26%), six in the FLT4 gene and six in the FOXC2 gene; most of the mutations (9/12, 75%) were new and none were identified in 100 healthy subjects or listed in the NCBI dbSNP. Two of the new mutations determine the synthesis of a prematurely truncated protein, while the other seven cause amino-acid changes that we classified in three distinct categories according to their degree of conservation in the course of evolution, and chemico-physical parameters of wild-type and mutant amino acids. A clear relation emerged between genotype and phenotype because 4 (80%) of the 5 probands with onset at birth showed FLT4 mutations and 4 (80%) of the 5 probands without distichiasis and with FOXC2 mutations had an amino-acid substitution outside the forkhead domain, in line with data recently reported by van Steensel MAM et al. (2009). Besides allelic heterogeneity shown by „private" mutations in each proband, the absence of mutations in almost 75% of familial cases of primary lymphedema also suggests genetic heterogeneity.

P12.204

Restrictive dermopathy-like phenotype caused by the homozygous mutation *LMNA* p.R435C due to partial uniparental disomy of chromosome 1

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Restrictive Dermopathy (RD) is a rare autosomal recessive disorder characterized by intrauterine growth retardation, tight and rigid skin with prominent superficial vessels, bone mineralization defects, dysplastic clavicles, arthrogryposis and early death soon after birth. RD can be caused by mutations in *LMNA* or *FACE1*. *FACE1* codes for the ZMPSTE24 protein, which is necessary for the processing of prelamin A. *LMNA* mutations may affect the cleavage site for ZMPSTE24 resulting in progeroid syndromes. We report a patient affected by a progeroid syndrome with RD-like features. Besides missing hairiness, stagnating weight and growth, RD-like features including skin swelling and solidification, acrocontractures, osteolysis and muscular hypotension were continuously progressive until the patient died at the age of 11 month. For mutational analysis, the complete coding region including intron/exon boundaries of the *LMNA* and *FACE1* genes was amplified and used for direct Sanger sequencing. As a result, the homozygous mutation *LMNA* p.R435C was found. Interestingly, this mutation is not located at the cleavage site necessary for processing of prelamin A by ZMPSTE24. This might explain the atypical phenotype compared to other published cases of RD. Sequencing of the not consanguineous parents showed that the mutation was present only in the mother in heterozygous state, but not in the father who was wild type. MLPA analysis confirmed that the patient had two copies of the gene. Direct Sanger sequencing of highly polymorphic markers on chromosome 1 showed a partial uniparental disomy of chromosome 1 (1q21.3 to 1q23.1) including the *LMNA* gene.

P12.205

Homozygosity mapping of two Omani Arab families with progressive myoclonic epilepsy

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Progressive myoclonic epilepsy (PME) refers to a group of neurodegenerative diseases which are clinically and genetically heterogeneous. Five specific disorders have identified as the most frequent causes of PME including: Unverricht-Lundborg disease (Baltic myclonus); myoclonus epilepsy and ragged red fibres (MERRF syndrome); Lafora body disease (LBD); neuronal ceroid lipofuscinoses (NCL); and type I sialidosis. The main clinical feature shared by this group of disorders is progressive neurodegeneration accompanied by myoclonias and epilepsy. As a part of a larger research project on the genetics of inherited neurological disorders in Oman, this study was carried out to investigate genetic abnormalities in two Omani families with PME. The study comprised of two families with total of five affected individuals initially diagnosed with PME . Age of onset ranged from 7-17 years. Symptoms included progressively increasing myoclonic jerks with action and stimulus sensitive myoclonus, generalized tonic-clonic seizures, and ataxia**.** Some patients suffered also from memory and mental impairment and visual deterioration. Genome-wide homozygosity mapping was performed using Illumina 33K SNP arrays. SNP microarray analysis identified a shared 1.7 Mb autozygosity region between the affected sibs on chromosome 12q22.3, flanked by the two markers; rs13048089 and rs3788151. This region encompasses 54 genes, among which is *CSTB* which known to cause myoclonic epilepsy of Unverricht and Lundborg (ULD, or EPM1). Other candidate genes associated with epilepsy in this region include *PDXK* and *TRAPPC10*. Sequencing of candidate genes is in progress.

P12.206

Mutation in CUL3 is the cause for Pseudohypoaldosteronism type II in a Turkish family.

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A rare Mendelian form of hypertension is Pseudohypoaldosteronism type II (PHAII), also known as Gordon hyperkalemia-hypertension syndrome (OMIM 145260). Mutations in *WNK1* and *WNK4*, and two recently identified genes (*KLHL3* and *CUL3*) have been reported as causes of monogenic hypertension. In a Turkish family (parents and 5 children) one daughter was suspected of PHAII. This daughter has been diagnosed at age 24 years with severe hypertension (200/130 mm Hg), hyperkalemia (6.5 mmol/l; normal range 3.5-5.1), and normal renal function. All other family members are healthy.

We hypothesized that a *de novo* mutation in the daughter is the most likely cause of her disease. Sanger sequencing of *WNK1* and *WNK4* and SNP array analysis (OmniExpress, Illumina) did not reveal any mutation or large chromosomal abnormality. Full exome sequencing (Nimblegen SeqCapEZV2, Illumina HiSeq2000) of all seven family members identified 34,733 unique variants in our patient. Filtering against dbSNP, 1000Genome project, variants found in unaffected family members, and selecting loss or gain of function mutations resulted in 481 possible disease-causing variants. By comparing the recent mutations reported by Boyden et al (Nature, Febr 2012), we found an identical *de novo* mutation in *CUL3*, a splice acceptor site mutation in intron 8 resulting in the skipping of exon 9. This results in an in-frame 57 amino acid deletion abrogating the function of CUL3.

This *de novo* splice acceptor site mutation found in a second patient with PHAII confirmed *CUL3* as a cause of PHAII in line with Boyden et al.

P12.207

RUNX2 overexpression provides evidence for the involvement of a pro-osteogenic signaling pathway in pseudoxanthoma elasticum

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Aim. Pseudoxanthoma elasticum (PXE) is characterized by oculocutaneous and cardiovascular manifestations, due to mineralization and fragmentation of elastic fibers. The pathophysiology of ectopic calcification is unclear and hypotheses include oxidative stress and an unidentified serum factor. PXE patients also have diminished vitamin K (VK) serum concentrations, leading to inefficient activation of the VK-dependent mineralization inhibitor matrix gla protein (MGP). MGP acts as inhibitor of the pro-calcifying Bone Morphogenetic Protein 2 (BMP2). One of several BMP2-associated pathways is the "BMP2-Smad-RUNX2" pathway, where RUNX2 acts as transcriptional regulator of proteins involved in mineralization, osteogenesis and apoptosis. We studied the role of this signaling pathway in PXE.

Methods & Results. Immunohistochemistry for BMP2, Smad 1-4-5-8 and RUNX2 in whiskers of Abcc6 knock-out mice and human PXE dermis showed positive labeling, co-localizing with mineralization compared to controls, which was confirmed via qPCR on human PXE fibroblasts. TUNEL assays revealed significant increase in apoptosis in PXE fibroblasts. Caspase 3 stains demonstrated co-localization of apoptosis with mineralization foci in all tissues. Comparable qPCR results and apoptosis rates were obtained on PXE serum inoculated control fibroblasts.

Discussion & Conclusion. Our results indicate the involvement of the pro-osteogenic BMP2-Smad-RUNX2 pathway in PXE. RUNX2 upregulation can explain the expression profile of proteins previously implicated in PXE, leading to ectopic mineralization or neovascularisation. By also demonstrating an effect of oxidative stress and PXE serum, three principal pathophysiological observations in PXE can be merged in this pathway, which may provide therapeutic options through small-molecule inhibitors of BMP receptors.

P12.208

Mutational analysis of an iranian patient with rhisomelic chondrodysplasia punctata type I (RCDP I) revealed a novel homozygous mutation in WD1 domain of *PEX7* gene

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Rhizomelic Chondrodysplasia Punctata (RCDP) is a peroxiomal biogenesis disorder which is categorized into three types. The genetic abnormality that causes RCDP type1 is *PEX7,* which is responsible for peroxisomal sorting of peroxisomal targeting signal type 2 containing proteins. Peroxisomal targeting signal type 2 consists of a nonapeptide that is located at the N-terminus of several peroxisomal matrix proteins.

In the present study, mutational analysis performed on an Iranian patient's fibroblast cells with RCDP distinct clinical phonotype. At the first step, total RNA extraction and cDNA synthesis carried out. RT-PCR product consisting whole length of *PEX7* cDNA was inserted into T/A cloning vector and sequenced. To evaluate functionality of detected mutation, mutant and normal CDS of *PEX7* was cloned into an expression vector PDB2 and co-transfected into fibroblast cells.

Sequence data revealed a missense homozygous mutation of G to A at nucleotide 257 in exon3 of *PEX7* coding sequence. Moreover, genomic analysis of *PEX7* gene confirmed the mutation in the mentioned location. This mutation caused one amino acid residue substitution of Cys to Tyr at codon 86 located on WD1 repeat domain region severely affected the functionality of *PEX7* protein. Back-transfection of vector containing mutant CDS of *PEX7* did not restore the normal peroxiome function in RCDP patient's fibroblasts unlike the native type of *PEX7*.

P12.209

Renal tubular dysgenesis: report of one case

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Renal tubular dysgenesis (RTD) is an autosomal recessive disease, which was described in stillborn siblings in 1983 by Allanson. In 2005 Gribouval et al. first reported that mutations in the genes coding renin-angiotensin system (RAS) are associated with RTD. The disease is characterised by an early onset and persistent fetal anuria that results to oligohydroamnion, Potter ,s syndrome, lung hypoplasia and calval hypoplasia. Inherited RTD represents a disease with a very severe course, which is lethal in most of the cases.

Specific pathomorphological change found in kidney with RTD are represented by either absence or poorly developed renal proximal tubules. Here we report a casuistic of a newborn with RTD - this is the first reported case of inherited RTD in Slovakia with evidence of a novel mutation in RAS. A male infant died eleven hours after birth because of a respiratory failure. Renal histology and following genetic analysis confirmed diagnosis of renal tubular dysgenesis. A novel homozygous R259C mutation in the gene coding angiotensin-converting enzyme (ACE) was found. This mutation was evaluated with the prediction tool PolyPhen-2. R259C mutation is predicted to be probably damaging with a score of 1.000. Consequent mutation analysis found the same heterozygous mutations in both parents, who are asymptomatic. Identification of this mutation now allows genetic counseling and prenatal diagnosis.

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P12.210

High Prevalence of Mutations in the CRB1 Gene in Spanish Patients with Congenital and Child-Hood Onset Retinal Dystrophies

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Purpose: Mutations in the CRB1 gene have been associated with severe con-

genital and early-onset forms of retinal dystrophies including Leber congenital amaurosis (LCA), retinitis pigmentosa (RP) and cone-rod dystrophies. The aim of this study was to determine the incidence of CRB1 mutations in Spanish patients with congenital and/or early-onset retinal dystrophies.
Methods: 91 LCA and 255 Early-Onset RP unrelated Spanish patients were first analyzed with an APEX based microarray (LCA or ARRP chip, Asper Ophthalmics). All mutations were confirmed by direct sequencing and familial segregation was also verified. In patients without any mutation or with only one mutation, high-resolution melting (HRM) and multiplex ligation-dependent probe amplification (MLPA) analysis were further performed to complete the mutational screening of the CRB1 gene. Haplotype analysis and whole genome homozygosity mapping were also performed in 143 families.
Results: Known variants in CRB1 were identified in 42 of 346 (12%) patients by genotyping microarray, of them 31 patients (10%) carried two pathogenic disease alleles.
Conclusions: This study proved that 10% of Spanish patients with early onset Retinal dystrophies carried mutations in CRB1, ranging from 7% of early-onset RP cases to 15% of LCA families. The most frequent mutation in our cohort was p.Cys948Tyr, which was present in 22% of the alleles (15/68) in 13 families.

P12.211

Next-generation sequencing of 53 known genes for retinitis pigmentosa and allied diseases identifies the causative mutations in the majority of 40 patients

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Retinitis pigmentosa (RP), Leber congenital amaurosis (LCA) and Stargardt disease (STGD) are genetically extremely heterogeneous conditions, making mutation analysis difficult. We developed a diagnostic next-generation sequencing (NGS) approach targeting all coding exons of 53 genes: 31 for autosomal recessive RP (arRP; 413 exons), 23 for autosomal dominant RP (adRP; 248 exons) and 15 LCA genes (196 exons). We investigated 40 patients (33 with arRP, sporadic RP or STGD; 1 with adRP; 6 with LCA/early-onset severe retinal dystrophy). Sequence variants were identified using an in-house bioinformatic pipeline and JSI SeqNext-Software, and verified by Sanger sequencing. Biallelic mutations in *ABCA4*, *RP1*, *CRB1*, *TULP1*, *EYS* and *PDE6B* were found in 2 - 5 patients each and accounted for 50% of cases. Biallelic mutations in *C2ORF71*, *MERTK*, *RPGRIP1*, *CEP290* and *RDH5* were identified in single individuals. In one patient, a heterozygous truncating mutation was identified in the autosomal recessive disease gene *SAG*. Two patients turned out to be affected by adRP that appeared sporadic due to incomplete penetrance of *PRPF31* mutations in first-degree relatives, and the only patient with a family history indicating adRP carried a novel heterozygous *RHO* mutation. In summary, we identified the genetic basis of disease in 29/40 patients (72.5%). The identification of dominant mutations in apparently recessive cases and the involvement of 14 genes (although mutations in 6 genes account for two thirds of cases) illustrate the benefit of massively parallel sequencing of all RP genes regardless of the assumed mode of inheritance.

P12.212

Clinical and molecular analysis of Rett syndrome patients from the University Hospital of Medical School from Ribeirao Preto - Brazil

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Rett Syndrome is a developmental disorder caused by mutations in the MECP2 gene located on X chromosome. Generally affects feminine individuals with an incidence of 1 in each 10.000-15.000 newborn girls. The syndrome leads to total or partial loss of cognitive, sensorial, emotional, motor and autonomic functions of the brain. Patients generally present deficits in the areas of learning, speaking, sensorial perception, mood management, movements, breathing, heart function, severe scoliosis, and even on the chewing, swallowing and digesting areas. These symptoms arise in 6 to 18 months old girls, after an apparently normal development, when the deficit of the psychomotor abilities becomes more evident. Laboratorial diagnosis can be done by screening for mutations along the coding region of the MECP2 gene and its adjacencies. This work focus on identifying and characterizing mutations on the MECP2 gene in patients clinically diagnosed as Rett syndrome, and to study the relationship between their genotype and phenotype. DNA was extracted from blood samples after parents signing the informed consent form, and High Resolution Melting technique and DNA sequencing were employed to screen for mutations. While searching for mutations in MECP2 exons, we have found a missence mutation (T158M) (GenBank X99686). All the four exons and the promoter region will be screened and genetic counseling will be offered to the families carrying mutations. This methodology is efficient for confirming the diagnosis of patients under suspect of Rett syndrome, a subdiagnosed pathology due to its complex clinical suspicion.
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P12.213

Comparison of MLPA, microarray and qPCR data in patients with Rubinstein-Taybi syndrome and CREBBP deletions

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There are mainly three techniques for DNA copy number analysis in the genome: (i) multiplex ligation-dependent probe amplification (MLPA), (ii) genome-wide microarray analysis, and (iii) quantitative PCR (qPCR). We aimed to compare the precision of MLPA and microarray results in breakpoint determination using a cohort of patients with RTS.
Using MLPA (Kit P313, MRC Holland), we identified 16 patients with Rubinstein-Taybi syndrome (RTS) and CREBBP deletions. Eleven patients (73%) had deletions extending beyond CREBBP: 7 patients (47%) showed (only) distally flanking deletions, 3 patients (20%) showed (only) proximally flanking deletions, and one patient (7%) demonstrated a large deletion flanking CREBBP on both sides, encompassing 860 kb and ~20 genes (from pter to qter: ZNF597-NAA60-CLUAP1- NLRC3-SLX4-TRAP1-DNASE1-TRAP1-CREBBP-ADCY9-SRL-TFAP4-GLIS2-PAM16-CORO7-VASN-DNAJA3-HMOX2-NMRAL1-C16orf5). Four patients (27%) showed both breakpoints within the CREBBP gene. Excluding one case due to insufficient DNA, data on 17 breakpoints within CREBBP could be precisely compared using MLPA, microarray analysis (Affymetrix 6.0), and qPCR. Comparison of MLPA and microarray data yielded consistent results for 9 breakpoints, differences of only 1 exon for five further cases, and for 2 and more exons in 3 cases with qPCR in most cases confirming the MLPA data. Taken together, genome-wide microarray analysis may at least in some cases give less precise data than other methods like MLPA or qPCR and may, in particular, overlook small deletions. The combination of the three techniques may improve the diagnosis and avoid later alternative and expensive diagnostics and/or management strategies.

P12.214

Twelve new mutations in the EP300 gene in patients with Rubinstein-Taybi syndrome.

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Rubinstein-Taybi syndrome (RSTS) is a rare disorder affecting approximately 1/100,000 newborns. The syndrome is characterized by mental and growth retardation and a particular dysmorphology mainly concerning the face, hands and feet. The most frequent cause of RSTS are mutations in the CREBBP gene, which are found in 30-50% of patients. Furthermore, microdeletions involving the CREBBP gene have been found in ~10% of patients. Mutations in EP300 have been published, but seem to be rare with a frequency of 1-3%.
In a cohort of 119 patients suspected of having RSTS, in 12 patients (6%) a pathogenic mutation in the EP300 gene was found. Eleven EP300 mutations were small sequence variants: three frameshift mutations, five nonsense mutations, one splice site mutation, one insertion/deletion mutation and one (de novo) missense mutation in the functional HAT domain. In addition, a deletion of exons 24 to 29 was detected. About one third of patients in this cohort (32%) could be explained by a mutation in the CREBBP gene, so in the majority of patients no mutation could be identified. In conclusion, these results show that mutations in the EP300 gene are a rare cause of Rubinstein-Taybi syndrome, but perhaps not as rare as previously thought.

P12.216

The identification of the SCA36 intronic expansion in Spain further highlights the role of dysfunctional RNA processing in neurodegeneration

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SCA 36 was recently shown to be caused by a GGCCTG repeat expansion in intron 1 of NOP56 in Japanese families. NOP56 encodes a component of the ribonucleoprotein complex and plays a role in transcription and splicing processes. We found the same mutation in ten families, including two very large kindreds from the coastal region in Northwestern Spain (Costa da Morte, Galicia). The screening of the NOP56 expansion, carried out with Southern Blot analysis and repeat-primed PCR, revealed expanded alleles ranging from 650-2500 repeats, within a unique haplotype. The most recent common founder chromosome was dated over 600 years ago. We have studied 66 mutation carriers and observed both further expansions and contractions of the repeat upon transmission. The main clinical characteristics of the disease are a late-onset cerebellar syndrome with upper and lower motor neuron signs, oculomotor abnormalities and sensorineural hearing loss. SCA36 represents the most frequent SCA type so far in our region, with epidemiological implication for South American countries, the main destiny of traditional Galician emigration, where thus additional SCA36 cases might be identified. Together with the recent description of the intronic C9ORF72 hexanucleotide expansion in amyotrophic lateral sclerosis, our findings further highlight the increasing recognition of a major role of abnormal RNA processing in neurodegeneration.Acknowledgements: Xunta de Galicia-Consellería de Sanidade (PGIDT05SAN26PR), Consellería de Economía e Industria (10PXIB9101280PR) and Consellería de Innovación (Lucas Labrada contract to MG-M). Instituto de Salud Carlos III, Madrid, Spain: Miguel Servet contract to MS and ISCIII-SERGAS stabilization contract to BQ.

P12.217

Genotype-phenotype correlations in epilepsy patients with the SCN1A point mutations

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SCN1A, the gene encoding the sodium channel alpha 1 subunit, is now one of the most important epilepsy genes. SCN1A-related seizure disorders encompass a spectrum ranging from simple febrile seizures (FS), generalized epilepsy with febrile seizures plus (GEFS+) to Dravet syndrome (DS) and intractable childhood epilepsy with generalized tonic-clonic seizures (ICEGTC). Mutations of this gene were also detected in less common phenotypes. More than 700 mutations in SCN1A gene have already been associated with DS/GEFS+, mainly sequence alterations (80%). All mutations' are dominant and most of them occur de novo, however familial cases also been described (5-10%). In such cases proband, usually shows the most severe form, while the remaining family members milder phenotypes.

We present analysis of the distribution and type of SCN1A point mutations, we have identified in 50 patients clinically diagnosed as DS/GEFS+ and referred for molecular testing. Most of them showed sporadic form of disease (each with different mutation), but we also identified familial forms (3 families). Because all identified familial cases have mutation at this same protein residue p.Arg1596 (p.Arg1596His and p.Arg1596Cys) we show not only phenotypic heterogeneity between family members
but also interfamilial disease course and clinical picture differences.

P12.218

Delineation of a novel syndrome caused by biallelic SEMA3A mutations

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Molecular karyotyping is commonly used to identify disease causing de novo copy number variants in patients with developmental delay and multiple congenital anomalies. In such a patient with multiple congenital anomalies we now observed an 150 kb deletion on chromosome 7q21.11 affecting the first exon of the axon guidance molecule gene SEMA3A. This deletion was inherited from the healthy father, but considering the function of SEMA3A and phenotypic similarity to the knock-out mice, we assumed a recessive defect. Sequencing of the SEMA3A gene in the patient revealed the de novo in-frame mutation p.Phe316_Lys317delinsThrSerSerAsnGlu. Cloning of the mutated allele in combination with two informative SNPs confirmed compound heterozygosity in the patient. While the altered protein structure was predicted to be benign, aberrant splicing resulting in a premature stop codon was proven by RT-PCR to occur in about half of the transcripts from this allele. Expression profiling in human fetal and adult cDNA panels, confirmed a high expression of SEMA3A in all brain regions as well as in adult and fetal heart and fetal skeletal muscle. We therefore report the first bona fide human mutations in the SEMA3A gene delineating a novel autosomal recessive disorder characterized by postnatal short stature with relative macrocephaly, camptodactyly, septal heart defect and several minor anomalies. Normal intellectual development in the patient was surprising but may be explained by the remaining 20% of SEMA3A expression level demonstrated by quantitative RT-PCR.

P12.219

Clinical, in-silico and experimental evidence for pathogenicity of two novel splice site mutations in the *SH3TC2* gene

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Introduction: Charcot-Marie-Tooth (CMT) neuropathy is the most common inherited neuromuscular disorder. CMT is genetically very heterogeneous. Mutations in the *SH3TC2* gene cause Charcot-Marie-Tooth neuropathy type 4C (CMT4C), a demyelinating form with autosomal recessive inheritance. Two novel splice site mutations in the *SH3TC2* gene have been studied (c.279G>A, c.3676-8G>A).

Patients and Methods: Mutation c.279G>A was detected on one allele in two unrelated families with CMT4C in combination with a known pathogenic mutation (c.2860 C>T in one family, c.505T>C in the other) on the second allele of *SH3TC*2. Variant c.3676-8G>A was detected in one patient on one allele of the *SH3TC*2 in combination with c.2860 C>T on the other allele.

In-silico tests were performed and Exon Trap experiments were undertaken to prove the effect of both mutations on proper splicing of SH3TC2. Fragments of *SH3TC2* were subcloned into pET01 Exon Trap vector (Mobitec) and transfected into COS-7 cells.

Results: Aberrant splicing was predicted by computer tests for both mutations, which was confirmed by Exon Trap analysis. For c.279G>A it was shown that 19 bases from intron 3 are retained in cDNA. For c.3676-8G>A it was shown that the mutation produces a novel splice acceptor site for exon 17 and complex changes in splicing were observed.

Conclusions: We present evidence that mutations c.279G>A and c.3676-8-G>A in the *SH3TC2* gene cause aberrant splicing and are therefore pathogenic and causal for CMT4C. This report broadens the spectrum of causal mutations in the *SH3TC2* gene.

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P12.220

Ciliogenesis associated signal transduction is altered by NEK1 mutations in short rib-polydactyly syndrome type Majewski

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Defects of ciliogenesis have been implicated in a wide range of human phenotypes and play a crucial role in different signal transduction pathways and cell cycle coordination. We recently identified nonsense and splice site mutations in *NEK1* as the underlying cause of short rib-polydactyly syndrome type Majewski (SRPS II) (Thiel et al. 2011). According to their phenotype the short rib-polydactyly syndromes (SRPS) are classified into four distinct types: Saldino-Noonan (I), Majewski (II), Verma-Naumhoff (III) and Beemer (IV) and include the phenotypically related asphyxiating thoracic dystrophy (ATD) and Ellis-van Crefeld syndromes (EVC). Here, mutations in *EVC1/2*, *IFT80*, and *DYNC2H1* have been observed.

Absence of full-length NEK1 leads to a severely reduction of primary cilia length and a significant decrease of ciliated fibroblasts. The primary cilium acts as a chemosensor for important developmental pathways like hedgehog, wnt and the platelet-derived growth factor (PDGF) pathways. Thus we performed quantitative real-time PCR experiments to elucidate expression level changes of the crucial genes of these pathways and the other known SRPS genes. The overall difference of expression levels was increased up to 3.8 fold in our patient fibroblasts in comparison with controls. We further evaluated the changes of expression levels under starvation conditions to initiate ciliogenesis. These results confirm that disturbed cilia formation by the loss of *NEK1* affect signal transduction in these pathways explaining the clinical variability.

P12.221

Shox2 and Tbx4 operate in a feedback loop during murine limb development

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The paralogous genes *SHOX* and *SHOX2* encode for homeodomain transcription factors with important functions during embryonal development. *SHOX* was identified as a human growth control gene as mutations in this gene lead to the skeletal deformities seen in Leri-Weill and Langer syndrome as well as idiopathic short stature. Although its closely related counterpart *SHOX2* could not be linked to any human phenotype so far, analysis of *Shox2* deficient mouse models revealed that *Shox2* also plays a crucial role in limb development where it controls neural, muscular and skeletal processes. To gain more insight into Shox2 dependent signalling pathways, we searched for target genes using microarray expression profiling and identified several interesting candidates such as different members of the T-Box transcription factor family as well as extracellular matrix protein encoding genes. For further analysis we focussed on the T-Box transcription factor *Tbx4* as a putative *Shox2* target gene which has essential functions in skeletal and muscular development of the limbs, similar to *Shox2*. We could demonstrate a dynamic regulatory effect of *Shox2* on *Tbx4* specifically in forelimbs. In turn, we uncovered *Tbx4* as a novel transcriptional activator of *Shox2* and therefore postulate *Shox2* as a feedback modulator of *Tbx4* during murine limb development.

P12.222

Counteracting effects on the allosteric control of SHP2's function drive selection of the recurrent Tyr62Asp and Tyr63Cys substitutions in Noonan syndrome

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Activating mutations in *PTPN11* cause Noonan syndrome (NS), the most common non-chromosomal disorder affecting development and growth. *PTPN11* encodes SHP2, an SH2 domain-containing protein tyrosine phosphatase that positively modulates RAS function. Here, we characterized functionally all possible amino acid substitutions arising from single-base changes affecting codons 62 and 63 to explore the molecular mechanisms lying behind the largely invariant occurrence of the Tyr62Asp and Tyr63Cys substitutions recurring in NS. We provide structural and biochemical data indicating that the autoinhibitory interaction between the N-SH2 and PTP domains is perturbed in both mutants as a result of an extensive structural rearrangement of the N-SH2 domain. Most mutations affecting Tyr^{63} exerted an unpredicted disrupting effect on the structure of the N-SH2 phosphopeptide-binding cleft mediating SHP2's interaction with signaling partners. Among all the amino acid changes affecting that codon, the disease-causing mutation was the only substitution that perturbed the stability of SHP2's inactive conformation without severely impairing proper N-SH2's phosphopeptide binding. On the other hand, the disruptive effect of the Tyr62Asp change on the autoinhibited conformation of the protein was balanced, in part, by less efficient binding properties of the mutant. Overall, our data demonstrate that the selection-by-function mechanism acting as driving force for *PTPN11* mutations affecting codons 62 and 63 implies balancing of counteracting effects operating on the allosteric control of SHP2's function.

P12.223

Novel mutations in a family with Maroteaux type of acromesomelic dysplasia and Sjögren's syndrome associated with neuropathy

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Sjogren's syndrome is a chronic autoimmune disorder and displays a strong female predominance. We identified a consanguineous family with one affected female with Maroteaux type of acromesomelic dysplasia (AMDM) and Sjögren's syndrome associated with neuropathy. These symptoms are inherited as autosomal recessive traits. Exome sequencing of one affected and one normal individual in the family using Illumina Hiseq2000 was subjected and identified a homozygous nonsense mutation on a known AMDM gene, NPR2 gene, for the AMDM syndrome. For Sjögren's syndrome associated with neuropathy, we identified a novel homozygous variant on a gene that is highly expressed in immune cells and is a neurogenesis inhibitor. This variant is not present in 376 Taiwanese controls and we are now screening for its allele frequency in other patients with Sjögren's syndrome associated with neuropathy.

P12.224

Spectrums and frequencies of SLC26A4 and SLC26A5 genes mutation among patients with non-syndromic hearing loss from different regions of Russia

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Background: The molecular etiology of hearing impairment in Russia has not been fully investigated. Study of GJB2, GJB6, GJB3, 12SrRNA, tRNASer(UCN) and MYO7A genes revealed that 55% of the patients with hearing loss carried GJB2 mutations in different regions of Russia. The SLC26A4 and SLC26A5 genes mutations are analyzed in this study.

Methods: Two hundred and fifty unrelated deaf patients were included. The all coding exons of SLC26A4 and first ten exons of SLC26A5 genes were sequenced in all 250 patients, including 130 patients carrying bi- and monoallelic recessive GJB2 mutations, two patients carrying a known GJB2 dominant mutation c.224G>A (p.Arg75Gln), as well as six patients with mtDNA (m.1555A>G, m.961insC(n), m.961delTinsC(n) and m.7444G>A) mutations.

Results: Eight patients (3,2%, 8/250) with non-syndromic hearing loss were found carrying SLC26A4 and SLC26A5 mutation and polymorphic variants. Among them, one patient with bi-allelic SLC26A4 mutations (c.85G>C (p.Glu29Gln) and c.149T>G (p.Leu50Arg)) had EVA by CT scan. One patient with non-syndromic hearing loss was heterozygous for mutations c.919-2-A>G in SLC26A4 gene. The most common SLC26A5 gene mutation, g.-53-2A>G, accounted for 0,4% (1/250) of all SLC26A4 mutant alleles. Two patients with non-syndromic hearing loss were heterozygous for polymorphic variant c.49548A>G (p.Gly740Ser) in SLC26A4, and one was heterozygous for polymorphic variant g.38190T>C in SLC26A5. The novel SLC26A4 gene mutation g.29607delA was identified in one patient with EVA.

Conclusion: Our results suggest that GJB2, SLC26A4 and SLC26A5 mutations together make up a major cause of congenital hearing loss in the different populations from Russia.

P12.225

FL-SMN and *PL3* expression in discordant spinal muscular atrophy (SMA) siblings and asymptomatic *SMN1* deletans

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Survival motor neuron1 gene (*SMN1*) is the main determinant for SMA disease while plastin3 (*PLS3*) is referred as a neuroprotective gene allowing genotype/phenotype discrepancy for healthy *SMN1* deletans. We compared the transcript levels of *FL-SMN* and *PLS3* in peripheral blood mononuclear cells (PBMCs) and EBV-immortalized cells from three individuals harbouring a homozygous deletion of *SMN1*. Two subjects are male and female discordant siblings haploidentical for the SMA locus (0 *SMN1*/4 *SMN2*), one subject is a asymptomatic unrelated female (0*SMN1*/5 *SMN2*). We analyzed the expression of *FL-SMN* and *PLS3* in those individuals by qRT-PCR in PBMC and EBV-immortalized cells. All three individuals similarly express both genes in PBMC suggesting that none of the two is responsible for neuroprotection of the asymptomatic females. In EBV-immortalized cells instead, we measured comparable expression levels only for *FL-SMN* transcripts across the subjects tested, while a dramatic reduction for PLS3 transcripts, ranging from 25 to 100-fold less, was observed in two out of three SMN1 deletans. A strong reduction of PLS3 expression could reflect an EBV-dependent event or particular feature of B lymphocytes. To address this question we compared transcript levels of PLS3 in B and NON-B cells from the two unrelated, phenotypically discordant subjects and observed a decrease in B cells only, ranging between 1,6 and 5-fold less than those in PBMC. We conclude that PLS3 transcript levels, at least in pheripheral blood is unrelated to health/disease condition of SMA deletans and that an EBV-dependent immortalization could alter gene expression.

P12.226

A novel SMAD3 variation in a family variably affected by symptoms of aneurysms-osteoarthritis syndrome

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Thoracic aortic aneurysm and dissection (TAAD) is a condition which can occur isolated or in combination with other syndromes, such as Marfan syndrome or Loeys-Dietz syndrome. Recently a new syndrome, termed aneurysms-osteoarthritis syndrome (AOS), has been described (van de Laar et al. 2011). AOS is an autosomal dominant disorder. The main features of AOS are aortic aneurysms and dissection, osteoarthritis and aneurysms of other vessels, such as the cerebral arteries. Mutations responsible for this disease were found in the SMAD3 gene. The SMAD3 protein is activated by TGF-ß via the TGF-ß receptors 1 and 2 and functions as a transcriptional modulator.
We report on a family in which the affected members present with diverse symptoms, including aortic aneurysms, skeletal and ophthalmological manifestations, and with variable expressivity. We identified a novel sequence variation, c.934G>A (p.Ala312Thr), in the SMAD3 gene. The variation is located in exon 7 in the MH2 domain of SMAD3, which is responsible for oligomerization of SMAD3 with SMAD4. The mutated alanine residue is highly conserved among other species and biometric analysis predicted this variation to be pathogenic. In one family member with skeletal manifestations we could not detect the SMAD3 variation. This could indicate that the SMAD3 variant acts as one of several genetic and non-genetic factors predisposing for the disease in this family.

P12.227

A restricted spectrum of mutations in the *SMAD4* tumor-suppressor gene underlies Myhre syndrome

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Myhre syndrome is a developmental disorder characterized by reduced growth, generalized muscular hypertrophy, facial dysmorphism, deafness, cognitive deficits, joint stiffness and skeletal anomalies. Here, by performing exome sequencing of a single affected individual coupled to a hypothesis-driven filtering strategy, we establish that heterozygous mutations in *SMAD4*, which encodes for a transducer mediating TGFβ and BMP signaling branches, underlie this rare Mendelian trait. Two recurrent *de novo SMAD4* mutations were identified in eight unrelated subjects. Both mutations were missense changes altering Ile^{500} within the evolutionary conserved MAD homology 2 domain, a well known mutational hot spot in malignancies. Structural analyses suggest that the substituted residues are likely to perturb the binding properties of the mutant protein to signaling partners. While *SMAD4* has been established as a tumor suppressor gene somatically mutated in pancreatic, gastrointestinal and skin cancers, and germline loss-of-function lesions and deletions of this gene have been documented to cause disorders predisposing to gastrointestinal cancer and vascular dysplasias, the present report identifies a previously unrecognized class of mutations in the gene with profound impact on development and growth.

P12.228

The mutation status of BMPR1A, SMAD4, PTEN and STK11 genes in Polish patients with hamartomatous polyposis syndromes

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The hamartomatous polyposis syndromes are a heterogeneous group of disorders that share an autosomal-dominant pattern of inheritance and are characterized by hamartomatous polyps of the gastrointestinal tract. These syndromes not only carry the risk of developing colorectal cancer, but also increase the risk of malignant transformation in other organs. In this study four hamartomatous polyposis syndromes (juvenile polyposis syndrome, Peutz-Jeghers syndrome, Cowden syndrome and mixed polyposis syndrome) were investigated. Mutations in SMAD4, BMPR1A, STK11 and PTEN are responsible for developing hamartomatous polyps. The study group consisted of 62 Polish families. Mutation screening methods and MLPA technique were used. The sequence analysis was performed for fragments with difference SSCP/duplex patterns and for verification of MLPA method. As a result of molecular analysis 32 pathogenic mutations in 34 patients were identified. Three mutations were detected in BMPR1A gene. In four families with juvenile polyposis syndrome has been identified pathogenic mutations in SMAD4. In STK11 gene was detected 12 types of mutations in 13 families with Peutz-Jeghers syndrome. Two substitutions were detected in families with Cowden syndrome. Moreover, numerous of polymorphic changes in both the coding and intronic sequences were observed. The majority of mutations are large changes and they represent 40% of all detected mutations. Mutations established in Polish population have heterogeneous nature. Only three mutations were recurrent. In addition, there was no strong genotype-phenotype correlation in all studied hamartomatous polyposis. In all cases, MLPA analysis should be performed as a first step to improve efficiency of molecular diagnostics.Supported by grant NN401014435

P12.229

Modifiers of Smith-Lemli-Opitz Syndrome and implication for mutation databases

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Background The Smith-Lemli-Opitz Syndrome (SLOS [MIM 270400]) is an autosomal recessive malformation syndrome that shows a great variability regarding severity. SLOS is caused by mutations in the Δ7sterol-reductase gene (DHCR7; E.C. 1.3.1.21) which disrupt cholesterol biosynthesis. Phenotypic variability of the disease is already known to be associated with maternal *ApoE* genotype. Recently a second modifier of this monogenic disease has been demonstrated which is ABCA1. The existing LOVD based mutation databases includes the *DHCR7* genotype for each phenotypical described patient. It includes the so-called severity score, *DHCR7* haplotypes, geographic origin, and cholesterol data if present.
Findings on modifier Regarding ApoE there are the known allelic variants ApoE2, E3, E4 determined in the mothers who play a role in phenotypic variability and cholesterol concentrations of the patient. Regarding ABCA1 it is the maternal genotype of the SNP p.Arg1587Lys that is associated with severity of the disease and in an already unknown manner with the viability of SLOS foetus.
Implication for mutation databases The aim of patient based mutation database is to interpret molecular findings concerning the accurate diagnosis, getting information about prognosis and possible useful therapies. Hence the existing database is complemented now by additional data about maternal ApoE genotype. Adding all known sequencing results (SNPs) of the concerned genes, and additionally molecular data of modifier genes makes particularly sense in atypical patients.

P12.230

Study of positive modifiers of spinal muscular atrophy severity in Russian patients.

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Spinal muscular atrophy (SMA) is autosomal recessive neuromuscular disorder caused by homozygous mutation within the *SMN1* gene. The ability of *SMN2* gene, a nearly identical copy gene of *SMN1,* to produce 10% of full-length transcript makes it principal positive disease modifier. The *SMN2* copy number correlates with patients' phenotypes and is used as reliable biomarker for SMA diagnostics. The second positive disease modifier is c.859G>C substitution in *SMN2* gene. The aim is to evaluate role of positive disease modifiers in Russian patients. We have developed fast and reliable test based on RFLP and sequence-specific primers to perform screening of c.859G>C mutation. Totally 145 individuals with mild phenotypes were tested. No patients with mutation were found which could be explained by population differences. *SMN2* gene dosage analysis was performed for 117 patients with I (39), II (50) and III (28) SMA types by means of real-time PCR. Most of type I patients had 2 *SMN2* copies (76,92%), 20,51% of patients had 3 copies and one person (2,56%) had 1 copy. Most of type II patients showed 3 copies (78%), 12% and 10% of patients had 4 and 2 copies accordingly. 50% of type III patients had 4 copies, 46,43% - 3 copies and only one person (3,57%) possess 2 copies. We observed marked differences in frequency of *SMN2* gene copies between patients with different SMA types. Our results confirm genotype-phenotype correlation between *SMN2* copy number and SMA severity and allow to use it as important biomarker for determination of disease phenotype.

P12.231

Investigating autosomal recessive cerebellar ataxias: clinical, biochemical and neuroimaging studies of 262 Brazilian patients

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Autosomal recessive cerebellar ataxias (ARCAs) comprise a heterogeneous group of inherited neurodegenerative disorders that affect the cerebellum, the spinocerebellar tract and/or the sensory tracts of the spinal cord. They lead to progressive cerebellar ataxia in association with other neurological or extra-neurological signs. The epidemiological features and the relative frequency of such disorders are quite unknown yet. We prospectively studied 262 suspected ARCA patients from Brazil between 2005 and 2011. All patients were evaluated in the neurogenetics clinics with a standard evaluation, neuroimaging studies (CT scan, brain MRI with spectroscopy), ophtalmological and auditory evaluations, neurophysiological studies (EEG, ERG, and EMG/NCV), hormone and biochemical tests, muscle biopsy with respiratory chain mitochondrial analysis, screening for inborn errors of metabolism (enzyme studies, peroxisomal and sterol panels, cholestanol dosage, organic acids, aminoacids chromatography), molecular studies for the FRDA expansion , mitochondrial point mutations and SCAs 1, 2,3, 6,7,10,12,17 - when indicated, nerve/skin biopsy for EM studies and karyotype were also performed. A conclusive diagnosis was established for 196 patients. The most frequent causes seen in our cohort were Friedreich ataxia, leukodystrophies, Joubert syndrome, ataxia with oculomotor apraxia types I and II, mitochondrial disorders and neurolipidoses. ARCAs are rare disorders with a wide differential diagnosis. Recognition of the most frequent genetic causes of ARCA can lead to a sequential evaluation capable of establishing a definitive diagnosis in the majority of patients; new techniques as SNP arrays and exome sequencing can be extreme useful in identification of new ARCAs, although they were not available for our study.

P12.232

Search for a novel gene locus for spinocerebellar ataxia in combination with leukodystrophy: Genome wide linkage, fine mapping and exclusion of candidate genes in a German family

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We investigated three brothers affected by severe, progressive gait and limb ataxia in combination with dysarthria and nystagmus starting early in childhood. MRI revealed white matter loss. Since the mother is also affected, but signs and symptoms started in her late twenties, we postulate autosomal dominant or X-chromosomal recessive inheritance.

After exclusion of all known genes mutated in spinocerebellar ataxia (SCA), we additionally excluded diseases such as Pelizaeus-Merzbacher Disease/ Paraplegia, Typ 2 (PLP) and Alexander Disease (GFAP).

Since we were not able to detect a disease-causing mutation in any of the analyzed genes, we performed genome wide linkage analysis. Ten genomic regions linked to the disease were identified. Among those, regions on 1q, 3p, 7q, 7q, 21q and Xq. We subsequently further delineated the critical regions and searched for copy number variations. Linked regions and candidate genes based on expression data and function will be presented.

P12.233

Influence of mutations in first nucleotides of exons on splicing of the btk gene

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Splicing is a crucial step of eukaryotic gene expression which takes place at loosely defined splice sites. Apart from alterations in conserved dinucleotides, it is difficult to predict effect of mutations located in splice sites just from sequence change itself. Mutations in the first nucleotide of exons affect splicing when found in AG-dependent 3‘ splice sites typically possessing weak and/or short polypyrimidine tracts while exons with AG-independent splice sites are resistant to this type of mutations. Recent study showed that mutations in the +1 G impaired splicing in exons with preceeding polypyrimidine stretches (PPS) from 4 nt do 10 nt long whereas those with PPS from 9 to 16 nt long were normally spliced.

We identfied three changes of first nucleotides of exons in our patients with *btk* mutations. Two of them were point mutations of +1 G located in 3‘ splice site with borderline PPS length (8 nt), though just one of them affected the process of splicing. The third mutation was a deletion of four nucleotides in a splice site with 10 nt long PPS (but the affected exon started with A nucleotide). This mutation did not influence splicing of the gene. We sought to examine how the most commonly used *in silico* splicing prediction tools would cope with the effect of these mutations on splicing. We found that all herein described mutations decreased predicted scores in NNSPLICE, MaxEnt and PSSM. However, with an exception of MaxEnt program, the score change was rather mild.

P12.234

Novel mutations in a family with spondylocostal dysostosis

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Spondylocostal dysostosis (SCDO) is a heterogeneous group of disorders with multiple vertebral segmentation defects that results in hemivertebrae, rib fusions and deletions. We characterized a SCDO family with two affected children inherited as an autosomal recessive mode in Taiwan. Four known genes causing autosomal recessive forms of SCDO were excluded to be disease-causing gene for this family by direct Sanger sequencing. Exome sequencing of this family using Illumina Hiseq2000 was subjected to identify novel homozygous variants and compound heterozygous variants. A novel homozygous insertion variant that results in a frameshift in amino acid sequence was identified in a gene that has caused other disorders with vertebral malsegmentation and much severe clinical manifestations. This variant is not present in 376 Taiwanese controls. This finding expends the phenotypic spectrum resulting from novel mutation of the gene.

P12.235

Genetic testing of Stargardt disease as a model for high-throughput analysis of heterogenous diseases

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Stargardt disease (STGD), a frequent maculopathy with juvenile onset, is caused by mutations in ABCA4, CNGB3, and ELOVL4. To date, more than 550 distinct ABCA4 mutations have been reported to cause disease. Nevertheless, the genetic heterogeneity of STGD and the complexity of the ABCA4 gene, which has 50 coding exons, often hinder routine application in genetic testing. We therefore developed an Affymetrix-based resequencing array that allows analysis of STGD-related genes in a cost- and time-saving procedure.

A challenging task is the interpretation of sequence data and the identification of disease-relevant variants. Consequently, we have generated an automated variant interpretation pipeline which is linked to ongoing international DNA variant project efforts such as the 1,000 Genomes Project or the NHLBI Exome Sequencing Project which currently has data from 3,510 individuals of European descent. In addition, the pipeline automatically queries in-silico prediction tools such as MutationTaster and PolyPhen-2. Neutral and intronic sequence changes not affecting the canonical splice-acceptor or splice-donor sites are analyzed with the Alamut decision-support software. So far, we have included a total of 158 STGD patients in our analysis. This identified 1,027 distinct variants, of which 20% (n=199) were defined as pathogenic or likely pathogenic and 91 as variants of uncertain significance (UVs). From the 120 unique possibly pathogenic variants, 35 (29%) are novel. These data provide an excellent starting point for functional assessment of the defined variants. Overall, our approach provides the basis for further tackling the demanding task of introducing multi-gene analysis in routine diagnostics.

P12.236

Alu elements cause most common mutation of STK11 gene in Peutz Jeghers patients

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The STK11 gene mutations cause the occurrence of the Peutz-Jeghers syndrome. These mutations are heterogeneous, but a significant proportion of them are large rearrangements. The occurrence of large mutations may be associated with the presence of interspersed repeats or microhomologies. In the STK11 gene, only one type of interspersed repeats is observed -Alu elements. Alu sequences represent 19% of the entire gene sequence. Among large mutations of STK11 gene the deletion of exons 2 and 3 has been described four times so far in different European populations. In two cases the deleted sequence was described in detail and in another two, deletions were identified only on the basis of the MLPA results. The deletion of exons 2 nad 3 was also observed by us in one of the cases of Polish families. The deleted sequence endpoints are localized in Alu elements. We can assume that Alu elements localized in regions including nucleotides c.280+5594 and c.464+384 are responsible for causing one of the most common recurrent mutation of STK11 gene in Peutz-Jeghers patients.

P12.237

Sudden death and next generation sequencing: characterization of heart disease using a 72-gene NGS panel

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Purpose - Genetic characterization of heart disease patients in a fast, comprehensive, and cost-effective manner using a 72 gene NGS approach, coupled with a bioinformatics pipeline.

Methods - A methodology was developed for resequencing 72 genes (44 genes associated with cardiomyopathies, arrhythmogenic right ventricular dysplasia, Marfan syndrome, aortic aneurysm, and 28 genes associated with Brugada syndrome, long QT and short QT syndromes, familial atrial fibrillation and catecholaminergic polymorphic ventricular tachycardia). The design included 750 Kb of exons, splicing regions, 5' UTR and 3' UTR. Targets were captured (SureSelect, Agilent), and then sequenced in a SOLiD v4 platform. The bioinformatics pipeline consisted of mapping and aligning reads against the GRCh37/hg19 sequence, classification and identification of point variations, structural variations, and small indels, as well as their involvement at the transcriptional level. Results were confirmed by Sanger sequencing. A set of 12 cases with a known mutation was used for validation studies.

47 patients were studied (20 cases with aortic aneurysm/Marfan syndrome, 2 cases with ARVC/D, 7 cases with hypertrophic cardiomyopathy, 1 case with dilated cardiomyopathy, 2 cases with familial cardiomyopathy, 6 cases with long QT syndrome, 1 case with Brugada syndrome, 3 cases with familial arrhythmia, and 5 cases with family history of sudden death).

Results - 91 relevant nucleotide changes were found: 14 pathogenic mutations; 77 unclassified variations, of which, using in silico predictions, 9 are likely pathogenic and 7 are unlikely pathogenic.

Conclusions - Targeted resequencing enables the efficient analysis of genes associated with heterogeneous heart diseases.

P12.238

Defects in the Ski complex, a multi protein complex involved in aberrant mRNAs decay, cause Syndromic Diarrhea

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Syndromic Diarrhea (SD) is a rare and severe disorder characterized by intractable diarrhea, dysmorphism, immunodeficiency and hair abnormalities. This syndrome has been recently associated with mutations in *TTC37* in 21 patients but several other individuals with typical SD present no variation in this gene. The function of TTC37 in humans is not known but it is reported in databases as being the human ortholog of Ski3p, one of the yeast Ski complex cofactors. The Ski complex is required for the exosome-mediated RNA surveillance including normal mRNAs regulation and non-functional mRNAs decay such as non-sense mediated mRNA decay, no-go decay or non-stop decay. Considering TTC37 homology with Ski3p, we assumed that other genes encoding Ski complex proteins might be responsible for SD in patients without variation in *TTC37* and confronted this hypothesis with the results of a linkage analysis performed in a consanguineous family with such a patient. We noticed, in a region of homozygosity, a gene encoding another cofactor of the Ski complex, SKIV2L. Direct sequencing of *SKIV2L* in seven patients presenting typical SD without variation in *TTC37* identified stop or frameshift mutations in all seven.

Although genetically heterogeneous, SD is extremely homogenous clinically suggesting that a defect in the Ski complex function is a key mechanism responsible for the clinical features.

Our results show that mutations in genes encoding cofactors of the human Ski complex cause SD, establishing for the first time, a link between defects of the exosome complex and a Mendelian disease.

P12.239

Mutational analysis for thoracic aortic aneurysm and dissection (TAAD) using a custom resequencing array

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TAAD is a severe cardiovascular complication that may occur in isolation (FTAAD) or as part of syndromic conditions such as Marfan Syndrome. To date, more than eight genes are known to harbour mutations causing TAAD. Subsequent analysis of these genes with standard methods is costly and parallel sequencing on a single platform has not yet been established.

We describe a 100-kb resequencing array, the MFSTAAD chip, which allows the simultaneous resequencing of the genes *ACTA2*, *COL3A1*, *FBN1*, *MYH11*, *NOTCH1*, *SLC2A10*, *TGFBR1* and *TGFBR2*. A total of 181 positive controls with known mutations such as point mutations, deletions and insertions were used to determine the analytical sensitivity of the assay and the mutation yield was evaluated in a cohort of 28 TAAD patients, 18 of these subjects were previously been tested negative for mutations in the genes *FBN1* and *TGFBR2*. An average call-rate of 99.9% was obtained using Sequence Pilot SeqC data analysis software. The assay showed a high analytical sensitivity for point mutations (100%) and an overall sensitivity of 85%. The largest deletion could be detected by a striking decline in the hybridization line. Upon analysis of the 28 TAAD patients, with this assay we could detect 4 known mutations and 6 possibly pathogenic point mutations (mutation yield: 32%).

In conclusion, the MFSTAAD chip is a feasible tool that can facilitate the genetic analysis of TAAD in routine genetic testing. Future modification for the detection of gross changes such as deletions and insertions will make this assay even more applicable.

P12.240

The first case of Tenascin-X deficient type Ehlers-Danlos syndrome in Japan

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Tenascin-X is a large extracellular matrix glycoprotein and is known to be associated with an autosomal recessive type of Ehlers-Danlos syndrome (EDS). Here we report the first case of Tenascin-X deficient type EDS in Japan. The patient of 39-year-old male was born in breech position, had cast immobilization for congenital hip dislocation until 9months of age, diagnosed as EDS for skin hyperextensibility, laxity in finger joints, softness of heels.

At the age of 18months he was treated for inguinal hernia and snaping finger of thumbs, left middle finger and right annular finger. Other skin manifestation is easy bruising, but the wound repair had been normal. Recurrent dislocations of the shoulders and sprains were seen from his childhood. His foot went flat when loaded. He had gone through keloid/ lump formation, hemorrhoid associated bleeding problems and diverticulitis several times into the adulthood. He was referred to our clinic after his fragile intestinal tract tissue ruptured during his operation of diverticulitis. A complete absence of tenascin-X was identified on triplicate testing of the proband's serum. *TNX-B* mutation analysis was performed and showed a homozygous 1-bp deletion in exon 25, encoding fibronectin type III repeat. The parental serum tenascin-X values were both on the lower side of the normal distribution and intermediate between the proband and the background population. Further analysis is currently underway.

P12.241

Leri's pleonosteosis results from a genetic defect causing dysregulated TGF-beta signalling

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The Transforming Growth Factor-beta (TGF-β) signalling pathway is key to many cellular processes and its dysregulation has been documented in a number of Mendelian phenotypes characterised by joint contractures and scleroderma, including stiff skin syndrome, Myhre syndrome, acromicric and geleophysic dysplasias.

Leri's pleonosteosis [MIM 151200] is a rare autosomal dominant condition characterized by flexion contractures of the interphalangeal joints, restricted motion of multiple joints, facial dysmorphism, bony overgrowths, short broad hands and feet and occasional sclerodermatous thickening of skin.

We genotyped the two most distantly related individuals in a large family with Leri's pleonosteosis by Affymetrix SNP6.0 array. Copy number analysis revealed a shared ~1Mb duplication of chromosome 8q22.1 that segregated with the phenotype and was confirmed by QPCR. The duplication was not present in polymorphism databases and over 500 controls. We identified an overlapping 8q22.1 duplication in an unrelated patient with Leri's pleonosteosis to confirm the causal relationship.

The minimum critical region consists of six genes, including *SDC2* encoding the transmembrane heparan sulphate proteoglycan, syndecan-2. We show that overexpression of *SDC2* causing altered TGF-β signaling in fibroblasts is the major molecular contributor to the clinical phenotype. We therefore add Leri's pleonosteosis as a further disorder to a spectrum of conditions characterized by dysregulation of TGF-β signaling and importantly, provide an insight into role of altered proteoglycan homeostasis in this pathway.

P12.242

Multiplex assay for the detection of common Mediterranean beta-thalassemia mutations

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Hemoglobinopathies are the most abundant group of genetic abnormalities in humans, caused by genetic defects affecting the globin genes encoding for the hemoglobin alpha and beta chains. In particular, a great variety of mutations present in heterozygous, homozygous and compound heterozygous states disturb the function of the HBB gene. Molecular characterisation of the causative genetic variants is an essential part of the diagnostic process. The unusually large number of individual mutations presents technical challenges. However, in any particular population, a limited number of genetic variants are responsible for the vast majority of hemoglobinopathy cases. Developing reliable, rapid and cost-effective molecular diagnostic assays targeting particular populations greatly facilitates routine hemoglobinopathy investigations. We developed a one-tube single-nucleotide primer extension assay for the detection of eight common Mediterranean beta-thalassaemia mutations: IVS-I-110 (G->A), IVS-I-1 (G->A), IVS-I-6 (T->C), Codon 39 (C->T), IVS-II-745 (C->G), Codon 5 (-CT); CCT(Pro)->C--, Codon 6 (-A); GAG(Glu)->G-G, and Codon 8 (-AA); AAG(Lys)->--G. According to available mutation frequency data, these eight sequence variations together account for a large proportion of the hemoglobinopathy cases in Macedonia (89%). The novel assay offers superior accuracy achieved through double mutation interrogation on both genomic strands. We validated the new assays using previously genotyped samples obtaining 100% agreement between independent genotyping methods. Our protocol, applicable in a range of Mediterranean countries, provides a cost-effective diagnostic tool of unmatched precision. It can be further adapted to particular populations by including/excluding assayed mutations. We facilitate future modifications by providing detailed information on assay design.

P12.243

The modifying effect of Xmn1-HBG2 on thalassemia phenotype is associated with its linked elements in beta globin LCR

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The core sequence of 5′HS4-beta globin locus control region and Xmn1-HBG2 site were analyzed and compared among 86 thalassemia patients with homozygous or compound heterozygous beta globin gene mutations and 101 normal individuals. Frequency of the G allele in the polymorphic palindromic sequence of 5′HS4 (TGGGGA/G CCCCA) and positive Xmn1-HBG2 profile was significantly higher in thalassemia patients compared to the normal population. Linkage disequilibrium was observed between the G allele and positive Xmn1-HBG2 profile in patient population. Furthermore, dominance of IVSII-1 in the mutation spectrum of the patients enabled us to identify linkage disequilibrium relationships between IVSII-1, positiveXmn1-HBG2 and the G allele at 5′HS4. The frequency of milder clinical phenotype was significantly higher in patients with GG/++than cases with AA/--genotypic pattern in 5′HS4/Xmn1-HBG2 loci. These data together with biochemical evidence suggesting a role for the A/G polymorphism at 5′HS4 palindromic site on modifying chromatin structure and in the absence of any evidence from functional studies relating the Xmn1-HBG2 site to the increased gamma chain expression, suggest that the phenotype modifying role long time assigned to Xmn1-HBG2 is possibly played by more functionally potent elements linked to it in LCR.

P12.244

A new TP63 mutation in a patient with cleft lip, split hand, and tibial agenesis

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TP63 gene, located at 3q27 chromosome region, encodes a transcription factor that plays an essential role in the development of epidermis, upper lip and limbs. Homozygous tp63 null mice exhibit craniofacial abnormalities, limb truncations, and absence of epidermal appendages. In humans, mutations in TP63 can give rise to a series of syndromes characterized by various combinations of ectodermal dysplasia, limb malformations and orofacial clefting. The propositus here reported is the first child of a non-consanguineous couple. Some individuals of the maternal family were referred, but not examined, to be affected by some of the malformations found in the propositus, suggesting an autosomal dominant inheritance. The main clinical features included left cleft lip, bilateral split hand, bilateral tibial agenesis, club feet and absence of the right hallux. No ectodermal abnormalities were observed. Despite of mild hypotonia in the first months, neuropsychomotor development was apparently normal. The analysis of copy number variations (CNVs) was performed using the Genome-Wide Human SNP Array 6.0 (Affymetrix). None potential pathogenic CNVs were found. Subsequently, TP63 gene was sequenced. A four-nucleotide insertion (AGAG) was detected at 5'UTR region of this gene resulting in a frameshift mutation. Considering the site of this mutation, it might have caused an alteration in the pattern of expression of TP63 gene. To our knowledge this is the first time that a mutation in the TP63 has been associated with this pattern of malformation - cleft lip, split hand and preaxial limb reduction defect in lower limbs.

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P12.245

Auto-regulation of the *THAP1* (DYT6) gene and THAP1-mediated activation of *SGCE* (DYT11) gene expression.

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Mutations in Thanatos-associated [THAP] domain-containing apoptosis-associated protein 1 (THAP1) cause a form of pure dystonia (DYT6). THAP1 encodes a transcription factor that regulates the expression of the DYT1 dystonia gene *TOR1A*. Here, we investigated whether THAP1 also influences the expression of the myoclonus-dystonia (DYT11) associated gene *SGCE* and its own expression. Using in-silico prediction and luciferase reporter gene assays, we characterized the *SGCE* and *THAP1* promoters. Interestingly, these two core promoters contained one or five THAP binding sequences (THABS). Luciferase reporter gene assays revealed that THAP1 activates the expression of *SGCE* and that this activation is disturbed by different THAP1 mutations. In addition, THAP1 represses its own expression. Binding of THAP1 to the core promoters was demonstrated using chromatin immunoprecipitation (ChIP). Further, THAP1 binding to the THABS within the *SGCE* promoter was confirmed by electromobility shift assay (EMSA). To test for *in-vivo* changes of expression levels, we re-programmed fibroblast cells from a *THAP1* mutation carrier (Leu159fs180X) and controls to pluripotent induced stem (iPS) cells that were differentiated into neurons. Quantitative PCR in these cells did not reveal a significant difference of *SGCE* expression in a THAP1 mutation carrier compared to wildtype samples. However, *THAP1* expression was increased in mutant THAP1 cells suggesting an autoregulation of THAP1. In conclusion, we identified two additional targets for *THAP1, SGCE* and *THAP1* itself, *in vitro* and confirmed *THAP1* in vivo. It is conceivable that the autoregulation compensates for alterations in the expression of other target genes to a certain degree.

P12.246

Transcriptome profiling during early neuronal differentiation in Lissencephaly associated with DCX mutations

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Genetic factors play a major role in a large proportion of patients with congenital neurological and neurodevelopmental disorders. Despite the progress related to genotype-phenotype associations, little is known about how specific gene mutations mediate abnormal cellular and molecular processes during early neuronal differentiation. Induced pluripotent stem cell (iPSC) technology has emerged as an indispensable tool to model genetically determined phenotypes at the cellular and molecular levels in non-accessible tissues. We have initiated an iPSC based effort to model different monogenic phenotypes derived from neuronal or neural crest cells, e.g. Lissencephaly. Lissencephaly is a neurodevelopmental disorder characterized by abnormal cerebral surface, mental retardation and seizures. The disease is caused by insufficient migration of maturing neurons, although disease mechanisms are not fully understood. We have generated iPSC from skin fibroblasts of two Lissencephaly patients with different doublecortin (*DCX*) mutations.
To study perturbations of neuronal differentiation and function in more detail we established iPSC lines from the Lissencephaly patients and healthy controls. All lines were characterized and showed the capacity to form all three germ layers in embryoid body differentiation assays. IPSC lines were differentiated into neuronal precursor cells (NPC) and further into neuronal cells. Total RNA samples are obtained from the iPSC and NPCs and sequenced using the SOLiD platform. The aim is to identify and compare transcriptome signatures during early neuronal differentiation.
We present how this "pipe-line" may be used as a sensitive method to characterize and mirror molecular abnormalities associated with *DCX* mutations at early stages of neuronal differentiation.

P12.247

A novel exon 2-skipped TNFR1 transcript: regulation by *TNFRSF1A* rs1800692 and possible role on TNFR-associated Periodic Syndrome (TRAPS) phenotype

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Binding of TNFα to one of its cell surface receptors, TNFR1 induces various cellular responses including cell death, differentiation or inflammation. Several polymorphisms in the *TNFRSF1A* gene, encoding TNFR1, have been repeatedly associated with diseases of the immune system, and heterozygous mutations are responsible for the dominant autoinflammatory disease, TNFR-associated periodic syndrome (TRAPS).
We identified the first spliced transcript named TNFR1-d2. This novel transcript, lacking exon 2, is expressed in a tissue-specific manner. To search for a possible regulatory element of *TNFRSF1A* expression, we checked for a possible effect of polymorphisms present along the *TNFRSF1A* sequence. We found the rs1800692 polymorphism (c.473-33C>T) in intron 4 of particular interest because we observed that the T/T genotype was absent in our TRAPS group. Splicing alternative assays showed that this polymorphism can modulate exon 2 skipping and luciferase assays revealed that intron 4 contains transcriptional regulatory element. Thus the exon 2 skipping in TNFR1-d2 may be controlled via transcriptional regulation in a cell-specific manner. Moreover, by *in vitro* studies, we showed that one particular mutation, identified among TRAPS patients involves a translation defect of TNFR1-d2 suggesting that its altered function may account for some of the inflammatory processes occurring in TRAPS.
We suggested that the transcript ratio, depending on the *TNFRSF1A* alleles, may partly account for the inter-individual variations observed in patients with a TRAPS phenotype and possibly in patients suffering from other inflammatory conditions and that, this new protein could have a role on the physiopathology of TRAPS.

P12.248

Frequency of *POLR1D*, *POLR1C* and *TCOF1* Mutations in a Large Cohort of Patients with Treacher Collins Syndrome

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Treacher Collins syndrome (TCS) is a disorder of craniofacial development characterised by a combination of bilateral downward slanting palpebral fissures, coloboma of the lower eyelid, micrognathia, malformation of the external ear and bilateral conductive hearing loss.
TCS type 1 is caused by dominant loss-of function mutations in the *TCOF1* gene. The Oxford Molecular Genetics Laboratory, UK, has provided a molecular diagnostic service for this gene since 2005. To date 119 referrals with a good clinical diagnosis of TCS have been analysed and pathogenic variants have been detected in approximately 70% of patients (of which around 4% were large deletions) [1]. In approximately 30% of referrals, no pathogenic variant was detected.
Mutations in the *POLR1D* and *POLR1C*, components of RNA polymerases I and III, have recently been reported in individuals with TCS [2]. Sequencing analysis of these genes was undertaken in a cohort of 26 patients strongly suspected of having TCS, in whom a pathogenic *TCOF1* variant was not detected. Probable pathogenic variants in the *POLR1D* gene were detected in 6 out of the 26 patients (23%) tested to date. The mutation spectrum includes novel frameshift, missense and nonsense variants, all predicted to result in loss of protein function. No pathogenic variants were detected in the *POLR1C* gene.
Data from our cohort suggests that approximately 7% of individuals with a strong clinical diagnosis of TCS may have a pathogenic mutation in the *POLR1D* gene.
[1] Bowman *et al*., 2012, EJHG, E-pub 8th Feb.
[2] Dauwerse *et al*., 2011, Nat.Genet. 43(1):20-22.

P12.249

A novel TRPS1 gene mutation in the family with Trichorhinophalangeal syndrome from Russia

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Trichorhinophalangeal syndrome I (MIM 190350) is a malformation syndrome characterized by the distinctive craniofacial and skeletal abnormalities and is inherited as an autosomal dominant. Patients have sparse scalp hair, bulbous tip of the nose, long flat philtrum, thin upper vermilion border, and protruding ears. Skeletal abnormalities include cone-shaped epiphyses at the phalanges, hip malformations, and short stature. We presented five patients from four generation family with TRPS I type transmitted as an autosomal-dominant trait from Russia. All patients had uniform symptoms. Proband 18-old man had the height 174 cm, typical symptoms: facial features included low-set, posteriorly rotated ears, prominent malar eminence and orbital ridge, bulbous nose, hypoplastic nasi alae nasi, hypotrichosis, and long philtrum. He also had brachymesophalangy, wide halluces, and flat arches. Radiographs showed short metacarpals, cone-shaped epiphyses of middle and proximal phalanges (2nd, 3rd, 4th fingers). This leads to varying degrees of brachydactyly without short stature. For this proband we conducted direct automated sequencing of zinc finger transcription factor gene TRPS1, encodes the 1281 amino acids protein TRPS1, participating in the regulation of chondrocytes and the perichondrium, as result the novel mutation c.2800G>A (Gly934Ser) in exon 6 had been found in the heterozygous state. By the additional research, this mutation was not detected among the 60 population samples (120 chromosomes). The same mutation was also detected in the DNA sample of proband's mother suffering TRPS I type.
Thus, these data support the fact that the detected change is a pathological mutation.

P12.250

Fragile X mental retardation 1 (FMR1) premutations: instability and associated phenotypes

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Fragile X syndrome (FXS) is the most common hereditary form of intellectual disability with an estimated frequency of 1/4000 males and 1/8000 females. FXS is caused by a $(CGG)_n$ expansion of over 200 repeats, in the 5'UTR of the *FMR1* gene, which as a result is methylated and gene silenced. Based on $(CGG)_n$ length, four classes of alleles can be distinguished: normal (5-44), intermediate (45-54), premutated (55-200) and fully mutated (>200; FM) alleles. Both *FMR1*-related primary ovarian insufficiency (FXPOI) and fragile X-associated tremor/ataxia syndrome (FXTAS) have been described in premutation carriers. To gain insights into instability of *FMR1* $(CGG)_n$ and associated phenotypes, we assessed repeat-length in 541 individuals from 128 Portuguese FXS families. We found 5.3% of intermediate, 29.9% of premutated and 26.6% of FM alleles. For a total of 115 transmissions of the maternal premutation, 26 (23%) with alleles ranging 60-98, the average expansion was 17 repeat units, whereas 89 (77%) with alleles 66-199, expanded to FM. In 44 transmissions of maternal FM, the offspring inherited the FM. For 10 paternal transmissions of premutations, ranging 56-120, all the daughters inherited a premutation, with an average expansion of 7 repeat units. We identified one male with FXTAS and two females with FXPOI among seven investigated premutation carriers; the remaining premutation individuals were not yet examined. In conclusion, in Portuguese FXS families, allele instability upon transmission is in agreement with previous reports, where the risk of premutation to FM expansion is linked to the premutation size of the transmitting mother.

P12.251

Molecular diagnostic of tuberous sclerosis by next-generation sequencing technology

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Tuberous sclerosis complex (TSC) is a rare genetic disorder, that belongs to neurocutaneous syndromes where both the skin and central nervous system are involved. It is characterized by an autosomal dominant pattern of inheritance and a variable penetrance. The symptoms of tuberous sclerosis vary from person to person and may include seizures, developmental delay, skin abnormalities, lung and kidney disease.

TSC is caused by a mutations in the genes *TSC1* or *TSC2*, which encode the protein hamartin and tuberin respectively. These proteins act as tumor growth suppressors, agents that regulate cell proliferation and differentiation.

Molecular analysis can detect mutations in about 85% of cases and is complicated by the size of both genes, absence of mutations hotspots and a high rate of *de novo* mutations. Untill now DGGE mutations scanning, direct sequence analysis in combination with deletions/duplications analysis of in *TSC1* or *TSC2* was performed in patients with suspected TSC.

In respect to the progress of new molecular genetic technologies we have adapted next-generation sequencing (NGS, Roche GSJ) into routine testing of TSC patients. All relevant areas of genes *TSC1* and *TSC2* were amplified by gene-specific primers with universal tail and marked by MID adaptors (Multiplex Identifier Adaptors), to differentiate between patients. We used Geneious software with ADINIS-MultipluginG to handle NGS data. Here we report on our experience of routine diagnostics for TSC profiting from the power of NGS.

P12.252

Targeted next-generation sequencing identifies a homozygous ABHD12 nonsense mutation in a family clinically diagnosed with Usher syndrome type 3

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Usher syndrome (USH) is an autosomal recessive disorder with congenital sensorineural hearing impairment (HI) and retinitis pigmentosa (RP). There are three clinical subtypes: USH1 presents with severe to profound HI, facultative vestibular impairment and early RP, whereas USH2 is characterized by moderate to severe HI and RP in adolescence. USH3 is rare and variable; it may resemble USH1 or USH2, and vestibular dysfunction may occur. Clarin-1 (*CLRN1*) is the only known USH3 gene (locus: *USH3A*). We have previously identified a consanguineous Lebanese family with an USH3 phenotype. Microarray SNP analysis identified three homozygosity-by-descent regions: 1q43-q44 (8.54 Mb), 20p13-p12.2 (10.48 Mb), and 20p11.23-q12 (19.35 Mb). We excluded mutations in ninein-like protein (*NINL*) on chromosome 20p11.21, an interactor of the USH protein complex. Using a capture array targeting all three candidate regions and next-generation sequencing, we subsequently identified a homozygous nonsense mutation, c.193C>T (p.Arg65X), in a neighboring gene, *ABHD12*. Of note, truncating *ABHD12* mutations have recently been shown to cause PHARC, a neurodegenerative disease with polyneuropathy, hearing loss, ataxia, retinitis pigmentosa, and early-onset cataract. Both patients from our family display HI, ataxia, RP, and cataracts, but no polyneuropathy. ABHD12 hydrolyzes 2-arachidonoyl glycerol, an endocannabinoid lipid transmitter that acts on cannabinoid receptors CB1 and CB2. This pathway is probably unrelated to the USH interactome. Consequently, the phenotype in our family rather represents a variant of PHARC, an entity that should be taken into account as differential diagnosis for Usher syndrome.

P12.253

Comprehensive molecular genetic analysis for Usher syndrome including conventional and next-generation sequencing and MLPA: Proposal of a diagnostic strategy

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Usher syndrome (USH) is an autosomal recessive disorder with congenital sensorineural hearing impairment (HI) and retinitis pigmentosa (RP). USH1 presents with severe to profound HI, facultative vestibular impairment and early RP, USH2 with moderate to severe HI and RP in adolescence. USH3 is rare and variable. Because of the mostly large size of the 10 USH genes, genetic confirmation of the diagnosis is the exception. We have applied a novel and efficient diagnostic strategy to 32 patients. For USH2, we sequenced *USH2A*, followed by *USH2A*-MLPA and next-generation sequencing (NGS) of all USH genes. For USH1 or atypical USH samples, we sequenced *MYO7A* (USH1B) before NGS was available to us; now, they directly undergo NGS. We found *USH2A* mutations in 18 out of 19 USH2 patients, including large intragenic deletions and duplications. NGS revealed *GPR98* (USH2C) mutations in one USH2 patient, but no USH gene mutations in the only patient with a monoallelic *USH2A* mutation. All three atypical USH patients and 4 of the 10 USH1 cases carried *MYO7A* mutations. *CDH23* mutations were found in one USH1 patient, and linkage analysis with subsequent sequencing and MLPA identified *PCDH15* mutations in two USH1 families from Syria. NGS for three *MYO7A*-negative USH1 patients is ongoing. In conclusion, our results with *USH2A* as the major USH2 gene and mutations more evenly distributed in USH1 support the following diagnostic procedure (in the order of listing): USH2: *USH2A* sequencing, *USH2A*-MLPA, NGS. USH1 and atypical USH: NGS, MLPA or linkage-based approaches where applicable.

P12.254

Mutations in KIAA1632 cause Vici syndrome, a multisystem disorder with defective autophagy

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Vici syndrome is a rare, recessively inherited multisystem disorder with markedly reduced life expectancy, characterised by callosal agenesis, cataracts, cardiomyopathy, combined immunodeficiency, hypopigmentation and an associated myopathy.

We sequenced the exomes of four affected individuals from three families and found homozygous and compound heterozygous mutations in *KIAA1632* in all four patients. Affected individuals from eleven additional Vici syndrome families were analysed by Sanger sequencing of *KIAA1632*. Mutations were detected in ten families. No *KIAA1632* mutations were identified in the remaining family nor in two further families with similar features, suggesting locus heterogeneity.

KIAA1632 was recently identified as the human homologue of the metazoan-specific autophagy gene *epg-5 (ectopic pgl granules family member 5)* (Tian et al) encoding a key protein of the autophagy pathway implicated in the formation of degradative autolysosomes. Immunohistochemical, histological and functional studies were consistent with homozygous or compound heterozygous null mutations in *KIAA1632* causing autophagy defects.

The remaining human homologues of the metazoan-specific autophagy genes identified in Tian et al, *VMP1* and *EI24*, were screened in the three Vici syndrome / Vici-like families without *KIAA1632* mutations. No mutations were identified.

Tian, Y., Z. Li, et al. (2010). „C. elegans screen identifies autophagy genes specific to multicellular organisms." Cell 141(6): 1042-1055.

P12.256

A novel mutation in the distant sonic hedgehog (SHH) cis-regulator ZRS in family with Werner mesomelic syndrome from Russia

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Werner mesomelic syndrome (WMS)-autosomal dominant tibial hemimelia-polysyndactyly-triphalangeal thumbs syndrome (MIM 188770). We present a family with 2 affected members in 2 generations from Russia. Propositus, 17-old age boy and his mother, have bilateral hypoplasia of the tibia with preaxial polydactyly in the feet and hands. The height of the boy is 128 cm and his mother is 135 cm. The right hand of the boy is characterized by 6 and the left hand by 7 isodactylous digits (on the left hand I and II digits are triphalangeal), the thumbs is nonopposable. Also boy has short legs due to tibial hypoplasia (length of tibias is 152 and 54 mm) and bilateral preaxial polydactyly of the feet with 7 triphalangeal digits. Radiologically and MRI legs showed thicken of fibula and dysplasia of hip. His mother on the hands has 5 digits, I digits is triphalangeal on both hands, the thumbs is nonopposable. On the feet she has 6 triphalangeal digits and tibial hypoplasia which lead to shortening of legs. The molecular-genetic study of boy and his mother identified heterozygosity for a 403G>C transversion in the zone of polarizing activity (ZPA) regulatory sequence (ZRS) in intron 5 of the LMBR1 gene in both patients. Population analysis of 150 unaffected people did not detect this mutation.

P12.258

Novel mutations in the POU3F4 gene in patients with X-linked hearing loss

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Up to half of the X-linked hearing loss cases are caused by mutations in the single exon gene POU3F4 which encodes a 361 aa POU domain transcription factor mainly expressed in the inner ear and central nervous system. Intragenic mutations as well as deletions of the coding region or the upstream region of POU3F4 have been reported. Most affected males show a profound sensorineural hearing loss with or without a conductive component (which can be masked by the sensorineural loss). Common features are stapedial fixation and temporal bone anomalies (including dilation of the internal auditory canal, visible in computed tomography (CT)) leading to a perilymphatic gusher following stapedectomy or during cochlear implantation.

We extracted DNA from blood samples of five patients with a characteristic temporal bone CT and sequenced the coding region of the POU3F4 gene and its flanking sequences. For the detection of larger deletions or duplications MLPA analysis was applied. In three patients we found intragenic mutations which have not been described so far: one missense mutation (c.844C>T, p.Arg282Trp), classified as probably pathogenic by biometric analysis, and two frame shift mutations (c.226delC, p.Leu76Trpfs*6; c.346dupG, p.Ala116Glyfs*77) leading to a truncated protein lacking both DNA-binding domains. The two other patients showed deletions of the POU3F4 coding exon.

Our study shows that POU3F4 mutations are frequently found in patients with X-linked hearing loss and that genetic testing may contribute to the confirmation of the clinical diagnosis.

P12.259

X-exome sequencing identifies the causal variant in a large pedigree with X-linked intellectual disability, truncal obesity, gynecomastia, hypogonadism and unusual face

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We characterized a large Dutch family with 7 males affected by a rare syndrome of X-linked intellectual disability (XLID), hypogonadism, gynaecomastia, truncal obesity, short stature, small head, short ears and recognizable craniofacial deformities. Eight females in this pedigree showed a much milder expression of the phenotype, comprising learning disorder and recognizable facial features. We performed X chromosome exome (X-exome) sequencing in five individuals from this family and identified a novel intronic variant in a histone deacetylation family member. The gene is located in the region for which linkage was established in this family with a maximum LOD-score of 4.93. The variant was shown to affect normal splicing, resulting in exon skipping and introduction of a premature stop. The mutation completely co-segregates with the phenotype in this family and is absent in Dutch controls or available exome databases. Interestingly, affected female carriers show a markedly skewed X-inactivation pattern in blood where all cells show the mutated chromosome X completely inactive. The mutated gene is important for epigenetic control of developmental processes and a knock-out model supports the facial phenotype present in our patients. In summary, we provide genetic evidence for the involvement of the histone deacetylation pathway in a syndromic form of XLID.

P12.260

Sequencing X-chromosome exomes: A diagnostic approach in non-syndromic intellectual disability

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Non-syndromic intellectual disability (ID) imposes a considerable diagnostic demand, as more than 100 genes have been associated with familial forms of ID. Since X-chromosomal genes greatly contribute at least in affected males, diagnostic tools for the detection of causal genetic changes are a prerequisite for molecular diagnoses.

In order to test, whether NGS provides us with a diagnostic platform in XLID, we have established a sequencing pipeline involving target enrichment for all coding sequences of the X chromosome. Sequencing of males patients with non-syndromic ID typically yielded in 60-100x coverage of X chromosomal genes and a robust base calling in these hemizygous individuals. Moreover, even drop-out of single exons could be monitored in our patients as measured as a significant reduction in read coverage per exon.

A first patient cohort of 12 XLID patients allowed us to work out the sensitivity of the system for the analysis of 88 XLID genes: More than 50 genes have been completely sequenced > 10x and only four genes had less than 90% of all coding bases covered less then 10x (ARX, IQSEC2, IKBKG, and NHS). Typically, less than 3-5 novel variants were found per patient, and further validation steps allowed us to reduce the list of potential disease-causing variants to 0-1 observations.

In summary, X-ome sequencing represents a robust tool for the detection of established XLID and thus closes a longstanding gap in the diagnostic repertoire for children with XLID.

P12.261

Two forms of rare short stature syndromes in Yakuts

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Hereditary short stature syndrome is one of the major concerns in Yakuts. We have identified 49 patients with short stature syndrome in 43 Yakut families with pre- and post-natal non-progressive growth failure, facial dysmorphism and normal intelligence. A genome-wide linkage analysis for these families revealed linkage to region 6p21.1 with the highest multipoint LOD score of 24.6 at D6S282. We applied a homozygosity mapping approach and narrowed the causative gene to the same locus of the 3-M syndrome (Huber et al, 2005). We found a novel homozygous 4582insT mutation in CUL7, which resulted in a frameshift and subsequent premature stop codon at 1553 (Q1553X). Second form of short stature is characterised by autosomal recessive inheritance, severe postnatal growth retardation, facial dysmorphism with senile face, small hands and feet, normal intelligence, Pelger-Hue¨t anomaly of leucocytes, and optic atrophy with loss of visual acuity and colour vision. This new syndrome is designated as short stature with optic atrophy and Pelger-Huet anomaly (SOPH) syndrome. Genomewide homozygosity mapping was conducted in 33 patients in 30 families. The disease locus was mapped to the 1.1 Mb region on chromosome 2p24.3, including the neuroblastoma amplified sequence (NBAS) gene. Subsequently, 33 of 34 patients were identified with SOPH syndrome and had a 5741G/A nucleotide substitution (R1914H) in the NBAS gene in the homozygous state. Immunohistochemical analysis revealed that the NBAS protein is well expressed in retinal ganglion cells, epidermal skin cells, and leucocyte cytoplasm in controls as well as a patient with SOPH syndrome.

P12.262

ZNF750 downregulation in keratinocytes promotes cell proliferation and decreases apoptosis

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Seborrheic dermatitis (SD) and Psoriasis are common dermatologic diseases with overlapping features. Each of the two dermatoses affects 2-3% of the population worldwide. The molecular mechanisms leading to excessive keratinocyte proliferation, the hallmark of both diseases, remain elusive. *ZNF750* mutations were previously reported to be associated with psoriasiform SD and with familial psoriasis. *ZNF750* encodes a putative transcription factor that is highlyexpressed in keratinocytes and represents a psoriasis candidate gene.

To understand ZNF750 function, we initially determined the sub-cellular localization of ZNF750 and assessed the effect of *ZNF750* silencing on cell proliferation and apoptosis in the human keratinocyte cell line, HaCaT. Immunofluorescence and subcellular fractionation followed by western blot analysis showed nuclear localization of ZNF750. In addition, using EGFP-tagged constructs, we identified which of the two ZNF750 nuclear localization signals is functional. As excessive proliferation of keratinocytes is a hallmark of both psoriasis and SD, we examined the effects of *ZNF750* silencing in HaCaT keratinocytes on cell proliferation (Ki67 assay) and apoptosis (annexin V assay). In comparison to controls, HaCaT cells in which *ZNF750* expression was down-regulated exhibited a 10-fold increase in cell proliferation and a 3-fold decrease in apoptosis.

We are currently performing genome wide expression microarray analyses of *ZNF750* silenced cells and ChIP-Seq analysis to identify downstream targets of ZNF750. Unraveling the role of ZNF750 in keratinocyte cell proliferation and determining its downstream pathways might open new insights to the events leading to excessive keratinocyte proliferation in psoriasiform SD and psoriasis.

P12.263

Distribution of the Q318X and R356W mutations of the CYP21A2 gene in Macedonian patients with classic 21-hydroxylase deficiency

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Background: Deficiency of 21-hydroxylase is present in 90-95% of all cases with congenital adrenal hyperplasia (CAH), an autosomal recessive disorder. Phenotype in all clinical forms of the disease: calssic salt wasting (SW) and simple virilizing (SV) and nonclassic late onset form (LO) corresponds to the genetic lesion in the CYP21A2 gene. The Q318X nonsense and R356W missense mutations at exon 8 that totally ablate 21-hydroxylase activity are most often associated with salt wasting disease.

Method: Using the PCR/ACRS method, we have performed direct molecular detection of the Q318X and R356W mutations in 35 Macedonian patients with classic clinical form of CAH (23 had SW and 12 had SV form), diagnosed according to standard clinical criteria at the Department of Endocrinology and Genetics, University Children's Clinic, Skopje, Republic of Macedonia.

Results: Mutations were detected in 20% (7/35) of all patients, Q318X in 5 (14.3%) and R356W in 2 (5.7%). In 17.4% (4/23) of the SW patients Q318X mutation was detected (three homozygotes and one heterozygote), and only one was heterozygote for R356W (4.3%). Among SV patients mutations Q318X and R356W were detected in one heterozygote (8.3%) each.

Conclusion: The R356W distribution in the Macedonian patients with classic CAH is comparable whereas the Q318X frequency was higher than reported in the most European populations. However, these findings further support a role of the severe mutations Q318X and R356W in the salt wasting form of the 21-hydroxylase deficiency.

P12.264

A genome-wide association study identifies risk loci for non-syndromic sagittal craniosynostosis on chromosomes 20 and 7

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Craniosynostosis, the premature fusion of one or more cranial sutures in the skull, is a common malformation, affecting 1 out of 2,500 live born babies. Sagittal craniosynostosis is the most common type, accounting for 40 to 58% of all cases. We conducted the first genome-wide association study (GWAS) for non-syndromic sagittal craniosynostosis using 130 European American case-parent trios. The strongest associations reached $p = 1.13 \times 10^{-14}$ (OR = 4.57) in the 3' UTR of BMP2 on chromosome 20 and $p = 1.61 \times 10^{-10}$ (OR=0.19) in an intron of BBS9 on chromosome 7. We replicated these associations ($p = 4.08 \times 10^{-32}$ and $p = 2.31 \times 10^{-15}$, respectively) in an independent European American population of 186 unrelated probands with non-syndromic sagittal craniosynostosis and 564 unaffected controls. We focused our studies on the locus on chromosome 20. We did not find coding region mutations of *BMP2* by direct sequencing; but by using quantitative real-time PCR, we found a significant increase in BMP2 expression in three of eight calvarial osteoblast cell lines derived from affected individuals as compared to control calvarial osteoblasts. ELISA assays and protein immunoblots showed that two of the same osteoblast lines have higher levels of BMP2 protein and increased phosphorylation of SMAD 1/5/8. In summary, we have identified two candidate loci for sagittal NSC and suggest that BMP2 plays a role in the genetic etiology of sagittal craniosynostosis.

P12.265

Improving genetic counselling in carriers of spinal muscular atrophy with two copies of the SMN1 gene.

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Autosomal recessive spinal muscular atrophy (SMA) is caused by mutations in the Survival Motor Neuron1 gene (*SMN1*) leading to loss of motor neurons of the spinal cord. Carrier frequency is around 1/50. Detection of carriers of *SMN1* deletions is crucial to define couples at risk for SMA offspring. One of the pitfalls in quantitative SMA carrier diagnosis is the presence of two SMN1 genes in cis (2/0 carriers). We analysed 2827 individuals (810 parents and 2017 other relatives) for SMA carrier diagnosis. In the group of parents, 786 (97%) showed one SMN1 copy. The remaining 24 showed two copies and based on the inheritance of the at-risk alleles and quantitative analyses of their parents or siblings, 18 individuals were considered 2/0 carriers (2.25%) and 6 "de novo" cases (0.75%). In the group of relatives we detected 600 carriers with one copy, 1362 two copies, 50 three copies and 5 four copies. Using the same criteria as in the parent group, 27 of the cases with two SMN1 copies (4.3%) were considered 2/0 carriers. In 414 individuals of the general population (partners of possible or confirmed carriers) 15 (3.6%) showed one copy, 342 two copies, 36 three copies and 4 four copies. We conclude that 2/0 carriers are detected using both, marker and quantitative SMN1 analyses. For better genetic counselling, cases from the general population can be identified after quantitative analyses of their parents when one parent show 3 SMN1 copies and the other one SMN1 copy (Supported by CIBERER/FIS 11-2606).

P13. Metabolic disorders

P13.01

Metabolarray®: targeted array-CGH for the detection of copy number changes in inherited metabolic disease

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Clinical molecular testing in inherited metabolic disease (IMD) is currently PCR-based precluding the identification of deletions which account for a variable fraction (1-25%) of mutant alleles depending on the gene involved. We have developed a high-resolution comparative genomic hybridization array (Metabolarray®) for the detection of CNV in 205 genes involved in IMD which are currently diagnosed in the laboratory. The array consists of 62,979 oligos spread genome wide, with 40,555 hybridizing to target genes with an average spacing of about 250 bp and 26,678 covering the rest of the genome. For validation, we have retrospectively analyzed a series of IMD patients carriers of exonic deletions previously genotyped by different methods (MLPA, SNP-arrays). All the heterozygous and homozygous deletions even of a single exon were detected using the Metabolarray®. We are applying this tool prospectively to a series of patients with incomplete genotype. In one propionic acidemia patient with discordant Mendelian inheritance, we have identified a novel 2 Kb deletion in the PCCB gene encompassing exons 4 and 5. Our results show the clinical utility of this new molecular tool in the genetic diagnosis of IMD, allowing the detection of CNV at the resolution of individual exons.

P13.02

Association of non-alcoholic fatty liver disease and hypercholesterolemia with mutations on the genes LEP, UGT1A1, ATP7B.

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Introduction: Leptin (Lep), a polypeptide hormone probably have been implicated in pathogenesis of non-alcoholic fatty liver disease (NAFLD) (Swellam M, et al, 2012). Wilson's disease (WD) is a severe disorder of copper misbalance, caused by mutations in a gene ATP7B and is accompanied by accumulation of copper in tissues, especially in the liver, that can manifest as liver pathology (including NAFLD). Gilbert syndrome could be involved in lipid metabolism
Aim: To determine whether the polymorphisms and mutations, mentioned below are associated with dyslipidemia in patients with steatohepatitis (non-alcoholic fatty liver disease).
Material and methods: The study population included 39 patients who had been screened for hypercholesterolemia (total cholesterol >5mmol/l) and steatohepatitis, that liver biopsy revealed. Determinants were the mutation *H1069Q* in ATP7B gene, $(TA)_7$ in gene *UGT1A1,* I and D alleles in *LEP* gene 3'UTR region. In control population there were 56 individuals.
Results: $(TA)_7$ allele frequency in affected individuals 0.5303 (p=0.47, OR 0.85, CI95% 0.46 - 1.58) - Deletion allele 0.39 (p=0.12, OR 0.63, CI 95% 0.35-1.14). *H1069Q* allele frequency 0.01, in control group it was not found. After linear regression analysis there were not found significant asssociation (p>0.05) with any of biochemical marker.
Conclusions: There have to be done larger study to evaluate genetic reason to find association for NAFLD and hypercholesterolemia.

P13.03

Deletion of *NT5E* promotes dislipidemia, intramyocellular lipid accumulation and results in peripheral insulin resistance

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Mutations in *NT5E* are the cause of nonfunctional CD73 in humans and subsequently result in calcification of lower-extremity arteries and hand and foot joint capsules. CD73 converts extracellular AMP to adenosine, which is known to inhibit lipolysis. It is unknown, however, whether adenosine formed by CD73 is functionally relevant in lipid homeostasis. We therefore explored the effect of CD73-derived adenosine on lipid metabolism of transgenic mice lacking CD73 ($CD73^{-/-}$) at the age of 6-8 months. Using ^{1}H MRI we found significantly decreased superficial fat content in $CD73^{-/-}$ mice (WT: 2.6±0.9 a.u.; $CD73^{-/-}$: 1.4±0.5 a.u.) accompanied by increased serum free fatty acids (WT: 203±65 µM; $CD73^{-/-}$: 354±141 µM), triglycerides (WT: 4.2±4.9 mg/dl; $CD73^{-/-}$: 15.4±12.2 mg/dl), blood glucose (WT: 111±14 mg/dl; $CD73^{-/-}$: 146±24 mg/dl) and serum insulin levels (WT: 1.20±1.15 µg/l; $CD73^{-/-}$: 7.06±5.51 µg/l). Consistent with insulin resistance, intramyocellular lipid levels as measured with localized ^{1}H MR spectroscopy were significantly increased (WT: 1.01±0.31 a.u.; $CD73^{-/-}$: 1.52±0.61 a.u.; n=10 each). Insulin-induced Akt phosphorylation was reduced in skeletal muscle of $CD73^{-/-}$ mice. Islets from WT mice did not express CD73 at the mRNA and protein level, and glucose-stimulated insulin release from pancreatic islets was not different between WT and $CD73^{-/-}$ mice. In contrast, high fat diet almost completely downregulated the expression of CD73 in WT mice, which was also observed in adipose tissue from ob/ob mice. Our findings suggest that adenosine generated by *NT5E*/CD73 is an important insulin independent modulator of lipid metabolism *in vivo*, whereas non-functional *NT5E*/CD73 results in peripheral insulin resistance.

P13.04

A novel cardiomyopathy syndrome due to dolichol kinase deficiency

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Congenital disorders of glycosylation are a growing group of inborn errors of protein glycosylation.Cardiac involvement is frequently observed in the most 14 common form, PMM2-CDG, especially hypertrophic cardiomyopathy. Dilated cardiomyopathy, however, has been only observed in a few CDG subtypes, usually with a lethal outcome. We report on cardiac pathology in nine patients from three unrelated Israeli families, diagnosed with dolichol kinase deficiency, due to novel, homozygous DK1 gene mutations. The cardiac symptoms varied from discrete, mild dilation to overt heart failure with death. Two children died unexpectedly with acute symptoms of heart failure before the diagnosis of DK1-CDG and heart transplantation could take place. Three other affected children with mild dilated cardiomyopathy at the time of the diagnosis deteriorated rapidly, two of them within days after an acute infection. They all went through successful heart transplantation; one died unexpectedly and 2 others arecurrently (after 1-5 years) clinically stable. The other 4 children diagnosed with mild dilated cardiomyopathy are doing well on supportive heart failure therapy. In most cases, the cardiac findings dominated the clinical picture, 33 without central nervous system or multisystem involvement, which is unique in CDG syndrome. We suggest to test for DK1-CDG in patients with dilated cardiomyopathy.Patients with discrete cardiomyopathy may remain stable on supportive treatment while others deteriorate rapidly. Our paper is the first comprehensive study on the phenotype of DK1-CDG and the first successful organ transplantation in CDG syndrome.

P13.05

Determing clinical revelance of gene conversion CYP21A2 and CYP21A1P

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Congenital Adrenal Hyperplasia (CAH) is a common autosomal recessive disorder caused mainly by mutations in the 21-hydroxylase gene (CYP21A2). The CYP21A2 is located at 6p21.3 about 30 kb apart from its pseudogene CAP21A1P, which encodes for an inactive protein due to the presence of 15 mutations. Gene and pseudogene are part of a tandemly repeated structure;

hence they are often targets of intergenic recombinations causing small lesions, deletions, duplications and gene conversions. Latter comprises fused genes with its 5`end and 3`end corresponding to CYP21A1P and CYP21A2 respectively, which are known to be pathogenic.

However, here we demonstrate three patients with a fusion between CYP21A2 and CYP21A1P observed by a suspicious MLPA result. In all patients exons 1-3 of the gene are duplicated whereas the corresponding region of the pseudogene is deleted. Direct sequencing reveals that a part of the gene (from upstream of the promoter to intron 3) indeed replaced the corresponding region of the pseudogene. Even if the gene promoter is present, the stop mutation in exon 7 of the pseudogene part generates a truncated protein. In addition two intact CYP21A2 copies are present.

In conclusion, the described chimeric gene CYP21A2/CYP21A1P is not disease causing, while at least one intact copy of the CYP21A2 gene is present. We recommend performing a long-range PCR in combination with direct sequencing subsequent to the observation of aberrant MLPA pattern to validate whether an intact gene copy is available.

P13.06

Defect of cobalamin intracellular metabolism (cblC, cblD and cblF defects) masquerading as diabetic ketoacidosis

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Inborn errors of metabolism, especially aminoacidopathies, manifesting as diabetic ketoacidosis or hyperglycemia are rare, only a few cases have been reported. We report a 13-month-old boy who presented with vomiting, dehydration, coma, hyperglycemia, high anion gap metabolic acidosis, and ketosis mimicking diabetic ketoacidosis (DKA). Treatment with parenteral fluid, electrolyte and insulin infusion lead to an improvement in hyperglycemia but persistence of metabolic acidosis and lack of improvement of neurologic findings led us to suspect an inborn error of metabolism. Urinary organic acid analysis revealed increased methylmalonic acid levels. In addition to this, he also had increased plasma and urine homocystine tested by high performance liquid chromatography (HPLC). There was some improvement in neurologic status and metabolic parameters after treatment with low-protein diet, vitamin B12, and L-carnitine but he ultimately succumbed to nosocomial sepsis. Methylmalonic aceidemia presenting with DKA like symptoms has been reported. But to the best of our knowledge, this is probably the first case report of late-onset combined methylmalonic acidemia and homocytinuria (cblC, cblD and cblF defects) masquerading as diabetic ketoacidosis. The early diagnosis of IEM is of utmost importance for the treatment, prognosis as well as genetic counseling for the family. High index of suspicion even in varied clinical presentation is the only way to diagnose these disorders in places where newborn screening is still not a routine practice.

P13.07

Genetic analysis of congenital disorders of glycosylation patients using candidate gene genomic capture and next generation sequencing

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Congenital disorders of glycosylation are a heterogeneous group of disorders caused by genetic defects in the protein glycosylation pathway. The clinical and subsequent biochemical diagnosis allow to classify the affected patients as CDG type I caused by defects in cytoplasmic and endoplasmic reticulum proteins or CDG type II caused by defects in the Golgi apparatus. Genetic diagnosis is required to identify the affected gene using a high-time consuming approach to sequence gene by gene. The aim of this study was to improve molecular diagnosis for congenital disorders of glycosylation by developing a customized array.We present the initial results obtained by combination of a targeted in solution capture from Agilent and subsequent next generation sequencing using the Solid platform. In this work 16 barcoded patients have been analyzed (seven CDGI and nine CDGII). On average, coverage was 45 to 60 fold and we have detected close to one-hundred SNV per patient. The SNV were filtered excluding common variants and also excluding synonymous, deep intronic variants and also UTR changes. The initial results have allowed the identification of pathogenic mutations in DPAGT1, RTF1 and COG7 genes. In summary the development of next generation sequencing panels in the genetic diagnostic laboratory allows a most efficient genetic diagnosis compared with the conventional gene-by-gene sequencing.

P13.08

Cystic fibrosis related diabetes-risk factors

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Background: Cystic fibrosis related diabetes (CFRD) is frequent in female with pancreatic insufficiency, carriers of so-called severe mutations . While risk factors for type 1 diabetes are well-known, CFRD risk factors who could be influenced are still looking. **Objectives**: Evaluate the risk factors for the development of CFRD in our patients. **Methods**: We performed retrospective seven years cohort study; 81 patients were evaluated. For all CFRD patients were obtained data about familial history of diabetes, positive antecedent of rickets and early diet. Biannual biochemical evaluation was completed in addition to clinical examination. **Results**: CFRD was diagnosed in eight patients (9.8%), 50% carriers of class I mutations. Prevalence was significantly higher in girls (75%) compared to boys. Median age at CFRD debut was 13.37 years and the mean age at CF diagnosis 7.27 years. We did not noticed positive family history for diabetes in any of patients. Five patients (62.5%) had rickets as toddlers, although vitamin D was given for prophylaxis . All of CFRD patients were nourished with cow`s milk formulas, one was breast fed for 2 months. Early introduction of gluten cereals (at 4 months) was documented in 7 patients (87.5%). Unfortunately 50% of CFRD patients died from pulmonary disease. **Conclusion**: Girls diagnosed late with CF, fed with cow`s milk and gluten nourishment in the early infancy, with positive history of vitamin D deficiency are more prone to develop CFRD. Early dietary intervention especially in female patients with "severe" genotype might be helpful.

P13.09

Metabolic characterization of common variants of the FTO and TCF7L2 loci by nutritional challenge tests

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Common single nucleotide polymorphisms in the FTO and TCF7L2 gene loci have been consistently associated with obesity and type 2 diabetes mellitus, respectively. The mechanisms underlying these associations remain poorly understood. Measuring metabolite response profiles by metabolomics during nutritional challenges may help to unravel how the interaction of genetic variants with lifestyle influences metabolism. We present an approach combining detailed phenotyping, nutritional and intravenous interventions and metabolomics analyses to investigate early metabolic alterations in healthy risk allele carriers. 77 non-obese male participants of the KORA S4/F4 cohort, aged 34 to 67 years, were recruited. 19/24 homozygous carriers of the FTO locus risk allele rs9939609, 16/17 carriers of the TCF7L2 locus risk allele rs7903146 and 20/24 homozygous controls performed nutritional and intravenous challenge tests, respectively. Nutritional challenges comprised an oral glucose tolerance test after overnight fasting, a standardized fast food meal, and a lipid tolerance test within a two-day study period. The intravenous challenge consisted of an intravenous glucose tolerance test and a subsequent euglycemic hyperinsulinemic glucose-clamp test. For metabolomics analysis, blood was sampled at three time points for each participant during each challenge and concentrations of 163 metabolites were determined using the AbsoluteIDQTM p150 kit (Biocrates Life Sciences AG). We show the initial analyses of metabolite-response profiles to the different challenges. The presented approach intends to functionally characterize genetic variants in the FTO and TCF7L2 loci and to identify novel markers of prediabetes. The presented design provides a framework for further analysis of additional risk variants.

P13.10
Investigation of CAT gene C1167T polymorphism in diabetic nephropathy

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Diabetic nephropathy (DN) is the most severe diabetic complication. Oxidative stress may play a role in its pathogenesis. Antioxidant defense seems to be modulated by genetic variability. The aim of this study was to investigate the association of CAT gene C1167T (rs769217) polymorphism with DN in type 1 diabetes.

Clinical data and blood samples were collected from 269 Romanian patients with type 1 diabetes. They were divided in two groups according to the presence of DN - 108 patients without DN and 161 patients with DN. Genomic DNA was extracted from peripheral blood leucocytes using commercial kits and the rs769217 polymorphism was assessed by TaqMan SNP assay on the ViiA7 Real-Time PCR system. Statistical analysis was performed using PLINK v1.07 software.

The sample population was in Hardy-Weinberg equilibrium. The frequency of minor allele (T) was 0.21 in DN group and 0.26 in the controls without DN. In raw analysis neither T allele (OR=0.762, [95%C.I. 0.512-1.135], p=0.18) or C allele (OR=1.312, [95%C.I. 0.881-1.954], p=0.18) conferred risk or protection for DN. We performed adjustment for a minimal additive model (age, sex, duration of diabetes and glycated hemoglobin A1c), but the results remained concordant with the raw analysis (ORT=0.773, [95%C.I. 0.5073-1.178], p=0.23].

In summary, CAT gene C1167T (rs769217) polymorphism does not seems to confer risk for DN in patients with type 1 diabetes.

P13.11
Type V Hyperlipoproteinemia with Neonatal Onset

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Introduction. Familial hyperlipoproteinemias in neonates are rarely encountered in clinical practice. The incidence of Frederickson type V familial hypertriglyceridemia is not estimated in childhood. The pattern of inheritance is autosomal dominant. **Case report**. We present a case of sixteen days old newborn admitted in our department for milky plasma detected in Maternity and association of severe bleeding disorders. Physical examination revealed pallor, dysmorphic face with xanthomas, mucosal bleeding, abdominal distension. Laboratory findings identified extremely high values for triglycerides (2500 mg/dl), cholesterol (1276 mg/dl initially then 336 mg/dl with HDLc 19 mg/dl, LDLc 3,87 mg/dl), total fats (5317 mg/dl), the test for chylomicrons positive, severe alteration of coagulation tests without proof of clotting factors deficiency, transitory thrombocytosis. Echocardiography revealed non-compaction cardiomyopathy. First -degree relatives have evidence of dyslipidemia above 90th percentile. **Conclusion.** Our case presented a rare familial hyperlipoproteinemia type V with neonatal onset associated with severe bleeding disorder and non-compaction cardiomyopathy.

P13.12
Development of a cell-based reporter assay for the analysis of regulatory interactions between FGF23/KLOTHO/FGFR1, small inhibitors and downstream targets

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The analysis of rare genetic disorders affecting phosphate homeostasis led to the identification of several proteins essential for the renal regulation of phosphate homeostasis: PHEX (XLH [MIM 307800]), FGF23 (ADHR [MIM 193100]), SLC34A3 (HHRH [MIM 241530]), DMP1 (ARHR1 [MIM 241520]), ENPP1 (ARHR2 [MIM613312]), GALNT3 (FTC [MIM 211900]), and KLOTHO (FTC [MIM 211900]). A key regulator of phosphate homeostasis is the fibroblast growth factor 23 (FGF23). It is mainly secreted from osteocytes, circulates in the blood, and binds to receptor heterodimers composed of FGF receptor 1 (FGFR1) and KLOTHO in the kidney. FGF23 activates KLOTHO/FGFR1 to inhibit renal phosphate reabsorption and to suppress 1,25-dihydroxyvitamin D3 synthesis. As a key signalling pathway mitogen-activated protein kinase (MAPK) pathway is employed. To analyse regulatory interactions between FGF23/KLOTHO/FGFR1, small inhibitory compounds and further downstream targets, we have developed FGF23-inducible HEK293 cells that stably express KLOTHO (HEK293-KL). Stable cell clones were picked, expanded, and expression of KLOTHO was confirmed by Western blot analysis. By investigating the activation of MAPK pathway we could show that HEK293-KL cells are FGF23-inducible. Moreover, we could inhibit the induction with FGF23 by the use of two small inhibitory molecules: (1) SU5402, an inhibitor of FGFR1 and (2) U0126, an inhibitor of MAPK pathway. Taken together, we have established a potent cell-based reporter assay, which can now be used to investigate FGF23/KLOTHO/FGFR1 receptor signalling and receptor complex inhibition in more detail. We will try to identify novel downstream targets which may be candidates for regulatory compounds involved in phosphate homeostasis.

P13.13
A novel splice mutation and a novel exon deletion in the AGL gene in a patient with Forbes-Cori disease (GSD III)

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Glycogen storage disease type III (GSD III) is an autosomal recessive disorder characterized by excessive accumulation of glycogen in the liver and in skeletal / cardiac muscles. The typical symptoms are hepatomegaly, hypoglycaemia and muscles weakness, also shown in our 4 years old male patient. GSD III is caused by a deficiency of the glycogen debranching enzyme (AGL). We performed screening for mutations in the AGL gene by sequencing all exons including flanking intronic sequences. The analysis revealed a heterozygous mutation at the donor splice site of exon 10. Considering the autosomal recessive inheritance pattern of GSD III, this mutation cannot solely be responsible for the present phenotype in the patient. So it was appropriated to search for possible deletions in the AGL gene. A gross deletion was excluded by Array-CGH. As there is no commercial MLPA kit for AGL available, we designed our own MLPA probes. Having established the assay we analyzed DNA from the patient. We found a heterozygous novel single exon deletion in the AGL gene. This result was confirmed by junction fragment analysis using flanking primers. Sequence analysis of the junction fragment revealed a 2.3 kb deletion and the intronic break points. The deletion could also be detected in paternal DNA and the splice mutation in maternal DNA. This demonstrates the compound heterozygosity of the two detected genetic alterations. Our results show that home-made MLPA tests are an appropriate method to detect causative exon deletions in genes since there is no commercial MLPA kit available.

P13.14
Efficacy of enzyme replacement therapy with velaglucerase alfa in patients with type 1 Gaucher disease and marked thrombocytopenia or splenomegaly

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Background. The responses of type 1 Gaucher disease (GD)-related thrombocytopenia and splenomegaly to enzyme replacement therapy (ERT) are linked to their pretreatment severity.

Methods. TKT032 and HGT-GCB-039 were parallel-group trials; eligible patients were ≥2 years old with untreated type 1 GD. In both trials, 1 treatment arm was allocated to velaglucerase alfa 60 U/kg ERT every other week (EOW). Patients completing either trial could enrol in a combined extension study, HGT-GCB-044.

Results. 27 type 1 GD patients received velaglucerase alfa 60 U/kg EOW in TKT032 or HGT-GCB-039 and HGT-GCB-044 over 24 months. 15/27 patients had a pretreatment (Baseline) platelet count <100×10^{9}/L; 6 of these 15 had a platelet count <60×10^{9}/L. All 15 had an intact spleen. 6/27 patients had severe Baseline splenomegaly (splenic volume >15 multiples of normal); all 6 had a platelet count <100×10^{9}/L. At 24 months, 14/15 (93%) patients had reached the platelet count therapeutic goal and 6/6 with severe Baseline splenomegaly had reached the splenic goal. 5/6 (83%) patients with a Baseline platelet count <60×10^{9}/L had a normal platelet count (≥120×10^{9}/L), including 2 with severe Baseline splenomegaly (Table).

Table. Individual platelet and splenic responses: 24 months of study drug

Base-line age, y	Gender	Platelet count, ×10⁹/L				Splenic volume, multiples of normal			
		Baseline age, y	Gender	Baseli-ne	12 m.	24 m.	24-m. change, %	Baseli-ne	24-m. change, %
7	M			44	124	148	236	31.6*	−81.7
9	M	66	92	134	105	35.0*	11.0	6.3	−82.0
18	M	77	188	228	196	8.9	4.1	3.2	−63.5
19	F	47	150	181	285	6.4	2.7	2.2	−66.2
23	F	50	102	152	204	10.8	5.1	3.5	−67.4
24	M	50	62	147	197	26.4*	16.0	6.2	−76.7
25	F	68	85	151	122	33.3*	7.7	4.3	−87.0
27	M	89	190	176	99	9.5	4.3	3.7	−61.6
29	M	62	75	75†	21	36.9*	17.2	12.3	−66.7
29	F	44	85	222	405	11.2	3.8	2.6	−76.4
31	F	99	193	192	94	5.7	3.4	2.7	−52.4
36	F	99	101	163	66	7.2	4.3	3.5	−50.9
42	M	64	94	109	70	19.1*	10.3	7.1	−63.0
44	F	90	155	192	113	8.5	5.1	4.0	−52.7
58	M	50	92	112	124	12.0	4.1	3.0	−75.3

*Patients with spleens >15 multiples of normal in volume at Baseline.
†Below goal.
Therapeutic goals by 2 years: splenic volume must decrease 50-60%; Baseline platelet count 60-120×10⁹/L must be ≥100×10⁹/L; Baseline platelet count <60×10⁹/L must increase 2-fold.

Conclusion. Clinically significant improvements in platelet count and splenic volume occurred in the first 24 months of velaglucerase alfa treatment among patients with type 1 GD and severe Baseline splenomegaly and/or a platelet count <100×10⁹/L (including those with a platelet count <60×10⁹/L).

P13.15

The genetic origin of glutaric aciduria type I in Belarus

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Glutaric aciduria type I (GA-I) is a rare organic aciduria caused by inherited deficiency of glutaryl-CoA dehydrogenase which is involved in the catabolic pathways of L-lysine, L-hydroxylysine and L-tryptophan. From 1975 more than 500 patients were diagnosed worldwide. More than 200 disease-causing mutations of GCDH gene (19p13.2) are known thus far, most mutations are unique to individual families. In some countries GA-I is included in the panel of diseases identified by expanded newborn screening.
In Belarus the possibility to diagnose GA-I appeared after the beginning of acylcarnitine analysis by tandem mass spectrometry (MS/MS). From 2007 4120 patients passed selective screening by MS/MS, and four unrelated patients, aged 7 months - 2 years, were diagnosed as having GA-I. The analysis of GCDH gene revealed one common mutation, p.R402W, covering 75% (6 from 8) of all mutant alleles. High prevalence of p.R402W also among Russian GA-I patients indicates possible Slavic origin of this mutant allele and suggests that screening for this mutation may be appropriate for the confirmation of biochemical and clinical diagnostic, identification of carrier status and prenatal diagnosis of GA-I in this region.

P13.16

Association of HFE gene mutations with HLA-A and -B alleles in patients with idiopathic hepathobiliar disorders

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Hereditary hemochromatosis (HH) is an autosomal recessive disease characterized by abnormal accumulation of iron in parenchymal organs leading ultimately to organ dysfunction. This is the most common inherited liver disease. HH gene (*HFE*) is located within the human leukocyte antigen (HLA) class I region on chromosome 6. It is suggested linkage disequilibrium between these genomic regions which results in certain clinical features of HH. The aim of the study was to establish the distribution of HLA-A and -B alleles in patients with idiopathic hepathobiliar disorders (IHD) in association with C282Y and H63D mutations in *HFE* gene.
The presence of *HFE* mutations was established in 70 patients with IHD (chronic idiopathic hepatitis, liver cirrhosis, hepatomegaly) and in 60 healthy controls. HLA typing was performed in 25 *HFE* mutation carriers and 20 non-carriers. *HFE* mutations were screened for by RFLP performed on PCR products. HLA alleles were detected using allele-specific PCR.
Heterozygous C282Y mutation found in 14.3% of patients with IHD was significantly higher than in control group (χ^2=4.625, P<0.05). The differences in H63D mutation frequencies were not reliable. An expected significant association between *HFE* mutations and HLA-A3 allele was established (χ^2=3.902, P<0.05). 4 out of 5 patients with chronic idiopathic hepatitis were HLA-A3/B7, HLA-A3/B62 and HLA-A3/B14 genotype carriers in association with both C282Y and H63D mutation.
We suggest that heterozygous status for C282Y mutation may be a risk factor for IHD. HLA-A3 allele and HLA-A3-containing genotypes in combination with *HFE* mutations may play important role in susceptibility to chronic idiopathic hepatitis.

P13.17

Diagnostic tests for the genetic defects of urate transporters

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Introduction: Primary hereditary renal hypouricemia is a genetic disorder affecting renal uric acid (UA) reabsorption with clinical features such as nephrolithiasis and exercise-induced acute renal failure. The known causes are: defects in the SLC22A12 gene, encoding the human urate transporter 1 (hURAT1), and also impairment of voltage urate transporter (URATv1), encoded by SLC2A9 (GLUT9) gene. Diagnosis is based on hypouricemia (< 119 µmol/l) and increased fractional excretion of UA (> 10%). To date more than one hundred Japanese patients with mutations in hURAT1 gene have been described. Hypouricemia is sometimes overlooked, therefore we have set up the flowchart for this disorder.
Methods**:** The patients were selected for molecular analysis from 640 Czech hypouricemic patients. These cases were found in 3 700 blood and urine samples. Serum and urinary UA and creatinine were determined. The sequence analysis of SLC22A12 and SLC2A9 genes were performed.
Results: Other secondary causes of hyperuricosuric hypouricemia were excluded. The estimations of : 1) serum UA, 2) excretion fraction of UA, 3) and analysis of hURAT1 and URATv1 genes follow. We have found 3 transition, 4 deletions in SLC22A12 gene and one nucleotide insertion in SLC2A9 gene in overall 7 Czech patients. Three patients had acute renal failure and urate nephrolithiasis.
Conclusions: Our finding of the defects in URATv1 gene gives further evidence that SLC2A9 is a causative gene of primary renal hypouricemia. Hereditary renal hypouricemia is still unrecognized disorder and probably not wide spread in East Asia only. (Supported by project PRVOUK, MOLMED of Charles University).

P13.18

Identification of two novel isoforms of the HNF1A gene

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Background
HNF1A is a transcription factor that plays a central role in the regulation of pancreatic beta cells. It controls the transcription of key genes such as insulin and GLUT2. Their mutations cause deregulation of certain processes leading to the onset of MODY3 diabetes. So far it has been described three HNF1A isoforms. We have identified two new isoforms in liver cells.
Methods
HNF1A cDNA was synthesized in HepG2, Capan 1, Hek and MCF7 cells (negative control). The reaction products were cloned to analyse the isoforms activity by luciferase assay in COS1 and HepG2 cells. We carried out a predictive analysis of the structures of two new isoforms and their location was determined by immunofluorescence in both cell types.
Results
We found two unreported transcripts: HNF1AΔ2, which lost exon 2 and the reading frame, and HNF1AinsIVS8, who never missed the reading frame and inserts 31 amino acids in the protein. Luciferase analysis show a 77% reduction in expression levels of isoform HNF1AΔ2 and a twofold increase in the activity of the isoform HNF1AinsIVS8 with respect to the Wt. The structure prediction shows a significant change in the case of isoform HNF1AinsIVS8. Immunofluorescence analysis shows that this isoform maintain the nuclear localization. This isoform is expressed in Hepg2 cell line but not in Capan1, HEK293T, JURKAT or MCF7. Analysis of HNF1AΔ2 isoform is underway.
Conclusion
We report two novel isoforms cloned by HNF1A gene that are expressed in HepG2 cell line and could help to better understand HNF1A activity.

P13.19
Molecular genetic analysis in patients with congenital hyperinsulinism of infancy: Identification of novel mutations in *ABCC8* and *KCNJ11* and implementation of genetic diagnosis in disease management

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Congenital hyperinsulinism (CHI) causes persistent hypoglycemia due to uncontrolled insulin secretion in newborns and infants. Since patients are at increased risk of neurological sequelae, a rapid diagnosis and disease management is mandatory. CHI is a heterogenous condition at the clinical and genetic level. Mutations in several genes including *ABCC8*, *KCNJ11*, *GLUD1*, *GCK*, *HADH*, *SLC14A1*, *HNF4A* and *UCP2* have been identified in approximately half of the patients in several patient cohorts. The most severe forms of CHI are caused by loss of function of the *ABCC8* and *KCNJ11* genes encoding subunits of the ATP-sensitive K(+) channel. They are usually associated with unresponsiveness of pancreatic ß-cells to medical treatment. Recently, it has been suggested to implement rapid genetic analysis of *ABCC8* and *KCNJ11* in newborns with diazoxide unresponsive CHI to improve patient management. We report sequence analysis in 37 CHI patients referred to us within one year. Mutations in *ABCC8* and *KCNJ11* were observed in 7/17 patients aged less than one year and in 5/20 patients at age of more than one year. Seven patients harbored homozygous or compound heterozygous mutations that cause diffuse form of CHI and five patients had paternally transmitted single heterozygous mutations predicted to cause focal form of CHI. Together, 14 different mutations were identified and 12 were novel with one recurrent mutation in two consanguineous families of Turkish descent. In conclusion, genetic analysis of *ABCC8* and *KCNJ11* in CHI newborns and infants revealed mutations in about one third of the patients and contributed to further patient management.

P13.20
Clinical signs of autosomal dominant inherited hypophosphatasia

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Hypophosphatasia (HPP) is a hereditary metabolic disorder of the bone. It is caused by impaired activity of the tissue-nonspecific isoenzyme of alkaline phosphatase. Five clinical forms are distinguished depending on the patient's age when clinical symptoms occur. Early onset of the disease normally accompanies a severe clinical course, in this case HPP is normally transmitted as an autosomal recessive trait. HPP due to a single mutation in the ALPL gene and inherited as an autosomal dominant trait can cause milder forms without clearly defined symptoms so far.

Results: Here we present clinical data of 9 families and 22 individuals with one mutation in the ALPL gene. Stress fracture and impaired healing of fractures are leading symptoms of dominant HPP. The majority of mutation carriers show signs of odontohypophosphatasia like premature tooth loss, tooth decay and caries. One third of the patients complained about joint pain, followed by pain of the muscles. Approximately a quarter of patients reported impaired physical fitness. Only few patients suffered from ostealgia.

The majority of patients showed decreased activity of the alkaline phosphatase. Eight missense mutations in the ALPL gene and no nonsense mutation were found.

Conclusion: The clinical signs of dominant HPP are not well known and despite of decreased activity of the alkaline phosphatase diagnosis of dominant HPP is rarely made. Therefore, these patients are often not treated in an appropriate way.

P13.21
The inherited metabolic diseases of liver in genetic practice

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Objectives: To investigate the role of inborn errors of metabolism (IEM) as a cause of liver disease and their mutation spectrum.

Patients and Methods: A group of 2837 patients with hepatomegalia/hepatosplenomegalia or jaundice of unknown etiology was investigated. The diagnosis of IMD was based on the results of biochemical investigation. The DNA assays included restriction analysis, allele-specific hybridization or direct sequencing.

Results and Conclusions: The IMD appeared the cause of liver disease in 68% (1929/2837) of investigated patients. The following IMD were detected: galactosemia - 20 cases (mutant alleles Q188R and K285N - 64%), inherited fructose intolerance - 2, glycogen storage disease type Ia - 2 (R43C - 50%), Ib - 4, III - 1, VI-1, alfa-1-antitrypsin deficiency - 43 (PiZZ- 13, PiMZ - 28, PiMS - 2), Wilson disease - 55 (H1069Q, 2299insC, I1102T, 3400delC - 76%), Nieman-Pick type C - 4, CESD - 3 (E8SJM - 50%), hereditary hemochromatosis - 5 (C282Y - 2, C282Y/H63D - 3), Gaucher disease type I - 8 and III - 2 (N370S, L444P, RecNciI - 77%), methylmalonic aciduria - 3, LCHAD deficiency - 3, VLCAD deficiency - 1, syndrome HHH - 3, CDG-syndrome -1, tyrosinemia - 3, Alpers syndrome - 1 (A467T/G268A), hepatocerebral mitochondrial depletion syndrome - 2, Zelweger syndrome - 2, citrullinemia type 1 - 1, Gilbert's syndrome - 1759. IEM are the frequent cause of liver disease and should be kept in mind working with such groups of patients.

P13.22
Maternally inherited Leigh syndrome in a Hungarian patient with the G13513A mutation

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Leigh syndrome (LS) is a progressive neurodegenerative disorder with symmetrical lesions in the brainstem and/or basal ganglia in infancy and childhood. Mutations in nuclear and mitochondrial genes of the energy metabolism have been associated with LS. The G13513A mutation in ND5, one of the 7 mtDNA encoded subunits of Complex I, was originally reported in MELAS, but later identified in children with LS.

We present the first Hungarian documented case of LS associated with the G13513A mtDNA mutation in a 3,5 year-old girl. Psychomotor delay was noted at the age of 1 year, unaided walking developed at 17 months, bilateral ptosis at 21 months, ataxia and intention tremor at 3 years of age. Elevated blood and CSF lactate, multiple lesions in the cerebellum, brain stem, caudate nucleus, internal capsule and thalami pointed to LS. Progressive deterioration from age 3 years on lead to death at age 3,5 years due to aspiration. Mutation analysis from blood showed a 60% heteroplasmy of the G13513A mutation, postmortem muscle and liver tissue revealed 70% mutation loads. No mutation was detected in blood from the mother.

The G13513A mutation has not been detected in the 350 Hungarian mitochondrial encephalomyopathy patients from our Biobank so far. Despite the variable phenotype, the course of disease and the absence of cardiologic manifestations differentiate our case from previous reported ones. Since the A13513G mutation is often observed as a de novo change it is important to carefully examine multiple tissues from the mother before genetic counseling can be given.

P13.23
Leigh Syndrome with mild neonatal Complex IV deficiency

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Isolated complex IV deficiency is a frequent cause of respiratory chain impairment and mitochondrial disease. Onset, nature and severity of the clinical presentation or the genotype of these patients are heterogeneous.

Here, we report clinical, biochemical and genetic data of a male infant with pathogenic mutations in *COX15*. The patient was born as the 2nd child to healthy, not related parents and has an healthy brother. He was hypotonic and presented with feeding difficulties, failure to thrive, psychomotor retardation, lactic acidosis and cardiomyopathy. His MRI was suggestive for Leigh Disease. The cardiomyopathy progressed rapidly and the patient died of heart failure at five months of age.

Oxidative phosphorylation (OXPHOS) enzyme activities were measured using spectrophotometrical analysis. Biochemical analysis of the COX activity showed a very mild decrease in fibroblasts and a low normal range value in muscle tissue. Other respiratory chain activities were normal. Functional integrity of the five complexes was evaluated using blue native polyacrylamide gel electrophoresis followed by in-gel activity staining in muscle tissue, and showed a decreased amount of fully assembled CI, CII, CIII and CIV, and the presence of subassembly products of CV.

Sequencing analysis of the coding exons of the *COX15* genes revealed that the patient is compound heterozygous p.Ser151X/p.Pro302Leu. Although the number of reported *COX15* mutant patients is limited to four, the p.Ser151X mutation was previously seen in two unrelated families with a predominant clinical presentation of cardiomyopathy. This mutation might represent a hot spot location in the *COX15* gene.

P13.24

Recurrent LMNA mutation in patients with familial partial lipodystrophy Dunnigan-type

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Familial partial lipodystrophy (FPLD) Dunnigan type is an autosomal dominant disorder with abnormal distribution of adipose tissue. After puberty, patients show excess fat on their face, neck, back, and absence of subcutaneous fat of the extremities, trunk and gluteal region resulting in a muscular appearance. Metabolic abnormalities include insulin-resistant diabetes mellitus, abnormal serum lipoproteins, and hypertension. FPLD is a heterogeneous disorder with mutations in LMNA and other genes including PPARG, AGPAT2, and PLIN1. We compared clinical and molecular findings in 15 patients presenting with FPLD. Sequencing of LMNA identified a heterozygous missense mutation in exon 8 (p.R482Q) in 5 female patients from 3 families. All 5 carriers of p.R482Q show a characteristic muscular habitus with abnormal fat distribution. Of note, 3 of 5 patients complained about muscle pain, which seems to be an additional characteristic feature. 3/5 patients had mild elevated HbA1c (mean 6.2 %). One patient only required insulin therapy since the age of 47 years. Patients showed dyslipoproteinaemia with hypertriglyceridemia (3/5), elevated cholesterol (4/5), low HDL (3/5), and elevated LDL (3/5). Additional features included hypertension (4/5), hepatic steatosis (2/5). None of the p.R482Q carriers but 8/10 of the other patients without LMNA mutations had pancreatitis. The overlap of metabolic features in FPLD and metabolic syndrome and the overrepresentation of female patients raise the possibility of significant under diagnosis of FPLD among patients with the reported metabolic changes. Especially men with a muscular habitus and less prominent metabolic changes may be suspected to have metabolic syndrome instead of FPLD.

P13.25

Is histidinemia always a “non-disease”? and why truncating mutations in the HAL gene appear so rare?

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Histidinemia is an autosomal recessive defect with an incidence of 1/12000-20000, due to a deficient activity of histidine-ammonia-lyase (HAL gene). Initial studies in the 1970s suggested that it might be associated to intellectual deficiency, epilepsy, autism or ataxia. Later assessment of cases identified through neonatal screening, who did not suffer from developmental delay or ID, led to consider histidinemia as a non-disease. A recent study has suggested however that it may be a risk factor for autism (Miyachi et al. 2009). Because of this lack of established clinical impact, interest in this trait has subsided and a single mutation study in the HAL gene revealed only 4 different missense mutations in a minority of tested cases ascertained through neonatal screening. Following the identification of histidinemia in a girl with developmental delay and ataxia, we initiated a mutation study. Preliminary results on 3 patients with ID revealed 5 different missense mutations, and one splice site mutation. In another case with no reported neurological phenotype, another homozygous missense variant was found, not predicted pathogenic by SIFT or Polyphen2. Up to now, truncating mutations appear underrepresented (one detected compared to 10 different missenses). The spontaneous histidinemic mouse mutant (with no obvious neurologic phenotype) is also due to a missense mutation. One may wonder whether phenotype in histidinemia depends on the level of residual activity, and whether total loss of function may be lethal or very severe. We are looking for collaborations to extend this study to other cases with or without neurological involvement.

P13.26

GI microbiota and epigenetic markers in metabolic syndrome and caloric restriction

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Microbial diversity, abundance and metabolic activities contribute to a highly individual GI tract microbiota. The metabolic syndrome is one condition, where host genetic factors, microbiota composition and microbiota-directed regulation of gene expressions combined contribute to pathogenesis. Intestinal symbiosis is maintained via the signaling activities of short chain fatty acids (SCFAs), bacterial LPS mediated immune reactions, but also via epigenetic mechanisms, e.g. causing a hyporesponsiveness of toll-like receptors (TLRs) towards the symbiotic and commensal constituents of the microbiota.

We analyzed changes in microbiota and epigenetic regulation of inflammatory mediators in type 2 diabetic (n = 25) volunteers under caloric restriction in comparison to lean (n = 18) and obese (n = 8) healthy controls. The abundance of bacteria and bacterial subgroups we measured in fecal samples with quantitative PCR (qPCR) of 16S rRNA coding regions.

Lactobacilli and Clostridium cluster XIVa differed significantly in type 2 diabetics compared to lean controls before intervention and after weight loss. Obese individuals had a higher Firmicutse to Bacteroidetes ratio than lean controls. In type 2 diabetics with weight loss the ratio of Firmicutes to Bacteroidetes increased throughout the study period.

In addition to changes in the microbiota composition, we also report that epigenetic mechanisms regulate gene promoters with relevance to inflammation, antioxidation and DNA-repair in type 2 diabetes and that diet and caloric restriction have epigenetic regulatory effects.

P13.27

High endocannabinoid levels are genetically determined and are associated with obese phenotype

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The endocannabinoid system (ECS) is involved in energy homeostasis and food intake. It was suggested that ECS hyperactivation may contribute to obesity development, however conflicting data are reported.

Aim of this study was to investigate the association of variants in the ECS receptor (CNR1) and degrading enzyme (fatty acid amide hydrolase, FAAH) genes with the obese phenotype and the plasma level of ECS mediators. 736 randomly selected subjects were submitted to SNPs genotyping and measurement of EC plasma levels. Seven SNPs in CNR1 region (rs12720071, rs806368, rs806370, rs1049353, rs806381, rs6454674, rs10485170) and the coding variant c.385C>A in FAAH gene (rs324420) were genotyped by MassARRAY platform. Circulating ECs, AEA, 2-AG, PEA and OEA were measured by an LC-MS/MS method validated according to FDA's guidance. Genotypes were compared to EC levels and to body mass index (BMI; kg/m^2), waist circumference and waist-to-hip ratio.

EC circulating levels were significantly higher in overweight and obese (BMI>25,0) compared to normal weight subjects (AEA, PEA, OEA p=0,00001; 2AG p=0,008). FAAH 385A allele carriers showed significantly increased EC levels, particularly OEA ($p=8x10^{-11}$) and PEA ($p=5x10^{-7}$), but they showed no significant association to obesity indexes. Coexistence of FAAH-predisposing genotype and increased EC levels also did not correlate with obesity. None of CNR1 SNPs showed association with obesity indexes, nor with EC increased levels. In conclusion, our data show that the FAAH 385A allele has a direct effect on EC elevated plasma levels, and that the detected hyperactivation of ECS may be a novel biomarker of obesity.

P13.28

Efficiency of metformin treatment for obesity and metabolic syndrome in children and adolescents depends on TCF7L2 genotype

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Despite a large body of research, currently there is no common strategy for drug therapy of obesity and metabolic syndrome (MS) in children and adolescents. At present, Metformin is the drug of choice for treating children with MS over 8 years old. However, a number of patients remain refractory to Metformin. We investigated the contribution of TCF7L2 IVS3 C>T polymorphism to response to Metformin in children with MS. 38 of 200 children with obesity in the age of 10 - 17 enrolled in this study were treated with Metformin. Patients were diagnosed with MS in accordance with guidelines developed by International Diabetes Federation in 2007.Among 38 of 200 children treated with Metformin, one (2.6%) was diagnosed with MS, and in another 37 abdominal-type obesity with concurrent abnormalities was manifested. Abnormalities included impaired glucose tolerance (in 35.1% of children), hyperinsulinemia (in 32.4%), atherogenic dyslipidemia (in 45.9%), hypertension (in 10.8%). 20 children taking Metformin were genotyped as C/C, 13 as C/T, and 5 as T/T. Postprandial glucose level in children with C/T and T/T genotypes was higher than in children with C/C genotype. During the treatment, the most pronounced improvements were observed for children with C/T genotype, namely, reduction in body weight ($p<0.001$), body mass index ($p<0.001$), waist circumference ($p<0.001$), fasting plasma ($p=0.017$) and postprandial ($p=0.003$) glucose levels, uric acid level ($p=0.05$), atherogenic index ($p=0.029$). Our data suggest that Metformin treatment efficiently promotes body weight reduction and normalization of carbohydrate metabolism in children, suffering from obesity and MS, with TCF7L2 C/T genotype.

P13.29

New probable pathogenic mutations in 22 tRNA mitochondrial genes in Iranian cytopathy patients

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Mitochondrial tRNA (*MTT*) gene mutations are an important cause of human morbidity and are associated with a wide range of pathology, from isolated organ-specific diseases such as myopathy, hearing loss, MELAS, MERRF, CPEO, Leigh syndrome through to multisystem disorders with encephalopathy. The aim of this study was to detect any type of mutations, polymorphisms and possible pathogenic variations in blood samples of Iranian cytopathy patients. We describe the result of extensive sequence analysis for tRNA genes in 18 patients selected according to several criteria. The result included: reported mutation as T5543C in tRNA^{Trp}, A8344G in tRNA^{Lys} , G12236A in tRNA^{Ts} and Several

known polymorphism such as A12308G were observed. These findings are besides to novel transitions, m.15930G>A in tRNA^{Thr} , m.5790C>A in tRNA^{Cys} and m.10467 in tRNA^{Arg}, in different patients who were negative for reported mtDNA mutations. These nucleotide were moderated and they were absent in 100 control suggesting its pathogenicity . So further investigation includes familial study, functional assay and nuclear genes analyses are needed.

P13.30

Large-scale deletions on 22q13.33 and 12q24.33 detected in patients with mitochondrial disorders

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Mitochondrial disorders (MD) represent a group of clinically and genetically heterogeneous diseases whose molecular-genetic diagnosis is challenging. The standard diagnostic approach is direct DNA sequencing of candidate genes. Nevertheless, this technique does not enable to detect large heterozygous deletions. SNP arrays with high marker density may be utilized. The **aim** of this study is to analyze large-scale deletions in group of 15 patients with MD of unknown etiology. Genome-Wide Human SNP 6.0 array (Affymetrix) was used for genotyping of DNA isolated from leucocytes of patients. In 20% of patients, large deletions as a cause of MD were found. In 2 of them, 175-kb, 87-kb resp. heterozygous deletions on 22q13.33 affecting *SCO2* and *TYMP* genes were detected. Missense point mutations in *TYMP*, *SCO2* genes resp. were identified on the other allele. Patient 1 with MNGIE phenotype inherited c.261G>T in *TYMP* from father and 175-kb deletion from mother. A novel *SCO2* mutation c.667G>A was found on maternal allele of patient 2 in combination with 87-kb deletion inherited from father. Interestingly, 175-kb deletion on 22q13.33 partially overlaps with two described copy-number variations (CNV; variation_5192 and variation_4139). In patient 3, homozygous 5-kb deletion on 12q24.33 affecting exon 4 of *PUS1* gene was revealed. **Conclusions:** Genome-Wide Human SNP 6.0 Array allowing detection of deletions larger than 700 bp was successfully used to determine genetic diagnosis in 3 out of 15 patients. *Supported by research project PRVOUK of the Charles University in Prague-First Faculty of Medicine (program MOL-MED) and grant IGA NT 11186-5/2010.*

P13.31

Molecular modifications and bioenergetics in relation to phenotype of MILS-NARP syndrome

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Objectives: Mitochondrial DNA mutations at m.8993T>G of the mtDNA ATPase 6 gene typically cause the Maternal Inherited Leigh Syndrome (MILS) and neurogenic muscle weakness, ataxia, retinitis pigmentosa (NARP). To elucidate the molecular-clinical correlations in MILS-NARP syndrome, the phenotype, biochemical parameters, mtDNA mutant loads, and bioenergentics were investigated in three generations of a pedigree harboring m.T8993G mutation.

Methods: Detailed neurological and ophthalmological phenotypes, biochemical and metabolic status, mutant load, cellular bioenergentic and molecular modification were investigated in members of three generations of pedigree with MILS-NARP syndrome.

Results: The ATP6 mutation was ubiquitously distributed in various tissues of the affected individuals. A remarkable high mutation load was demonstrated in individuals with MILS and Retinitis Pigmentosa (RP), respectively. While a mutant load ranging from 5% to 97% was noted in those individuals with mild or absent clinical symptoms. Interestingly, the individual affected with MILS showed remarkable elevation in the levels of lactate, pyruvate, and alanine, and deficiency of carnitine, impaired cellular bioenergetics, and molecular compensation with glycolysis. While the fibroblasts from RP showed molecular modification through the nuclear respiratory chain complex pathway.

Conclusion: The correlation between the mutant load in tissues and the severity of phenotype in MILS-NARP is very complex, and the genetic background may play an important role in modulating the bioenergetics, biochemical defects and clinical outcome. Our results emphasizes the complexity of mechanism contributing in the phenotypic expression of the m.8993T>G mutation and the need for caution in predictive counseling in such patients.

P13.32

A constant and similar assembly defect of mitochondrial respiratory chain complex I allows rapid identification of NDUFS4 mutations in patients with Leigh syndrome

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Isolated complex I deficiency is a frequent cause of respiratory chain defects in childhood. In this study, we report our systematic approach with Blue native PAGE (BN-PAGE) to study mitochondrial respiratory chain assembly in skin fibroblasts from patients with Leigh syndrome and CI deficiency. We describe five new NDUFS4 patients with a similar and constant abnormal BN-PAGE profile and present a meta-analysis of the literature. All NDUFS4 mutations that have been tested with BN-PAGE result in a constant and similar abnormal assembly profile with a complete loss of the fully assembled complex I usually due to a truncated protein and the loss of its canonical cAMP dependent protein kinase phosphorylation consensus site. We also report the association of abnormal brain MRI images with this characteristic BN-PAGE profile as the hallmarks of NDUFS4 mutations and the first founder NDUFS4 mutations in the North-African population.

P13.33

Mutations in the *GCK* gene are the most common cause of MODY-Diabetes in a cohort of over 600 patients in Germany

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Maturity Onset Diabetes of the Young (MODY) is an autosomal dominant form of diabetes mellitus. The clinical phenotype is mild in comparison to type 1 Diabetes but diabetic complications can occur. In patients with MODY the insulin production or blood glucose level sensing is impaired. According to the literature, mutations in *HNF1A* are the most common causes of MODY. Here we describe the results of a diagnostic mutation screening in MODY genes conducted in the years 2008-2011 in a cohort of over 600 patients.

Material and methods: 619 probands mostly of Caucasian origin with clinical suspicion of MODY were referred to our laboratory for molecular genetic analysis. The analysis was performed stepwise, beginning with the genes known to be more commonly involved in the pathophysiology of MODY. Based on the proband's clinical picture some clinicians delineated the diagnostics to specific MODY subtypes.

The causative genes for MODY subtypes HNF4A, GCK, HNF1A, IPF1 and HNF1B were directly sequenced. MLPA-analysis was performed to detect large deletions and duplications.

Results and discussion: In the 619 patients, altogether 163 mutations were identified (Table). These data show that in our patient cohort mutations in the *GCK* gene are the most common cause of MODY. These results differ from published data and are likely to be more widely applicable to the German population.

Gene	Patients analysed (N)	Mutations detected (N)	% of all mutation-positive samples
HNF4A	238	10	6%
GCK	494	88	54%
HNF1A	489	52	31%
IPF1	76	1	0,5%
HNF1B	208	12	7%

P13.34

Clinical features in 17 patients with Mucopolysaccharidosis

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The mucopolysaccharidosis (MPS) are a large group of inherited lysosomal storage disorders. Each mucopolysaccharidosis subtype is caused by a deficiency in the activity of a single specific lysosomal enzyme which required for glycosaminoglycan degradation. The deficiency of these enzyme results in the storage of the glycosaminoglycans in several tissues.

The clinical presentation and the natural course of the patients with MPS may be different among subtypes which are influenced by the presence of the genetic background including functional polymorphisms and environmental problems. The general features are course facies, corneal clouding, developmental delay, mental retardation, skeletal and joint abnormalities, and cardiac abnormalities.

We report frequency of the clinical symptoms of the MPS patients of our pediatric genetic clinic. Between January 1995 and December 2011, aged 2-18 years, seventeen children (11 boys, 6 girls) were examined. The MPS III, MPS IV, and MPS I were the most frequent subtypes amongst the MPS patients, respectively. The patients were most frequently representing coarse facies, skeletal abnormalities, mental retardation and hepatomegaly.

Although MPS is a rare disorder, it is important to consider the MPS in the differential diagnosis of patients presenting coarse facies, skeletal abnormalities, neurodevelopmental disabilities and hepatomegaly. We could not forget the importance of the screening for oligosaccharides in children with neurodevelopmental delay with mild phenotypic symptoms. Early diagnosis and effective medical management in some types can improve patient outcomes and may reduce the disease burden on patients and caregivers. Furthermore, it allows genetic counseling.

P13.35

Cystic fibrosis mutation detection- difficulties and traps

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Background: A characteristic aspect for Romania is the CF mutations heterogeneity which leads to a reduced percentage of genotype identification. **Objective:** Assessment of a mixed panel efficacy for CF mutation detection in Romanian patients. **Methods**: We evaluated retrospectively 40 patients (pts) with typical CF, registered in the National CF Center Timisoara. The genetic tests were performed using a mixed panel - (29 mutations) - ARM and another kit for 38 mutations-PCR. 18 mutations were common to the two kits; the total number of identifiable alleles was 49. **Results:** The first panel identified in order of frequency: ΔF508, G542X, N1303K, 621 + 1 G>T, I148T, representing 17.2% from panel 1 mutation. We found the following patients genotypes: 21 pts homozygous for F508del, 10 pts with F508del/ x , 5 pts F508del/G542X , 1 patient F508del/ N1303K . In 3 patients with compound genotype non-F508del , the other alella could not be identified, complementary genetic testing done in parents have ruled out the possibility of homozygous non-F508del genotype. In 13 patients (32.5%) we could not fully identify the genotype, thus they were further tested with panel 2. **Conclusions:** Superposition of kits with identified mutations in CF Romanian patients is low, although kits contain the most frequent mutations used in Europe. Genetic heterogeneity in Romania limits significantly the possibility of detection of both alleles, the diagnosis rate of heterozygote being reduced. The question of using additional kits or methods like CF gene sequencing raises the issue of a high cost.

P13.36

Niemann-Pick type C disease: mutation analysis and genetic counselling in Czech and Slovak families

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Niemann-Pick type C disease (NPC, OMIM #257220, #607625) is a severe autosomal recessive neuro-visceral disorder characterized by progressive neurological deterioration and hepatosplenomegaly. The most prominent biochemical feature is complex lipid storage of free cholesterol and glycolipids. Molecular defects in two late endosomal/lysosomal proteins (NPC1 or NPC2) cause symptoms of NPC.

Fifty four Czech and Slovak NPC patiens were diagnosed by histochemical and biochemical methods. Prevalence based on these data is 1:109,000, significantly higher than the reported pan-ethnic prevalence of 1:150,000. Probands from 37 families were available for molecular genetic analysis. Mutations in NPC2 gene were proved only in one family. Thirty nine patients from 36 families belonging to NPC1 complementation group had highly variable clinical manifestations - from infantile neurological form to adult solely visceral form. By Sanger sequencing we have identified 32 different mutations on 69 NPC1 alleles, 14 of them being novel. MLPA method did not reveal any deletion in NPC1 gene of three patients, in which only one mutation was identified by sequencing. The most common mutations were p.R1186H, p.S954L and p.P1007A (16, 10 and 7 alleles, respectively).

In one family an unrelated partner of a carrier was tested during the preconception genetic counselling for mutations in four exons (18, 19, 20, 23) which encompassed 60% of all identified mutations. Unexpectedly, recurrent mutation p. P1007A (exon 20) was identified. This case shows that molecular genetic analysis of unrelated partners may be important also in very rare disorders.

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P13.37

Clinical, Biochemical and Molecular Diagnosis of Niemann-Pick Disease type C, in three Iranian Patients, and two Novel Mutation in the NPC1 gene

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Niemann-Pick type C is a rare autosomal recessive neurometabolic disorder, characterized by progressive neurodegeneration. It is a pan-ethnic disease with estimated prevalence of around 1 in 120 000 to 150 000 worldwide. Age of onset may vary from the perinatal period to adulthood. Two different genes, *NPC1 or NPC2,* have been identified to cause disease, 95% is due to the *NPC1* gene (18q11), its product is a large membrane glycoprotein that consists of 1278-amino-acids, and 5% have mutations in the *NPC2 gene (14q24.3)* which encodes a small protein that consists of 132-amino-acid a binds to cholesterol with high affinity. Clinical characteristic is hily variable. In the classic form, patients appear to be normal in the first 1 to 2 years, they gradually develop neurologic abnormalities (ataxia, grand mal seizures, and myoclonic jerks), dystonia, vertical supranuclear gaze palsy, dementia, and psychiatric manifestations, hepatosplenomegaly is less striking than NP-A/B.

Diagnosis based on clinical suspicions and foamy histiocytes in BM aspirates, white matter involvement on MRI scanning, formal esterification studies. Diagnosis would be confirmed by performing filipin staining on skin fibroblast cell cultures and mutation analysis in NPC1 and NPC2 genes.

Here we report 3 Iranian affected cases that were confirmed by filipin staining and mutation analysis. We detected mutations in NPC1 gene in all of them, in two patients the mutations were novel and have not been reported before. Two of the patients are under treatment with Miglustat and now their general conditions are stable especially with amelioration of visceromegaly.

P13.38

Evaluation of the innate immunity in mucopolysaccharidosis: analysis of the functional activity of phagocytes

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Mucopolysaccharidoses (MPS) are a group of inherited metabolic disorders characterized by the deficient activity of catabolic enzymes in the lysosomes and its consequent abnormal accumulation of deposits of glycosaminoglycans (GAGs). The lysosomal dysfuction caused by this irregular storage is responsible for the clinical manifestations seen in MPS.
Once the lysosome is also important for normal functioning of the immune system, playing a key role in the expression of cellular membrane receptors, the presentation of antigens, the secretion of cytokines and phagocytosis, we presume that these processes may be impaired in patients with MPS. The presence of recurrent respiratory infections in these individuals may be a clinical clue of the immune dysregulation in MPS.
We studied the leukocyte oxidative burst activity and chemotactic function of neutrophilic granulocytes and phagocytic activity and expression of mieloperoxidase in phagocytes by flow cytometric immunoassay of 15 patients with MPS types I, II, IV and VI.
All patients demonstrated normal phagocytic activity and normal chemotactic function of neutrophilic granulocytes. Normal levels of reactive oxygen metabolites after the stimulation with PMA and opsonized *Escherichia coli* and normal expression of myeloperoxidase by granulocyte and monocytes. In our *in vitro* tests using widely available commercial kits, we were not able to find either quantitative deficiency or functional defects of granulocytes and monocytes. This is the first study in the literature of evaluation of the innate immunity in patients with MPS.

P13.39

The mutant phenylalaninhydroxylase gene is more often found out in alcoholics

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It has been investigated 156 patients with alcoholism of both sexes at the age of 25-60 years on presence of mutation in the PAH gene. By PCR method the R408W mutation is established in 11 out of 156 alcoholic patients (7,05%) and 10 out of 417 volunteers of the same age (2,39%). Metabolism of Phe appeared to be impaired in chronic alcoholism as shown by an increase in concentration of Phe in blood serum as well as by elevation urinary excretion of Phe and phenylpyruvic acid. The most distinct impairment in Phe metabolism was observed in alcohol withdrawal syndrome (delirium tremens). This study aimed to investigate whether the mayor (about 60%) R408W mutation in phenylalaninehydroxylase (PAH) gene influences chronic alcoholism and delirium tremens cases. The data obtained indicate that decreased by R408W mutation activity of PAH and development of alcoholism and especially delirium tremens are impaired. So, genetic rearrangements in PAH are the contributing factor to development of alcoholism.

P13.41

Genome-wide association study identified MEP1A in relation to insulin metabolism in Polycystic Ovary Syndrome

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There is a clear association between polycystic ovary syndrome (PCOS) and cardiovascular events, which might be based on disorders of glucose metabolism. Accumulating evidence suggests that insulin resistance and hyperinsulinemia affect 65-70% of PCOS patients. Vitamin D has been shown to influence both insulin resistance and other symptoms of PCOS. In a genome-wide association study (GWAS) of cardiovascular patients, we found MEP1A as a potential candidate gene in PCOS.MEP1A variants were replicated in 586 PCOS women and 105 controls. Metabolic, hormonal, functional and anthropometric parameters were determined. In cell culture experiments, human hepatocellular carcinoma (HepG2) cells were treated with insulin, vitamin D and parathyroid hormone (PTH) for the expression of MEP1A. In the replication cohort, MEP1A variants were not associated with the incidence of PCOS per se. However, the SNPs showed a significant association with insulin metabolism in overweight/obese PCOS. MEP1A GG-carriers showed a significantly increased HOMA index ($p = 0.005$), elevated fasting insulin ($p = 0.006$), and stimulated insulin after 30min ($p = 0.003$), 1h ($p = 0.003$) and 2h ($p = 0.009$). HepG2 cell experiments showed a relation of MEP1A to the expression of bone genes and vitamin D. MEP1A is a possible target for disease modifying in PCOS and potential new therapeutic options. It might contribute to the involvement of vitamin D deficiency in abnormalities of glucose metabolism and insulin sensitivity. Whether MEP1A is a potential risk factor for PCOS and how it is associated with gene function will be further investigated.

P13.42

Homozygous 669-698del in exon 12 of HMBS gene in a Spanish patient with acute intermittent porphyria

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Acute intermittent porphyria (AIP, MIM # 176000) is an autosomal dominant disease caused by a partial deficiency of hydroxymethylbilane synthase (HMBS; EC 4.3.1.8). It is characterized by acute attacks of neurovisceral dysfunction, often precipitated by several factors. Early detection of AIP carriers is very important for the prevention of acute attacks. The diagnosis of AIP is based on clinical symptoms and increased urinary porphyrin precursors, δ-aminolevulinic acid (ALA) and porphobilinogen (PBG), in combination with the HMBS activity assay.
We report a case of a female patient of 35 years old who came from a southeastern region of Spain, with abdominal pain, neurospsyquiatric symptoms, hyponatraemia and tachycardia. Neurological checkup showed axonal polyneuropathy with proximal afectation in all the four limbs. In the past, she had two episodes of muscular weakness of limbs. Abdominal examination did not reveal any abnormality. There was no sensory impairment. Laboratory investigations showed the TLC of 6,300/cmm with a DLC of P-52%, L-33%, M-13% and E-2%; hemoglobin was 7,9 g/dL. Urine was strongly positive for ALA (172 mg/24h) and PBG (3,3 mg/24h).
Genomic DNA was isolated from PBL, and all 15 exons and flanking regions of the HMBS gene were amplified by PCR and sequenced in an ABIPRISM 3100 Genetic Analyzer. Patient was carrier of a homozygous 30 pb deletion in exon 12 of HMBS gene. This mutation, 669-668del, causes a mutant protein that lacks of 10 amino acids (p.Glu223-Leu232) and has been described previously in Spanish AIP of southern ancestral origin with a possible founder effect.

P13.43

Novel variants in the *CYP51* gene found in Caucasian mothers and neonates with potential to contribute to spontaneous preterm birth

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Cholesterol is an essential component of cellular membranes, a precursor of steroid hormones, oxysterols, and bile acids. It is involved in many signaling pathways including the sonic hedgehog (Shh) pathway. Sterols play crucial role in maintaining pregnancy, and a large amount of cholesterol is required during ontogenesis and embryogenesis. Defects in cholesterol synthesis or intracellular transport result in serious malformations and mutations in some cholesterol synthesis genes are associated with preterm delivery (PTD).
Here we investigated for the first time variants in fetal and maternal lanosterol 14α-demethylase (*CYP51A1)*, a key gene of post-squalene cholesterol synthesis, and we examined their contribution to PTD. Ten amplicons covering exons, untranslated regions (UTR) and intron-exon borders have been investigated in 188 Caucasian women who had a spontaneous preterm delivery and 188 unrelated preterm infants born at a gestational age <37 weeks. The study included neonates from singleton pregnancies, 94 of each gender.
We identified 22 polymorphisms, where 11 represent rare, novel variants. Three novel variants are heterozygous missense mutations in exons 1, 3 and 4. According to PolyPhen2 the mutation in exon 3 causes a probably dama-

ging amino acid substitution in the substrate recognition site, resulting in a T/G change causing a Y145D transversion. This amplicon was sequenced further in 1000 premature infants. The low frequency of the novel *CYP51* polymorphism suggests that this polymorphism has little contribution to PTD. TaqMan genotyping of common variants in larger population is in progress, together with further sequencing of the 5' and 3'-UTRs.

P13.44

Yeast, a simple and effective tool to study COQ gene mutations causing primary CoQ10 deficiency

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Primary coenzyme Q10 (CoQ) deficiency is associated to different phenotypes mainly affecting SNC, skeletal muscle or kidney and it is caused by mutations in genes involved in CoQ biosynthesis. Mutations in COQ6, encoding a monooxygenase required for CoQ biosynthesis, have been reported in patients affected by early-onset steroid-resistant nephrotic syndrome (SRSN) with sensorineural deafness.

We employed a yeast model to evaluate the role of different isoforms of COQ6 and to study the functional consequences of the mutated alleles on protein function. Human COQ6 encodes for at least two isoforms. In yeast there is only one COQ6 isoform and its deletion causes the loss of the ability to grow in non-fermentative carbon source (glycerol).

We proved that human isoform a but not isoform b can complement the deleted yeast. We then modeled the "human" mutations on the corresponding residues of the yeast gene that are conserved throughout evolution.

The yeast model proves to be simple and effective to validate COQ6 mutations. All alleles, except for a nonsense mutation, show some residual activity (as shown both by growth and CoQ6 content analysis); analysis of the CoQ/DemetoxyCoQ ratio in the mutants did not detect an altered ratio (as in the case of COQ2 mutations) suggesting that the mutations affect only COQ6 catalytic activity but not the structure of the entire CoQ biosynthetic complex.

Together these data show that all patients thus far identified still retain some residual endogenous CoQ biosynthesis, supporting the notion that complete lack of CoQ biosynthesis is embryonically lethal.

P13.45

Characterisation of a novel metabolic defect in prolin synthesis and pathomechanism in autosomal recessive cutis laxa syndrome type 2B

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Mutations in the Pyrroline-5-carboxylate reductase 1 (PYCR1; EC 1.5.1.2) gene have been recently discovered as the underlying etiology of patients diagnosed with autosomal recessive cutis laxa syndrome (ARCL-2B). Intriguingly this unique metabolic defect in proline synthesis, leading to a recognizable dysmorphology syndrome, appears to be a mitochondrial disorder as well, based on careful evaluation of the phenotype.

PYCR1 is a mitochondrial enzyme, catalyzing the NAD(P)H-dependent conversion of pyrroline-5-carboxylate to proline. This disease is closely linked with P5CS deficiency, caused by mutations in *ALDH18-A1*, a gene coding for an enzyme catalyzing an earlier step in de novo proline synthesis. Both disorders are associated with progeroid features, lax joints, dysmorphic features, microcephaly, intrauterine growth retardation and developmental delay.

While patients diagnosed with P5CS deficiency have variable hyperammonemia and abnormal amino acid levels no obvious metabolic abnormalities have been described so far in *PYCR1* patients. Here we report on the phenotypic and metabolic characteristics of 5 novel patients with cutis laxa syndrome diagnosed with recessive *PYCR1* mutations. Mitochondrial function, respiratory complex activity and oxygen consumption have been evaluated in patient cell lines and in HeLa cells after knockdown of *PYCR1*. Proline synthesis and its metabolic consequences were studied in different body fluids of patients by NMR analysis and in fibroblast cell culture media. Our results suggest that *PYCR1* mutations lead to a metabolic defect altering the intracellular endogenous proline buffer, changing the balance in NAD(H) concentration and mitochondrial membrane gradient, leading to early apoptosis.

P13.46

MECP2-related disorders and molecular investigation: Iranian aspect

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MECP2-related disorders include classic Rett syndrome, variant or atypical Rett syndrome, and mild learning disabilities in females and neonatal encephalopathy and mental retardation syndromes in males. Classic Rett syndrome is a progressive neurologic disorder in girls characterized by normal birth and apparently normal psychomotor development during the first six to 18 months of life. The girls then enter a short period of developmental stagnation followed by rapid regression in language and motor skills. Females with classic Rett syndrome typically survive into adulthood, but the incidence of sudden, unexplained death is significantly higher than in controls of similar age. Atypical Rett syndrome is increasingly observed as MECP2 mutations have been identified in individuals previously diagnosed with autism, mild learning disability, clinically suspected but molecularly unconfirmed Angelman syndrome, or mental retardation with spasticity or tremor.

In this study PCR and whole MECP2 gene sequencing was performed in 57 cases clinically suspected to RETT syndrom. The result showed five reported mutations in 7 patients. Two of them had C>T (Arg 168 Term), the other two patients showed C>T (Gln 406 Term) and each of these alterations C>T (Arg 106 Trp), C>T (Arg 270 Term), G>A (Val 288 Met) were found in 1 patients, respectively. Investigation about the new alterations which have been seen in 12 patients without any reported mutations was under reviewed. The rest of our cases showed no alteration related to disease.

P13.47

High frequency of common mutation g.1541G>A in SCO2 gene responsible for cytochrome c oxidase deficiency in Polish population.

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Mutations in the *SCO2* gene (22q13) lead to severe COX deficiency observed mainly in muscles, heart and brain. SCO2 is one of the ancillary proteins necessary for correct assembling and functioning of cytochrome c oxidase (COX). It is involved in the transport and incorporation of the copper ions to the CuA enzymatic site on COXII subunit. A common substitution, g.1541G>A (p.E140K), is identified at least on one allele in all reported patients.

The aim of this study was to ascertain the frequency of g.1541G>A mutation in Polish population.

We examined 3080 anonymous newborn's samples (dry blood spots - Guthrie cards). Genotyping with Taqman's probe (based on Real Time equipment) was used to identify of the specific mutation. Presence of the mutation in selected samples were confirmed by direct sequencing. The study revealed the presence of the common mutation in 21 carriers coming from 13 provinces (out of 16). The mean frequency was determined as 1:147 life birth with the highest result in lodzkie voivodeship.

The obtained data enabled us to establish the high frequency of the common mutation (g.1541G>A) in Poland and assess its regional variety. We hope for that our study increase awareness of the severe clinical consequences of cytochrome c oxidase deficiency.

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P13.48

Frequency distribution of *NQO1*2* and *SULT1A1*2* alleles in Polish population

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The *SULT1A1* gene encodes a phenol sulfotransferase, which belongs to the enzyme superfamily involved in the sulfonation of xenobiotics, hormones and drugs. Quinone oxidoreductase encoded by *NQO1* gene is a detoxification enzyme that catalyses the reduction of a wide range of substrates. Both enzymes participate in the biotransformation pathway of popular anaesthetic drug - propofol, catalyzing the secondary step of its metabolism. Changes in this metabolism step, related to variations in *SULT1A1* and *NQO1* genes may lead to adverse effects after propofol use. Large interindividual variability in these enzymes activity has been shown and several polymorphisms in genes coding these enzymes have been described. Most of this variability is related to the polymorphism P187S in *NQO1* gene and R213H amino acid substitution in *SULT1A1* gene, which are responsible for decreased enzyme activity. The aim of our study was to determine the frequency distribution of these two alleles *SULT1A1*2*, *NQO1*2* in Polish patients under propofol anaesthesia. We analyzed 232 alleles using pyrosequencing as a rapid genotyping method. The frequency of the *SULT1A1*2* allele was 19,3% and *NQO1*2* was 14,2%. Disturbed enzyme activity in biotransformation pathway may lead to increased risk of propofol toxicity. The current analysis is an important initial step in bringing these polymorphisms into an optimal planning of anaesthesia based on the modified pharmacodynamic response of propofol.

P13.49

ATP7B expression measurement improves detection rate of newly diagnosed patients with Wilson Disease

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Background: Wilson Disease (WD) is an autosomal recessive disorder leading to toxic accumulation of copper mainly in liver tissue. Currently, more than 370 mutations are known in the disease-related *ATP7B* gene (OMIM#606882). However, standard investigations to diagnose WD occasionally fail, e.g. disease-causing *ATP7B*-mutations sometimes remain undetected by direct sequencing.
We therefore aimed to evaluate quantification of *ATP7B* mRNA as a new tool to improve WD diagnostics.
Methods: Total RNA was extracted from snap-frozen and from FFPE liver tissue. After cDNA synthesis, real-time PCR was performed using HybProbes and TATA Box-binding protein (TBP) as reference gene. Expression determination was done by calibrator-normalized relative quantification with efficiency correction. *ATP7B*-expression of 12 snap-frozen liver specimens from WD-patients was compared to that of 22 patients with hepatocellular carcinoma (HCC), 9 patients with biliary atresia, 8 clinically healthy donors and 12 patients with other liver diseases. Furthermore, *ATP7B*-expression in FFPE liver tissue from 8 WD-patients was compared to that of 4 hepatitis B and 5 biliary atresia patients.
Results: *ATP7B* mRNA expression in snap-frozen liver tissue from WD-patients was significantly lower (median 1.7) than in the control groups (median: HCC 5.3; biliary atresia 4.9; healthy 7.4; other 6.5). Comparable results were found in FFPE tissue (median: WD 1.08; hepatitis B 3.50; biliary atresia 2.65).
Conclusion: Our findings suggest that quantification of *ATP7B* mRNA provide a novel tool for the diagnosis of WD in patients where no *ATP7B* mutation is found. Prospective studies in larger patient cohorts are necessary to validate our results for clinical practice.

P14. Therapy for genetic disorders

P14.03

Effect and tolerability of agalsidase alfa in patients with Fabry disease who were treatment-naïve or formerly treated with agalsidase beta

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Background: Agalsidase alfa (agalα) effect and tolerability were assessed in patients with Fabry disease (FD; treatment-naïve or previously agalsidase beta-treated [switch]).
Methods: An ongoing, multicenter, open-label, treatment protocol (HGT-REP-059) evaluated estimated glomerular filtration rate (eGFR), left ventricular mass index (LVMI), biomarkers (globotriaosylceramide [Gb3]; globotriaosylsphingosine [lyso-Gb3]), and tolerability after 1-year intravenous agalα (0.2mg/kg every other week).
Results: 29 naïve (14 male/15 female; median age [range] 38.7yrs [12-74]) and 62 switch patients (33 male/29 female; 47.0yrs [5-84]) were assessed. Baseline median years since FD diagnosis were numerically lower in naïve (1.1 [0.1-27.4]) versus switch patients (7.1 [0.9-49.3]), suggesting lower naïve patient disease burden.
12-month change from baseline
Mean (95%CI)
Naïve Switch
eGFR, mL/min/1.73m2 -1.3 (-10.2, 7.6) -3.2 (-6.6, 0.2)**
LVMI, g/m2.7 2.2 (-1.2, 5.6) 0.0 (-4.7, 4.7)
Plasma Gb3, nmol/mL -6.7 (-9.1, -4.3)** -2.6 (-3.6, -1.6)**
Creatinine-normalized urine Gb3, nmol/mg -2.1 (-4.2, 0.0)* -0.9 (-1.6, -0.1)**
Plasma lyso-Gb3, nM -37.8 (-58.1, -17.4)** -3.0 (-7.3, 1.2)
*p<0.05, **p<0.01
28 (96.6%) naïve and 56 (90.3%) switch patients experienced ≥1 treatment-emergent adverse events (AEs; mostly mild/moderate; possibly/probably drug-related: 12 [41.4%] naïve, 26 [41.9%] switch). 7 (24.1%) naïve and 19 (30.6%) switch patients had serious AEs (1 possibly drug-related: transient ischemic attack). 2 naïve patients discontinued agalα (possibly/probably drug-related); 1 switch patient died (not drug-related).
Conclusion: After 12-months agalα, no statistically significant change in LVMI (naïve/ switch) or eGFR (naïve) occurred. Switch patients showed an eGFR drop of -3.2 mL/min/1.73m2. Plasma and creatinine-normalized urine Gb3 (naïve/switch) and lyso-Gb3 (naïve) dropped significantly. Agalα was generally well tolerated.

P14.04

Generating a Mouse Model for Familial Dysautonomia Disease

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Familial Dysautonomia (FD) is an autosomal recessive congenital neuropathy that occurs almost exclusively in the Ashkenazi Jewish population. The major mutation leading to FD is a T to C mutation at position 6 of intron 20 of the *IKBKAP* gene; this mutation causes aberrant splicing of the *IKBKAP* gene product in a tissue-specific manner and reduces production of the IKAP protein in the nervous system. The aim of this study was to establish a humanized *IKBKAP* knock-in mouse model that exhibits some of the characteristics of the human FD disease such as alternative splicing of exon 20 of *IKBKAP* gene. We established two homozygous humanized mice strains carrying human exon20 and its two flanking introns: one possesses the FD mutation and the other one lacks it. The IKBKAP$^{\mathrm{FD}\mathit{loxP}/\mathrm{FD}\mathit{loxP}}$ mouse demonstrates a unique tissue-specific alternative splicing pattern of the *IKBKAP* gene.
We also generated two other humanized knock-in mice strains similar to the strain described above except that they contain a *Neomycin* cassette in intron 20. The IKBKAP$^{\mathrm{FD}\mathit{loxPNeo}/+}$ mice were supplemented with phosphatidylserine (PS), a food supplement that was previously shown in our lab to increase mRNA and protein levels of *IKBKAP* in cell lines generated from FD patients. We demonstrated that PS treatment increased *IKBAKP* mRNA levels in various tissues of the IKBKAP$^{\mathrm{FD}\mathit{loxPNeo}/+}$ mice.
Here we show that the IKBAKP$^{\mathrm{FD}\mathit{loxP}/\mathrm{FD}\mathit{loxP}}$ mice have great potential for use as a model to evaluate the effects of PS and other potential therapeutic agents on the mRNA and protein levels of *IKBKAP*. Hence, this mouse model provides a medical breakthrough in the research of FD for preliminary evaluation of drugs that may improve the clinical status of FD patients.

P14.05

Polymorphism in gene encoding drugs and xenobiotic metabolizing enzyme CYP2D6 as a risk factor for drug response in colchicine unresponsive FMF patients

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Familial Mediterranean Fever (FMF) is a recessively inherited autoinflammatory disorder characterized by recurrent attacks of fever and serositis. Although colchicine is the standard therapy for preventing attacks and suppressing inflammation, 5%-10% of compliant patients are colchicine-resistant. We report the effect of *CYP2D6* in FMF with colchicine unresponsiveness. The genetic polymorphism results in different *CYP2D6* xenobiotic metabolisms phenotypes namely extensive metabolizers (EM), intermediate metabolizers (IM), and poor metabolizers (PM). Total genomic DNA samples from 60 FMF patients of colchicine unresponsiveness were used for *MEFV* and *CYP2D6* genes profile analyses. Target genes were genotyped by multiplex PCR based reverse hybridization stripAssay method. 12 (20 %) patients were poor metaboliser, 48 (80 %) were intermediate metaboliser for *CYP2D6* gene. Every one patient of five was poor metaboliser and was also colchicine unresponsive. Our results indicate that *CYP2D6* polymorphisms were associated with colchicine resistance in nonresponder FMF patients during the common therapy protocol.

P14.06

The Metabotropic Glutamate Receptor Theory in Fragile X Syndrome: testing the safety and efficacy of AFQ056/Mavoglurant in adults and adolescents

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Fragile X syndrome is the most common cause of inherited mental retardation and is associated with behavioral problems including hyperactivity, attention deficit disorder and autism. It is caused by the expansion of a CGG repeat in the *FMR1* gene, leading to hypermethylation and transcriptional silencing of *FMR1*, and absent or reduced levels of the translational repressor FMR1 protein (FMRP). The metabotropic glutamate receptor (mGluR) theory hypothesizes that without FMRP, uncontrolled protein synthesis occurs in response to activation of synaptic mGluRs and may lead to the clinical symptoms of FXS.

Randomized controlled data suggest that the mGluR5-antagonist AFQ056/Mavoglurant might improve behavioral symptoms of FXS, especially in patients with fully-methylated FMR-1 promoter regions *(*30 points improvement on Aberrant Behavior Checklist-Community edition (ABC-C) vs. placebo, n=7).

Novartis currently conducts the largest clinical development program in FXS, testing the efficacy and safety of AFQ056/Mavoglurant. It is the first program conducted in Europe (Denmark, France, Germany, Italy, Spain, Switzerland, Sweden, UK) and across multiple languages and cultures. Adults (18-45 years) and adolescents (12-17 years) are randomized in two separate studies to up to 4 months treatment with AFQ056/Mavoglurant or placebo. ABC-C (primary outcome), other behavioral scales and safety parameters are measured. After completing these studies, patients can enroll into open-label, long-term studies with AFQ056/Mavoglurant for ≥24 months.

In summary, the AFQ056/Mavoglurant program is testing the mGluR theory in FXS and attempts to replicate the promising results seen previously. It is actively recruiting patients worldwide. Studies in smaller children and over longer treatment periods are planned.

P14.07

The mGluR5 antagonist AFQ056 does not affect methylation and transcription of the mutant FMR1 gene in vitro

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Fragile X syndrome (FXS), the leading cause of inherited mental retardation, is due to expansion and methylation of a CGG sequence in the FMR1 gene, which result in its silencing and consequent absence of FMRP protein. This absence causes loss of repression of metabotropic glutamate receptor 5 (mGluR5)-mediated pathways resulting in the behavioral and cognitive impairments associated with FXS. In a randomized, double-blind trial it was recently demonstrated a beneficial effect of AFQ056, a selective inhibitor of metabotrobic glutamate receptor type 5 (mGluR5), on fully methylated FXS patients respect to partially methylated FXS ones. To determine whether AFQ056 may have secondary effects on the methylation and transcription of FMR1, here we treated three FXS lymphoblastoid cell lines and one normal control male line. A quantitative RT-PCR was performed to assess transcriptional reactivation of the FMR1 gene. To assess the methylation status of the FMR1 gene promoter it was carried out a bisulphite sequencing analysis. Both FMR1-mRNA levels and DNA methylation were unmodified with respect to untreated controls. These results demonstrate that the AFQ056 effect on fully methylated FXS patients is not due to a secondary effect on DNA methylation and consequent transcriptional activation of FMR1.
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P14.08

A phase II randomized placebo-controlled double-blind pilot clinical trial to test the safety and effectiveness of Ascorbic acid and Alpha-tocopherol on behavioral and learning problems in the Fragile X syndrome

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Introduction and Objectives:
Fragile X Syndrome (FXS) is a neurodevelopmental disorder affecting intelligence and behaviour.The treatments available today are unable to normalize these symptoms. We demonstrated that an excess of oxidative stress is present in FXS-mouse brain and a treatment with antioxidants could reverse hallmarks of the FXS-mouse phenotype. We propose a combination of ascorbic acid and alpha-tocopherol to improve learning abilities in young male patients.

Material and Methods:
Phase II randomized placebo-controlled double-blind pilot clinical trial to treat 30 selected FX male patients(15 treated patients and 15 in placebo) in two groups: A:6 to 12 and B:13 to 18 year olds. Mean age (SD) 11.67 (4.20) [treated subgroup: 12.13 (3.44); placebo subgroup: 11.71 (4.86)].
A questionnaire to evaluate clinical data and neuropsychological tests was performed at the beginning of the trial (T0) and at 12 weeks of placebo versus treatment (T1). The principal variable: the Wechsler Intelligence Scale for Children (WISC-R) tested by simple linear regression ($p<0.05$).

Results:
Significant improvements in direct scores in total manipulative and total verbal subscales were observed in young patients compared to the placebo group only when they were not taking psychoactive medication.

Conclusions:
Clinical trials for Fragile X Syndrome are necessary due to the absence of effective therapies and the side effects of available psychopharmacological treatments. We present our positive results about improvement in learning problems measured with the WISC-R scale after 12 weeks of treatment with a combination of two well known vitamins with a potent antioxidant capability, ascorbic acid and alpha-tocopherol.

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P14.09

Current efforts towards population screening and therapeutic drug discovery for Friedreich Ataxia

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Background: Friedreich ataxia (FRDA) is a neurodegenerative disease characterized by progressive ataxia and cardiomyopathy with an incidence of 1:50,000. FRDA is typically diagnosed by identifying GAA-repeat expansions, or mutations, in FXN that cause reduced frataxin expression. We describe a Luminex immunoassay to measure frataxin in whole blood (WB) and dried blood spots (DBS) for population screening and therapeutic monitoring of FRDA. In addition we adapted this assay to a MesoScale Discovery platform and completed an initial screen of a Library of Pharmogologically Active Compounds (LOPAC 1280) in FRDA patient cells. Results: Recovery for frataxin is 99% from WB and DBS. Intra-assay imprecision is 4.9-13% CV and inter-assay imprecision is 9.8-15.8% CV. The LOD is 0.07 ng/mL and reportable range is 2-200 ng/mL. The reference range for adult and pediatric normals is 15-82 ng/mL (median: 33) for DBS and WB. FRDA carriers (n=30) have frataxin levels of 12-30 ng/mL (median: 18) and FRDA patients (n=82) of <26 mg/mL (median: 6). Frataxin was stable for over 6 months at 22°C, 4°C and -70°C. An initial screen of LOPAC 1280 has yet to uncover a positive hit but it also has not provided evidence that EPO, pentamidine, or bisbenzimide enhance frataxin expression as observed by others in different experimental settings. Conclusions: We validated a high-throughput assay for frataxin that is applicable to diagnosis and population screening and modified the method for FRDA drug discovery. Ongoing work includes expanding the drug screen beyond LOPAC 1280.

P14.10

Two-year efficacy and safety of velaglucerase alfa in patients with type 1 Gaucher disease switching from imiglucerase: Phase III trial HGT-GCB-039 and extension

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Objective: To determine the efficacy and safety of velaglucerase alfa in type 1 Gaucher disease (GD1) patients switched to velaglucerase alfa in an open-label extension study (HGT-GCB-044 [ClinicalTrials.gov, NCT00635427]) after receiving imiglucerase in a double-blind, randomized, Phase III trial (HGT-GCB-039 [NCT00553631]).
Methods: In HGT-GCB-039, treatment-naïve GD1 patients aged ≥2 years received velaglucerase alfa or imiglucerase as a continuous 60-minute intravenous infusion (60 U/kg every other week [EOW]; 9 months). Imiglucerase-treated patients completing HGT-GCB-039 could enroll in ongoing extension study HGT-GCB-044, switching to velaglucerase alfa (60 U/kg EOW). Assessments were conducted after 15 months in HGT-GCB-044 (total of 2 years' enzyme replacement therapy).
Results: Sixteen patients receiving imiglucerase in HGT-GCB-039 entered HGT-GCB-044 (median age, 27 years; male, n=7; splenectomized, n=10). Mean changes from baseline (HGT-GCB-039 entry) at 9 months and 2 years, respectively, were 1.40 g/dL and 1.98 g/dL for hemoglobin concentration, 149×10^9/L and 164×10^9/L for platelet count, −1.18% and −1.67% bodyweight for liver volume, and −2.79% and −3.63% bodyweight for spleen volume. Adverse events (AEs) reported by ≥20% patients: arthralgia and influenza in HGT-GCB-039; headache and upper respiratory tract infection in HGT-GCB-044. Three serious AEs occurred in 3 patients (none study drug-related or life-threatening). Anti-velaglucerase alfa antibodies were not detected in any patient receiving velaglucerase alfa, including 3 patients who developed anti-imiglucerase antibodies in HGT-GCB-039.
Conclusions: GD1 patients continued to improve across 4 key clinical parameters after switching from imiglucerase (9 months' treatment) to velaglucerase alfa (15 months' subsequent treatment). No patient exposed to velaglucerase alfa developed anti-velaglucerase alfa antibodies.

P14.11

Evaluating the effects of olesoxime, a mitochondria-targeting drug on the behavioral and neuropathological phenotype of a Huntington Disease rat model

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Olesoxime, a cholesterol-oxime is a neuroprotective compound and was found to exert its beneficial effects by an improvement of mitochondrial function. Since mitochondrial dysfunction also plays a prominent role in Huntington disease (HD), we evaluated its effects on the behavioral and neuropathological phenotype of the BACHD rat, a full-length mutant huntingtin (mhtt) model of HD. Behavioral observations were carried out during a 12-months study period and neuropathology was investigated subsequently. BACHD rats displayed clasping behavior and had severe problems to run on a rotating rod already at 6 weeks of age, indicating early-onset motor dysfunction. A simple swimming test carried out at 8 months of age revealed a deficit in reversal learning. Furthermore, BACHD rats showed metabolic abnormalities, deteriorating with disease progression. However, no difference was found between rats treated with olesoxime and untreated rats. NMRI revealed a specific reduction in cerebral and not cerebellar volume in the BACHD rats. Immunostaining of brain slices further showed cytoplasmic aggregation and intranuclear accumulation of mhtt in most cerebral brain regions as well as a decreased width of axonal bundles within the corpus striatum. Treatment with olesoxime increased overall brain volume in BACHD but also in wild type rats, and by trend reduced nuclear mhtt accumulations within the prelimbic cortex. It remains unclear, how olesoxime exerts these effects and whether there is an interaction between olesoxime and mhtt. However, the beneficial effects found ex vivo did not lead to an improvement of the behavioral phenotype of the BACHD rat.

P14.12

The investigation of the ITPA and XDH genes variants in Polish IBD patients treated with thiopurine drugs

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Thiopurine drugs are widely used in the treatment of inflammatory bowel disease (IBD). However, even among patients treated with azathioprine (AZA) or 6-mercaptopurine varied responses to treatment were observed. Thiopurines in as many as 30% of patients are not effective and in 20 % of patients side effects occur. Identification of genetic factors is essential in optimizing the individual treatment in IBD patients. In the metabolism of thiopurines besides well-established thiopurine methyltransferase, also other enzymes as inosine triphosphate pyrophosphohydrolase (ITPase) and xanthine oxidase (XO) are involved, which are encoded by ITPA and XDH genes. Two sequence variations in the ITPA gene have been described as associated with ITPase deficiency (P32T, IVS2+21A>C). Changes R149C and T910L in the XDH gene are the most common alleles responsible for a decreased enzyme activity. The aim of our study was to determine the frequency of these 4 sequence variations. We established pyrosequencing method. We tested 91 of Polish IBD patients treated with thiopurine drugs, where 11 did not have any therapeutic effect of thiopurine treatment and where adverse drug reactions existed. Allele frequencies estimated in our study for ITPA gene were lower to the determined in other Caucasian populations. Allele P32T was identified with frequency of 4.95%. The frequency of IVS2+21A>C allele was 8.24%. Investigated alleles in the XDH gene were not observed in this study group of Polish IBD patients and in 200 healthy Polish individuals additionally, what suggested probably no role of this alleles on thiopurine metabolism in the Polish population.

P14.13

The effect of losartan on the TGF-beta pathway in vascular smooth muscle cells of patients with bicuspid and thoracic aortic aneurysms

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Introduction The renin-angiotensin system is believed to participate in the development of thoracic aortic aneurysms (TAA) by targeting the TGF-beta pathway. Blockade of the renin-angiotensin system by the angiotensin II Type 1 receptor (AT_1) antagonist, losartan, has been shown to prevent aneurysm growth in Marfan syndrome. We therefore aimed to investigate the effect of losartan on the TGF-beta pathway in vascular smooth muscle cells (VSCM).
Methods and Results Aortic cells were cultivated and characterized as VSCM. The cells were grown to 80 percent confluency and incubated in serum-free media for 24 h. Next, 1 µM losartan was added to the media followed by incubation for 24 h. The cellular RNA was extracted, reverse transcribed into cDNA, and the expression of 84 genes related to the TGF-beta pathway quantified using the RT^2 Profiler PCR Array from Qiagen. Several genes related to the TGF-beta pathway demonstrated significant changes in expression after losartan treatment, although none of these changes were above two fold. Among these were *Edoglin* (-1.14, p = 0.005), *SOX4* (-1.25, p = 0.006), and *CDC25A* (-1.24, p = 0.0008).
Discussion Our study revealed the complex relationship between the renin-angiotensin system and the TGF-beta pathway in aortic smooth muscle cells. The relatively mild changes in gene expression may indicate that the losartan dosage used is not sufficient to fully antagonize the AT_1 receptor in VSMC obtained from bicuspid aneurysmal tissue.

P14.14

Losartan therapy in operated patients with Marfan syndrome after aortic aneurysm: prevention of re-dissection of the aorta

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Background. Aortic root dilation, dissection and rupture are major clinical problems in Marfan syndrome. Marfan patients require specialized interdisciplinary care for optimal out-come and life quality. We study the efficacy and tolerability of angiotensin II receptor blockade Losartan to prevent progressive dilation of aortic root and dissection in other parts of the aorta in patients with Marfan syndrome operated on aneurysms of ascending aorta.
Methods. We examined 41 patients with Marfan syndrome after aortic valve-

replacing root operations. All patients are meeting strict diagnostic criteria for Marfan syndrome based on the original Ghent nosology and underwent aortic valve-replacing root operations. We collected clinical data, biological samples (DNA/blood samples, aorta/valve tissues), and echocardiographic. We make two groups: one treated and one non-treated with losartan.
Results. In the 20 patients who received losartan experienced better control of blood pressure during the day, previously normalized clinical parameters, quality of life, none of the patients did not require repeat surgery. The changes in aortic distensibility and cross-sectional compliance were similar between the two groups. Mean aortic diameter increased 0.5 mm/year, while in patients who have not received losartan 1.6 mm/year. Left ventricular function was better in patients treated with losartan.
Conclusions. The results of this clinical study could lead to profound modification of the management of aortic risk and complications in patients with Marfan syndrome. The uniqueness in our study is related to the fact that the additive effect of losartan evaluated in patients with Marfan syndrome who have already undergone surgery.

P14.15
The differentiation of human mesenchymal stem cell in to neural cells

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The differentiation of human mesenchymal stem cell to neuron like cell on engineered nanofibrous scafolds have a great potential for bionanomaterial- cell transplautation therapy of neuro degenerative disease and injuries of the nerous system.
We investigated the potential of human bone marrow derived mesenchymol stem cell (MSCS) for neural differentiation in vitro on polycaprolacton (PCL) nanofibrons scaffolds.Studies in this fild have led to the realization that invivo cells intract with the extra cellular matrix , composed of nanofibrous at sub- micron scale , which not only provides the mechanical support to the cells but also plays akey role in regulation of cellular behavior with stem cells is emeraging as an important tool in the development of tissue engineering and regenerative medicine.
PCL characterizations were carried out using SEM , contact angle and tensile instrument.
The differentiation of MSC was carried out using neural inducing factors including Retinoic acid , epidermal growth factor (EGF) and fibroblast growth factor (FGF-2) and Ibmx in DMEM/F12.
Scaning electron microscopy results showed normal morphology and proli fration of Mesenchymal cell on PCL nanofibrous scaffolds.
The expression of neural protein markers was analyzed by immunocytochemistry. The differentiated Mesenchymol cell on nanofibre scaffold were found to express the neural protein , - tubulin III and Map2 ,On day 14 a fter culture by immuno- fluorescent.
Our studies on the differentiation of MSC to Neural cells on nanofibrous scaffold suggest their potentioral application towards nerve regeneration.

P14.16
Partial mechano-sensoric blindness to micrometer topography in *NF1* haploinsufficient cultured fibroblasts indicates a new function of neurofibromin in mechanotransduction

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Cells sense their physical environment and translate geometries into biochemical signals enabling them to adapt to cues in their surroundings. In this study, cell responses to surface topography of micro-structured polydimethylsiloxane substrates were investigated in cultured human cells differing in *NF1*. Age matched dermal fibroblasts from five patients with Neurofibromatosis type 1 (*NF1*+/-) and five controls (*NF1*+/+) were tested. As response indicator the mean cell orientation along micro-structured grooves with heights of 175 or 200 nm and distance of 2 µm was systematically examined by analysis of microscopy images using ImageJ. The tested *NF1* haploinsufficient fibroblasts were significantly less affected by the topography than those from healthy donors. Incubation of the *NF1*+/- fibroblasts with the farnesyltransferase inhibitor FTI-277 disrupting constitutive H-Ras-specific activation of MAP kinase ameliorates significantly the cell orientation. These data indicate that the response to surface topography can be altered by *NF1* haploinsuffiency resulting in a partial mechano-sensoric blindness in cultured fibroblasts. In further studies this new function of Neurofibromin in mechanotransduction will tested in more detail.

P14.17
Artificial over expression of NT3 and its receptor TrkC in bone marrow stromal cells

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The adult mammalian nervous system has a very limited capacity to replace neurons lost following an injury. In the last few decades, transplantation of neural-like cells derived from stem cells has been carried out as a potential treatment for neurodegenerative diseases. The potential success of the transplantation process, however, depends on the functional activity of the grafted cells in their new environment. Bone marrow stromal cells (BMSCs) which are capable of differentiating into neural cells, has been employed for cell and gene therapy of neurodegenerative diseases. Neurotrophin-3 (NT-3) is a member of the neurotrophin family of growth factors, best characterized by its survival- and differentiation-inducing effects on developing neurons.Our previous studies revealed that BMSCs do not express NT-3 and its receptor, TrkC, either before or after differentiation into neural like cells. Since during the development of the brain, NT-3 expresses earlier than other NTs, we used electroporation technique to introduce NT-3 and TrkC genes into the BMSCs by pDsRed-N1 and pCMV vector, respectively. PDsRed-N1 is a mammalian expression vector that encodes a variant of red fluorescent protein. Vectors transformed into E coli DH5α. Expression of NT-3 and TrkC confirmed using fluorescence microscopy and RT-PCR method. Our results showed that BMSCs have the potential for being genetically manipulated and electroporation can serve as a fast, easy, and efficient method for it. We successfully obtained cells could express RFP, NT3 and TrkC. Due to the expression of RFP, the fate of cells can be easily traced after transplantation into tissues.

P14.18
Induction of embryonic microRNA (miR) cluster 302 and pluripotency genes in human mesenchymal stroma cells under hypoxic culture conditions

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Multiple ways and cells sources for the generation of induced pluripotent stem (iPS) cells using for analysis of pathogenesis and personalized treatment are currently under investigation. We hypothesized that due to their multipotency human mesenchymal stroma cells (hMSC) could be more easily reprogrammed than other somatic cells. Therefore, we investigated whether cultivation of MSCs under hypoxic culture conditions, known to support pluripotency in embryonic stem (ES) cells, would suffice to induce pluripotency in MSCs. In particular we analysed the expression of pluripotency-associated genes and the microRNAs (miRs) 302, specific for pluripotent embryonic stem cells, as parameter for reprogramming. Therefore primary human MSCs obtained from bone marrow aspirates and the MSC cell line L87 were cultured under hypoxic (5%O$_2$) and normoxic (20%O$_2$) culture conditions with and without FGF2 supplementation. We found that under hypoxic culture condition in the presence of FGF2, MSCs showed higher proliferative capacities and decreased senescence. FGF2 induced *OCT4* and *NANOG* analyzed by real-time-RT-PCR. In combination with hypoxia this effect was potentiated. If not already present, *KLF4* and *c-MYC* were likewise induced. Most importantly, in contrast to many retroviral methods, the combination of hypoxia and FGF2 led to an induction of the miR302 cluster similar to that found in ES cells leading to the repression of their predicted targets at day 14. In summary, we showed that hypoxia is a safe, easily applicable and sufficient tool to induce pluripotency-associated genes and miRs in multipotent MSCs. This may serve as a first step to generate clinical-grade iPS-cells.

P14.19

Bezafibrate as treatment option in patients with mitochondrial complex I deficiency

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Mitochondriopathies are inherited metabolic disorders with a severe clinical phenotype affecting different organ systems. A majority of them affect the respiratory chain and the cellular ATP production but even these clinical manifestations are highly variable, extending from early childhood encephalopathies to adult-onset myopathies. In spite of rapid progress identifying the molecular cause, curative therapeutic options are barely available. Bastin et al. (2008) and Wenz at al. (2008) provided evidence from in vitro studies and mouse models that activation of the PPAR/PGC-1-alpha pathway with bezafibrate could be a new therapeutic approach. In order to verify this effect, we collected 28 fibroblast cell lines from patients with isolated complex-I deficiency and a defined molecular diagnosis. The complex I activity in patient fibroblasts ranged from 10% to 80% compared to controls. Bezafibrate treatment led to significant improvement of complex I activity in more than 50% of the cell lines. In a subgroup of 13 cell lines, the treatment effect was additionally analyzed on genome-wide expression levels, revealing increased expression of genes involved in lipid and fatty acid metabolism and transport. In 5 cell lines which responded to bezafibrate treatment with significant increase of complex I activity, we found increased amounts of complex I assembled in supercomplexes by two-dimensional blue native SDS-PAGE experiments. These results support bezafibrate as a promising treatment option in a well defined subgroup of patients. They have to be verified in other mitochondrial disorders like COX-deficiency.

P14.20

Vascular malformations and soft tissue tumours in segmental overgrowth disorders respond to mTOR inhibitors, in spite of normal PTEN and AKT1 function

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Vascular malformations are common among patients with segmental overgrowth disorders. Very recently, a recurrent activating AKT1 mutation was identified in Proteus syndrome lesions and loss-of-function mutations of PTEN are known to cause SOLAMEN syndrome. Genetically, both aberrations are predicted to increase downstream phosphoinositide 3-kinase (PI3K) signalling pathway activity. A significant number of patients, however, do not show mutations in either PTEN, or AKT1, and the pathogenesis of hamartomatous tumours and associated vascular malformations remains unresolved. Over the past years, we provided clinical care and molecular diagnosis for patients affected by vascular and hamartomatous tumours. No constitutive PTEN mutation was detected in a cohort of eleven children with segmental overgrowth disorders, mainly classifiable as Proteus, or Proteus-like syndrome. Molecular analyses of tumour tissues failed to demonstrate somatic activation of AKT1. Mosaic mutations were neither found in dermal fibroblasts derived from affected regions, nor in vascular malformations, nor in soft tissue tumours. Nevertheless, experimental therapy with mTOR inhibitors was able to induce rapid remission in three of three treated patients, proving efficacy against diverse lesions, including intestinal haemangioma, intra-thoracal lipomatosis, and retro-orbital lymphangioma. In view of the poor outcome and high complication rate of surgical approaches, mTOR inhibition therefore holds the promise of a well-tolerated first line medical alternative for this group of disorders, regardless of mutational status of proven disease genes. Our results provide clear evidence for genetic heterogeneity in segmental overgrowth disorders, especially in Proteus, and Proteus-like syndrome. Preliminary molecular data suggest a common pathogenic mechanism involving the PI3K/PTEN/AKT/mTOR pathway.

P14.21

Testing Riluzole in a conditional mouse model of SCA3

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Spinocerebellar ataxia type 3 (SCA3) or Machado-Joseph disease (MJD) is an inherited neurodegenerative disorder caused by the expansion of a CAG repeat within the *MJD-1* gene resulting in a polyglutamine repeat in the encoded protein ataxin-3. SCA3/MJD therefore belongs to the group of polyglutamine diseases. Up to now, no treatment is available for this disease. In a recent study, a positive effect of riluzole (benzothiazol, Rilutek), was observed in a heterogenous group of patients suffering from different types of cerebellar ataxias after just eight weeks of treatment with riluzole. However, in the mentioned study, only short-term effects were analyzed which may be just symptomatic. In order to analyze whether riluzole may also be beneficial for SCA3 and whether also long-term effects of riluzole treatment can be observed, we treated our recently generated inducible mouse model of SCA3 with riluzole. This mouse model allows us not only to measure a possible effect of riluzole on disease progression but also to quantify this effect by comparison with an “optimal treatment” (i.e. turning off the expression of ataxin-3). We started the treatment once significant deficits in the rotarod performance were obvious in the generated mice and followed the outcome of the treatment using Rotarod and measurement of home cage activity. Mice were sacrificed at different time points and brain tissue was analyzed for inclusions and neuropathology using Western-Blot and immunohistochemistry. Tissue was also analyzed on RNA level to exclude that riluzole influences the expression of the transgenic ataxin-3.

P14.22

The prevalence of VKORC1 1639 G>A and CYP2C9*2*3 genotypes in patients that requiring anticoagulant therapy in Turkish population

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The VKORC1 and CYP2C9 genotypes were investigate in anticoagulant therapy requiring patients in two different Turkish populations. Cohort included 292 patients that need anticoagulant therapy with the history of deep vein thrombosis and/ or pulmonary artery thromboembolism.Genomic DNA was isolated from peripheral blood samples and StripAssay reverse hybridization technique was used for genotype analysis. Genotypes for CYP2C9 were detected as; 165 (56.5%) for CYP2C9*1/*1, 67 (23.0%) for CYP2C9*1/*2, 25 (8.6%) for CYP2C9*1/*3, 9 (3.0%) for CYP2C9*2/*2, 21 (7.2%) for CYP2C9*2/*3, 5(1.7%) for CYP2C9*3/*3 for CYP2C9 and the allele frequencies were; 0.723 for allele*1, 0.182 for allele*2 and 0.095 for allele*3 respectively. Genotypes for VKORC1 were detected as; 64 (21.9%) for GG, 220 (75.4%) for GA and 8 (2.7%) for AA alleles. The G allele frequency was detected as 0.596 and A allele frequency was 0.404. The VKORC1 1639 G>A and CYP2C9 mutation prevalence and allele frequency of current results showed similar great variable profiles when compared to the other results from Turkish population.

P14.23

Sodium butyrate and Valproic acid as a splicing restoring agents in erythroid cells of β-thalassaemic patients

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β-thalassaemia is a common autosomal recessive disorder in humans caused by a defect in β-globin chain synthesis . Over two hundred different mutations have been found to cause β-thalassaemia, with the most common being splicing mutations (1). Most of these mutations activate aberrant cryptic splicing of 5’ or 3’ splice sites without abolishing completely normal splicing. Some mutations allow a significant level of normal splicing (e.g. IVSI-6), leading to thalassaemia intermedia, while others reduce normal splicing to low (e.g. IVSI-110) or very low levels (e.g. IVSI-5 and IVS2-654) and lead to blood transfusion dependency in the homozygote forms. In most of these cases, the normal splicing sites are not mutated, resulting in competition between the normal and aberrant splice sites and the production of variable amounts of normal mRNA . Modulation of splicing can be achieved by activation or suppression of transacting factors such as SR proteins and hnRNPs through drugs. Here, we demonstrate restoration of IVSI-110 mutation obtained by sodium butyrate, iso butyramide and valproic acid, histone deacetylase inhibitors, known to upregulate the expression of splicing factors. These results highlight the therapeutic potential of splicing modulation for genetic diseases caused by splicing mutations.

P15. Laboratory and quality management

P15.01

A novel StripAssay for the detection of genetic factors modulating the risk of developing AA amyloidosis

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Systemic reactive (AA) amyloidosis represents the most important complication within TNF receptor associated periodic syndrome (TRAPS), familial Mediterranean fever (FMF) and other autoinflammatory syndromes, progressively leading to endstage renal failure. The homozygous condition of the serum amyloid A (SAA) variant SAA1.1 is significantly associated with the occurrence of AA amyloidosis in TRAPS patients (unpublished results). Likewise in FMF patients the *MEFV* mutation c.2080A>G (M694V) correlates with amyloidosis and the presence of a SAA1.1 / SAA1.1 genotype increases the clinical severity (age at disease onset, amyloidosis, arthritis). An association between mannose-binding lectin 2 (*MBL2*) alleles and AA amyloidosis in TRAPS patients is not conclusively established, but the analysis of *MBL2* variants is of considerable diagnostic interest also in closely related fields, including rheumatoid arthritis or innate immunity. Still under discussion of being a modulating risk factor for TRAPS, the *TNFRSF1A* intron 4 polymorphism c.473-33C>T is involved in *TNFRSF1A* expression.

We developed and validated a reverse-hybridization assay (StripAssay) for detection of genetic factors modulating the risk of developing AA Amyloidosis. *MBL2* and *TNFRSF1A* variants can be identified as well, but their role within amyloidosis needs to be cleared. Our panel of markers encompasses c.473-33C>T (*TNFRSF1A*); c.2080A>G (*MEFV*), c.209C>T, c.224T>C (*SAA1*); g.4447 C>G, g.4776 C>G, g.5000 C>T, c.154C>T, c.161G>A, c.170G>A (*MBL2*).

The StripAssay is based on multiplex PCR and reverse-hybridization of biotin-labeled amplification products to a parallel array of allele-specific oligonucleotides immobilized on membrane teststrips. The test follows a simple protocol and requires only small amounts of DNA for comprehensive genetic analysis.

P15.02

Accreditation of cytogenetic laboratories

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The accreditation of a laboratory is the formal recognition of its competence to perform specific tests. It requires regular inspections by qualified external assessors. In medical laboratories, the usual standard is ISO 15189.

This report presents the experience of 75 inspections in 40 cytogenetic laboratories in Germany. The requirements concern: technical aspects such as accommodation, equipment, methods, pre- and post-examination procedures, reporting, quality control, and competence of staff.

In total, 226 non-conformities were found. On average, 5.6 (range 0-12) non-conformities were reported at first accreditation and 2.4 and 3.2 at subsequent second and third controls, respectively.

The highest number of non-conformities (26.6%) was reported for examination procedures. Of these, 33% concerned validation, 28% documentation and 23% procedure itself.

Laboratory equipment was the reason for 25.6% of non-conformities; insufficient documented or expired consumables accounted for 28%, maintenance and surveillance for 26% and data security for 19% of these.

About 19% of the observations concerned accommodation and environment, of which almost 40% concerned security aspects and 23% inadequate storage.

Inadequate reporting was the reason for 16.8% of the non-conformities of which 55% were due to incorrect use of ISCN.

Staff, pre- and post-examination procedures, and quality control were found only rarely among the non-conformities.

The observations show an improvement of the performance of accredited laboratories. The laboratories with a high number of non-conformities in the first inspection profited from the external evaluation to improve their organisation.

(The study includes data that have been reported earlier: Chromosome Res. 2009; 17: 23-24).

P15.03

Comparing Ion Torrent's PGM™ Sequencer and the 5500 SOLiD™ System with Sanger sequencing: Next generation resequencing of the *CFTR* gene using PCR and sample multiplexing

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Almost 2000 mutations have been described in the Cystic Fibrosis (CF) associated CFTR gene. In a few populations the mutation spectrum is well defined and screening tests for the most frequent mutations exist. Sequencing of the entire CFTR gene would be preferable especially in less well characterized populations, but Sanger sequencing is expensive and tedious. Thus, we tested two next generation sequencing (NGS) platforms (Ion Torrent Personal Genome Machine™ (PGM™) and 5500 SOLiD™ System) concerning their throughput, ease of use and analytical sensitivity.

Testing of 20 human samples with different known CFTR genotypes (validated by Sanger sequencing on 3500xL Genetic Analyzer, Applied Biosystems), using a Multiplicom™ multiplexing assay for the CFTR gene, sample barcoding and subsequent NGS with the 5500 SOLiD™ System and the semiconductor-based IonTorrent PGM™ Sequencer.

Sanger sequencing identified a total of 33 mutations and 53 SNPs in the 20 samples. Using the SeqNext software module (JSI medical systems GmbH), all mutations except one insertion/deletion mutation were identified correctly with data from both NGS systems. The SNP detection rate was highly dependent on the coverage reached for a particular region of interest. For sequence coverage of 100-fold or higher the detection rate reached 100% for both NGS systems.

If a sufficiently high coverage can be realized, Ion Torrent and Solid NGS sequencing produce reliable data. Bioinformatic analysis of more complicated variants still needs to be improved.

The products referred to in this abstract are for Research Use only, not intended for animal or human therapeutic or diagnostic use.

P15.04

Scanning and genotyping of CFTR mutations and common SNPs using snapback primers

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Complete genotyping of CFTR mutations is currently performed exclusively by sequencing, a costly and time-consuming procedure. High-resolution melting analysis with snapback primers can be used to genotype all common mutations and scan for rare mutations more rapidly and economically. There are 23 common mutations listed by the American College of Medical Genetics and 7 common SNPs in the CFTR gene. Twenty seven CFTR gene exons were amplified in 39 exon/intron fragments due to length considerations for some of the exons. High-resolution melting was performed on all 39 fragments on the LightScanner® to scan the intramolecular amplicon melting curves for common and rare mutations or SNPs. The twenty exon/intron fragments that may contain common mutations or SNPs were amplified using snapback primers. After scanning, these amplicons were then diluted in H2O to favor intermolecular hybridization, denatured by heating, cooled, then melted again on the LightScanner®. The resulting snapback probe melting peaks detect intra-molecular hybridization and identify the genotype at the loci all of common mutations or SNPs. Twenty six CFTR mutation cell lines from Coriell, 25 pretested CF patient and 80 none patient samples were used for blinded testing. All of the loci of common mutations and SNPs were genotyped correctly by high-resolution melting with snapback primers. The method genotyped all but 0.3% samples require sequencing. Genotyping CFTR mutations by using snapback primer with high-resolution melting analysis is reliable, rapid and inexpensive. The fewer than 1% amplicons that were not directly genotyped by the method were detected for subsequent sequencing.

P15.06

Pyrosequencing Assay Panels For Genotyping Disease-Associated Polymorphisms/Mutations

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Background: Molecular diagnostics are increasingly used to guide diagnosis and therapy of human diseases. The detection of key polymorphic variants/ mutations in human genes provides valuable information about diagnosis, risks or therapeutic regimens.

Familial Mediterranean Fever (FMF) is an autosomal-recessive disease which affects Armenian, Arab, Jewish, Greek, Turkish and Italian populations. Beta thalassemia (BTAL) is a blood disorder that reduces the production of hemoglobin. BTAL is common in Mediterranean countries such as Greece, Italy, Spain, Cyprus, but occurs as well in North Africa, the Middle East, India, and Eastern Europe. Thrombosis is a blood clot inside a blood vessel, obstructing or stopping the flow of blood through the circulatory system. Thrombotic diseases are one of the main causes of mortality worldwide.

Methods: Pyrosequencing assays were developed targeting key mutations/ polymorphisms in genes associated with thrombosis (*Factor II, Factor V, MTHFR, PAI-1*), FMF (*MEFV*) and BTAL (*HBB*). The important disease related deletions, insertions, point mutations and polymorphisms in these genes were determined by pyrosequencing. Plasmids coding for FMF, BTAL and thrombosis variants were used to study assay precision.

Results: All assays allow flexible, fast and reliable genotyping of the mutations in DNA isolated from whole blood with excellent concordance to Sanger sequencing.

Conclusions: Pyrosequencing was shown to be highly suitable to detect, identify and quantify important polymorphisms/mutations in human disease-associated genes. The newly developed assays have a significant lower turn-around time and are easier to interpret raw-data compared to the current gold standard Sanger sequencing and maintain the flexibility of a sequencing-based method.

P15.07

Guidelines for the genetic diagnosis of hereditary recurrent fevers

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Hereditary recurrent fevers (HRFs) are a group of monogenic autoinflammatory diseases characterized by recurrent bouts of fever and serosal inflammation that are caused by pathogenic variants in genes important for the regulation of innate immunity. The discovery of the molecular defects responsible for these diseases has initiated genetic diagnostics in many countries around the world including the Middle East, Europe, USA, Japan and Australia. However, diverse testing methods and reporting practices are employed, and there is a clear need for consensus guidelines for the HRF genetic testing.

Draft guidelines were prepared based on current practice deduced from previous HRF External Quality Assurance (EQA) schemes and data from the literature. The draft document was disseminated through the EMQN (European Molecular genetics Quality Network) for broader consultation and amendment. A workshop was held in Bruges (Belgium) on September 18 and 19, 2011 to ratify the draft into a final consensus document.

An agreed set of best practice guidelines was proposed for genetic diagnostic testing of HRFs, for reporting the genetic results, and for defining their clinical significance.

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P15.09

Automated analysis by using real-time PCR assays of mutations: Factor V Leiden, *F12C46T* and rs7025486 of DAB2IP gene

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Several genetic factors implicated in the pathogenesis of venous thromboembolism have been reported in the literature. The aim of this study was to automate the methods of genetic analysis of mutations: Factor V Leiden, and *F12C46T* and rs7025486 of *DAB2IP* gene. Methods: DNA extraction was performed by MagnaPure Compact (Roche). We designed an assay of TaqMan allelic discrimination by real-time PCR 7500 (Applied Biosystem). Were selected DNA samples from patients (n=50) from whom the mutations were previously genotyped by other assays. Factor V Leiden and *F12C46T* mutations were performed by real-time PCR with allele-specific probes with the Light Cylcer 2.0 analyzer (Roche). The rs7025486 mutation of *DAB2IP* gene (c.18554G>A) was performed by direct sequencing with Analyzer Genetic 3500 (Applied Biosystems). Results: We designed assays using real-time PCR 7500 with allele-specific *Taqman* probes in 96-well plate format samples. In all samples we obtained the same genotype for each mutation with both methods (sensitivity and specificity of 100 %). However, the automated process by real-time PCR genotyping plate allows us to analyze a large number of samples in each run (n = 92) including matched controls (n = 4). If this protocol were employed using automated DNA extraction, the full process would last 2 hours until the report of the results. Conclusion: T*aqMan* allelic discrimination assay provides a good alternative tool in detection of SNPs associated with venous thrombosis. Red RECAVA RD 06/0014/0016. Generalitat de CatalunyaAG-AUR 20095GR1147.

P15.10

Quality assurance practices in Europe: a survey of molecular genetic testing laboratories

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In the 2000s, a number of initiatives were taken internationally to improve quality in genetic testing services. To contribute to and update the limited literature available related to this topic, we surveyed 910 human molecular genetic testing laboratories, of which 291 (32%) from 29 European countries responded. The majority of laboratories were in the public sector (81%), affiliated with a university hospital (60%). Only a minority of laboratories were accredited (23%), and 26% were certified. 22% of laboratories did not participate in external quality assessment and 28% did not use reference materials. The main motivations given for accreditation were to improve laboratory profile (85%) and national recognition (84%). Nearly all respondents (95%) would prefer working in an accredited laboratory. In accredited laboratories, participation in external quality assessment ($p<0.0001$), use of reference materials ($p=0.0014$) and availability of continuous education on medical/scientific subjects ($p=0.023$), specific tasks ($p=0.0018$), and quality assurance ($p<0.0001$) were significantly higher than in non-accredited laboratories. Restriction of the development of new techniques ($p=0.023$) and the improvement of work satisfaction ($p=0.0002$) were significantly overestimated by non-accredited laboratories. By using a quality implementation score, we showed that accredited laboratories (average score 92) comply better than certified laboratories (average score 69, $p<0.001$), and certified laboratories better than other laboratories (average score 44, $p<0.001$), with regard to the implementation of quality indicators. We conclude that quality practices vary widely in European genetic testing laboratories. This leads to a potentially dangerous situation in which the quality of genetic testing is not consistently assured.

P15.11

Quality assurance of PCR based molecular genetic tests through elimination of thermocycler variability during assay validation

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Validation and verification of PCR methods and procedures before their use in clinical molecular genetic testing is essential for producing correct and clinically relevant results.

Over the past decades many validation studies and external quality assessments have addressed the difficulties of producing and reproducing PCR, qPCR and HRM molecular diagnostic results. Non consistent PCR results are often attributed to different purities of template DNA and reagents used, but seldomly to variations in thermocycler performance as thermocyclers are perceived as a constant rather than a variable.

In this study the thermal performance of over 10.000 thermocyclers has been evaluated. The temperature calibration data show a large spread, both between and within thermocyclers. This variability is expressed in inconsistent, false negative, lower positive or incorrect PCR results.

Typically, thermocyclers can not be adjusted and therefore solutions must be sought to deal with this variability in the design and validation of PCR assays. Currently, accredited laboratories consider PCR assays as sufficiently validated when an empirical validation study on a limited number of thermocyclers has produced comparable results. However, the large variability as measured during this study reveals the severely underestimated risk of using limited numbers of thermocyclers for empirical validation studies. Through this detailed study we have generated guidelines for easy, straightforward, analytical validation of PCR based molecular genetic tests. These guidelines are accepted by auditors around the world.

P15.12

The Unified Sample Identifier - A universal sample coding system to manage large numbers of biological samples

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Since the introduction of high-throughput DNA genotyping and sequencing technologies, the number of DNA samples analyzed for genetically complex phenotypes has tremendously increased. Furthermore it has become very important to combine samples from different sites to further increase statistical power to detect small genetic effects. This has led to serious challenges concerning the individual sample coding. Often encountered problems which hamper the unambiguous identification of individual samples include samples having the same identifier by chance as well as spelling errors in the sample identifier.

To address these problems we have developed an universal sample coding system the Unified Sample Identifier (USI) over the last years. The USI is based on the ISO certified IBAN system which is used in the international banking system since more than 30 years. The USI includes a checksum making it very resistant to spelling errors and data about project, study, sample type and aliquot, but does not include any information on the affection status, diagnosis, gender and ethnicity.

Here we present data on the implementation of the USI in our database system, the use of automatically generated barcodes and the evaluation of the checksum system. We especially analyzed the probable risk of collisions - different lab codes sharing the same check sum - in the normal lab context. We checked for random one letter changes first and focused on common human letter recognition issues in a second analysis. Based on these results we are further optimizing the algorithms for automatic correction of coding errors.

P15.13

About sequence quality: impact on clinical applications

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Advance of sequencing technologies is accelerating with a surprisingly fast rhythm, and it is clear that next-generation sequencing (NGS) should be in clinic in near future. Recent survey shows that the introduction of desktop sequencers like Ion Torrent PGM and Illumina MiSeq will be a primary growth driver for this new market.

Nevertheless, it doesn't necessary mean the end of Sanger sequencing: recent comparative studies show that performance of NGS platforms varies considerably and different NGS instruments produce different types of errors. In short, the current NGS workflows are too heterogeneous for their technical characteristics to conceive any standardized clinical platform. Also several surveys show a recent development of new markets for Sanger sequencing instruments designed for diagnosis, especially in China and one can imagine easily potential weight of this market.

Sanger sequencing remains the sequencing "gold-standard" for accuracy, reliability and easy of use: used to validate the results of NGS.

However, even in Sanger sequencing the sequence quality definition remains fuzzy and too empirical for clinical applications.

We realized a heuristic analysis of more than 31 000 sequence traces generated in the clinical diagnosis and demonstrated that the sequence traces qualities are inherently variable in routine practices and the most commonly used criterion "average quality value" alone is too inaccurate and not sufficient for clinical uses.

We demonstrated that with a combination of three parameters (average quality value, relative sequence intensity and electropherogram profile), it is possible to determine accurately the quality of any sequence.

P15.14

SNPflow - A laboratory information system for automated data processing and quality management of SNP genotyping results

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High-throughput genotyping projects in large epidemiological study populations require sophisticated laboratory information management systems (LIMS). Small to medium sized genotyping facilities often lack such LIMS and thus have to rely on basic R or VBA scripts for the quality control of their genotyping experiments. Since modern genetic epidemiological studies often comprise >10,000 individuals, automated workflows are, however, required to handle and review the large amount of genotypic data generated by modern multiplex genotyping approaches such as Sequenom iPLEX. To address this issue, we developed the web application "SNPflow". This solution provides automated data management and quality control for genotyping experiments employing the ABI 7900 HT-platform (e.g. TaqMan, KASPar Assays) or the Sequenom iPLEX platform. It automatically merges single raw output files of different DNA plates, converts them to ready-to-use genotype lists and stores the quality-controlled genotypes in a dedicated MySQL database. For each SNP assay QC values such as call rates, discordance rates and Hardy Weinberg Equilibrium (HWE) are calculated and a comparison of the observed genotype frequencies with the HapMap data is generated. It even provides a fast overview on these QC values for dozens of SNPs in one list. All data can be finally exported in well-arranged files for further analysis. Our software is, to the best of our knowledge, the first completely freely available, web-based application for an automated quality control of Sequenom iPLEX genotypes and can be accessed at genepi.i-med.ac.at/snpflow. It significantly improved the handling of and the overview on our genotyping projects in various cohorts.

P15.15

Experience and outcome of the EMQN External Quality Assessment Scheme for molecular analysis of Spinal Muscular Atrophy (SMA)

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Spinal muscular atrophy is a hereditary motor neuron disease characterized by proximal muscle weakness due to degeneration of the anterior horn cells of the spinal cord. Approximately 94% of patients with SMA reveal homozygous absence of the *SMN1*gene. However, small intragenic mutations are not detected by *SMN1* gene dosage analysis and the presence of two normal *SMN1* copies on one chromosome 5 can mask a deletion of *SMN1* on the other chromosome 5 (carrier). Subsequently, *de novo* mutation rate is relatively high. Accurate risk assessment and clinical genetic counseling are particularly important due to the genetic complexity and high SMA carrier frequency. This external quality assessment scheme was designed to assess laboratories' abilities to correctly genotype cases suspected of having SMA and to identify carriers of SMA. Each year three DNA samples (accompanied by mock clinical information) were distributed to approximately 70 laboratories from more than 30 countries after validation of the *SMN1* and *SMN2* gene copy numbers. Each laboratory sent in their written reports, usually accompanied by raw lab data. Each of the written reports is marked for genotyping and subsequent interpretation according to predetermined evaluation criteria. Most laboratories used MLPA to quantify *SMN1* gene copy number for diagnostic and carrier analysis. The genotyping error rate has increased concomitant to a decreased number of labs with full interpretation score. A full report of the results of the SMA schemes and recommendations for best practice guidelines for molecular analysis of SMA are presented.

ESHG Published Abstracts

J01. Genetic counseling, including Psychosocial aspects, Genetics education, Genetic services, and Public policy

J01.01

Genetic counseling: association of microdeletion 22q11.2 and Fragile X syndromes in one family

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Association of few genetic disorders in one family is not rare. Quality of genetic counseling for the outcome prognoses (model of inheritance, risk) depends on the accuracy of nosologic diagnose.
We present results of clinical, genetic, laboratory, prenatal and morphological investigations of the family, affected by del22q11.2 and Fragile X syndromes (FraX), detected by FISH and DNA studies. Parents were young, healthy, nonconsanguineous. G1: population risks, standard combined prenatal screening (SCPS) results were unremarkable, healthy girl was born. G2: SCPS data were normal at 1-st trimester, but heart defect, suspected via sonography at 16 weeks, was confirmed at 20 weeks of gestation. Fetus karyotype: 46,XX. Pregnancy was terminated due to fetus malformations. Morphological examination showed heart defects (membranous VSD, bicuspid aortic valve, aortic stenosis, hypoplastic aorta ascendens, interrupted arch), thymus aplasia, cleft palate. Presumably DiGeorge syndrome (del22q11.2) was confirmed by FISH (autopsy, lymphocytes of umbilical cord blood). Karyotype: ishdel(22)(q11.2q11.2)(N25-)dn. Parents had normal karyotypes (GTG, FISH), low risk of del22q11.2 for offspring. G3: normal SCPS, but pregnant had mentally retarded non-examined brothers (halfsibs) and needed an outcome prognoses. FraX was detected by PCR in both (mother was obligate carrier of CGGn expansion). The daughter was not examined because fetus (female) had low risk. G4. Pregnant underwent DNA examination of FRM1 gene because of possible risk of FraX for fetus (male). Expansion of CGGn was excluded (low risk), healthy boy born. Next pregnancy prognosis: population risks, SCPS. Scheme of genetic counseling and prenatal diagnostics protocols, made for each pregnancy will be presented.

J01.02

Are DentalFaculty Knowledgeable About Genetics and Understand its Implication forClinical Dental Practice and Education?

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Objective: In a companion study, dental students at the West Virginia University School of Dentistry (WVU SOD) were lacking "Knowledge, Skills, and Attitudes Required for Oral Health Professionals to Care for Patients with Genetic Conditions" utilizing the Report of Panel 3 of the Macy Study. The aim is to investigate the genetic knowledge, skills and attitudes of West Virginia (WVU SOD) faculty utilizing the same report for specific questions.
Methods: All full time dental faculty (32) were invited to answer 16, primarily Likert style questions (1= Strongly Agree to 5 =Strongly Disagree). Questions included Knowledge: of genetic transmission; molecular biology of the human genome; principals of population genetics; Skills: to perform a head/neck exam with special attention to signs of major genetic disorders; recognize when to refer a patient for genetic screening, testing, and counseling; Attitude: to understand the potential for genetics to contribute to the development of new approaches to prevention, diagnosis and treatment.
Results: 15 (46.9 %) participated. When it came to their Knowledge of transmission, biology of the human genome, principals of population genetics and Skills to perform a head/neck exam and when to refer, 62.2%, 51.2%, 95.5% and 69.1% disagreed respectively. Attitude, however, revealed that 66.7% agreed they understood the potential for genetics to contribute to new approaches of disease with 86.7 % interested in a CE update.
Conclusion: Although baseline knowledge and skills of the WVU SOD faculty were lacking, they recognized the importance of this new technology and were interested in a CE update.

J01.03

Importance of prenatal diagnosis of chromosomal abnormalities in genetic counseling

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Background: The role of genetic counseling in prevention of chromosomal abnormalities as genetic diseases is emphasized in the study.
Methods: Retro-prospective investigation included medico-genetic counseling of 572 pregnant women in 2006-2010. Were analyzed data regarding ultrasound examination, nuchal translucency, outflow tracts, biochemical screening, double test, fetal karyotype in the second trimester of pregnancy.
Results: Prenatal diagnosis identified severe fetal pathologies to pregnant women. Chromosomal abnormalities spontaneously occur, regardless of degree of genetic risk, as an outcome of „de novo" mutations. Diagnosis of chromosomal abnormalities to fetuses by cytogenetic methods (amniocentesis) contributed to the detection of deviations in fetal karyotype before their birth.
The age of pregnant women of low, medium and high genetic risk groups was from 17 to 44 years (average age 26,7 ± 5,1 years).
The study of fetal karyotype in amniocentesis (at 16-18 weeks of gestation) allowed to identify numerical and structural chromosomal abnormalities in 70 patients (12,2 %). From it: Down syndrome (n=28), Patau syndrome (n=4), Edwards syndrome (n=13), Turner syndrome (n=6), Klinefelter syndrome (n=5), other chromosomal abnormalities (n=14). Data analysis of fetal ultrasound indicated that the most frequent in the structure of the serious fetal pathologies are the abnormalities of cardio-vascular system (7.0%), followed by the abnormalities of central nervous system (3.2%), osteomuscular system anomalies (2.63%), renal system (2.7%) and digestive system (1.06%).
Conclusion: Using of prenatal diagnosis methods (fetal ultrasound, biochemical screening, karyotyping) in genetic counseling helps to reduce the frequency of chromosomal abnormalities and congenital malformations.

J01.04

The Role of Genetic Counselling in the Prevention of Hereditary Haemochromatosis: the perception of health professionals requesting HFE genotyping in Portugal

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AIM - To understand physician's main motivations behind requests for molecular tests of hereditary haemochromatosis and for consultation of genetic counselling, accessing to current medical practices of screening and early diagnosis of hereditary haemochromatosis and considering whether these can be improved to increase the effectiveness of prevention.
RESULTS - There is still a lack of awareness by physicians (especially by general practitioners) about the patient cases that should be sent for genetic counselling or for molecular test in hereditary haemochromatosis. This can compromise the prevention of the disease (early diagnosis and treatment) and a primordial family-based screening.
CONCLUSION - It's necessary to disclose more information about hereditary haemochromatosis among health professionals in order to improve strategies for screening and diagnosis.

J01.05

Pregnant women disorders and indications of legal abortion in Iran

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Maternal mortality in the world has decreased because high level of health and medical science. But some medical conditions such as cogenital heart disease in pregnancy can cause maternal mortality.
During pregnancy, pregnant women suffer from some diseases it can lead to infirmity, before sixteen weeks from last menstrual period, therefore the legal abortion is recommended . This subject in Iran would be done by requesting from judicial authorities. It would be accepted by three experts who are related to pregnant women disorders, then the legal abortion is issued by the legal medicine organization and eventually the legal abortion will be done by Obstetrics and gynecologist in public hospital.
This case is about a twenty four years old woman who three years ago due to aortic valve stenosis after the first pregnancy, before sixteen weeks of pregnancy, abortion was permitted and performed but she desire to have children regardless of comment her doctor was pregnant and died during

delivery. A recent research has shown that there are important conflicts in main branches of Islam that is related to the legal abortion but any disorders which can lead to infirmity or critical condition for pregnant woman is an issue related to the legal medicine organization in Iran and the legal abortion is issued.

J01.06
Assessing teaching and learning Medical Genetics

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In the last years I have tried to improve the outcome of teaching genetics to medical first year students. This time I have questioned whether useful or not to send the lessons by e-mail.

Students from the Medical and Phramacy University „Carol Davila" in Bucharest, were given twice the same two open questions during the first term. 132 students participated in the practicals I taught in the academic year 2010/2011. 6 of them did not come to any of the two tests and 5 did not come to the second testing. In this academic year from 106 students 6 were absent at both tests. This year's students received the lessons by e-mail.

In this study I compared only the marks obtained by my students during the second testing in the two different academic years.

In case of this year's students the higher marks were more frequent (table). In both years the students received twice the questions, so that the repetition could not bias the result. The comparison was made only for the second testing, when most of this year's students became aware of having received by e-mail everything I had previously taught.

This study assessed the use of written material received from the teacher and it showed that the purpose was achieved, students having better results after learning individually, what was taught during oral presentations.

Comparison between students achievements in the academic years 2010/ 2011 and 2011/ 2012

Marks obtained by students	0	1	2	3	4	5	6	7	8	9	10
Students marks in 2010/ 2011 (%)	0.83	0	7.44	6.61	9.09	10.74	13.22	13.22	9.92	11.57	17.36
Students marks in 2011/ 2012 (%)	3	1	6	1	5	9	7	10	18	18	22

J01.07
Twinning antecedents in families of patients with Down Syndrome

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Twinning has been reported in association with many chromosome abnormalities . It has been suggested that the incidence of Down Syndrome (D.S.)is significantly higher in multiple pregnancies than among singletons. The aim of this study is to describe the association of twin pregnancies in families of patients with D.S.. Contained within this report is a detailed clinical and genealogical history of 72 patients with Down Syndrome. Statistics show that 41 (56,9%) with 47,XY,+21 karyotype and 31 (43,1%) with 47,XX,+21 karyotype. The mean maternal age was 35,7 (age range 22-43 years) and the mean paternal age was 39,1 (age range 19-58 years). The antecedent of multiple pregnancies was found in 11 (15,2%) families. The rate of twinning antecedents in D.S. families is significantly higher, compared with 2131 families in the control group. It is very important for families with twinning antecedents to have genetic counselling in order to make prenatal diagnosis.

J01.08
Promoting genetic counselling in South Africa by creating a comprehensive web-based genetics resource

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Genetic counselling in South Africa is a developing field with 16 practising genetic counsellors. Annually, approximately 57,000 individuals with genetic defects are born in South Africa, while genetic counsellors see around 2,000 clients. To date, no genetic counselling information and/or genetics resource website exists in South Africa even though internet usage is rapidly increasing with 14% of the population accessing the internet in 2011 compared to the 5.5% in 2000. More than 80% of the population have mobile phones that can potentially access the internet.

The growth of, and access to genetic counselling in South Africa is hindered by the lack of awareness and knowledge by the public and healthcare providers. To address this need, a new website http://www.geneticcounselling.co.za was created. The ultimate goals of this website are; to build an online genetic counselling community thereby connecting professionals, patients and the generally inquisitive, to be the first comprehensive web-based genetics resource and to promote and increase awareness of genetic counselling in South Africa.

The website is intended to be accessible and informative to both the lay public and medical professional. The focus is on genetic counselling and its available services but the site also includes pages on knowledge share (customised talks, corporate consults and continual professional development), resources (our pamphlets, and links to external information sites), local and international news and events, and local support groups. In due course we intend to use this as the foundation to build the ultimate genetics resource in South Africa.

J01.09
Using internet social media to enhance public understanding of genetics and provide support for the psychosocial concerns of genetic carriers.

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Communication is crucial to disseminating information and facilitating understanding. The Internet age provides unprecedented opportunity for communication of 'virtually' anything at all. This presentation will introduce a selection of current social media tools that are, or can be, used to facilitate supportive communication among previously isolated individuals and families from around the world who are dealing with relatively rare genetic health concerns. Several examples from the well over 500 online social networks will be considered in depth. The use of multilingual tools such as Facebook and Twitter will be summarized.

Although many individual genetic disorders have a presence on the Internet, their focus is understandably on what can be done for those with the disorder. But reproductive decision making is fraught for parents of affected children, as well as for persons aware of their genetic risk prior to starting a family. One site, MyBlueGenome^{SM} (.org), aims to address issues of interest to genetic carriers regardless of the particular disorder of concern. Instead of dealing deeply with one disorder, it deals broadly with one aspect common to many disorders: coping with carrier status. The emphasis is on those psychosocial dimensions that require long-term engagement and the support of peers who have been through similar experiences. Resources such as understandable summaries of research articles for the lay public and links to related sites can do much to expand the genetic knowledge available to the public.

J01.10
Pharmacogenomics and public health: Implementing populationalized medicine

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Pharmacogenomics is most commonly used in personalized medicine to individualize therapy for a patient where it holds the potential to increase therapy benefits and minimize adverse drug reactions. Applying this technology to a population would produce the same benefits, in addition to saving already scarce health resources. The objective of this study is to review what has been researched and published in the fields of pharmacogenomics and public health, regarding how the two sciences can constructively intersect. Literature addressing pharmacogenomics in terms of global burden infectious diseases, public health initiatives, and public policy were researched in the PubMed database. Six major themes were identified and further discussed: interactions between public health and pharmacogenomics, pharmacogenomics and drugs, pharmacogenomics and diseases, benefits of including pharmacogenomics in public health policy, keys for implementing pharmacogenomics into public health policy, and points for consideration. Pharmacogenomics can prove to be a beneficial addition to a public health policy by maximizing therapeutic benefits, decreasing adverse drug reactions, and allowing for a better allocation of resources. Indirect health benefits can also be realized through economic advantages and international collaboration. The pharmacogenomics research surrounding major infectious diseases (malaria, tuberculosis, and HIV/AIDS) is limited, although important discoveries have been made. The implementation of this information and its implications are far reaching, and deserve extensive consideration. In order to realize the full benefits of this technology, support is needed from the private, public and governmental sectors in order to ensure the appropriateness, acceptability and affordability of this technology within a society.

J02. Clinical genetics and Dysmorphology

J02.01
Detection of polymorphism µ-opioid receptor in addicted people to opiate

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Addiction is a social problem in Iran and other countries. Studies have shown that addiction may be related to some factors including environment, genes and fetal developmental events. This study has evaluated the effect of gene and gene mutations in the mu opioid receptor (MOR). One of the most important genes involved in addiction is the mu opioid receptor (MOR) gene located on the human chromosome number 6. This gene has multiple exons including exon number 3. Various mutations have been detected in this exon which are involved in the effects of MOR gene. This study has investigated the presence of single nucleotide polymorphisms in the exon 3 of MOR gene in Iranian people. 213 male and female individuals divided into two groups of addicted and non-addicted subjects participated in the study. After preparing blood samples from subjects, DNA was extracted with G-DEX kit. MOR gene exon 3 was amplified with thermocycler equipment. PCR products were sequenced. And analysis by DNA MAN software, a 877 G>A mutation and 759 C>T mutation was detected. Both of these samples were belonged to non-addicted male subjects. Furthermore, in addicted, a 1043 G>C mutation was found. After analysis by x2 analysis. no significant relationship between addiction and genotype and significant relationship between addiction and sex was obtained($P<0.05$). This is the time which 1043 G>C mutation has been reported in Iran.

J02.02
Phenotypic variant of Alpers syndrome in a Hungarian family

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Alpers-Huttenlocher syndrome is an autosomal recessive mitochondrial DNA depletion syndrome that has been associated with pathogenic mutations in the mitochondrial polymerase γ (*POLG1*) gene. Comprised oxidative phosphorylation leads to progressive hepatoencephalopathy manifesting in severe, often intractable seizures, myocloni and stroke-like episodes, psychomotor regression and variable hepatic dysfunction. Symptoms usually appear following normal early development, initial symptoms are often infection-induced. The phenotypic spectrum associated with *POLG1* mutations is very heterogeneous, even intrafamilial phenotypic variablity is observed.

We present a case of a 19 months-old boy in whom myocloni, lethargy and elevated liver enzymes following a viral infection and a positive family history raised the possibility of Alpers syndrome. His sister died at 18 months after an adenoviral infection provoked fulminant hepatoencephalopathy, however, no diagnosis was achived at that time. *POLG1* mutation analyis in our patient revealed compound heterozygosity for the common c.1399 G>A (p.Ala467Thr) mutation and a novel, so far unpublished missense mutation, c.3589 T>C (p.Cys1127Arg) in the polymerase domain. During the 11 months of follow-up no progression or neurologic deterioration and no hepatopathy has been observed in our patient, despite abnormal EEG findings. The fulminant course in his sister might have been precipitated by several environmental factors, including valproate therapy and adenovirus infection.

Our case points to the difficulty of phenotype-genotype correlation in *POLG1* mutation associated disorders. We recommend investigation of mtDNA depletion and multiple deletions from muscle tissue and analysis of the *POLG1* gene in all children with progressive neurodegeneration accompanied by episodic, therapy resistent epilepsy and hepatic involvement.

J02.03
Deletion of SHANK3 gene detected by MLPA in a patient with autistic features and hyperactivity

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Autism is a severe neurodevelopmental disorder and one of the most heritable neuropsychiatric syndromes, with a male to female ratio of 4:1. The diagnosis of autism is based on impairments in reciprocal social interaction and communication, and restricted and stereotyped patterns of interests and activities, with abnormal development apparent within the first 3 years of life. Autistic spectrum disorders (ASDs) include: autistic disorder, childhood disintegrative disorder, pervasive developmental disorder-not otherwise specified, Asperger syndrome and Rett syndrome. There is substantial evidence from twin and family studies to support the involvement of genetic factors in ASDs.

We report a case of 3 years old boy with echolalia, irrelevant talk, not comprehensible. Muttered to himself occasionally, poor social interaction and avoided eye contact. He was unable to follow commands and responded to his name very rarely. The child was born after 8 yrs of a non consanguineous marriage, gestation was controlled and it was a full term caesarean delivery. The provisional clinical diagnosis was autistic features with hyperactivity. Karyotype was normal, X-fragile syndrome analysis was normal too and was performed a genetic analysis using MLPA (SALSA®MLPA® Subtelomeric Screening P070, X-linked mental retardation P106 and Microdeletion Syndromes P245). Analysis results showed a deletion in SHANK3 gene in 22q13.3. Parents were not carrier of this deletion.

Several studies have described similar results and these findings have led to the hypothesis that haploinsufficiency of SHANK3 may cause the behavioural phenotypic consequences of severe expressive language delay, severe/profound mental retardation and at times autism.

J02.04
Beckwith-Wiedemann Syndrome in a newborn caused by aberrant methylation in KvDMR domain in 11p15

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Beckwith-Wiedemann syndrome (BWS) is an overgrowth disorder usually (but not always) present at birth characterized by an increased risk of childhood cancer and certain congenital features. Five common features are used to define BWS are: macroglossia, macrosomia, midline abdominal wall defects (omphalocele, umbilical hernia, diastasis recti), ear creases or ear pits, and neonatal hypoglycemia. This condition is caused ba a genetic or epigenetic alteration within two domains of imprinted growth regulatory genes in chromosome 11p15, leading to deregulated expression of the imprinted genes within this region. Approximately 60-70% of the patients have imprinting abnormalities, other causes are uniparental disomy, mutations in the CDKN1C gene as well as small deletions and translocations.

We report a case of a premature newborn of 7 months old referred to Neuropaediatric Service. Parents were moroccan origin and without consanguinity, with two previous normal children. Pregnancy was controlled and it was diagnosed an omphalocele at 20 week. Birth was premature at 34 week with a weight 3,100 g, length 43,5 cm, cranial perimeter 34 cm and Apgar 8-9. There was a defective closure in abdominal wall of 3 cm with intestinal loops recovered with the umbilical cord. Karyotype was normal and he was diagnosed as a phenotype of BWS. Genetic analysis was performed using DNA from PBL and MLPA (SALSA® MS-MLPA® BWS/RSS ME030-C1). Methylation analysis showed an aberration methylation of KvDMR domain in the 11p15 region, confirming diagnosis of BWS. MS-MLPA results a low cost technique that is suitable for detecting imprinting alterations.

J02.05
Late diagnosis of non-syndromatic intrahepatic biliary atresia. Case presentation

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Biliary atresia (BA) is a progressive obliteration of intra or extrahepatic biliary system occurring in neonatal period. During the perinatal period an exogen factor influences the innate immune system of a genetically predisposed individual inducing an uncontrollable immune response with consecutive atresia of the intra/extrahepatic bile ducts.Genetic factors that could account for the disease are assessed by recent studies.GWAS identified a susceptibility locus for BA on 10q24.2, while other authors suggested region of potential disease susceptibility on 2q37.3.

The authors present a case of a 3 months female uninvestigated by the time of admission.The infant was admitted for sclero-tegumentary jaundice present since the first day of life and grow retardation.The family medical history was insignificant.Clinical examination showed cutaneous trophic disturbances and hepatosplenomegaly.The infant didn't associated phenothypic particularities or others malformations.Biological assessment showed increased conjugated bilirubin, increased colestasis enzymes, hipertransaminasemia, hipercholesterolemia and negative serology for maternofetal infections.The abdominal ultrasound combined with billiary scintigraphy and liver biopsy confirmed the diagnosis of intrahepatic BA.The differential diagnosis was made with Alagille syndrome-an autosomal dominant disorder that associates intrahepatic BA with heart, skeleton, eyes malformations and characteristic facial appearance.

Besides medical treatment ursodeoxycholic acid, bile acid sequestrants and parenteral liposoluble vitamins, the patient was proposed for liver transplantation.

The particularity of this case was the late stage of BA diagnosis associating chronic cholestatic hepatitis (Knodell 14/Fibrosis 3).The diagnosis should be established in the first weeks of life at every infant with prolonged colestatic jaundice by increasing the awareness about this condition to primary care physicians.

J02.06

Clinical features of a child with chromosomal abberation 46,XY,der(9),t(9;12)(p24;p11.2)

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In this paper we report on a case of 17-months-old male child with chromosomal abberation 46,XY,der(9),t(9;12)(p24;p11.2). This is first child from first pregnancy. Ultrasound examination during pregnancy showed normal status. Invasive prenatal diagnosis wasn't done. Diagnosis of chromosomal abberation was made after child was born. Karyotype of mother was normal: 46,XX. Karyotype of father was presented balanced translocation: 46,XY,(9;12)(p24;p11.2). This child had: hypertelorism, epicanthic fold, high forehead, thick eyebrow, anteverted nostrils, delayed skeletal maturation, small hand, mental retardation. Parents are counseled that they have risk in every next pregnancy of having fetal chromosomal aberations and they are counseled for importance of having invasive prenatal procedure (amniocentesis or chorionic villi sampling) for diagnosis of unbalanced chromosomal abberations because husband has balanced translocation. In this situation it is importante for paretns to understand the risk for future pregnancy.

J02.07

Phenotypic description of interstitial deletion of 6q in a 13-year old girl

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Approximately half of the dysmorphic patients have remained undiagnosed. However, with implication of CGH and aCGH into clinical studies, most of these cases are being diagnosed as chromosomal abnormality. Here, we report, to best of our knowledge, the largest interstitial deletion of 6q with translocations of short arms of chromosomes 2 and 10.

13-year-old girl, born to nonconsanguineous couple, had referred to us with developmental delay/mental retardation, deafness, insomnia, dysphagia, characteristic expressionless glance and dysmorphic findings of skeletal system. We, first, cytogenetically detected distal deletion of 6q with translocation of chromosomes 2 and 10.Then we performed subtelomeric FISH analysis which revealed intact subtelomeric signals of both 6pter and 6qter. Hence, we determined that the chromosomal abnormality of our patient is interstitial deletion of long arm of chromosome 6 between bands of 6q16 to 6q25 with t(2;10)(p14;p13). The next step is to perform aCGH to identify deletion size and exact breakpoints in order to correlate dysmorphic findings with genotype of patient.

J02.08

First Alive Case of Yunis-Varón syndrome in Iran,

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The Yunis-Varón syndrome (YVS) represents a rare autosomal recessive syndrome of easy recognition characterized by cleidocraneal dysplasia, ossification defects, absence of thumbs and halluces, distal aphalangia, ectodermal anomalies, generalized Hypotrichosis, and poor outcome. It is a multisystem disorder which, affect the skeletal, ectodermal, and cardio respiratory system. The molecular basis is unknown. Here, we report a 3.5 month old female neonate with Yunis-Varón syndrome, born to a consanguineously married, with normal parent. The mother was 39 year old gravid 6, and father was 46 year old. They have recurrent abortion in pervious pregnancies, and 2 daughters. In examination the baby has dysmorphic features, who had microgonathia, wide fontanel, Prominent eyes, Poor sucking, Congenital heart disease, Asymmetric face, Ambigus genitalia, Reduction anomaly in right hand, hypoplastic distal phalanges of 3th fingers, and thumbs, hip dislocation, and partial absence of clavicles(cleidocranial dysplasia).Her karyotype analysis is normal.

J02.09

A girl with possible Harboyan syndrome-a connection between corneal clouding and endolymph based hearing loss ?

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She was born two weeks after term after an uneventful pregnancy. At the age of six months it was noticed that she had corneal clouding. Cornea was oedematously thickened centrally. She made good eye contact. Visual acuity was reduced; she could not see her parents five meters away, but play with toys that she kept close to her eyes. She developed normally.

At the age of two she was diagnosed with Corneal endothelial dystrophy (CHED2). She was homozygous for the c.2240+1G>A in the SLC4A11 gene on chromosome 20p13p12. It is a congenital dystrophy of cornea probably due to failure in the function of sodium borate transporter. Swelling of the endothelial cells leads to destruction of cornea.

Ion transporter SLC4A11 has been associated with sodium-dependent transport of borate and flux of sodium and hydroxyl across cell membranes. The maintenance of ion concentration influences several organs, including the cornea, the
ear and the kidney.

At the age of four it was found that she had impaired hearing at the routine screening. Her hearing loss was mild, bilateral and basin shaped. Perceptive deafness is thought to be associated with ion concentration in the endolymph of the inner ear.

The hearing loss in Harboyan syndrome is progressive. One person in Great Britain is reported to have this mutation, but in a heterozygous state. We are presenting this girl hoping to learn about the hearing loss.

J02.10

New diagnostic problems in clinical genetics

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The major goal of clinical evaluation in genetics is to establish the etiological diagnosis, the cornerstone for providing genetic counseling. Historically, the percentage of diagnoses established by geneticists has been low compared to other medical specialties in which a phenotypic diagnosis may be enough. With the recent advent of microarray-based comparative genomic hybridization (aCGH) which allows the rapid detection of submicroscopic genomic deletions and duplications, the number of etiological diagnoses established has progressively increased. However, there are many old and new diagnostic problems, frequently neglected, that contribute to make it difficult to establish the diagnosis.

At the Latin-American Association of Genetics (ALAG)'s meeting in Mexico in 1994, we first presented a classification delineating four groups of problems. For teaching and educational purposes, we recognized problems due to the patient, the disease, the observer, and the environment. This systematic review and illustration of different diagnostic problems creates awareness and improves the effectiveness of medical practice. With the new technologies, big advances have been made but also new problems have been recognized. In 2008 this classification was expanded to include a fifth group: problems of informatic due to the interpretation of microarray (aCGH) results mainly secondary to the incomplete knowledge about CNV and phenotype-genotype correlation. The application of aCGH to diagnosis is evolving and requires intense communication among clinical geneticists and the laboratory specialists about their specific clinical queries or troublesome cases. In this presentation we will review the diagnostic process and will illustrate and discuss different diagnostic problems.

J02.11

Variable expression of Ehlers-Danlos syndrome in sibs

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Ehlers-Danlos syndrome (EDS) is a diverse group of syndromes caused by defect synthesis of collagen. It affects mostly skin, joints and blood vessels.

There are six major subtypes described so far, with variable mode of inheritance. Additional subtypes are described linked with other comorbidities.
We describe two sibs with major features of EDS. Two sibs - older sister and younger brother share the same facial features - broad face with coarse facial features, sagging cheeks, long, smooth filtrum, sparse hair, joint laxity, thin lax skin with skin wrinkling, hyperextensibility, poor skin healing with forming an atrophic scars. Both children had mitral valve prolaps. The girl , now aged 25 years started to lose her hair progressively, have mild form of kyphoscoloisis and experiencing menstrual irregularities. The boy develops seizures at the age of 14 years. EEG revealed spike-wave complexes. CT scan and magnetic imaging showed demyelinisation that progressed in years. During the last several years his mental and motor abilities deteriorated. Also the kyphoscoliosis that has been noticed at puberty became significant. The diagnosis in both children was established by skin biopsy and histology evaluation so far, showing destruction of collagen fibers.
Last classification of EDS is made upon major signs and symptoms that are present in the patient. Intrafamilial variability is described. In this case kyphoscoliotic type with variable expression can be suspected . Since both children had aged appearance that progresses with age, also progeriod form is assumed as well. Further evaluation is needed in this family.

J02.12

Examination of SCN1B in Iranian epileptic patient

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Epilepsy is a common chronic neurological disorder that is characterized by recurrent unprovoked seizures. Molecular studies of candidate genes can help us to define a correct differential diagnosis. So we studied SCN1B gene in Iranian patient with Idiopathic Epilepsy includes Febrile Seizure, Generalized Epilepsy with Febrile Seizure (GEFS+) or Dravet syndrome, diagnosed clinically, to explain genotype-phenotype correlation. Materials and Method: We screened 34 selected epileptic unrelated Iranian propends for all coding regions of SCN1B by PCR amplification and direct Sequencing. All families and propends were previously screened for SCN1A and mtDNA mutations. Results: PCR amplification of whole coding regions and splicing site of SCN1B followed by direct sequencing revealed tow novel sequence variation in patients (p.248 R>S, p.210 L>P) which did not detected in the healthy normal family members. Conclusions: According to final results it seems that these two novels SCN1B variations are not causative mutation in epileptic patients but they can act as genetic predisposition factors in epileptic phenotypes which introduce susceptibility especially in response to antiepileptic drugs.

J02.13

Clinical and genetic studies in in Ewing sarcoma

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Throughout the latest years the results in treatment for familial Ewing's sarcoma, in the metastasis-free stages, have considerably improved. The prognosis remains somber for the metastatic disease patients at the first diagnosis time point or in case of relapse after therapy. Inefficient chemotherapy and the relapse are the major mortality causes in Ewing's sarcoma patients. For improving the therapy outcome in patients with potential relapse, the discovery of reliable markers that predict the tumor "behavior", help making the diagnosis or identifying the therapeutic molecular targets when relapse, is mandatory.
The study group includes 5 cases with Ewing syndrome, diagnosed and treated in the Pediatric Oncology & Hematology Clinic in Timisoara. The diagnostic protocol has included genetic testing (cytogenetic and molecular) and morphopatology testing (histomorphology, immunohistochemistry).
This article's scope is to highlight this way, the present concepts of the sarcoma genesis in Ewing's sarcoma and the comparison of different molecular markers which could affect its prognosis or contribute to the development of future therapies. A better understanding of the cells of origin and the molecular pathways that regulates carcinogenicity in Ewing's sarcoma could help in finding new therapies in Ewing's sarcoma.

J02.14

Facio-audio-symphalangism Syndrome in a patient with partial 17q22monosomy involving NOG gene

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Multiple Synostosis Syndrome (SYNS - also called Facio-audio-symphalangism) is an AD disorder characterized by fusion of the proximal interphalangeal joints of the hands, multiple and progressive joint fusions in the hands, feet, cervical vertebrae, hips, brachydactyly, subluxation of the radial heads, associated with facial anomalies, strabismus and conductive hearing impairment. It is commonly caused by NOG (17q22) mutations, as a part of the so-called "NOG-related Symphalangism Spectrum Disorders". We report a 11-years old boy presenting with synostosis of the proximal interphalangeal joints of the fifth finger of the hands, tall stature, facial anomalies (broad nasal bridge, hypoplastic alae nasi, thin upper lip, prognatism), webbed second and third toes, pes valgus, synostosis of cervical vertebrae C3-C4, subluxation of the radial heads, conductive hearing loss; he had normal mental development. CT-scan of temporal bones and brain MRI showed normal data. A clinical diagnosis of SYNS was proposed. It is noteworthy that tall stature has been described in 2 other cases of SYNS. Search for mutations in the NOG gene resulted negative. Array-CGH analysis with a resolution of 100 Kb revealed a partial monosomy of 17q22, extending for about 320 kb, including the NOG gene. Deletions of the 17q22 have been associated to a more complex phenotype, including microcephaly, developmental delay, heart malformations, limbs anomalies (including symphalangism), tracheoesophageal fistula and hearing loss (either sensorineural or conductive). To our knowledge, this is the first case of SYNS due to a specific NOG gene deletion.

J02.15

Fibrochondrogenesis in a 26-week fetus: Hepatic fibrosis

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Fibrochondrogenesis is a rare condition, neonatally and perinatally lethal osteocondrodysplasia with an autosomal recessive mode of inheritance. The disease is clinically characterized by a flat midface with a small nose and anteverted nares, significant shortening of all limb segments and a small bell-shaped thorax with a tuberant abdomen. We report a 26-week male fetus in which the diagnosis of lethal osteochondrodysplasia was suspected on prenatal ultrasound. After termination of pregnancy, fibrochondrogenesis was confirmed in the postpartum physical and radiological examination, the histopathological study showed us the presence of hepatic fibrosis in addition to fibrochondrogenesis. Here we present a new case of fibrochondrogenesis with hepatic fibrosis which is not previously described. We discussed the molecular pathogenesis of the case.

J02.16

Fibrodysplasia Ossificans Progressiva (FOP) in Cyprus: First case report and management issues

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Fibrodysplasia Ossificans Progressiva (FOP; MIM#135100), is a very rare and severely disabling autosomal dominant genetic disorder characterised by congenital malformation of the great toes and progressive heterotopic endochondral ossification in specific anatomic patterns. Bone forms at extraskeletal sites within tissues such as skeletal muscles, tendons, ligaments, fasciae and aponeuroses. Attempts to surgically remove heterotopic bone risk provoking explosive and painful new bone growth. Although most cases occur in individuals with no prior family history of FOP, autosomal dominant inheritance has been observed in a small number of families.
We report a 27-year-old lady with FOP, the first case ever reported in Cyprus. She presented with a long standing history of joint stiffness, episodic painful inflammatory soft tissue swellings over the arms and back and increasing mobility difficulties starting from her early teens. Analysis revealed that she was heterozygous for the c.617G>A (p.Arg206His) *ACVR1* mutation, reported in all classically affected FOP patients. She has recently started having reduced

temporomandibular joint mobility and chewing difficulties. The case illustrates management complexities for rare disorders particularly in relatively small centres.

J02.17

Identification of a novel de novo mutation in *FLNA* in a patient with late onset temporal lobe epilepsy

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Cortical malformations associated with defects in neuronal migration result in severe developmental consequences including intractable epilepsy and intellectual disability. However, female carriers of X-linked disorders may present very subtle clinical manifestations.

We present the case of previously healthy 34 year old woman, daughter of consanguineous parents, referred to our clinic due to late onset partial focal epilepsy. The initial presentation consisted of lightheadedness, dysphoric episodes and other unspecific symptoms, sometimes followed by loss-of-consciousness. The diagnosis of temporal lobe epilepsy was suggested. An MRI scan of the brain identified bilateral symmetric periventricular nodular heterotopias.

There was no relevant family history. Since the patient was planning a pregnancy, she was referred for genetic counseling at the Medical Genetics Department. Due to her parents' consanguinity, we performed linkage analysis for the ARFGEF2 locus, which revealed that the patient was homozygous for all informative markers. We proceeded to sequence analysis of this gene, which identified no mutation.

We then performed sequencing of FLNA, associated with X-linked periventricular nodular heterotopia. A *de novo* missense mutation p.Pro97Ser was identified in exon 2. This mutation has not been described previously, and is predicted to be disease causing.

The knowledge of this mutation will allow specific genetic counseling to this patient, who is at risk of having severely affected sons. This report also highlights the importance of considering de novo mutation in consanguineous families.

J02.18

Clinical and genetical study of patients from Republic Bashkortostan with novel mutation in GJB1 gene.

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Hereditary motor-sensory neuropathy IX occurs in 13,7 % of all HMSN cases in the Republic of Bashkortostan. Two out of three GJBI missense mutations were previously described: p.Pro87Ala (259C>G) and p.Arg22Gln (c.65G>A). The p.Thr86Ile (c.257C>T) mutation is novel. In the family with novel mutation the disease began in two males in their second decade of life and was characterized by severe impairment of the peripheral nerves with CNS involvement. Their clinical picture was presented by progressive weakness and wasting of distal extremities, reduced sensation of proprioception, sensitive ataxia, bilateral pes equinovarus deformity. Generalized postural tremor, muscle fasciculations in the hands were seen in one of the patients. Median motor conduction velocity (MCV) was 33 m/s, the M-amplitude was 3 mV. In one of female patients, first signs of the disease also appeared in her second decade of life. Her clinical picture was presented by bilateral pes equinovarus deformity, distal hypoestesia, mild weakness of distal parts of the hands, moderate weakness of the legs. Four female patients had no health complaints. Meanwhile, physical examination showed the absence of the Achilles reflexes in two of them. In the remaining two patients - neuropathy signs were identified by electroneuromyography alone.

J02.19

Is 8860 variation a rare polymorphism or associated as a secondary effect in HCM disease?

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Introduction: mtDNA defects, both deletions and point mutations, have been associated with hypertrophic cardiomyopathies. The aim of this study was to establish a spectrum for mtDNA mutations in Iranian hypertrophic cardio - myopathy (HCM) patients. Material and methods: The control group was chosen among the special medical centre visitors who did not have hypertrophic cardiomyopathy or any related heart disease. Hypertrophic cardiomyopathy (HCM) is widely accepted as a pluricausal or multifactorial disease. Because of the linkage between energy metabolism in the mitochondria and cardiac muscle contraction, it is reasonable to assume that mitochondrial abnormalities may be responsible for some forms of HCM. Point mutations and deletions in the two hot spot regions of mtDNA were investigated by PCR and sequencing methods. Results: Some unreported point mutations have been found in this study but no deletion was detected. Meanwhile some of these point mutations have been investigated among HCM patients for the first time. Conclusions: A8860G transition was detected in a high proportion, raising the question whether this rare polymorphism is associated as a secondary effect in HCM disease.

J02.20

Hemifacial microsomia: case report - from diagnosis to management

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Hemifacial microsomia (HFM) is the most frequently encountered form of isolated facial asymmetry and is the second most common facial anomaly. The prevalence is 1 in 3000 up to 1 in 5600 births. HFM is an early vascular disruption, possible associated with chromosomal anomalies. Males appear to be more affected than females and the right side of the face is affected more frequent. We report a 13 year old male patient with right hemifacial microsomia and discuss the management steps from diagnosis to treatment aiming to improve the facial and occlusal aspects. The orthodontic diagnostic was class II division 1 malocclusion with frontal open bite. The maxilla was narrowed on the involved side with decreased palatal width and unilateral crossbite. Three-dimensional CT reconstruction showed hypoplastic, malformed right mandibular body, minimal underdevelopment of the condyle and unilateral aplasia of the mandibular ramus, with absence of the glenoid fossa. On the involved side: zygomatic arch was incomplete, maxilla, squamous temporal and malar bone were small; ear presented malformed lobule with rest of pinna absent and bony atresia of external auditory canal; hypoplasia of facial muscles has also been observed. Orthodontic treatment and partial aesthetic solving of the disability with silicone implant on the affected part improved the patient's facial appearance. The treatment of patients with HFM requires an interdisciplinary approach including at least maxillofacial surgery, plastic surgery and orthodontics. Co-operation not only within the team, but also with the patients and their families is essential in order to achieve the best results.

J02.21

Hereditary multiple exostoses - clinical study of nine illustrative cases

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Hereditary multiple exostoses (HME) is a rare medical condition in which multiple bony spurs/lumps (exostoses/osteochondromas) develop on the bones of a child. The prevalence is estimated at 1:50,000 and seems to be higher in males (male/female ratio 1.5:1).

We present a clinical study of 9 cases of HME (6 male and 3 female) diagnosed in our Medical Genetics Center, to discuss suggestive features, particularities, long term follow-up, management and genetic counseling. This exploratory descriptive aims also to study the impact on daily living activities and quality of life.

According to literature, most commonly involved bones are the femur(30%), radius/ulna(26%), tibia(20%) and fibula (13%). Hand deformity, resulting from shortened metacarpals, is common. Axial sites, such as the pelvis, scapula, ribs and spine are more commonly the location of degeneration of osteochondromas to chondrosarcoma.

In our study exostoses are present in all patients. The most frequently involved bones were tibia and fibula - 6 cases (66.7%), ribs and scapula 4 cases (44.4%), humerus, radius/ulna and fist 3 cases (33.3%), the femur and knee are affected in 2 cases (22.2%), the spine and elbow in 1 case (11.1%). 3 cases were familial. No tumoral degeneration was identified.

Other associated anomalies are: scoliosis, lower-back pain, moderate pectus excavatum, tumefaction of the elbow joint, brachydactyly, clinodactyly of fifth finger, long fourth finger, genu valgum.

In conclusion, we present the clinical study of 9 illustrative HME cases, discussing particularities and consequences on daily living and quality of life, as well as management and genetic counseling issues.

J02.22
Clinical and imagistic correlations among pathological forms of holoprosencephaly

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Introduction:
The absence or the incomplete cleavage of prosencephalon into diencephalon and telencephalon in the 4-8 th weeks of fetal life, leads to holoprosencephaly. This is often associated with severe facial anomalies, microcephaly, hipertelorism and labio-palatine clefting.
There are multiple and various genetic implications (chromosomal deletions 2p21; 7q36; trisomy 13) which are associated with multiple environmental factors.
In this paper the authors aim to process the clinical and imagistic data resulted from the study of 4 clinical cases.
Material and method:
The study lot consisted of 4 newborn: 3 on term newborn with GA (gestational age)= 39-40 weeks and BW (birth weight)= 3500-4000 g and 1 new born with IUGR (intrauterine growth restriction).
On term newborn presented labio-palatine clefting; two of them presented cardiac malformations (Atrial septal defect and Ventricular septal defect); they were classified as having Semilobar Holoprosencephaly.
Newborn diagnosed with Semilobar Holoprosencephaly had altered phenotype: hipertelorism (2 clinical cases); labio-palatine clefting (3 clinical cases); micrognathia; flattened nose and upper implanted ear.
Cranial ultrasound and MRI showed anomalies of the interhemisferic split, agenesis of the posterior region of corpus callosum, various degrees of fusion of the lateral ventricles, the absence of cavum septum pellucida.
Conclusions:
Semilobar Holoprosencephaly is a severe form of illness due to the brain malformation which determines growth anomalies and severe neurological retardation.

J02.23
MLPA analysis in 25 Brazilians individuals with Holoprosencephaly

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Holoprosecephaly is a common disorder of the developing forebrain in humans, occurring with a frequency of 1:250 conceptuses. The etiology is heterogeneous and complex, as this developmental disorder can be due to environmental factors, chromosomal aberrations, or genetic anomalies. SHH, ZIC2, SIX3 and TGIF genes are the four major genes implicated in the susceptibility to HPE, but have only been found to explain 25% of the genetic cases, including mutations and microdeletion. We analyzed a cohort 26 individuals within the holoprosencephaly spectrum thought Multiplex Ligation Dependent Probe Amplification (MLPA) technique using P187 Holoprosencephaly Kit (MRHC-Holland®). All individuals have been previously analyzed for mutation in SHH, ZIC2, SIX3 and TGIF genes. We found one case with deletion in SHH gene. Numerous isolated HPE case reported have shown that most of the chromosomes have been implicated, emphasizing the genetic heterogeneity of HPE. Considering this multigenic aspect of the disease, investigation of HPE loci and identification of new HPE genes must be continued. Mutations and deletions in HPE genes do not always lead to physical signs of HPE, however this information may be helpful for genetic counseling purposes.

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J02.24
Findings in a routine setup of molecular diagnostics in Hypertrophic and Dilated Cardiomyopathy

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Background: Many findings has been published regarding the genetic background of hypertrophic (HCM) and dilated (DCM) cardiomyopathies. Hundred of mutations in a vast number of disease genes could be identified. However, most data were established using well defined study populations. In contrast, only few data are available about routine patients without study based preselection bias.
Materials and methods: We studied n=104 consecutive patients with HCM and n=22 cases with DCM. In HCM a panel of the most frequent disease genes MYH7, MYBPC3, TNNT2, and TNNI3 encoding for ß-myosin heavy chain, myosin binding protein C, cardiac troponin T and cardiac troponin I respectively, were analyzed using direct terminatorsequencing technology (ABI-BigDye-*Terminator*v1; 3130*XL*). In DCM a panel of the most frequent disease genes LMNA (encoding for Lamin A/C), MYBPC3, MYH7 and TNNT2 respectively, underwent analyses.
Results: In HCM of 104 pts in n=54 pts (52%) using the HCM diagnostic panel a mutation in one of the disease genes could be found, whereas in DCM of 22 pts in n=3 pts (14%) using the DCM diagnostic panel could be detected.
Conclusions: In molecular diagnostics of serial HCM and DCM patients using specific panels of the most frequent disease genes in HCM more than a half of all patients and in DCM more than 10% of all cases could be genotyped positively. This is comparable to study based data. These findings indicate the positive transfer of scientific data into clinical routine use in favour of a better patients care in the field of genetic counseling.

J02.25
KID syndrome: multiple joint contractures in Lithuanian patient with *GJB2* gene mutation

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Keratitis-ichthyosis-deafness (KID) syndrome is a rare congenital ectodermal disorder, characterized by the presence of localized erythematous scaly skin lesions, sensorineural deafness and severe bilateral keratitis. We present the first Lithuanian patient with a heterozygous missense mutation c.148G>T (p.Asp50Tyr) in *GJB2*. The boy has typical clinical features for KID syndrome consisting of diffuse hyperkeratotic erythroderma, bilateral keratoconjunctivitis, moderate bilateral sensorineural hearing loss, paronychia with nail dystrophy, alopecia totalis. Besides, he developed multiple joint contractures at the age of 3. To our knowledge, these findings have not been reported previously. We propose that the multiple joint contractures observed in our patient with p.Asp50Tyr are a severe subtype of Keratitis-ichthyosis-deafness syndrome, thus expanding the spectrum of connexin-associated keratodermias.

J02.26
Possible mosaicism in a case of lingual plexiform neurofibroma

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Introduction
Neurofibromatosis type I is typically an autosomal dominant inherited disorder with complete penetrance and variable expressivity, generated by the neurofibromin mutation, a tumor suppressor gene located on chromosome 17q11.2.
50% of the cases are mutations de novo, with no other affected family members. Somatic mosaicism accounts for many sporadic cases.
The classic NF1 is characterized by multiple neuro-fibromatous tumors, including the plexiform neurofibroma, cafe-au-lait spots, freckling of the groin or the axilla, Lisch nodules in the eye and skeletal abnormalities.
The patients have increased susceptibility to develop other benign or malignant tumors, so they need regular follow-up to detect malignant degeneration, an early recurrence or appearance of other manifestations.
A particular aspect is described in some patients, presenting only one clinical criterion of diagnostic, possibly generated by a mosaic form and having, in this situation, serious implications for the patient's evolution and its family.
Case presentation
A 29 years old male was hospitalized for a large tumor located on the right hemi tongue. There were no other clinical findings in physical examination. The pathological personal antecedents and the family history were negative.
Histological examination demonstrated the presence of a lingual plexiform neurofibroma.
Conclusion
Despite their occurrence in the head and neck region, neural sheath tumors are rare in the oral cavity; oral manifestations are reported much often in patients affected by neurofibromatosis.
Because of this, we think our patient may present a form of mosaicism and needs genetic testing for NF1 mutations, considering the possible implications.

J02.27

Investigation of Familial Mediterranean fever (FMF) in 25 Iranian patients

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Familial Mediterranean fever (FMF) is an Autosomal recessive disorder characterized by recurrent Attacks of fever and inflammation in the peritoneum, synovium, or pleura, accompanied by pain. FMF is caused by mutations in MEFV gene that is located at 16p13.3 and encodes a protein, pyrin or marenostrin.

In this study , PCR and sequencing method was performed for four high rate mutation exons of MEFV gene (2,3,5,10) in 25 unrelated Iranian patients. The most frequent homozygote mutation was R202Q (in %24), followed by M694V (in 16%), and M680I (in 8%). Eight percent were compound heterozygotes for three mutations (V726A, E167D, F479L).

J02.28

Proband with Hunter syndrome: ten years later

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Mucopolysaccharidosis II, Hunter syndrome (MPS II, OMIM 309900, Xq28, IDS, OMIM 300823), is a rare disorder caused by deficiency of the lysosomal enzyme iduronate sulfatase ((EC 3.1.6.13), leading to progressive accumulation of glycosaminoglucans in nearly all cell types, tissues, and organs. We reported on this case of HS in EJHG, v.11, Suppl 1, P. 166, 2003. Now proband is 18 yr aged. His intelligence is normal and the youth is the tenth grader of a comprehensive school. Proband has progressive coarsening of facial features, short stature (124cm) and underweight (32kg), skeletal deformities of thorax and feet, protuberant abdomen, hypermetropic astigmatism (S=D), chronic purulent ethmoiditis, cerebral ventricular dilation, cardiac valvular disease (myxomatosis of mitral valve and mitral regurgitation, cardiomyopathy), hepatosplenomegaly. Joint mobility is decreased, and the fingers have clawlike deformities. Laboratory findings and imaging studies were found to show moderate abnormalities. Unilateral auditory prosthetics was made. Care for our patient with HS involves a multidisciplinary approach and includes pediatrician, neurologist, orthopedist, otolaryngologist, ophthalmologist, geneticist etc. To our regret ERT was not available.

J02.29

Novel mutations detected in the NF1-gene

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Mutations in the NF1 gene are the cause of Neurofibromatosis type1, the most common tumor-predisposing disorder in humans. As the mutation rate in the NF1-gene is among the highest known, analysis of the NF1 gene continues to reveal novel mutations in many patients. Because of the large size of the gene, the lack of a mutation hot spot and the diversity of pathogenic mutations found NF1 analyses have long been laborious and sometimes yielded unsatisfactory results. Newer techniques are more efficient and allow detection of a causative mutation in the great majority of NF1-patients. We report on 7 novel NF1 mutations not yet described in the HGMD mutation database (version 9.12.2011). Among them a spectrum of different mutation types was found: 3 splice site mutations, caused by a small deletion (c.654+2delT), a duplication (c.2325+1dupG) and a base change (c.7126+1G>A) respectively, all concerning splice site consensus sequence positions. Furthermore we detected 2 missense mutations (c.3503G>A, c.6718C>T), 1 nonsense mutation (c.4720C>T) and 1 duplication leading to a frameshift (c.6676dupA). Two of the patients were adults, for whom genetic analysis served the confirmation of the clinical diagnosis. The patients who benefit most from the ameliorated mutation detection, however, are young patients with only little clinical manifestations and hence uncertain diagnosis. This was the case for five of the patients, who at the time of diagnosis were 11 years old (1 patient) or younger than two years (4 patients).

J02.30

Genetic testing for Neurofibromatosis (NF1). When and why?

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The diagnosis of Neurofibromatosis (NF1), a common autosomal dominant disorder affecting 1/3500 individuals, is based on well-defined clinical criteria in adults which are unsatisfactory in children. The responsible gene is the *NF1* tumor-suppressor coding for the neurofibromin. Approximately 50% of cases represent new mutations.

We describe the phenotypic and genetic variations in 39 Greek NF1 patients and point out the significance of early genetic testing, aged 7-months to 34-years (21 males/18 females).13 patients underwent molecular analysis of the *NF1* by cDNA sequencing of all exons and were found positive, 6 of them were younger than four years. If no mutation was detected the presence/absence of deletions was verified by MLPA. Our patients presented the following NF1 clinical features: café-au-lait spots in 34/39, two or more neurofibromas in 15/39, axillary or inguinal freckling in 9/39, optic glioma in 2/39, two or more Lisch nodules in 5/39, bony lesions in 13/39 and a first-degree relative affected with NF1 in 15/39. 5/39 patients had tumors (except neurofibromas) and mental retardation presented in 7/39 individuals older than 3 years. 15/39 patients had abnormalities in the brain MRI. 4/13 patients analyzed, carried novel mutations, 4/13 had missense, 2/13 frameshift, 6/13 nonsense and 1/13 a large deletion.

From our small group of NF1-patients we must strongly recommend the implementation of molecular testing at an early age as clinical diagnosis is difficult in young children. The sooner the molecular analysis is performed the more beneficial it is for the family counselling and the follow-up of the patients.

J02.31

A particular presentation in a possible case of segmental neurofibromatosis Type I

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Neurofibromatosis type I is an autosomal dominant condition, with birth incidence 1/3500 and a high degree of variability of clinical expression.

Segmental or regional neurofibromatosis type I (NF1) is diagnosed in individuals who have features of NF 1, restricted to a part of the body and has an incidence approximately 1/40.000. Some of those patients displaying only pigmentary changes or dermal neurofibromas, and others having both features .

In some cases the unusual distribution of features is probably just a chance occurrence in a individual with NF1. In other individuals segmental neurofibromatosis type I represents mosaicism for a somatic NF 1 mutation.

We report a case of a 30 years old woman who associates plexiform neurofibroma in lumbar region,and has also a pigmented area with asymmetrical distribution in lumbar region, flanks, and downwards on superior parts of the legs. She is the first affected person in her family. Because the tumor growth is progressive and patient associates also pain the surgical removal is the first priority.Patient wants to have children after this intervention, but counselling is very difficult, knowing that have been reported cases with segmental NF1 whose children have typical NF1. Prenatal diagnosis is controversial and limited , because even in presence of molecular diagnosis severity of the disease cannot be predicted in affected fetus

J02.32

Congenital heart defects in oculo-auriculo-vertebral spectrum

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The oculo-auriculo-vertebral spectrum (OAVS) is a non-random association of hemifacial microsomia with mandibular hypoplasia, ear malformations, preauricular tags and ocular dermoid cysts. Most cases are sporadic. The underlying genetic mechanism remains unknown. Congenital heart defects (CHD) have been reported in 5-60% of the patients.

We have analysed the types of CHD in 18 children with OAVS recorded in the files of Iasi Medical Genetics Center between January 2006 and December 2011. There were 7 girls and 11 boys. The diagnosis was based on the presence of the characteristic features of the disorder. CHD were present in 8 children - atrial septal defect (5/18), Fallot tetralogy (2/18) and ventricular septal defects (1/18). The prevalence of heart defects was higher in boys (54,54%) than in girls (28,5%). We found no correlation between the severity of the clinical manifestations and the association with CHD. In the literature ventricular septal defect and Fallot tetralogy are the most frequent CHD associated with OAVS. The comparison of our data and the literature data will be presented in detail.

In conclusion we present a study of 18 cases with oculo-auriculo-vertebral spectrum, 44,44% of them having heart defects. Cardiac defects are commonly associated to OAVS and males are more frequently affected than females. Echocardiography should be a routine investigation in patients with OAVS.

J02.33

Oral-Facial-Digital Syndrome (type I): A case report.

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Oral-facial-digital syndrome type 1 (OFDI; OFD1; OMIM 311200) is a rare developmental disorder transmitted as an X-linked dominant condition with embryonic male lethality. OFD1 is characterized by malformation of the oral cavity, face, and digits. Central nervous system (CNS) abnormalities and cystic kidney disease can also be part of this condition. Lesions in the mouth include median pseudoclefting of the upper lip, clefts of the palate and tonge, lingual tumors and dental anomalies (missing or supernumerary teeth, enamel hypoplasia, and teeth malpositions). Dysmorphic features affecting the face include hypertelorism, frontal bossing, micrognathia, facial asymmetry, alar hypoplasia and broadened nasal ridge. The digital abnormalities are syndactyly, clinodactyly, brachydactyly and, rarely, pre or postaxial polydactytly. Less frequently expressed phenotypic anomalies include skin milia, alopecia, deafness and trembling.It is considered to be a ciliopathy caused by mutations in the OFD1 gene. A variety of mutations have been described, and a genotype-phenotype correlation has been suggested.This disorder is due to mutations in the OFD1 gene that encodes a centrosomal protein localized at the basal bodies at the origin of primary cilia.

The proband was a female newborn, sporadic case with suspected OFD1. This newborn had many of the typical manifestations, including frontal bossing, micrognathia,lingual tumors, cleft tonge, clynodactily and others less frequently signs findings : alopecia and skin milia. We extended the pedigree to three proband's generations, performing a thorough physical examination .In the light of this case, the author discuss the variability phenotypic expression of OFD1 gene and the genetic counseling in this family

J02.34

Pathological findings of a male fetus with familial Pelizaeus-Merzbacher disease caused by a 320.6Kb Xq22.2 duplication

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Pelizaeus-Merzbacher disease (PMD) is a disorder with clinical variability, ranging from the severe connatal form to the classical form. We present the clinical and molecular findings of two affected males, three carrier females and an aborted male fetus with familial PMD. The two male probands exhibited severe PMD phenotype and intellectual disability (ID). High resolution oligonucleotide based array comparative genomic hybridization (aCGH) identified Xq22.2 duplication of 320.6Kb [102641391-102961998, hg18], including the proteolipid protein 1 gene(*PLP1*) and surrounding chromosomal region. Postmortem examination was completed in the aborted male fetus. To our knowledge this is the first report of specific neuropathological lesions in a fetus with familial *PLP1* duplication. The observed early degenerative brain lesions occurring before the onset of myelination suggest that the *PLP1* gene has a more complex role in human brain development exceeding its structural function in myelin formation.

J02.35

A case with Rhizomelic chondrodysplasia punctata

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Rhizomelic chondrodysplasia punctata (RCDP) is an autosomal recessive peroxisomal disorder with a phenotype of proximal shortening of humerus and femur, punctate calcifications around the large cartilage, possible calcification of the intervertebral discs, cataract, severe mental deficiency,and postnatal growth retardation. It usually results in death in the first decade of life. Characteristic biochemical criteria were present: decreased plasmalogens, elevated levels of plasma phytanic acid, and normal levels of very long chain fatty acids. Three variants: RCDP1, RCDP2 and RCDP3 are caused by mutations in the *PEX7*, *GNPAT* and *AGPS,* respectively. The clinical picture in RCDP2 and RCDP3 is similar with RCDP1, which is the most frequent type. To distinguish the subtypes, enzyme analysis is necessary from the skin fibroblasts.

Here, we report a 7-month-old female with bilateral cataract, punctate calcifications around the large cartilage, and postnatal growth retardation. Plasma phytanic acid level was elevated and long chain fatty acids levels were normal. The clinical phenotype and biochemical tests were consistent with RCDP and we analyzed our patient for *PEX7* mutations. Sequence analysis of all exons and intron-exon boundaries of *PEX7* showed no mutation. Enzyme analysis for subtyping and mutation analysis of the corresponding genes, *GNPAT* and *AGPS* are in progress.

J02.36

A Case of Sirenomelia Sequence with Aprosencephaly

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Sirenomelia sequence is a rare congenital anomaly. This is also known as "mermaid syndrome" because of typical feature of lower limb. Sirenomelia sequence is characterized with a single midline lower limb. Our case is an infant delivered at 34 gestational weeks by spontaneous vaginal delivery from a 34 years old gravida 2, para 0. Parents are not relative. Infant has one femur, one tibia and one phalanx at lower extremity. Calcaneus, metatarsals and other bones of the footh are absent. Patient has anal atresia and renal agenesy. Determinig of sex was impossible since external genitalia was absent. Ultrasonographic examination revealed aprosencphaly. Altough some risk factors (e.g maternal diabetes) have been suggested, etiolgy of sirenomelia sequence is debated. In this report, we describe a premature infant with sirenomelia sequence because of very rare presentation.

J02.37

Gross deletion in the SLC22A5 gene

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Systemic primary carnitine deficiency is an autosomal recessive disorder of fatty acid oxidation. Disorder is affected by mutation in SLC22A5 gene, which consists of 10 exons, maps to chromosome 5q31 and encodes the novel organic cation transporter (OCTN2). Affected patients can have a predominant metabolic or cardiac presentation.

We have studied the patient with systemic primary carnitine deficiency. Primers for sequence analysis of coding regions and flanking intronic SLC22A5 gene were chosen. PCR products of exons 8, 9, 10 were not received. Exons 8, 9, 10 were amplified in diplex PCR with the control marker - exon 13 PAH gene, mapping outside the SLC22A5 gene. PCR product was found only from the control marker.

Therefore, a homozygous novel mutation - the deletion of exons 8, 9, 10 of the SLC22A5 gene was found.

As a result diagnosis of systemic primary carnitine deficiency was confirmed for the first time in Russia by molecular genetic methods.

J02.38

Coexistence of Townes-Brocks syndrome and Albinism in a case

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Townes-Brocks syndrome (TBS) is a genetic disorder. The most common features of this syndrome are anal atresia, abnormally shaped ears, and hand malformations that most often affect the thumb. Most people with this condition have at least two of these three major features. Albinism is one of the archetypal inborn errors of metabolism. It is usually defined as a congenital hypopigmentation of the skin, hair, or eyes. In this report, we present 9 months male case having dismorphic features. The patient's mother and father were not consanguineous. Cytogenetic analysis was normal. The patient had an anal atresia, low and simple ears, ear tag, simian line and short palpebral fissure. In addition to these findings, he has also a congenital hypopigmentation of the skin, hair and eye, indicating albinism. This finding was seen in his mother diagnosed as albinism. This is the first case having coexistence of TBS and albinism in the literature. It is not clear whether this syndrome is associated with the other or independent event.

J02.39

The wide phenotypic variability of Tricho-rino-phalangeal syndrome type I - a five cases study

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Tricho-rino-phalangeal syndrome (TRFS) is a rare autosomal dominant syndrome, characterized by short stature, typical facial dysmorphism and skeletal abnormalities.
We present 5 cases of TRFS in order to illustrate this rare disorder, to present particularities and long term follow-up and to discuss management and genetic counseling.
Case 1: growth retardation, dysmorphic face (sparse/fine/depigmented hair, medial flare of eyebrows, bulbous nasal tip, hypoplastic nostrils, thin lips, microretrognathia), brachydactyly. Associated anomalies: scaphocephaly, hemangioma, cryptorchidism and right inguinal hernia.
Case 2: growth retardation, dysmorphic face (sparse/fine hair, lateral thinning of eyebrows, bulbous nose, hypoplastic nostrils, long/deeply grooved philtrum, thin lips), broad thumb, preaxial polydactyly (foot). Associated anomalies: umbilical hernia.
Case 3: short stature, dysmorphic face (sparse/fine/depigmented hair, lateral thinning of eyebrows, bulbous nose, long philtrum, thin lips, microdontia, micrognathia), thick nails. Hand X-ray: metacarpal shortening, cone-shaped epiphyses (middle/distal phalanges), delayed bone age. Associated anomalies: empty sella, posterior fossa arachnoid cyst (head CT), hypothyroidism.
Case 4: growth retardation, dysmorphic face (sparse/fine/depigmented hair, hypoplastic nostrils, long philtrum, high palate, abnormal tooth position, retrognathia), brachydactyly, kyphosis, scapulae alatae, mild mental retardation. Hand and forearm X-ray: slightly curved radius, clinodactyly.
Case 5: low weight, dysmorphic face (sparse scalp hair, abnormal columella, long/deeply grooved philtrum, thin lips, micrognathia, large ears), mild hypotonia. Associated anomalies: trigonocephaly, left inguinal hernia.
In conclusion, we present five cases of TRFS, in order to illustrate this rare genetic disorder, to discuss phenotypic variability and particularities found in our patients, as well as long term follow-up, management and genetic counseling.

J02.40

Karyotype and treatment response correlations in Turner Syndrome

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Aim: To assess treatment response to growth hormone (GH) and estrogenic therapy in girls with Turner syndrome (TS) and find possible correlations with karyotype. **Methods:** Nine girls diagnosed with TS that received treatment with GH for at least 2 years. We evaluated differences in height standard deviation score (HSDS), at baseline, at one and two years from baseline; and pubertal stage development in patients with or without estrogen therapy. **Results:** Median age was 12.1+/-3.7 years. Chromosomal analysis revealed six girls (66%) with pure 45X monosomy, while 3 (33%) had mosaic form. The patients had a baseline mean HSDS=-3.29±0.65. After 2 years of GH treatment only one patient achieved HSDS>-2 value for normal girls. Mean difference in HSDS after 1 year was 0.27+/-0.31; after 2 years it was -0.17+/-0.22 ($p=0.047$). Regarding estrogen replacement therapy: two patients from study did not reach puberty onset age; four of them had spontaneous puberty without treatment; two girls responded well to estrogen therapy, while one did not. We found weak correlations between pure monosomy and the necessity for estrogen therapy ($r=0.5$;$p=0.17$) and between pure monosomy and mean HSDS difference after 2 years with GH ($r=-0.35$; $p=0.34$). **Conclusions:** Results showed great variability in treatment response in girls with TS, in both GH and estrogen treatment, which could not be strongly correlated with karyotype. Larger studies are needed to assess these important correlations that could guide therapy for these patients in the future.

J02.41

Primary polytopic developmental anomalies - a particular form of VACTERL association?

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Background: VACTERL association occurs sporadic (16 cases/100,000 live births), more common in males. Low recurrence risk and heterogeneous causality are characteristic. The presence of at least three congenital malformations (Vertebral defects, Anal atresia, Cardiac anomalies, Tracheoesophageal fistula, Esophageal atresia, Renal anomalies, Limb defects) would specify diagnosis. Limb anomalies restricted to upper ones and cardiac septal defects are common. Intrauterine growth retardation and difficult weight gaining are noticed. Neurocognitive impairment is uncharacteristic. The management includes surgical correction of life-threatening abnormalities and long-term follow-up of their sequelae. Material and methods: We present a 5 months old male infant with multiple congenital anomalies (absence of right forearm, atrial septal defect, esophageal atresia, proximal tracheoesophageal fistula) admitted for pneumonia. He is the product of a full-term pregnancy complicated with hydramnios and threatened miscarriage. Birth weight was 2,600 g. Primary defects in family members and exposure to environmental factors were denied. Full assessment (history, clinical examination, biological and imagistic tests, neurological, cardiovascular and genetic evaluation) was done. Results: Productive cough, stridor, intermittent expiratory wheezing and mild weight deficit were noticed. Psychomotor acquisitions were age-appropriate. Barium swallow radiograph diagnosed gastroesphageal reflux and ruled out esophageal stricture. Child's complex pathology and mother's depressive disorder altered quality of family life. Conclusions: VACTERL association is probable in this case. Amniotic band syndrome with congenital amputation of the right forearm has been considered. Efficient orthopedic backing, genetic and family counseling, physical and occupational therapy are needed long term. Multiple and prolonged hospitalizations for recurrent pneumonia may worsen the prognosis.

J02.42

The evaluation of stress in 40 molecularly-confirmed patients with Williams-Beuren syndrome

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Psychological studies in Williams-Beuren syndrome (WBS) describe varying degrees of mental retardation, weakness in visuospatial/executive function, overfriendliness, empathy, a fluent speech and hyperacusis. They also show stereotyped behaviors, aggressiveness and some psychiatric disorders. These characteristics may increase the vulnerability of these patients to stress. The objective of this study is to determine the real extent of the stress in individuals with WBS and to identify the major events in their lives that might modulate a stress reaction.
A standard questionnaire based on DSM-IV-TR, the Brazilian version of intelligence measurement scales (WISCIII/WAIS-III) and objective stress scales (Lipp's Children Stress Scale or Lipp's Adult Stress Scale) were applied to 40 individuals with WBS. The stress scales were also applied to 40 normal individuals.
The major events related to stress reactions in the patients with WBS were excessive noise (60%), discrimination (58%) and excessive homework (35%). The average IQ in WBS was 68.5(SD: 8.89). Patients with WBS presented statistically significant ($p<0.001$) higher levels of stress (mean: 39.5) when compared to controls (mean: 24). No difference in subgroups of WBS patients stratified by gender (p: 0.74), level of IQ (p: 0.935) or whether they attended special education (p: 0.14) was observed.
Patients with WBS are at risk for stress. Hyperacusis was the most common stressor and, then, should be properly addressed in an attempt to improve the quality of life of patients with WBS.

J02.43

Meningomyelocele in the offspring of a patient with Waardenburg type 1 syndrome: a genetic counselling dilemna

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We describe two cases of Waardenburg syndrome type 1, one being diagnosed in a 5 y-old male with moderate mixed deafness, *dystopia canthorum*, causing subtle skin pigmentary changes of upper limbs and the other one in the unrelated 31 y-old husband of a 29 y-old female after termination of a 22 weeks pregnancy for lumb sacral meningomyelocoele and Arnold-Chiari malformation diagnosed by serial ultrasound screening. Family history of the genitor was unremarkable, except for a recent diagnosis of moderate deafness, in the context of familial premature graying of hair before 25 years of age (mother, sister, brother). On clinical examination, he has *dys-*

topia canthorum, suggesting a diagnosis of autosomal dominant Waardenburg syndrome type1.Neural tube defects are thought to occur sporadically as the consequence of multifactorial inheritance. Based on this assumption, a low recurrence risk is usually given (<3%), and folic acid supplementation (4mg/d) in the periconceptional period recommended in the future pregnant woman. Rare occurrences of NTD due to mendelian disorders have been described. They include, among others, *MTHFR* homozygous mutations, and Meckel syndrome with respect to autosomal recessive inheritance, *Van Gogh* and *PAX3* mutations when considering autosomal dominant inheritance. If the diagnosis of Waardenburg type 1 syndrome is eventually established in the genitor of the malformed fetus, a recurrence risk of 10% (empirical value) has to be taken into consideration, three fold-higher than the *a priori* 3% risk in the present case. We recommend to be aware of premature graying, dystopia canthorum and/or deafness when counseling for NTD.

J02.44

Novel synonymic transthyretin gene mutation N98N in cardiomyopathy patient from St. Petersburg, Russia

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Over the past several years we have been searching transthyretin (TTR) gene mutations in patients with cardiomyopathies from St. Petersburg (Russia). In our previous work TTR gene mutations H90N, V30M and deletion (del9) were found in patients with restrictive amyloid cardiomyopathy. In the present investigation new TTR gene mutation was identified in patient with hypertrophic cardiomyopathy without amyloidosis. Screening TTR gene for mutations was provided with SSCP-analysis followed by sequencing. Nucleotide substitution in position 6819 C>T according to NCBI reference sequence NC_000018.9 (c.354C>T according to NCBI reference sequence NM_00371.3) was found. This mutation leads to TTR codon substitution in the 98 position (from AAC to AAT, p.N98N (p.N118N according to the mRNA sequence)) in the 4-th exon of TTR gene which doesn‘t lead to the aminoacid substitution in the TTR polypeptide sequence. The N98N mutation was detected in heterozygous state. The mutation revealed in this study was not previously identified in other populations and was not previously described in literature and databases. The causal relationship of this mutation with the disease is an object for further discussion.

J02.45

Is there Influence of the Genetic Variations Associated with Thrombophilia on Sports Success?

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Regular physical activity reduces risk of thrombosis development. However cases of thrombosis in different sports are described. There is unresolved question, what influence of the genetic variations associated with thrombophilia on sports success.

The study was approved by Ethics Committee of the Ural State University of Physical Culture (USUPC). All participants gave written informed consent to genotyping. Junior group was consisted of 245 persons, who participated in sport competition and training on regular basis. Sportsmen group was consisted of 300 athletes from different sports (sub-elite level - 47%, elite level - 53%). Healthy sedentary control group was consisted of 255 students, employees of the USUPC. All participants were unrelated Caucasians living in Ural region of Russia. DNA was isolated from buccal epithelium. Genotyping was done using a TaqMan® SNP Genotyping Assays by use StepOne™ Real-Time PCR System (AppliedBiosystems, USA). The genotyping results were analyzed by using TaqMan® Genotyper Software (AppliedBiosystems).

Frequencies of heterozygote carriers of Leiden and prothrombine mutations in control group were identical - 2,4 %. Frequency of T/T genotype of C677T variation of *MTHFR* gene in control group was 9,8 %. Frequencies of studied sequence variations at juniors and athletes either didn‘t differ, or were a little above, than in control. Apparently, variations rs1799963 in *F2* gene, rs6025 in *F5* gene, rs1801133 in *MTHFR* gene, associated with thrombophilia, don‘t render strong negative influence on sports success. Possibly, prothrombotic action of the investigated variations is compensated by adaptive changes of a hemostasis as response on aerobic trainings.

J02.46

Left Dominant Arrhythmogenic Cardiomiopathy caused by a novel nosense mutation.

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INTRODUCTION

Some mutations in the desmoplakin gene generate an autosomal dominant inheritance pattern related to the involvement of the left ventricle (LV) in ARVC.

METHODS

It has made the study of 28 patients (14 women and 14 males), belonging to 3 families affected by ARVC. This cohort was obtained from a screening of 64 ARVC patients. The analysis of the 28 individuals was performed by sequencing of exons and flanking intronic regions for the DSP gene.

RESULTS AND CONCLUSIONS

We found a gene variant (Q447X) that is a heterozygous nonsense type not previously described. Is a C to T transition that generates a stop codon resulting in a peptide 85% smaller than the wild type and an autosomal dominant inheritance pattern with high penetrance (91%). In most cases, stop codon mutations are disease cause.

Eleven of the 28 studied patients were mutation carriers and ten of them were affected by ARVC. The rest were healthy patients. The eleven carrier patients consisted of eight women and three men.

The amino acid 447 is located in one of the globular head domains of desmoplakin to participate in the binding of this protein with plakoglobin and plakophilin. It is noteworthy that several mutations in this gene have been associated with the development of arrhythmogenic left ventricular dominance and even isolated involvement of the ventricle and simulating idiopathic dilated cardiomyopathy. We conclude this gene variant Q447X could be possibly the cause of ARVC with predominant LV.

J02.47

R14Del, a Dutch phospholamban mutation in a spanish family. Genotype-phenotype aspects.

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INTRODUCTION

Through genetic screening of dilated cardiomyopathy patients, we identified a previously described deletion of arginine 14 (PLN-R14Del).

METHODS

Nine individuals were evaluated using dHPLC and bidirectional sequencing of the exon and intron regions flanking the PLN gene.

RESULTS

Seven of the nine patients studied were mutation carriers although only two of them met diagnostic criteria of dilated cardiomyopathy: the proband and her asymptomatic maternal grandmother. Five carriers´s ECG showed strikingly low voltage QRS complex, despite no echocardiographic abnormalities in 3 (mother and 2 maternal aunts). Apart from proband all carriers were asymptomatic with no history of arrhythmia evidenced. Proband´s father belongs to another family affected by Hypertrophic cardiomyopathy, although the father himself only express mild left ventricular hypertrophy with normal ECG.

R14Del mutation was described in 40 families to date. There is information available from 68 carriers. This mutation was identified as a mutation with founder effect in up to 14% of cases in a cohort of Dutch patients with dilated and arrhythmogenic cardiomyopathy. Carriers from this cohort characterized by low voltage QRS and negative T waves in lateral leads similar to that evidenced in carriers of some desmosomal mutations.

CONCLUSION

Low voltage ECG has a high sensitivity to identify PLN mutation carriers. Echocardiographic phenotype can be mild or normal in most carriers of R14Del mutation who can remain asymptomatic throughout life. Severe phenotype can be consequence of double mutations. Interpretation of genotype-phenotype correlations should be done in the context of large family trees and complete cardiac evaluation.

J02.48

Age-related penetrance in genetic carriers of hypertrophic cardiomyopathy

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Introduction and Purpose: The aim is study the age-related penetrance of HCM in patients with different MYBPC3, MYH7 and TNNT2 mutations to determinate whether the age at diagnostic depends on genetic background.
Methods: We included 195 HCM causal mutation carriers (55% males, age 40±16 years); 64.8% had clinical manifestations of the disease. All patients were diagnosed in inheritance cardiomyopathy consultation, in a reference hospital. 146 patients were carriers of at least one mutation in MYBPC3 (IVS23+1G>A (72), Arg891fs (37), A107fsX116 (26), A216T (11), V896M (4)), 21 were carriers of a mutation in MYH7 (T1377M (21), D928N (4), E1348Q (8), E1356Q (4), R1382Q (4)) and 8 patients were carriers of R278C in TNNT2. IVS23+1G>A, the most prevalent mutation, was present in 18 unrelated families. We performed time-to-diagnosis analysis according to the affected gene and the most prevalent mutations.
Results: No differences in time to diagnosis were detected between the most prevalent mutations. Median age at diagnosis was 46±2 years old for IVS23+1G>A, 44±3 years old (Arg891fs), 43±2 years old (A107fsX116), 44±7 years old (T1377M) and 51±9 years old (A216T); log rank $p=0.963$. Similarly, there were no differences according to the 3 analyzed genes (log rank $p=0.935$). Median age at diagnosis for the whole was 47±2 yrs. Conclusions: Mutations in MYBPC3 encoding myosin binding protein C could be considered more benign form of HCM than initially was considered. Now, genetic diagnosis reveals that HCM-phenotype can appear later in life, reaching near full penetrance in the elderly.

J02.49

Imprinting defect in patients with Albright's Hereditary Osteodystrophy and platelet Gs hypofunction

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Pseudohypoparathyroidism (PHP) indicates a group of heterogeneous disorders whose common feature is represented by impaired signaling of hormones that activate Gsalpha, encoded by the imprinted GNAS gene. PHP-Ib patients have isolated PTH resistance and GNAS epigenetic defects while PHP-Ia cases present with hormone resistance and characteristic features jointly termed as Albright's Hereditary Osteodystrophy (AHO) due to maternally inherited GNAS mutations or similar epigenetic defects as found for PHP-Ib. Pseudopseudohypoparathyroidism (PPHP) patients with an AHO phenotype and no hormone resistance and progressive osseous heteroplasia (POH) cases have inactivating paternally inherited GNAS mutations.
We here describe 16 PPHP subjects and 1 POH patient with platelet Gs hypofunction but lacking Gsalpha mutations. The methylation for the three differentially methylated GNAS regions was quantified via Sequenom EpiTYPER. Patients showed significant hypermethylation of the XL amplicon compared to controls (36±3 vs. 29±3%; $p<0.001$); a pattern that is reversed to XL hypomethylation found in PHPIb. Methylation for NESP and ExonA/B was significantly different for some but not all patients, though most patients have site-specific CpG methylation abnormalities in these amplicons. Since some AHO features are present in other imprinting disorders, the methylation of IGF2, H19, SNURF and GRB10 was quantified. Surprisingly, significant IGF2 hypermethylation (20±10 vs. 14±7%; $p<0.05$) and SNURF hypomethylation (23±6 vs. 32±6%; $p<0.001$) was found in patients vs. controls, while H19 and GRB10 methylation was normal.
In conclusion, this is the first report of epigenetic defects in PPHP and POH though additional studies are needed to correlate epigenotype with the clinical phenotype.

J02.50

Contiguous Gene Deletion of ERCC8 and NDUFAF2; Case Report

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Here we describe a patient with clinical manifestation of Leigh's disease including persistent lactic acidosis and chronic encephalopathy. In addition, there was associated dysmorphic facial features and abnormal brain structure. Molecular karyotyping detected a homozygous deletion of 11 oligonuleotide probes at 5q12.1, spanning approximately 248 kilobases. The deleted region contains two known genes, ERCC8 (OMIM # 609412) and NDUFA2 (OMIM # 609653). Mutations of ERCC8 are associated with Cockayne syndrome, and mutations in NDUFA2 are associated with mitochondrial complex I deficiency. Both of the parents were confirmed to be heterozygous for the same deletion. We believe that this chromosomal deletion contributed to the complex phenotype on this patient. Ultraviolet light toxicity assay and mitochondrial study are still in progress.

J02.51

An Egyptian patient with cholestasis lymphoedema syndrome (Aagenaes syndrome)

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Lymphoedema cholestasis syndrome (Aagenaes syndrome) is a rare autosomal recessive disease consisting of hereditary, recurrent cholestatic liver disease and generalized lymphoedema from birth or childhood. The disease was first described by Aagenaes et al. in 1968 in Norway. Since then, most patients reported are originally from the same part of Norway. Fewer than 40 cases have been described elsewhere. To the best of our knowledge, none was described in Arab countries. Here, we describe the clinical and laboratory characteristics of the first Egyptian patient with Aagenaes syndrome.
He is a 3.5 year old boy, the second in birth order of first cousin marriage after uncomplicated pregnancy. He had an older brother who developed jaundice soon after birth and died at the age of 35 days without any available investigation. Our patient has severe form of the disease with progressive cirrhosis and relatively low GGT and cholesterol levels. He also developed progressive arthritis, a feature which was not described before in this syndrome. Although molecular analysis was not done yet, we suggest that our patient could have a different severe form of the disease associated with arthritis that has a different locus than LCS1 similar to the Serbian Romani patient described by Frühwirth et al., 2003.

J02.52

An atypical case of Langer-Giedion-syndrome: the role of additional chromosomal abnormalities

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Langer-Giedion syndrome (LGS), is defined as a contiguous gene disorder caused by the loss of functional copies of TRPS1 and EXT1 genes usually secondary to 8q microdeletion.
This condition combines features of trichorhinophalangeal syndrome type I (sparse scalp hair, bushy eyebrows, bulbous nose, long philtrum, cone shaped epiphyses, short stature), multiple exostosis, mild to moderate mental deficiency (MD).
We report a case of a 4-year-old girl presenting with facial dysmorphisms and skeletal abnormalities, short stature, congenital heart disease (CHD), central nervous system (SNC) anomalies, severe MD. A diagnosis of LGS was suspected.
The HR karyotype showed a reciprocal balanced translocation between: 2p24 and 11p15 chromosomes. The parental high resolution karyotype was normal. Array-CGH analysis revealed an interstitial deletion involving chromosome: 8q23.3-q24.11.
Our patient shares with LGS: microcephaly, sparse hair, dysmorphic facial features, growth retardation, multiple exostosis.
The presence of CHD, CNS anomalies (hypoplasia/agenesis of corpus callosum (CCA), pituitary gland dysmoprhism), severe MD has never been described in patients with LGS.
The Array-CGH confirmed clinical diagnosis. Up to date, this is the smallest deletion causing LGS.
The patient also showed a balanced chromosomal translocation involving 2p24 region where maps ASXL2 gene. Recently, a patient carrying a balanced translocation involving the same 2p24 region has been described showing CCA and MD.
We speculate that ASXL2 gene disruption might be responsible for the more severe neurological phenotype described in the current patient.

J02.53

An Italian case of X linked reticulate pigmentary disorder with systemic manifestations (XLPDR)

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The X- linked reticulate pigmentary disorder with systemic manifestations (XLPDR) is a rare skin disorder affecting males and mildly females, and it's characterized by a hyperpigmentation disorder of the skin and several visceral problem. This disorder in females shows brown pigmentation of the skin which follows the lines of Blaschko, while it is appears as reticulate sheets in males. Males also suffers of systemic manifestations as severe gastrointestinal disorders in infancy with failure to thrive, corneal dystrophy with severe photophobia, chronic respiratory disease and, in the most sever cases, early death No causative mutation has been identify yet.

We report a case of a 3-year-old boy with clinical features that are consistent with the diagnosis of XLPDR match the one typically described, characterized by brown diffuse and reticulate hyperpigmentation of the skin reflux, hypohidrosis ,

growth retardation, electrolyte imbalance, dry skin, unruly hair, eyebrow flare, digital clubbing, photophobia due to corneal diskeratosis. Retinitis pigmentosa, lymphoedema and failure to thrive. The boy has been suffering also of recurrent

pneumonia and severe colitis and diarrhea The family history shows that the mother of the boy is affected by incontinentia pigmenti and the boy's sister is apparently unaffected, none of the other member of the family show symptoms of the disease. So far this case is the 6th case of XLPDR described in literature.

J02.54

Lymphoma in a patient with Noonan syndrome

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Noonan syndrome (NS) is a well recognizable syndrome characterized by short stature, facial dysmorphism, heart disease and cryptorchidism. Gain of function mutations in the PTPN11 oncogene are identified in half of the patients. Apart from the T73I mutation, there is no clear relationship between PTPN11 mutations and increased cancer risk. GH treatment has been proved to improve growth in these patients

We report on a 30 year old man who had been diagnosed in infancy with Dubowitz syndrome and due to short stature, had received treatment with growth hormone. On examination, he has typical facial features of NS, short stature, normal intelligence and has recently developed a lymphoma with good response to chemotherapy. His 2 year old son has clinical features consistent with NS (short stature, typical facial features, cryptorchidism and normal development). Both are heterozygotes for the E139D (c.417C>G) mutation in exon 4 of the PTPN11 gene.

The E139D mutation is not known to increase cancer risk in NS patients, but has been previously reported in two patients with NS who developed a cerebral glioma. Both our patients have had normal cerebral MRIs.

This is the first report of this mutation related to a lymphoma in a NS patient. It is difficult to assess what the relationship between the E139D mutation and the GH treatment have with the development of the lymphoma or if this is a casual finding. With current evidence, the risk of treating our proband with GH seems to outweigh its benefits.

J02.55

Characterization of a 8q21.11 microdeletion syndrome associated with intellectual disability and a recognizable phenotype.

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We report eight unrelated individuals with intellectual disability and overlapping submicroscopic deletions of 8q21.11 (0.66-13.55 Mb in size). The deletion was familial in one and simplex in seven individuals. The phenotype was remarkably similar and consisted of a round face with full cheeks, high forehead, ptosis, cornea opacities, underdeveloped alae, short philtrum, cupid's bow of the upper lip, down-turned corners of the mouth, micrognathia, low-set and prominent ears, and mild finger and toe anomalies (camptodactyly; syndactyly; broadening of first rays). Intellectual disability, hypotonia, decreased balance, sensorineural hearing loss, and unusual behavior were frequently observed. High resolution oligonucleotide array showed different proximal and distal breakpoints in all of them. Sequencing studies in three of the individuals revealed that proximal and distal breakpoints were located in unique sequences with no apparent homology. The smallest region of overlap was a 539.7 kb interval encompassing three genes: a *Zinc Finger Homeobox 4* (*ZFHX4*), one micro RNA of unknown function and one non-functional pseudogen. *ZFHX4* encodes a transcription factor expressed in adult human brain, skeletal muscle and liver. It has been suggested to be a candidate gene for congenital bilateral isolated ptosis. Our results suggest that the 8q21.11 submicroscopic deletion represents a clinically recognizable entity and that a haploinsufficient gene or genes within the minimal deletion region could underlie this syndrome.

J03. Cytogenetics

J03.01

Characterization and isolation of a beta neurotoxin- Bt from scorpion venom

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Scorpion venoms are a particularly rich source of neurotoxic proteins/peptides that interact in a highly specific fashion with discrete subtypes of ion channels in excitable and non-excitable cells. Scorpion venom has historically been one of the richest sources of polypeptide toxins with unusually high degrees of specific actions on and interactions with the ion-channel membrane proteins of both excitable and non-excitable cells. In this study, we report the characterization and isolation of a beta neurotoxin- Bt, from the venom glands of scorpion Androctonus crasicauda venom Kuzestan (Iran). Scorpions were collected from the Khuzestan province and transported to the reference laboratory of the Razi Institute . cDNA was synthesized with extracted total RNA as template and modified oligo(dT) as primer. Using RT-PCR techniques, a 281 bp cDNA fragment encoding a beta neurotoxin active on mammals or on insects have been isolated from the telsons of scorpion Androctonus australis. All cDNA sequences displayed one major open reading frame of about 310 nucleotides. The deduced precursor open-reading frame is composed of 59 amino acid residues that consist of a signal peptide of approximately 22 amino acid residues. This polypeptide has a molecular mass of 9324.86 kDa with an Isoelecric point of 4.96 which is closely packed by three disulfide bridges. Amino acid alignment and secondary structure prediction revealed that the peptide deduced from cloned cDNA is a functional homolog of potassium channel-blocking neurotoxins from the venoms of other scorpions.

Keywords: *Adnroctonus crasicauda*, beta toxin, scorpion

J03.02

Chromosome abnormalities in children

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For the purpose of studying of frequency of chromosomal abnormalities cytogenetic research of lymphocytes of peripheral blood was carried out of 1546 children.

The results of research study had found out in the 212 (13,7%) cases. Aneuploydia was found in the 186 cases (87,8 %) and structural abnormalities

were revealed in the 26 cases (12,2 %).
Among aneuploydia were detected Down‘s syndrome in the 117 (63 %), Turner‘s syndrome in the 25 (13,4 %), Klinefelter‘s syndrome in the 23 (12,4 %), Edwards‘s syndrome in the 9 (4,8 %), an androgen insensitivity syndrome (AIS) in the 5 (2,7 %), 47, in the 4 (2,2 %), the Patau‘s syndrome in the 1 (0,5 %), 47, in the 1 (0,5 %), the 69, in the 1 (0,5 %) cases.
Were carried out following abnormalities: del(5)(p15.1), 46,XY,del(2)(p15p21), 46„add(7)(q31), 47„add(X)(q27) 46„t(6;13)(q26;q13), 46„t(3;13)(q23;q32), 46,XX,t(14;18)(q13;q23), 46,XX,t(10;16)(q23;q11.2), inv(9)(p11q13), 46,XX,t(8;20)(q22;p18.1), 46,XX,t(9;12)(q34;q24.1), inv(3)(q23p26), 46„t(10;12)(q23;q15), 45„der(13;14)(q10;q10), 45„der(14;15)(q10;q10), 46,X,i(X)(q10), 46,XX,t(7;9)(q11;q31), 46,XX,t(7;13)(q11.2;p11.1), 46„t (X;5)(q23;p15.2), 46„t(X;1)(q27;p22) among 26 structural chromosomal abnormalities.

J03.03

Human Infertility: the importance of cytogenetic analysis

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Infertility is a failure to conceive after at least one year of unprotected intercourse. It has been estimated that approximately 15% of the population in industrially developed countries are affected. Reproductive difficulties are associated intimately with cytogenetic abnormalities that could be structural aberrations such as translocations, inversions and supernumerary chromosome (#); or, constitutional aneuploidies such Klinefelter syndrome, 47, XYY, Turner syndrome and 47, XXX.
The authors present two cases (two couples) that have been followed in infertility consultation. Cytogenetic study and semen analysis were required. The technical procedures (blood culture, GTL and CTL banding, FISH assay) and cytogenetic analysis was performed according standards protocols and guidelines. Semen analysis was done according to World Health Organization Laboratory Manual (5th Edition-2010). In the first case we found a normal female karyotype (46, XX) and a male karyotype with a reciprocal translocation involving the short arm of #4 and the long arm of #22 [46, XY, t(4;22)(p16.1;q11)]; in the semen analysis a low spermatozoa concentration was detected and concerning morphology a border line value was found (4%). In the second case the female karyotype presented a paracentric inversion of #14 [46, XX, inv(14) (q13q24.3)] and the male has a normal karyotype (46, XY) with a normal semen analysis.
This study strongly point out the importance of cytogenetic analysis of infertile couples to allow an appropriate genetic counseling.

J03.04

Clinical features and a de novo t(1;12)(p22;p12-13) in a 22-year-old man with myotonic distrophy

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We report on a 22-year-old patient presenting with attached ear lobule, thight skin, long height, short phalanges, gynecomasty, mucsle weakness, short philtrum, long neck, webbed neck. The clinic features are overlapped with myotonic distrophy. Standard cytogenetic analysis showed a de novo translocation, 46,XY,t(1;12)(p22;p12-13) karyotype. Three hypotheses have been postulated to explain such phenotype abnormalities, including a break in a gene, a positional effect and a cryptic deletion or duplication. According to the literatüre, coexistence of t(1;12)(p22;p12-13) karyotype with myotonic distrophy features has not been reported. This is the first case presenting both features and karyotype. As for myotonic distrophy, there are two types in adult onset. This identity may not be associated with the present karyotype. Therefore, it will be clarified of these two findings. This case places further emphasis on the importance of cytogenetics analysis in the study of de novo translocations with this abnormal phenotype. In conclusion, fine mapping and cloning of cytogenetically translocation chromosome breakpoints in patients with multiple malformations are a promising strategy for the isolation of new genes that may be related with myotonic distrophy. Now we have foced on the patient for the fonctional study.

J03.05

Partial trisomy 16q and pericentric inversion of chromosome 9 in a young patient with a complex phenotype

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Here we report a duplication of chromosome 16 at q11.2 to q21 and the pericentric inversion of chromosome 9 at p11 to q13 identified by routine karyotyping in a one month old male patient. Pure duplications of 16q have only been reported in a small number of individuals. Partial trisomy for the long arm of chromosome16 is a rare condition,uncommonly identified in children and adults. Cytogenetic aberrations on chromosome 9 have been reported to be one of the most frequent abnormalities. The pericentric inversion of chromosome 9 inv(9)(p11q13) is one of the most common balanced structural chromosomal aberrations found in 1 to 3% of the general population.
Physical examination of the ours patient revealed mild dysmorphic features including low set ears with slightly redundant/extracrease of the helix bilaterally, micrognathia, deep creases in the sole soft he feet and mild bilateral 5th finger clinodactyly. Conventional cytogenetics (G banding) revealed a male karyotype with an apparent duplication in the long arm of chromosome16 and the pericentric inversion of chromosome 9 [46,XY,dup(16)(q11.2q21), inv(9)(p11q13)] in all the 30 metaphase plates screened.

J03.06

A case of inverted duplication deletion of the short arm chromosome 5 in an infant girl without "cri du chat"

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Proband is the first child of healthy unrelated parents. During pregnancy there were no complications, and the dates of prenatal screening were normal. Her birth weight was 3310g, length - 53 cm, occipitofrontal circumference - 34 cm. She did not have unusual cry. At the age of 1 year 3 months her motor development was slow: she could poise her head, but she couldn't sit or crawl. According to physical examination she had muscular hypotonia, dolyhocephaly, enlargement of fontanelle (3,5x4 cm), her weight was 8200g (<3cd), length - 74cm (<3cd). She showed minor dysmorphic features including: prominent forehead, preauricular pigs, epichantus folds, strabismus, coloboma of right iris, wide nasal bridge, long filtrum, carinate deformation of chest, thin skin, venous rete at the chest. Abdominal, transfontanellar and cardiac ultrasounds revealed right pyelectasis, enlargement of right corns of lateral ventriculuses of brain, patent ductus arteriosus, ventricular septum defect and ectopic of mitral valve cords.
The standard cytogenetic analyses revealed the additional chromosomal material on the short arm of chromosome 5. Parental karyotypes were normal. The FISH-analyses with the subtelomere probes for short and long arms of chromosome 5 (TelVision 5p, TelVision 5q, Vysis Abbott) revealed absence of signal on the p-arm with additional chromosomal material. M-FISH (24xCyte, MetaSystems) analyses revealed that additional material was due to chromosome 5. Consequently the karyotype of patient was interpretaited as inverted duplication with loss of subtelomeric region: 46,XX,der(5)del(5)(p15.3)dup(5)(p15.3p14)dn. This case confirms assumption that inverted duplications deletions are not very rare forms of chromosomal rearrangements.

J03.07

Fragile sites in recurrent pregnancy loss

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Introduction: Early pregnancy loss in the first trimester is the most common complication affecting at least 15 - 20% of the all clinically recognized pregnancy. Chromosomal structural abnormality in either parent is an important cause of recurrent pregnancy loss but increased spontaneous chromosome instability was reported in couples with recurrent spontaneous abortions but without constitutional chromosome aberrations
Objective: In this report we present the cytogenetic findings of the expression of fragile sites in couples with two or more spontaneous abortions.
Material and methods: We studied 636 couples with recurrent spontaneous abortions (>3) referred to Maternal - Fetal Medicine and Assisted Reproduction of Life memorial Hospital. No subject presented with obvious phenotype of chromosomal rearrangements.
Cytogenetic investigations were carried out from peripheral blood lymphocytes using standard techniques. The routine analyses was performed on G banded chromosomal preparations. Karyotypes of the fetuses were not studied.
Results: Autosomal fragile sites were found in 8 cases (0,62%). The fragile site was not typically folate-sensitive, being expressed in standard medium.
Conclusion

The role of fragile sites in causing abortion is still very difficult to asses. Fragile sites may possibly predispose to chromosome breakage and rearrangements in meiosis and consequent infertility

J03.08
Heterochromatin variants of human chromosome 9 and the reproduction failure

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Pericentric inversion of chromosome 9 - inv(9) - is considered to be clinically insignificant heterochromatin variant of human karyotype. However, various author repeatedly mention possible association of inv(9) and selected pathologies, especially with reproduction failure. This can cause some consultation dilemma, especially when inv(9) is identified in potential gamete donor.

Some authors also suggest the same role for other variants of the heterochromatin region of the human chromosome 9 (like 9qh+ or 9qh) as well.

Using the data from our cytogenetic laboratory - we analyzed the clinical indications among 383 patients with heterochromatin variant of chromosome 9 and we have found the reproduction failure to be the most common diagnose (more then 43 %). That was far more, then was the incidence of reproduction failure in our control group of patients with normal karyotype. This difference was also statistically significant.

We have confirmed heterochromatin variants of chromosome 9 as relatively common finding, this time in population in the Czech Republic. The clinical significance, however, remains subject of discussion. Possible association of heterochromatin variants of human chromosome 9 with reproduction failure had quite low statistical significance and will require further investigation.

J03.09
Cytogenetic abnormalities detected in patients with non-obstructive azoospermia and severe oligozoospermia inNorth-West of Iran

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Introduction-Chromosomal anomalies have been postulated to be as one of the principal genetic factors in male infertility. find the frequency and types of major chromosomal abnormalities with nonobstructive azoospermia and severe oligozoospermia in men who were referred because of primary infertilityto give appropriate genetic counseling before assisted reproduction techniques in north-west of Iran, and investigate the general characteristics in this infertile male population,was objective of study.

Material & methods-A total of 50 infertile males (35 were azoospermic, 15severe oligospermic) were studied for the cytogenetic evaluation prior to use of assisted reproduction techniques. Also, 60 fertile males as a control group were studied. Karyotyping was performed on peripheral blood lymphocytes according to the standard methods.

Results -The total prevalence of chromosomal abnormalities was found to be 16% (8/50), includingfive patients (10%) were 47,XXY;one patient (2%)46 ,XY/47,XXY;one patient (2%) 46,XY/45,XO. All of them were azoospermic males , and one patient (2%) 46,XY,del(Y)(q11.2) that was Oligozoospermic, And in control males except one case that was 46,XYY and fertile man,all control males had no chromosomal abnormalities.

Conclusions-Comparison of our results with the review of the literature shows a relatively similar incidence of chromosomal anomalies in infertile men.The occurrence of chromosomal abnormalities among infertile males strongly suggests the need for routine genetic testing and counseling prior to the employment of assisted reproduction techniques.

J03.10
Cytogenetic biomonitoring of general population of FB&H using micronucleus assay test

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The aim of this study is to determine values of Micronucleus Assay from peripheral blood lymphocytes of 200 healthy examinees both gender from general population of Federation of Bosnia and Herzegovina. We have determine micronucleus frequency and number of cells with micronucleus for each examinee, average (median) frequency of MN for each group of examinees divided into groups by the age (20-30y; 30-40y; 40-50y; 50-60y; 60-70y), as well as the gender (20M: 20F) and their smoking habits. 200 was the total number of analyzed examinees.

For each sample were analyzed 1000 binuclear lymphocytes and determined total number of MN, cells with MN as well as their distribution (number in cells). MN was determined according to proposed HUMN criteria.

Statistical analyze revealed following conclusions:

1. There is significant difference among number of MN found among genders ($p<0,05$).
2. There is highly significant difference in frequency number of MN among different age groups ($p<0,001$).
3. There is significant difference in frequency of 2 micronucleus and smoking habits of examinees while that difference is not present for 1 micronucleus frequency.

The results of this study considering age, gender and smoking habits are in accordance with results determined for general population of healthy people in other cytogenetic laboratories else in the world.

J03.11
Multiples and different chromosome aberrations in a healthy female patient

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It is widely known that the presence of a chromosome alteration in a patient has usually associated different congenital abnormalities, nevertheless the vertical transmission of a chromosomopathy has also been described to be associated with no phenotypic effects, defined as "variants".

In fact, once a chromosome alteration is found in a patient, such a chromosomopathy is present in all cells, unless it is a mosaicism (with two or three different cell lines) or it is a chromosomal instability syndrome, such as Nijmegen syndrome.

Here we present a very atypical case where multiples and different chromosome alteration were diagnosed in a peripheral blood karyotype of a 32 years old healthy female patient, who wanted to have a baby. As an antecedent she refers to have had a Hodking lymphoma 8 years ago, which was treated with chemotherapy for 6 months and with radiotherapy for 21 sesions (30 greys). Once she was cured, she decided to have a baby although the chromosome findings in the lab where frightened scared, showing multiples and different chromosome aberrations in 20% of cells. The karyotype was repeated and the same findings were observed. The question is: Could it be any relation between the lymphoma treatment which happened 8 years ago and the multiple chromosome alterations? If so, is there any risk for a pregnancy in our patient? Should she avoid her ovocites and consider a donor? Is there any other patient describe with a similar karyotype? Shall we check her hematology looking for another lymphoma?

J03.12
Incidence And Clinical Significance Of Pericentric Inversion Of Chromosome 9.

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Aim: The aim of the study was to study the frequency of inv(9) and its clinical correlation with human genetic diseases

Background: Pericentric inversion of the chromosome 9 is such a common occurrence that some cytogeneticists would consider them as normal variants. The frequency estimated to be 1 to 3% in the general population. Despite being categorised as a minor chromosomal rearrangement which does not correlate with abnormal phenotypes, there have been many controversial reports indicating that it may lead to abnormal clinical conditions such as subfertility, recurrent abortions, leukemia, dysmorphic features and psychosis.

Materials and Methods: We studied retrospectively the incidence and clinical significance of pericentric inv(9) from the collected peripheral blood karyotypes of 1,800 cases being referred to our department with suspected genetic diseases over a 3-year period.

Results: Pericentric inv(9) was detected in 21 cases (1%). Ten cases were adult patients, four (40%) of them were with obstetric and fertility problems, five adult patients (50%) had a sibling or offspring with inv(9) and 1 adult patient (10%) had the diagnosis of acute myeloid leukemia. Eleven cases (52%) were paediatric patients with dysmorphic features and congenital anomalies.

Conclusion:

The significance of inv(9) is still mostly unknown and hence to understand the clinical significance of the pericentric inversion of Chromosome 9 it will be required reporting of new additional cases with detailed chromosomal studies.

J03.13

Cytogenetics abnormalities of spontaneous abortion

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The loss of the desired pregnancy is always a stressful event for both partners/spouses, and therefore it is always important to establish the cause that led to the loss of pregnancy and how to achieve success in the next pregnancy and get a healthy child.

Research objectives: To determine the frequency, distribution and type of pathological karyotypes of spontaneous abortions.

Materials and methods: The study group consists of partners who underwent karyotyping of spontaneously aborted fetuses. The analysis of 549 samples of spontaneous abortions revealed 19.85% of chromosomal aberrations. **Conclusion**: Most frequent chromosomal aberration in spontaneous abortion group was the Turner syndrome, followed by triploidy, trisomy of chromosome 18, trisomy of chromosome 15 and Down's syndrome. Rare chromosomal aberrations were frequently present in earlier gestation ages. Women with spontaneous abortion, which is caused by a chromosomal aberration often have a history of adverse outcome of previous pregnancies.

J03.14

Probability rates of different pregnancy outcomes and meiotic segregation analysis of spermatozoa in carriers of t(1;11)(p36.22;q12.2)

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The unique rearrangement i.e. t(1;11)(p36.22;q12.2). ish t(1;11)(RP11-1115A15-,RP11-476D13-,RP11-498B2-,RP11-807G9-,RP11-496H15-,RP11-575L21-,RP11-874A11+;RP11-855010-,RP11-881M11+,799F14+) was found in two relatively large pedigrees of carriers studied due to occurrence of three miscarriages (pedigree 1) and the birth of newborn with hydrocephalus and myelomeningocele (pedigree 2). The same hybridization pattern was found in both families indicating similar, if not the same, rearrangement. STS marker walking analyses using hybrid containing derivative chromosome 1 did not allow us to define the 1p36 and 11q12.2 breakpoints in this rearrangement at the sequence level. Segregation analysis of cumulative data of pedigrees was performed by indirect method of Stengel-Rutkowski and showed that probability rate for unbalanced child at birth was 0/40 i.e. <0.9.% after ascertainment correction, the risk for stillbirths/early newborn deaths was 1/40 i. e. 2.5%±2.5.% and for miscarriages was 15/40 (37.5%±7.6 %). We didn't found any differences between males and females carriers.

Meiotic segregation pattern after the sperm analysis by three-colour FISH method of one male carrier from pedigree showed all possible combinations after 2:2 and 3:1 segregations. The most common segregation types were alternate and adjacent I (similar frequency). Low frequency was observed in adjacent II type, in opposite to 3:1 segregation with the high proportion of unbalanced gametes. However we suggest, that only one form of chromosome imbalances i.e. monosomy 1p36.22→pter with trisomy 11q12.2→qter may be observed in progeny at birth but with limited survival after delivery.

Obtained results of risk estimation of different pregnancy outcomes for carriers of t(1;11)(p36.22;q12.2) may be used in genetic counseling of carriers of this rearrangement.

J03.15

Case of diagnostics of a rare syndrome 18p-.

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Partial monosomies of short and long shoulders of a chromosome 18 are clinically distinguished syndromes. Considering a rarity of these syndromes, data on new cases deserve special attention. We result a case of diagnostics of a partial monosomy of a short shoulder of 18 chromosomes at the woman of 24-th years who have addressed for medicogenetic consultation. The patient had an expressed psychoneurological semiology: an intellectual development delay (F70), organic disorder of the person. At survey became perceptible phenotypic signs: mycrocephalia; hypertelorism; ptosis; mandible hypoplasia; dysplasia of auricles; an asthenic constitution; the low growth and the lowered mass of a body; kyphoscoliosis of thoracal department of a backbone; short neck; cross-section-longitudinal platypodia; clinodactyly of little fingers; hypoplasia of trailer phalanxes of brushes; hypermobility of interphalanx and radiocarpal joints; recurvation of ulnar joints; hypomyotonia.

From integuments were taped: the expressed dryness, ecdysis, diffusive pigmentation, signs of a follicular hyperkeratosis. The accompanying somatic pathology is diagnosed for the patient: a peptic ulcer duodenum and an iron deficiency anemia. Developmental anomalies in the given observation it did not become perceptible.

The proband karyotype has been identified as 46,XX,del(18)(:p11.31→qter). From parents of the patient it was possible to survey only mother, it has a normal karyotype.The phenotype of our patient corresponds to clinical descriptions of patients with a partial monosomy of a short shoulder of 18 chromosomes which were published earlier. However, along with characteristic craniofacial dismorphias and a delay of mental development, expression of somatic and dermal implications pays attention to itself.

J03.16

A case of 16p subtelomeric duplication with vascular anomalies

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We report a patient, a boy 12 months old, with karyotype 46,XY,der(4), recognized by standard cytogenetic techniques, presenting with facial features, neurological impairment and pulmonary hypertension. He was the first child of healthy nonconsanguineous parents. Family history was unremarkable. Distinct facial anomalies included microcephaly, high frontal hairline, blond thin hair, bilateral blepharophimosis and palpebral ptosis, short nose, everted upper lip, cleft palate, micrognathia, cupped anteverted ears. He had also hypoplastic distal phalanges and bilateral inguinal hernia. Pulmonary hypertension with tricuspidal regurgitation and cavernous liver hemangioma were found in our patient. Subtelomeric analysis by Multiplex Ligation-dependent Probe Amplification (MLPA) technique (a set of probes for testing subtelomeric imbalances in the SALSA P070 and P036B human telomere test kits, MRC-Holland, Amsterdam, Netherlands) demonstrated a duplication of the subtelomeric region of chromosome 16p and a deletion of the subtelomeric region of chromosome 4q, suggesting a translocation between 4q and 16p. A duplication of the subtelomeric region of 16p in the parents was excluded by MLPA technique. The imbalance of our patient resulted de novo. In comnclusion, we have confirmed the clinical features of patients with dup16p, involving the terminal 16p13.1-p13.3 region. Vascular anomalies have been previously described in association with dup16p. Thus, pulmonary vascular disease and other vascular anomalies can be a feature of dup16p, suggesting that this subtelomeric region in some patients could be related to vascular anomalies.

J03.17

Cytogenetic effects in Chernobyl accident liquidators in delayed terms following radiation exposure

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Genome response to radiation exposure caused by mutagenic effects both in the exposed targeted cells as well as in the unexposed bystander cells. In delayed terms following radiation exposure during cytogenetic examination of Chernobyl accident liquidators the frequency of all types of chromosome aberrations in their lymphocytes with the help of G-banding chromosomes staining had been established. The elevated chromosome aberrations frequency in Chernobyl accident liquidators lymphocytes exposed in doses 270-690 mGy formed due to translocations that are stored in the generations of irradiated target cells and chromatid breaks induced by bystander-type effect in the untreated cells had been established. The frequency of deletions, dicentrics and centric rings had no significant difference from control that was result of their elimination in time. In lymphocytes of Chernobyl liquidators exposed to radiation in doses 1010-2540 mGy the level of chromosome aberrations exceeded the population's one at the expense of high frequency of stable cytogenetic markers of radiation exposure, rings chromosomes and chromatid breaks. The results obtained confirmed the persistance of bystander-type cytogenetic effects in somatic cells of exposed persons for many years following radiation exposure. Our data confirm the need to asses the frequency of stable chromosome aberrations as basic cytogenetic markers of radiation exposure under the cytogenetic dosimetry in the late terms following human irradiation and incorrect using such indicators as the „frequency of aberrant cells" and „mean level of chromosome aberrations" that may be overestimated because of chromosome instability markers (chromatid type aberrations) due to the induction of bystander-type effect.

J03.18
Clinical characterization of five patients with microdeletion at 15q13.2-q13.3

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Individuals with 15q13.3 microdeletion may have wide range of clinical manifestations including intellectual disability (ID), cardiac malformations, seizures, autism and schizophrenia. Deletion of *CHRNA7* gene in this region is causative for the majority of neurodevelopmental phenotypes in the 15q13.3 microdeletion syndrome. Subsets of persons with the deletion have no obvious clinical findings. During 2009-2011 the chromosomal microarray analysis (CMA) was performed in 596 individuals due to their clinical indications. In four individuals 15q13.3 microdeletion was found. In one patient 15q13.2-q13.3 microdeletion was diagnosed previously during research study of the children with ID. Here we present the clinical features of five patients with 15q13.2-q13.3 microdeletion.

All our patients (aged 6-17 years, among them two sibs) had normal growth parameters, except one boy with larger deletion 15q13.2-q13.3 who had short stature and microcephaly. Mild or unspecified ID, speech delay and mild facial dysmorphism was noticed in all patients. Abnormal EEG was found in three of them (60%) and one boy has severe treatment resistant generalized epilepsy (20%). Positive family history for epilepsy was documented in two families. Therefore, it is highly possible that their epilepsy may be also caused by 15q13.3 microdeletion (not investigated yet) as in 1-2% of individuals with generalized epilepsy 15q13.3 microdeletion is found. Cardiac anomaly was occurred in three children (60%).

Microdeletion 15q13.3 is one of the most common microdeletions found by CMA in individuals with ID. In our cohorts of patients it was detected in 0.7% of investigated individuals.

J03.19
Gene polymorphisms and the frequency of chromosomal aberrations in persons exposed to long-term occupational irradiation (Microarray for using)

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There has been studied the correlation between SNP and the frequency and range of chromosomal aberrations in 96 employees (male) of Siberian Group of Chemical Enterprises, exposed to chronic γ-radiation: European, Age - 45-65 year, Cumulative Dose 100-300 mSv. At all employees, the analysis of frequency and range of chromosomal aberrations has been carried out (not less than 300 metaphases). Genotyping were performed by using the microarray „Cancer_SNP_Panel" (http://www.illumina.com/Documents/products/datasheets/datasheet_cancer_snp_panel.pdf). Panel contains 1 421 SNP of 406 genes.

SNP with Call Rate below 80% (25 SNP), SNP with the frequency of rare alleles below 10% (134 SNP), 80 SNP with genotype frequencies, that do not meet Hardy-Weinberg equilibrium, were excluded from the analysis. All in all p-value of 1 262 SNP were calculated with Bonferroni correction for recessive model („SNPassoc" program). There were analyzed the frequencies: aberrant cells; single chromatid breaks; paired fragments; point fragments; dicentric and ring chromosomes; translocations; chromatid exchanges.

Studies have shown that 23 SNP (mutant genotype) (rs2725349, rs2392221, rs1041163, rs2114443, rs6083, rs7462102, rs1760904, rs1051690, rs4986894, rs488133, rs5742694, rs2373721, rs5742667, rs1269486, rs1149901, rs2873950, rs1574154, rs10934500, rs4688046, rs889162, rs230532, rs312016 rs4807542), associated with the high frequency of different types of chromosomal aberrations, including 11 SNP associated with more than one type of chromosomal aberrations.

J03.20
Whole Genome Array- CGH analysis in patients with Developmental Delay/ Mental Retardation /Congenital Malformations in Saudi Arabia

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Microarray - based Comparative Genomic Hybridization (a-CGH) has enabled wide investigation of the genome at high resolution and has been implemented in different centers as a clinical diagnostic tool. Chromosomal imbalances are implicated in the etiology of Developmental Delay (DD)/Mental Retardation (MR). However, most of these cases could not be diagnosed by conventional cytogenetic techniques. We aimed to establish (a-CGH) technique and assess its potential as a diagnostic tool of chromosomal imbalances and to detect chromosomal aberrations in patients with DD/ MR. Subjects & Methods: A cohort of 47 patients diagnosed as having DD/ MR with or without congenital malformations were referred to the CEGMR for cytogenetic analyses. We used both conventional cytogenetic G-banding and Fluorescent in-situ hybridization techniques, besides we applied (a-CGH) high resolution Agilent scanner with 1X244 K array format, and Affymetrix 2.7 M cytogenetics array. Chromosomal aberrations could be detected in 6/47 (13%) patients by G-banding technique and 4/47 (8%) by FISH technique, however, 14/47 (30%) were diagnosed by a-CGH techniques. The following microdeletion syndromes were detected: (Del 15 (q11.2); Del 15 (q13-14); Del 22 (q11.2); Del 7 (q11.23); Del 18 (q21 q23); Del 1 (p36); and duplications: dup 18p (tetrasomy); dup 15 (q11 q23); dup 18 (q23).We noticed the increased number of CNVs detected by a-CGH which need further investigation for contribution to phenotypes. Our results indicate the strength of high resolution genomic arrays in diagnosing cases of unknown etiology and in detection of contiguous genomic alterations in the wide spectrum of cases with DD/mental retardation.

J03.21
Clinical correlation of mentally retarded individuals suffering from pervasive developmental disorders (PDDs) in South Indian population

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Aim and objective: To clinically correlate and analyse the relation between mentally retarded individuals suffering from PDDs.

Methodology: From 130 cases suffering with PDDs a sample of 30 individuals showing MR were chosen according to diagnostic and statistical manual of mental disorders (DSM-IV). We performed cytogenetic analysis by measuring the extent of chromosomal aberrations (CA) along with cytokinesis-block micronucleus (CBMN) assay in human leucocytes. This assay was performed to measure more specifically the DNA damage occurring in peripheral blood leucocytes. Buccal cells were collected and analysed for measuring DNA damage using micronucleus assay. We also checked three genes namely CNTNAP2, SHANK3 and MECP2 for mutations and its association with MR through molecular analysis by Polymerase Chain Reaction (PCR), Single Stranded Confirmation Polymorphism (SSCP) and sequencing.

Result: Cytogenetic analysis (i.e) chromosomal aberrations study ,CBMN assay in leucocytes and micronucleus assay using buccal cells showed significant variations in all MR cases, when compared with control samples. Molecular analysis revealed mutations in CNTNAP2, SHANK3 and MECP2 through PCR, SSCP and Sequencing.

Conclusion: The study showed a variety of cytogenetic abnormality along with significant changes in base pair content in the three genes considered for this study in the MR individuals. More number of these studies with a much larger sample size has to be conducted. So that it helps in biomonitoring and creating awareness in the population. Such studies will help in developing a simpler diagnostic method that can help identify the prevalence of MR in PDDs.

J03.22
Cytogenetics and molecular analysis of beedi workers among Vellore population

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Beedi is a thin South Asian cigarette made of 0.2-0.3g of tobacco flake wrapped in Tendu (Diospyrox melanoxylon) leaf and secured with colored thread at both ends. This work is based on the study of the beedi workers who are extensively engaged in beedi making in Elagiri village, Vellore for generations. Workers roll an average of 500-1000 beedies per day, handling 225-450 grams of tobacco flake, and inhaling tobacco dust and other volatile components. Our aim is to study the induced chromosomal abnormalities by tobacco, a potent carcinogen from cytogenetic and molecular aspects.

Cytogenetic analysis was performed to check the chromosomal aberrations in the beedi workers. Totally 27 workers samples and 15 controls are collected; the result shows that there is a significant increase in the aberrant cells to the workers than the control. Also there is an increased evidence of chromosomal aberration to the workers having the habit of smoking than the non-smokers. The aberration differences observed is due to smoking while the other group got tobacco exposure indirectly through tobacco dust while manufacturing.

In molecular analysis, DNA was extracted from the blood samples followed by PCR amplification with primers specific for the cyp1a1 gene (Exon-1). The PCR amplification product was determined by 1% agarose gel electrophoresis, then SSCP (Single Strand Conformational Polymorphism) was carried out to detect mutation by deletion or addition in bands. In this study 3 deletions were observed out of 10 beedi workers when compared with control.

J03.23

Study of methylparathion on bone marrow cells from mice, in vivo: Micronucleus assay

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Methylparathion is a largely used pesticide. To exert its biological effect it must be biotransformed into methylparaoxon. Previous work in our laboratory, using the chromosome aberrations assay in human lymphocytes in vitro has shown that while methylparathion had no clatogenic or aneugenic effect on chromosomes, methylparaoxon was responsible for the alterations in structure observed. The objective of the present work was to study the effect of methylparathion on chromosomes of mice bone marrow in vivo, using the micronucleus assay. Animals were separated into four groups. In the first group, 6 animals received methylparathion intraperitoneally during five consecutive days in a concentration equivalent to 25% of the LD_{50}. In the 6th day, animals were sacrificed, their femurs removed, the bone marrows collected and smears were made for slides preparation. After 24h cells were stained with Giemsa Gurr (2%) and analyzed under optical microscope. As positive control, 6 animals received cyclophosphamide (50mg/mL) once. Six animals were injected intraperitoneally with the solvent (corn oil) and 6 animals not exposed to any drug served as negative control for the experiment. In the test group, 12000 cells were observed and 199 showed micronuclei. In the group exposed to the solvent, 12000 cells were analyzed and none had micronucleus. In the positive control group, 12000 cells were observed and 102 had micronuclei and in the negative control group, of 12000 cells observed, none had micronucleus. The chi-square test for independence showed that our results were extremely significant (P<0.0001). They suggest that methylparathion is responsible for the micronuclei observed.

J04. Reproductive genetics

J04.01

Genetic and demographic aspects in Kaluga region and among the families with reproductive losses

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The national composition of Kaluga region is chiefly represented by Russians (93.5%).

Beginning from 2000 the parity of childbirth in Kaluga region changed as follows: the number of primiparas decreased by 5 %, while the number of women giving second birth increased by 3%, and the number of women with three childbirths increased by 2%. The number of women with 4 and more childbirths remained unchanged (4%).

Within the last 11 years Kaluga region birth rate increased by 30%, this fact being accompanied by the increase of childbearing women's average age from 25,05 to 26,85 years.

In the group of 957 families with reproductive losses the average age of women ranges from 26,92 to 28,5 years, which is distinctly higher than general population rate. The expected Down syndrome frequency among the families with reproductive losses amounts to 14,46 per 10000 neonates, general population rate being 11,51. This fact may initiate age-related fetal pathology and cause present and future reproductive losses. The average age of the men from the families with reproductive losses amounts to 29,46, which turns out to be lower than general population rate (30 years). Based on this, the fact described above should not influence the frequency of age-related fetal pathology in the families with reproductive losses.

Thus, we obtained certain evidences of possible influence of the age of women from the group with reproductive losses on the increase of age-related neonates pathology frequency and possible increase of repeated reproductive losses among them due to fetus trisomies.

J04.02

Frequency of mutation FV Leiden among the women of reproductive age living in the Novosibirsk region, and communication of this mutation with reproductive losses

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Factor FV Leiden mutation (replacement G on A at nucleotide position 1691 gene *F5*) is the autosomal dominant disorder that predisposes affected persons to venous thromboses and may have an increase risk for pregnancy-related venous thromboembolism finally leads to pregnancy loss.

In control group of 238 women of genital age with the normal obstetric anamnesis, living in the Novosibirsk region and selected by epidemiological criteria, frequency of 1691A gene *F5* has made 0,0044 (in the absence of homozygous genotypes). Results of inspection of 303 women, which anamnesis has been burdened by spontaneous interruption of pregnancy on term till 24 weeks, testify about authentic to higher frequency of 1691A gene *F5*. In this group its frequency has made 0,0396, thus two women have homozygous genotypes. The received results confirm the importance replacement G on A at nucleotide position 1691 gene *F5* as one of genetic factors contributing to early reproductive losses and confirm the big prognostic value of molekuljarno-genetic testing of the FV Leiden mutation at mediko-genetic consultation of families in which there were early reproductive losses, and families which plan pregnancy.

J04.03

Study of Oct4 and Sox2 Genes expression in Haematopoietic Stem Cells

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Every tissue in body needs to regenerate itself after injury. Stem cells because of their abilities of differentiation and self-renewal can expand and give cells to the tissues. Stem cells have various types; one of them is hematopoietic stem cells; in addition to differentiation of hematopoietic cells hsc can also differentiate in to neural, mascular, and cartilage cells. This unique possibility can help us in investigation of new treatments for cancers. So recognition of mechanisms in this process has multipular importance. In the present study expression of sox2 and oct4 genes -known as main genes in self renewal - were considered with RT-PCR from cord blood CD133+ cells. Our results show that both genes expressed in the first and 8th day of expantion but at 12th day despite of sox2 expression; oct4 was not expressd. Because of necessity of oct4 for keeping multipotency; loss of expression will cause to differentiation which seems to appear after 12th day.Our study demonstrated that mechanism of self-renewal in CD133+ cells are similar to embryonic stem cells or embryonic carcinoma cells and different with mesenchyme CD105+34-.

J04.04

The role of the genetic factors in the recurrent miscarriage

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Recurrent miscarriage (RM) is a serious problem of the modern obstetrics. Adrenal hyperandrogenism, which come from congenital adrenal hyperplasia (CAH), is one of the common reasons of RM. We analyzed 10 mutations of the CYP21A2 gene (P30L, I2splice, del8bp, I172N, V236E, V281L, Q318X, R356W, P453S, delA2) and the presence of two types of chimeric genes in two groups of women. The population group (first group), consisted of 82 women, and the second one included 102 women with RM.

Mutations in the 21-hydroxylase gene were detected in 15,5% (17/102) of women with RM and in 2,5% (2/80) of women from population group (p = 0.0013).

The chimeric genes were identified in 25% (25/102) of women in the second group and in 8,5% (7/80) of women in the first group (p = 0.0093).

We have studied 76 women of population group and 73 women with RM by means of the Real time-PCR method, in order to detect the CYP21A2 gene and CYP21A1P pseudogene copies. We found a statistically significant difference between both groups in gene duplications and deletions of pseudogene (p = 0.0235, p = 0.0345, respectively).

Thus the presence of mutations in CYP21A2 gene and chimeric genes has a substantial impact on development of recurrent miscarriage. It is notable that both studied groups differ from each other by the number of pseudogene copies, that might be due to the presence of unknown mutations. Further studies are needed to clarify the role of CYP21A2 gene in the development of RM.

J04.05

Interaction between maternal KIR and fetal HLA-C in success of pregnancy

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Introduction: Killer-cell Immunoglobulin-like receptors (KIR) expressed by natural killer (NK) cells at the site of placentation, can bind to human leukocyte antigen (HLA-C) molecules on trophoblast cells. Both systems are genetically highly polymorphic, in which their interactions result in release of varieties of cytokines and chemokines. The factors modulate placental relationship between mother and her fetus. Thus, it has been hypothesized that each of the particular maternal KIR/fetal HLA-C genotype combinations have different effect in pregnancy success.

Materials & Methods: The patients were 92 couples and 8 women with three or more recurrent miscarriage (RM) with no physiologic or pathologic reason for their problem. Also, 100 healthy porous women were selected as control group. DNA were isolated from the whole blood specimens and genotyped for HLA-C groups and 5 KIR genes (*KIR2DS1, KIR2DS2, KIR2DL1, KIR2DL2, KIR2DL3*) using PCR-sequences-specific primer method (SSP).

Results: Statistical analysis shown, that frequency of the HLA-C2 group is raised in the affected female compared with porous women. The frequencies of activating KIRs in the male of partner of RM were similar to controls women while it had been decreased in the affected female compared with porous women although these don't reach significant.

Conclusion: Our findings support the idea that interaction between maternal KIR on NK cells and paternal HLA-C expressed trophoblast cells, affect the successful placentation.

J04.06

Investigation of association between FABP9 gene mutations and sperm morphological defects in a group of Iranian Men

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Introduction : The male germ cell-specific fatty acid-binding protein 9 (FABP9/PERF15) is the major constituent of the murine sperm perforatorium and perinuclear theca. Because of its cytoskeletal association and sequence homology to myelin P2 (FABP8), it has been suggested that FABP9 tethers sperm membranes to the underlying cytoskeleton. Furthermore, its upregulation in apoptotic testicular germ cells and its increased phosphorylation status during capacitation suggested multiple important functions for FABP9. Also recently it is shown that it can affect sperm morphology in mice . According to these data , we designed a study to evaluate if mutations of this gene can affect sperm morphology in human.

Material and methods : The DNA was extracted from peripheral blood from the infertile males that their sperm count was normal but the number of morphologically abnormal sperms on their semen was above normal. Then the exon sequences of the FABP9 gene was amplified by PCR. These exons were sequenced and then analyzed for detection of potentially mutations.

Results : We couldn't detect any mutation in the four exons, intron 3 and splice sites of the FABP9 gene in our 100 samples which we studied.

Conclusion : As there was no mutation in the exons of this gene in our samples, we can conclude that the mutations of this gene may have a neglectable effects on sperm morphological defects and the infertility caused by that. However, because we didn't analyze the promoter and introns 1 and 2 of this gene, we couldn't say this surely.

J04.07

Human karyotype changes associated with hereditary thrombophilia

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Background: Thrombophilia is a inappropriate tendency to thrombus formation. In recent years numerous studies were conducted in the field of thrombophilia, in an attempt to prevent the consequences of thrombotic disease.

Methods: Between 02.2010-01.2012 in Oncomed Center we evaluated 104 patients with thrombophilia. 94 were women and 10 men. In 38 women were conducted karyotype.

Results: The study group showed a rate of 92.1% associated mutations. Only 7.9% of patients had a single form of thrombophilia. Thus, 89.5% showed PAI mutation, MTHFR C677T mutation - 52.6% , A1298C MTHFR mutation- 21.1% , double mutation MTHFR in heterozygous form - 4 patients , factor XIII mutation- 12 patients, fibrinogen mutation in 4 and factor V Leyden, factor II, protein S deficiency and GPIIb / IIIa each in 2 patients each.

Of the 38 patients, 28 had different chromosomal polymorphisms. The most common were the presence of satellites in 12 patients: chromosome 13 , 21, 14, 15 and 22. The remaining 16 patients had combined changes, both satellites and constituent heterocromatina. Constituent heterocromatina was present on chromosome 1, 9 and 16, all with PAI1 mutation. 26 patients achieved pregnancy. No differences were found between patients without changes in karyotype and those with this chromosomal satellites, but the patients with combined changes, only 6 (37.5%) have achieved pregnancy.

Conclusions: Both chromosomal changes and status of thrombophilia cause repeated reproductive failures. Further studies are needed to specify the combination of mutations at greatest risk and how therapeutic dose should be adjusted to be effective.

J04.08

Detection of AZF Factor of Y Chromosome

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Molecular genetic study of AZF region deletions of Y chromosome was carried out in order to investigate reproductive disorders in men. The material of the study were 177 men with a history of reproductive disorders (57% astenoteratozoospermia, oligoastenoteratozoospermia in 32% and azoospermia in 11%). Cytogenetic and molecular genetic studies were carried out.

Normal karyotype - 46, XY was diagnosed in 159 cases. The 18 cases (10%) showed chromosomal abnormalities: 16 patients -47, XXY - Klinefelter's syndrome, in one case- disomy of Y chromosome-47, X YY, and one structural abnormality - 45,XY,der(14,15)(q10;q10).

The 88 men with infertility and normal karyotype - 46, XY received molecular genetic study. Genomic DNA was extracted from peripheral blood by «Promega» set, USA. Multiplex polymerase chain reaction (multiplex PCR) was performed using Taq-polymerase. 9 STS-markers, specific to AZF-locus, were used, the results of amplification were evaluated by electrophoresis in 7% polyacrylamide gel. 7 patients had deletions of AZF locus, of which AZFa in the 15%, AZFb in the 15%, AZFa+b in the 15%, AZFc in the 55%. The obtained results allowed to establish the genetic cause of spermatogenic disorders, to avoid unnecessary treatment and the tactic of IVF / ICSI program.

J04.09

Use of Y chromosome specific repeat sequencing for sexing by PCR and Single cell PCR

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Embryo sexing is one of the important ways for sex selection of offspring. This is a potential method to considerably improve animal breeding and the efficiency of dairy and meat production. A novel repeated sequence specific to male cattle has been identified and named S4. S4 is a 1.5 Kb repeating unit contains various internal repeated sequence. S4 is localized on long arm of the Y chromosome in the region near to ZFY genes. Aim: The objective of this study was to establish a simple, sensitive, reliable, reproducible and cost effective PCR based technique for sexing. Materials and Methods: Genomic DNA was extracted from the whole blood samples of male and female cattle. PCR and single cell PCR were performed using specific primers for this region. Result: By this PCR based methods we could differentiate between female and male genomic DNA. Discussion: With this technique we can distinct male from female using as much as 0.1pg DNA. Using this method we could determine the sex of embryo (4 blastomers). This method may optimize for the quantitative detection of Y chromosomes in semen.

J04.10

Effect of Oxidative Stress on MicroRNAs Expression in Testis, Sperm Quality and Testis Histopathology in Mature Mouse

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Male infertility is responsible for approximately 50% of infertility in the world. Reactive oxygen species (ROS) is one of the causative agents of infertility in males which effects on sperm quality and function. In this study, the effects of oxidative stress induced by tertiary-butyl hydroperoxide (TBHP) were investigated on sperm quality, testis tissue and miRNAs expression.

Adult male mice strain Balb/c was randomly selected from mouse colony. After a primary study to determine LD_{50}, TBHP was injected at the concentration of 1:10 LD_{50} for 2 weeks. The mice were sacrificed and their testis tissues were used for cell viability, macroscopic-histopathology analysis, ROS assay and miRNAs expression. Epididymis was also surveyed for sperm analysis by CASA system.

The sperm motility, count and viability were decreased in the TBHP treated mice in comparison of the control mice. The flowcytometry analysis showed a significant increase in H_2O_2 and $O_2^{\bullet -}$ levels in both testis and sperm 2 weeks after intra-peritoneal injection. Body weights revealed no treatment-related effects but atrophy of testis and decrease of testis cells viability, the expression of mmu-miR-34a and mmu-miR-181b was observed. Results showed that exposure to TBHP can lead to morphological changes in somniferous tubules.

TBHP-induced oxidative stress caused to decrease in sperm vitality and motility and testis cells viability. Results indicated that oxidative stress induction in testis reduced its normal function. That is due to an increased level of H_2O_2 and $O_2^{\bullet -}$ in testis and their deleterious effects on genomic levels.

Keywords: male infertility, oxidative stress, miRNA.

J04.11

No association between gr/gr deletions and non-obstructive azoospermiain Iranian males

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Objective: Genes located in the azoospermia factor (AZF) region including AZFa, AZFb, AZFc and AZFd on the long arm of Y chromosome play an important role in spermatogenesis. Microdeletions in these regions have been seen in 10% of infertile males with azoospermia or oligozoospermia. Partial deletions of the AZFc region were also reported to be a significant risk factor for oligo/azoospermia. In this study, we estimated the frequency of partial AZFc microdeletions in Iranian azoospermic men with spermatogenic failure and in fertile controls.

Methods: A total of 150 Iranian azoospermic infertile men were selected for the molecular study of Y chromosome microdeletions. Patients without classical AZFa, AZFb and AZFc deletions and with elevated serum FSH levels were analyzed for detection of partial deletions in the AZFc region. 100 fertile men were also studied as the control group.

The presence or absence of the AZFc gr/gr subdeletion in all subjects was tested by multiplex PCR using sY1191, sY1291, sY1206, sY1201, sY142, sY1258, sY1197, sY1054 and sY1161 STS markers. The unique absence of sY1291 product was considered as a gr/gr deletion.

Results: The prevalence of gr/gr deletions in patients and controls were 8.45% (12/142 cases) and 10 % (10/100 cases) respectively. Statistical analysis showed no significant differences in the frequency of gr/gr deletions between the patient and control groups (p>0.05).

Conclusion: The present study revealed no evidence of association between the occurrence of gr/gr deletion and male infertility in Iranian azoospermic infertile men.

J04.12

Genetic polymorphisms and predisposition to a polycystic ovary syndrome

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Background: Polycystic ovary syndrome (PCOS) is one of the most common reproductive health problems of women. It is characterized by hyperandrogenia, oligoovulation or anovulation and polycystic ovaries. Although a major genetic contribution is suspected, no particular genes or family of genes have been confirmed to be causal for PCOS.

Methods: 34 women with PCOS and 32 healthy female controls were genotyped by RFLP and SSCP techniques for polymorphisms PvuII T/C and XbaI A/G and (TA)n repeats in ER*α* gene; C polymorphism in STK11 gene; RsaIG/A - in ERβ gene, and Asn680Ser and Thr307Ala in FSHR genes - respectively. Both selected groups (clinical and control) meet all requirements for conducting associative studies.

Results: No differences were found between the alleles distribution in patients and controls for STK11 gene, PvuII T/C in ERα, RsaI G/A in ERβ, Asn680Ser and Thr307Ala polymorphisms in the FSHR. XbaI A/G polymorphism in ER*α* gene was related to a higher risk for PCOS since the prevalence of A alleles was 67.2% in PCOS group vs. 46.9% in the controls (p=0.032). Women with two shorter (TA)n alleles in the ER*α* gene were found more often among PCOS patients in comparison to healthy women (44.1% vs. 18.8%, p=0.036).

Conclusions: Our study suggested that the XbaI A/G and (TA)n polymorphisms of the ER*α* gene could be related to the development and clinical features of PCOS. Larger studies in different ethnic groups are needed to establish the precise role of the estrogen receptor polymorphisms for the ovarian function.

J04.13

Comparison between Molecular and Histopathological Methods for Assessment of Spermatogenesis in Non-Obstructive Azoospermic men

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In Non-Obstructive Azoospermic (NOA) men, evaluation of spermatogenesis is performed with histopathological techniques as a gold standard. Pathological assessment of testes could be imprecise due to randomized biopsy and presence of focal spermatogenesis. NOA men showed a variety of defects in spermatogenesis stages, therefore molecular analysis of the stage-specific of gene expression in the testis could be confirmatory of histopathological techniques in evaluation of spermatogenesis in NOA men.

In this study, spermatogenesis status evaluated through expression of germ cell specific genes (DAZ, TSPY1, SPTRX3 and SPTRX1) in testicular tissue of azoospermic men. Histophatological evaluation was performed using H&E routine method. Semi-nested RT-PCR was performed on synthesized cDNA. The molecular results prepared from gene expression were compared with the histopathological findings using Kappa test.

RT-PCR results showed a significance difference (Kappa coefficient=0.009, P value = 0.894) with the histopathological results. TSPY1, DAZ, SPTRX3 and SPTRX1 were expressed in 94%, 94%, 17.6% and 52.9% respectively in azoospermic men diagnosed as Germ cell aplasia. Detection of DAZ, TSPY1 and SPTRX1 transcripts in testicular tissue can be used to predict the presence of mature spermatid / sperm in the testis especially in men diagnosed as spermatogenesis arrest using histopathological technique and may provide the better chance of finding the mature sperm to use through ART.

J04.14

Complex molecular study of Klinefelter syndrome

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We examined 46 KS patients with following karyotypes: 47,XXY (n=37), 47,XXY,t(3;8)(q23;p21) (n=1), 47,XY,derX (n=1), mos 46,XY/47,XXY (n=4), mos 47,XXY/48,XXXY/46,XY (n=1), mos 46,XX/47,XXY (n=1) and 48,XXYY (n=1).

Molecular study included the analysis of Y chromosome microdeletion, CAG-repeat of exon 1 of the Androgen Receptor (AR/HUMARA), X chromosome inactivation (XCI) and PCR amplification of 12 STR loci of the X chromosome (Investigator Argus X-12 Kit, Qiagen).

No classic (complete) AZF deletion was found. Partial AZFc deletions (b2/b3, n=4; gr/gr, n=1; del sY1197 and sY1206, n=1) were detected in 6 of 46 (13%) patients. AR CAG-repeats number varied from 16 to 30, alleles with 26-30 repeats were found in 7 of 43 (16.3%) patients. Homozygosity and heterozygosity for CAG-allele was revealed in 17 and 26 of 43 examined individuals, respectively. Eleven homozygous for AR-allele patients were tested for 12 STR loci. We found that X chromosomes were different in 3 cases (two 47,XXY and one 48,XXYY), and X-haplotypes were the same in 8 cases. XCI was evaluated in 21 of 26 AR-allele heterozygous individuals. Skewed XCI with the rate 70-80% was found in 3 of 21 (14.3%) 47,XXY men, 4 patients presented XCI rate (67-69%) near to skewed.

In contrast to partial deletions classic AZF deletions no found in KS. No strong genetic and phenotypic correlation was revealed between KS patients with or without Y chromosome microdeletion, normal or high AR CAG-repeats, and random/skewed XCI. However severe oligozoospermia was detected only between KS patients with normal CAG-repeats, not-skewed XCI and without AZF deletion.

J04.15

Male infertility and polymorphisms in CREM, LRP8, ABCA1, SMPD1 genes.

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The lipid metabolism genes play essential role in mouse spermatogenesis, but their role in human infertility has not been fully established. Products of CREM gene are essential for the initiation of spermatid maturation and development. Mice lacking CREM gene exhibit specific arrest of round spermatid development, fenotype similar to some human infertility conditions. Few studies have shown that CREM deficiency is associated with spermatogenic disorders in man. ATP-binding cassette transporter 1 (ABCA1) mediates lipid efflux from Serotoli cells and influences male fertility. Apolipoprotein E receptor-2 (LRP8) and acid sphingomyelinase (SMPD1) play important role in sperm development, maturation and function in mice. This case-control study investigated associations between polymorphisms in the CREM, LRP8, ABCA1 and SMPD1 genes of lipid metabolism and male infertility.
Screening of eight polymorphisms (CREM: rs1531550,rs17499247, LRP8: rs17108177,rs3737983, ABCA1: rs2230806,rs2066714, SMPD1: rs1542705,rs1050239) was performed in 522 Slovenian and Serbian men with azoospermia and/or oligoasthenospermia and in 445 Slovenian and Serbian controls.
Distribution of genotypes and alleles of investigated polymorphisms were in accordance with Hardy-Weinberg equilibrium in different groups or with the total population. We have found significant differences in genotype frequencies of ABCA1 gene rs2066714 polymorphism ($p<0,002$) between group of patients with azoospermia and control group. However, no significant differences in genotype frequencies of other tested polymorphisms (CREM,LRP8,SMPD1) and male infertility were observed (azoospermia and/or oligoasthenospermia).
The ABCA1 gene rs2066714 polymorphism might represent a possible risk factor for infertility susceptibility in Slovenian and Serbian men. Further studies with a larger sample size are needed to confirm these findings.

J04.16

A novel nonsense mutation in HSD17B3 gene in a Tunisian patient with sexual ambiguity

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17 β-hydroxysteroid dehydrogenase type 3 isoenzyme (HSD17B3) is present almost exclusively in the testes and converts delta 4 androstenedione (Δ4 androstenedione) to testosterone. Mutations in the *HSD17B3* gene cause HSD17B3 deficiency and result in 46 XY disorders of sex development (DSD).
The present paper is the first to report on 46,XY DSD case due to HSD17B3 deficiency in Tunisia. The two years old patient belongs to a consanguineous family, her clinical presentation and endocrinology evaluation showed a sexual ambiguity (Prader IV) and testosterone/Δ4 androstenedione ratio equal to 0.16, reflecting a defect in HSD17B3 enzyme activity (normal range is>0.8). The karyotype was realized by standard G banding technique and was 46,XY and the mutational analysis identified a novel homozygous nonsense mutation in *HSD17B3* gene in the exon 9 (c.618 C >A) leading to the substitution p.C206X. The protein lacked 105 amino acids in the C-terminal region. According to the multiple alignments, the missing 105 amino acids of the C-terminal region were almost conserved in different species. This conservation together with the absence of the mutation in 50 controls support the pathogencity and explain the 46,XY DSD observed in our patient.
In conclusion, we detected and reported, for the first time, a novel nonsense mutation leading to 17BHSD3 deficiency in a Tunisian patient. Based on the present data, the screening of this mutation could help contribute to the rapid diagnosis of HSD17B3 deficiency. The genetic confirmation of mutation in *HSD17B3* gene provides crucial information for genetic counseling and prenatal diagnosis.

J04.17

Genetic investigation of mov10L1 gene in azoospermic men with complete maturation arrest

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Thousands of genes are involved in spermatogenesis. Alterations in any of these genes could be one cause of infertility in men. Mov10L1 gene is one of the genes that are expressed specifically in germ cells. Genetic disruption of this gene in mouse stops spermatogenesis during Meiosis I and causes Azoospermia.
In this study, the genetic changes of mov10L1 gene analyzed in a population of 30 infertile patients with a complete cessation of spermatogenesis as the patient group and 70 fertile men who had at least one child as the control group.
After DNA extraction from blood samples of selected individuals, PCR-SSCP method was done to classificate individuals and ultimately sequencing was used to confirm genetic changes of the mentioned area.
Analyzing the data shows, from 30 azoospermic patients, 6 patients had (C25A) and (A101G) changes in exon 18and also(G105A) change in exon 18 was seen in only three of patients . Change (A101G) causes the Arginine amino acid convert to Glutamine at its protein level, while the other two Changes are nonsense polymorphisms. The changes were not observed in the control group.
Based on the results, it is expected that mutations and polymorphisms in mov10L1 gene could be a genetic factor in the incidence of infertility in men which requires further studies.

J05. Prenatal and perinatal genetics

J05.01

An analysis of 2000 cases of amniocentesis in our hospital over 30 years

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Amniocentesis is one of the prenatal diagnosis which is an invasive procedure. In 1966, Steele et al. performed amniocentesis first and they succeeded the fetal chromosome analysis. Our hospital performed the first amniocentesis in 1976. Since then, we have done more than 2000 cases over 30 years. When we started amniocentesis, we examined amnio fluid and fetus by feeling with the hands because ultrasonography had not developed enough. In addition, obstetrician had to cultivate cells because there was no processing section. Ultrasonography has become a common tool and it makes us to perform amniocentesis easier. We could perform amniocentesis with more information such as placentation, fetus and leiomyoma. We also could order chromosome analysis to a commercial company.
Amniocentesis is getting easier and more people would like having it, so we have to consider more carefully about the ethical and social aspects of the prenatal diagnostic examinations. Before performing prenatal diagnostic test, we have to give some information such as the maternal and fetal risk and complications and also have to discuss together what the clients would like to do after knowing the results.
In this report, we show patient's motives, age, gestational weeks and chromosome results of amniocentesis in our hospital and discuss about the ideal way of amniocentesis.

J05.02

Laboratory investigation of affected fetuses in beta-thalassemia

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introduction
The beta thalassemia is the group of blood disorder in which the function of one or both beta hemoglobin genes is affected. About 13 beta-globin mutations encompass 70 - 90% of mutation spectrum in Iran .The total annul Iran

with more than 1800 affected individuals, represents one of the areas in the word with an unusually high prevalence of beta thalassemia.
Material and Method
Couples of beta-thalassemia were referred to Pasteur Institute of Iran from Primary Health Care(PHC)center. Fetal samples of chronic villous were collected according to the gestational age .DNA extraction was performed according to standard methods by Roche kit. We used PCR -ARMS and RFLP and direct sequencing technologies, for detection mutations of fetuses.
Result
We detected mutations from 360 couples who were beta thalassemia carriers. 192 of these couples referred for prenatal diagnosis. 28 families had more than one pregnancy. The Result of our study shown that 25/2% of fetuses were affected. 24/8% were normal and remained were carrier. 60% of fetuses were compound heterozygous and 40% were compound homozygous. The expected compound homozygous were (IVS II-1/ IVS II-1) &(IVS I-5/ IVS I-5) and the expected compound heterozygous were (IVS II-1/IVSI-6),(IVS II-1/ IVS I-5),(IVS II-1/CD 8/9),(IVS II-1/CD30).
Discussion
Since the Iranian population is mixture of different ethnic group, it is necessary to determine the frequency and distribution of mutations. It helps us to diagnosis prenatal and preventing to birth of affected fetus in couples at -risk of having an affected child.

J05.03

Detection of Fragile x chromosome mutation in males with developmental delay / mental retardation

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The fragile X syndrome gene (FMR1) contains a highly variable repeat of the nucleotide triplet (CGG). A variety of clinical conditions is associated with the expanded allele sizes that predominantly affect males. Fragile X syndrome is caused by a large expansion of CGG repeat (full mutation) which silences the FMR1 gene and stops the protein (FMRP) production. An important feature of the syndrome is mental impairment which may include mental retardation, autism, etc. It is therefore important to exclude FXS whenever patients are in diagnostic procedure for developmental delay affecting mental capabilities.
For the detection of the FXS patients, a PCR-based technique, designed as an exclusion test, was used. This technique is useful for the detection of normal variants of the CGG repeat number in males and heterozygous females. The definitive diagnosis of FXS based on molecular genetic analysis, Southern blot technique, which is labour intensive and time consuming.
We analyzed DNA samples from 97 male subjects from North Eastern Slovenia referred for genetic testing because of developmental delay / mental retardation. The PCR amplification for FXS was successful in 92 patients out of 97 (94.8%). The presence of normal CGG sequence variations was detected in 91 subjects; in one patient (1.08%, 1/92) FXS was suspected and subsequently confirmed by Southern blot analysis.
Presented methodology, based only on PCR assay as screening test, is suitable as a preliminary test to exclude FXS in males or heterozygous females. With this approach we detected full mutation in 1.08% of males.

J05.04

Prenatal invasive diagnostics chromosomal pathology

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The prime value among actions for the prevention of a birth of children with a hereditary pathology belongs to prenatal diagnostics the leading part in which is taken away to invasive methods.
In 3432 cases for the purpose of diagnostics of a chromosomal pathology at a fetus in the first and second trimesters of pregnancy for prenatal karyotyping Indications for prenatal research were: the age of the pregnant woman of 35 years also is more senior, a deviation of indicators of the biochemical serum markers, ultrasonic markers of a chromosomal pathology at a fetus.
In 95 % cases (3260) have defined the normal karyotype - 46, /46, has been diagnosed. Percent of detestability of chromosomal aberrations - 5,0%. At prenatal diagnostics of a fetus it has been taped - 172 cases of a pathology. From them 83,1% cases of aneuploidy (143), the 16,9% structural aberrations: translocations, duplications, inversions (29).
Structure of a chromosomal pathology: the Down's syndrome - 47,5 % (68), Edwards's syndrome - 20,3 % (29), the Patau's syndrome - 6,3 % (9), the Turner's syndrome - 8,4 % (12), the Klainfelter's syndrome - 3,5 % (5), triplo X - 4,9 % (7),the other aneuploidy - 9,1 % (13).
Above cited data most visually reflects necessity of carrying out of prenatal invasive diagnostics at pregnant women of group of high risk on chromosomal anomalies.

J05.05

Quantitative Real-time PCR for non-invasive rapid and reliable diagnosis of Turner Syndrome

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The aim of the study is a method of non-invasive for prenatal diagnosis, which is based on quantitative real-time PCR. Materials and Method: Genomic DNA were extracted from peripheral blood lymphocytes of Turner syndrome subjects (n=15), and normal controls (n=10) that were tested by quantitative real-time PCR. This technique was applied by a MGB TaqMan probe based real-time PCR assay for rapid diagnosis of monosomy X-linked status in Turner syndrome. In the present study, we have measured and determined the gene dosage of FVIII (target gene on X chromosome) relative to PMP22 (reference gene on 17p11.2). Results: The formula ratio = 2-ΔΔCt applied for the calculation of the FVIII/PMP22 ratio. The gene dosage ratio was quel 1.005±0.00342 and 0.4867±0.00797 for normal individuals and Turner syndrome, respectively. Conclusion: This technique, including quantitative real-time PCR can be used as a rapid and standard and reliable method for rapid detection of prenatal diagnosis of monosomy X-linked.

J05.06

Investigation of Hb variants among patients referred to Pasteur institute of Iran

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Background and Objective: More than 700 hemoglobin variants have been described, of which clinically most important ones requiring diagnosis are HbS ,HbC ,HbE ,HbD Panjab and Hb O Arab. Here We have investigated the frequencies and types of Hb variants in β-globin gene among patients referred from primary health care to Pasteur institute of Iran.
Materials and methods: After obtaining informed consent, the blood samples were collected in tubes containing EDTA. Genomic DNA was extracted using the salting out method. ARMS- PCR was used for molecular characterization of HbS. The region containing exon 3 was amplified for HbD and the PCR product of this amplicon was digested by EcoRI restriction enzyme. DNA sequencing techniques were used to analyze for samples without finding any mutations.
Results and discussion:
In this study we showed that Hb D was the most common Hb variant with(50%) and after that Hb S with(36%), in order of frequency by Hb C(5.5%),Hb E(2.7%),Hb monoro(2.7%),Hb G(2.7%).These Hb variants other than Hb S are probably rare(except Hb D) because they do not provide any selective advantage for any severe disease and usually do not any health problem even in homozygous states.

J05.07

The relationship between polymorphisms & sites of beta-globin gene

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Introduction
The thalassemia syndrome the most common monogenic disorders in the world . Polymorphism in biology occurs when two or more clearly different phenotypes exist in the same population of a species . The term is also used somewhat differently by molecular biologists to describe certain point mutations in the genotype, such as SNPs(IVSII-666 , CD2,…). IVSII-666 and CD2 are located in the second intron and first exon of the β-globin gene respectively. In this research we investigated relationship between polymorphisms and haplotypes.
Materials& Method:
Informed consent was obtained in all cases before the collection of blood samples.DNA extraction was performed by salting out method.175 unrelated families of heterozygous thalassemia that referred to Pasteur Institute of Iran including carrier of α and β-thalassemia that didn't find any mutation was investigated. we were determined by direct sequencing using Big Dye

from ABI. With RFLP was studied at 3 sites within the beta-globin gene cluster, including 3`ψ βHincII, βAvaII, βHinfI.

Results:

total of them 20% had different polymorphisms there were relationship between IVSII-666 and CD2 polymorphisms with three sites within the β-globin gene cluster**.** This results were shown that about 90% pattern of AveII /β site in IVSII-666 , CD2 was -/+ . about 70% pattern
of 3`ψHincII /β site in IVSII-666 , CD2 was -/+ and in HinfI/β was +/+

Discussion:

With sequencing method we found pattern of 3 sites in the cluster of beta globin. So, These relations can help us in Prenatal Diagnostic.

J05.08

Two cases of 22q11 deletion syndrome prenatal detection

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22q11 deletion syndrome (22q11DS) is a well known syndrome with the occurrence of 1 in 4000 life births. Up to 75% of 22q11DS detected due to presence of congenital heart defect (CHD), mostly conotruncal. It is widely described and detected in pre- and postnatal diagnosis. Despite ultrasound (US) techniques improvement and specialists' awareness of the possible fetal phenotype suggestive for this deletion, 22q11DS prenatal diagnosis remains complicated because of atypical phenotype that may present and difficulties of the syndrome's earliest detection. We evaluated two groups of pregnant women referred to invasive procedures in 1st (22 cases) and 2nd (27 cases) trimesters due to US findings. Two fetuses were found to carry 22q11 deletion. Indications of the 1st trimester were increased nuchal translucency (NT) -19 (none detected) and lymphatic formations in the neck area - 3 (1 detected). In the 2nd trimester one of 20 fetuses with CHD had 22q11 deletion and none of 7 with intrauterine growth retardation and/or palatal malformations was found to carry 22q11 deletion. 22q11DS prenatal diagnostics in 2nd trimester could be proposed when US reveals CHD, e.g. conotruncal, while in 1st trimester the main US-findings are increased NT, lymphatic malformations (edemas, cysts), which may later transform to CHD. For now isolated NT is admitted as unreliable US-key for 22q11DS, so we suppose that such a rare symptom as lymphatic formations could be another indication for 22q11DS investigation in early diagnostics. Further elaboration of diagnostic criteria's for early deletion detection can help genetic consultation of these families.

J05.09

Prenatal case of Pallister-Killian syndrome in conjunction with unbalanced t(15;18)

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A 28-year-old primigravida was carried cordocentesis after an expert fetal ultrasound at 22 weeks of gestation which revealed increased nuchal translucency, hypoplasia of nasal bone, smoothed profile, intrauterine growth retardation (2-3 weeks), micromelia of predominantly rhizomelic type, a left severe congenital diaphragmatic hernia, right-sided displacement of the heart, hypoplasia of the left lung, small for the gestation age ears. GTG-banding karyotype in cultured fetal blood lymphocytes showed 46,XX,der(15). mFISH (24XCyte, MetaSystems) revealed an additional material of chromosome 18 on q-arm of chromosome 15. FISH with subtelomere 18p/q DNA-probes (TelVysion, Vysis, Abbott) showed presence of 18q-material. Paternal karyotype was normal and mother was found to have balanced translocation 46,XX,t(15;18)(q24;q21). Since ultrasound phenotype was also rather suggestive for Pallister-Killian syndrome (PKS), FISH-analysis was performed with CEP12 DNA-probe (Vysis, Abbott) on non-cultured fetal blood. Three copies of D12Z1 loci were revealed in 10% of cells. Two metaphase spreads with supernumerary i(12)(p10) also were found by FISH-analysis of cultured lymphocytes with XCAP 12 short DNA-probe (MetaSytems). Thus fetal karyotype was determined as 46,XX,der(15)t(15;18)(q24;q21)mat[58]/47,XX,der(15)t(15;18)(q24;q21)mat,+i(12)(p10)[2]. Considering severe life prognosis, the family opted for termination of pregnancy after genetic counseling. Postmortem examination corroborated ultrasound findings. Prenatal detection of PKS remains significant and also complicated because of well known tissue specific mosaicism and low presence of additional isochromosome 12p in cultured lymphocytes. Thus, if fetal phenotype is suspected of PKS, despite of normal karyotype in cultured lymphocytes, it is necessary to use FISH with chromosome-specific 12 DNA-probes on direct blood samples or another fetal tissues.

J05.10

Mutation spectrum of CD40LG gene in Russian families with X-linked HIGM

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Defects in CD40L gene are the cause of X-linked immunodeficiency with hyper-IgM type 1 (HIGM1). HIGM1 characterized high or normal by levels IgM and low IgA, IgG and IgE concentrations. The clinical manifestations of HIGM1 include current infection of respiratory ways, an intermediate pneumonia, a chronic diarrhea, oral ulcers, sclerosing cholangitis and a hepatitis. Gene CD40L includes 5 exons, 4 introns, and mapped to Xq26. In the given work we describe nine patients of a various family tree which have various mutations in CD40L gene. A search of mutations was performed by direct DNA sequencing analysis of all exons and exon-intron junctions and PCR-RFLP. The mutations identified in this research include one combined mutation (c.[744C >A;745C >A]), two splice site mutations (c.156+2T>C, c.346+1G>A), and four deletions/insertions defects (c.13_14delTA, c.158_161delTAGA, c.207_208insA, c.532delT). Two additional patients with the large deletions included exons 1-2 and exons 4-5, but exact borders of defects are not defined. As a whole, this supervision confirm heterogeneity of mutations in HIGM1. The splice site mutation c.346+1G>A and deletion c.158_161delTAGA are in hotspot for these mutations in CD40LG. Mothers of all patients were heterozygous for a mutations. Also, seven prenatal diagnostics of HIGM1 have been made.

J05.11

Unbalanced translocation with two partial monosomies in fetus with 45 chromosomes

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Constitutive unbalanced translocations are generally inherited from one parent carrying balanced translocation, and described as derivative or recombinant chromosome carrying partial monosomy of one and partial trisomy of the other chromosome. However, transmission of partial monosomy of both chromosomes of a parental balanced translocation is a very rare phenomenon. In the present case, increased fronto-nasal angle detected through routine ultrasound imaging as a measure of antenatal screening was the only deformity to consider fetal karyotyping. FISH test was also performed for quick testing of the numerical status of chromosomes 13, 18, 21, X and Y, which appeared normal. However, conventional G-banding karyotype appeared with 45 chromosomes and monosomy 21. Since interphase FISH presented two normal signals of 21, a critical analysis following high resolution banding was performed and could trace the second 21 rearranged on 9p. Therefore, the fetus was carrying 45,XX,der(9)t(9;21)(p22;q11.2) karyotype with a constitutive abnormality. Subsequently parental karyotyping confirmed mother being the carrier of t(9;21)(p22;q11.2) transmitted the unbalanced translocation. The present fetus had 45 chromosomes without the other derivative chromosome resulting in partial monosomy of both chromosomes. Therefore, it is apparent that meiotic nondisjuction, most likely maternal, resulted in aneuploid condition. Had the child inherited the normal 21 from the mother, the child would have had partial trisomy 21q which might have produced clinical expressions of Down syndrome. This is the first case to describe two partial monosomies in an aneuploid fetus with 45,XX,der9t(9p22;21q11.2). The study also describes the complementation of FISH and G-banding techniques in prenatal diagnosis.

J05.12

Gene expression profile as a prenatal test for Down syndrome

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The effect of supernumerary chromosome 21 in Down syndrome (DS) on global profile of gene expression in various cell types has been demonstrated in several previously performed studies. However, diagnostic utility of these transcriptional alterations has been poorly studied. For this reason, we performed global expression profiling to find differentially expressed genes (DEG) and subsequently performed targeted validation and diagnostic performance evaluation on a larger group of case and control samples.

Initially, transcriptomic profiles of amniocytes from 10 fetuses with trisomy 21 and 9 euploid fetuses were determined using Agilent 4x44K expression microarrays. DEG were discovered using linear regression modelling implemented in limma package. Subsequently, top DEG were validated using RT-PCR quantitation on independent sample of 16 cases with DS and 32 controls. The classification was performed using support vector machine classification kernel and evaluated using leave-one-out cross validation ap-

proach.
Global profiling has revealed 1292 DEG in trisomy 21 samples at the adjusted p-value cut-off set at 0.05. Of these, 8 upregulated and 1 downregulated genes were incorporated in the validation core set, and the significant differences in expression were confirmed in all but one gene in core set. Classification performance of this core gene set attained discriminatory rate with AUC values reaching 0.985.
In conclusion, we demonstrate good classification performance of a small expression biomarker set. As expressional alterations reflect both, causal and reactive cellular mechanisms, such biomarker may thus have potential in diagnosis of a wide array of heterogeneous diseases that result from genetic, epigenetic or environmental disturbances.

J05.13
The impact of gene-gene interaction in the development of necrotising enterocolitis in the neonates

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Background: Necrotising enterocolitis (NEC) is a widespread disability in the neonates but its developing mechanism is not completely investigated.
The **aim** of our study was to evaluate gene-gene interaction in the development of NEC in the neonates.
Methods: We conducted a case-control study of 69 neonates with NEC and 110 healthy neonates (control group). The *I/D, A1166C, G308A, C677T* polymorphism of *ACE, AT2R1, TNF-a, MTHFR* genes were detected using PCR and RFLP analysis. Statistical analysis was performed to assess the effects of all analyzed genes and their combinations (Statistica 6.0) and MDR model (MDR_2.0)).
Results: We observed significant differences of several investigated genotypes between neonates with NEC and neonates from control group [table1]. The statistical model including all investigated genes had the higher predictive value (Percentage Correct=69.3, p <0.0001), but we found no additive interaction between investigated genes.
Table 1. The results of statistic analyses

Gene (genotype)	x^2	p	OR	95% CI
ACE (ID)	2,54	>0.05	1,64	0,89-3,00
ACE (DD)	12,48	<0.001	3,6	1,73-7,49
TNFa (AG)	5,37	<0.05	2,12	1,12-4,04
TNFa (AA)	4,7	<0.05	5,14	1,01-26,3
MTHFR (CT)	10,2	<0.05	2,72	1,46-5,06
MTHFR (TT)	4,25	<0.05	3,15	1,01-9,83
AT2R1 (AC)	1,67	>0.05	1,57	0,79-3,11
AT2R1 (CC)	6,16	<0.05	6,1	3,23-30,26

Conclusion: *DD* genotype of *ACE* gene*, CC* genotype of *AT2R1* gene*, AG, AA* genotypes of *TNF-a* gene, *TT, CT* genotypes of *MTHFR* gene in the neonates is independent risk factors with high predictive value for the NEC development. The further research with including other prognostic factors may be useful for new approaches to early diagnostics.

J05.14
The association of genes polymorphism with necessity and duration of oxygen supplementation in respiratory distress syndrome cases

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Background: The role of genes polymorphism in the development and course of perinatal lung disorder in the neonates is studied insufficiently.
The **aim** of this study was to evaluate the influence of genes polymorphism on neonatal course in neonates with respiratory distress syndrome (RDS).
Methods: We conducted a case-control study of 151 neonates with RDS (including preterm, full-term) and 110 healthy neonates (control group). The *I/D, A1166C, G308A, C677T* polymorphism of *ACE, AT2R1, TNF-a, MTHFR* genes were analyzed using PCR and RFLP analysis. Statistical analysis was performed to assess the analyzed influence (logistic regression, Statistica 8.0).
Results: The risk of RDS was significantly increased for neonates with mutant allele of all investigated genes, excluding *AC* genotype of *AT2R1* gene. We found the influence of certain genotypes on neonatal adaptation course among neonates with RDS [table1].
Table 1. The influence of gene polymorphism on neonatal adaptation among RDS cases

Medical interventions	Genes polymorphism associations			
	Medical interventions	ACE I/D	AT2R1 A1166C	MTHFR C677T
Necessity of emergency care		p<0,01	p>0,05	p<0,01
Duration of hospitalization	p<0,02	p>0,05	p<0,03	p<0,01
Necessity of oxygen therapy	p<0,001	p>0,05	p<0,03	p<0,001
Duration of oxygen therapy	p<0,04	p>0,05	p<0,01	p<0,03

Conclusion: The investigated polymorphism of *ACE, TNF-a, MTHFR* genes is involved in the RDS development and necessity of medical interventions. The further analyzes may be useful for new personalized approaches in the treatment.

J06. Cancer genetics

J06.01
Association of 3a4 *1g gene Polymorphism in the development of Acute Leukemia

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Acute leukemia is a progressive malignant disease of the blood-forming organs, marked by distorted proliferation and development of leukocytes and their precursors in the blood and bone marrow. Human cytochrome P450 3A enzyme catalyzes the metabolism of exogenous and endogenous compounds. SNPs in the gene encoding this enzyme are known to be associated with altered expression and function. Hence the genetic variation within the CYP3A4 gene may contribute to interindividual variability in drug metabolism.
The present study consists of 392 acute leukemia cases collected from MNJ Regional Cancer Institute and Nizam institute of Medical Sciences, Hyderabad as well as 264 age and sex matched healthy controls. The CYP 3A4 *1G polymorphism was analyzed by PCR-RFLP technique.
Heterozygous GA genotype frequency was slightly elevated in acute leukemia patients (54.0%) when compared with controls (49.0%) indicating the presence of G allele might predispose to acute leukemia. When the data was stratified with respect to type if leukemia, the elevation GA genotype frequency was observed only in AML group.
Attempt was also made to evaluate the interaction of genotype with confounding epidemiological variables like age at onset, occupation, habits and area of living as well with clinical variables like WBC count, Platelet count and complete remission rate.

J06.02
Antimutation and anticancer effects of Morin in human cutaneous T cell lymphoma

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At present cancer is one of the mortality factors in the world and treating it debilitates the patient.Therefore,prevention can be considered as important as treatment in cancer.Diet can play a vital role in cancer prevention. Nowadayes the scientists are looking for natural food which can prevent the cancer occurrence.The purpose of this research is to examine antimutagenicity and
anticancer effects of morin in Cutaneous Human T cell Lymphoma(Sezary syndrome).
In this Experimental study HUT-78 cell line were cultured in %90 RPMI1640 , supplemented with10%fetal calf serum,l-glutamine,peniciline,streptomycin and then incubated at 37°cfor2days.The cancer cell line was treated by different morin concentrations and cellular vital capacity was determined by MTT.The morin was subsequently evaluated in terms of antimutagenicity and anti cancer properties by a standard reverse mutation assay[Ames test This
was performed with histidine auxotroph strain of Salmonella typhimurium[TA100] .Thus, it requires histidine from a foreign supply to ensure its growth.The aforementioned strain gives rise
to reverted colonies when expose to carcinogen substance [Sodium Azide].
During MTT,Tcell lymphoma cancerous cells reveaed to have a meaningful cell death when compared with controls[p<0.001].In Ames test morin prevented the reverted mutations and the
hindrance percent of morin was 98.16 % this value in anticancer test was was 99.23 %. These results have revealed morin has anticancer and antimu-

tagenesis effects in the Sezary Syndrome in vitro.

J06.03

Dose- and Time-Dependent Response of Human Leukemia Cell Line, NB4, to Arsenic Trioxide Treatment

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Background: Although arsenic trioxide was shown to be a potential drug in treatment of APL, most notably in patients with relapsed APL, the nderlying mechanisms remains unclear. In this study, the cytotoxicity effect of ATO on APL cancer cells was evaluated.
Material and methods: In this basic-applied study, the human leukemia (NB4) cell line was used as a model to evaluate the cytoxicity effects of arsenic trioxide in APL cells. NB4 cells were exposed to different concentrations of ATO (0.5, 1, 2 µM) for 2, 4 and 6 days and dimethylthiazol diphenyl tetrazolium bromide (MTT) assay was applied on them.
Results: Data obtained from this assay indicated that arsenic trioxide significantly reduced the viability
of NB4 cells and inhibited cell growth in a time and dose dependent manner.
Conclusion: Findings from the present study indicate that arsenic trioxide is highly cytotoxic to human leukemia cells, supporting its use as an effective therapeutic agent in the management of acute promyelocytic leukemia.

J06.04

Is The BCL-2 Prompter (-938C>A) Polymorphism Associate with IRANIAN Breast Cancer Patients Susceptibility?

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Background and aim: Breast cancer is the first most common
cancer among the females in Iranian cancer patients. Apoptosis and cellular proliferation play role an important role during normal mammary development &carcinogenesis of the mammary gland.Bcl-2 is the one of the most important anti apoptotic genes.Bcl2 gene has been demonstrated with breast cancer development and a single nucleotide polymorphism (SNP-938C >A) has been identified recently.The aim of this study was to identify whether Bcl-2(-938C>A) polymorphism which is located in the inhibitory P2 promoter of Bcl-2 is associate with breast cancer ,as well as clinicopathological characteristics. Materials and methods: patients and tissue specimens: tissue samples were obtained from 34 consecutive patients with BC from IRANIAN National Tumor Bank ,National Cancer Institute, Imam Khomaini Hospital Complex,
Medical Tehran University,Tehran,Iran. Histopathological examinations were performed, and all tumors were confirmed as adenocarcinoma. Mutational analysis of Bcl-2 (-938C> A) in tumour samples: fresh tumours and their adjacent were extracted for genomic DNA using the QIAamp Mini Kit . We searched for
-938C>A in this gene.The Bcl2 (-938C>A) analysis were made by means of PCR sequencing. Result and conclusion:
10 of 34 (29%) samples were analyzed. The frequency of polymorphism in Bcl2 (-938C>A) was not present in the samples (0%).It also was not a significant associated with histopathological grade, age or cancer stage in Iranian Breast Cancer Patients. Although some study have been shown in BC,Bcl-2(-938C>A) polymorphism, our study was not shown and more samples should
be investigated in Iranian Breast Cancer patients.

J06.05

High prevalence of A15326G alteration in mitochondrial complex III in Iranian breast cancer patients.

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Mitochondria play a decisive role in the regulation of apoptosis and thereby exhibiting major changes in their structure and function. A decrease in mitochondrial membrane potential is an early universal event of apoptosis. Numerous somatic mitochondrial DNA (mtDNA) mutations have been found in various types of neoplasms, including breast cancer. Cytochrome b (CytB) of mitochondrial electron transport complex III is encoded in mtDNA 14747-15887 and has also been reported mutated in a large variety of human tumors as breast cancer. Establishing the mtDNA mutation pattern in breast cancer cells may enhance the specificity of cancer diagnostics, detection and prediction of cancer growth rate and/or patients' outcomes; and therefore be used as a new molecular cancer bio-marker. Mitochondrial DNA of 24 patients comprising the coding complex III was analyzed by PCR-sequencing methods. The aim of this study is to summarize data on mtDNA mutation involvement in breast cancer and estimate effects of resulting amino acid changes on mitochondrial protein function. Also T14783C, T14798C, A14820C, G14831A, C14872T, C14929T, C14953T, G15043A, G15110A, T15115C, G15148A, A15158G, A15203G, A15283G, G15301A, C15452A, A15488T, T15514C, G15617A, G15768A, T15793C polymorphisms were found in cyt b.
The polymorphism A15326G resulting in the change of T194A in the Cytb protein was found in the large majority (>95%) of patients. So we think this polymorphism may use as biomarker in breast cancer but more investigation needs to prove it.

J06.06

C14766T polymorphism in mitochondrial complex III as biomarker in Iranian breast cancer patients ?

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In most pathways of apoptosis, the release of mitochondrial cytochrome b and apoptosis-inducing factor are also key events in initiating the cascade of reactions leading to apoptotic cell death. Mitochondria play a decisive role in the regulation of apoptosis. Mitochondrial dysfunction is relevant to the genesis of many types of cancers , including breast cancer. Numerous somatic mitochondrial DNA (mtDNA) mutations have been found in various types of neoplasms, including breast cancer. Cytochrome b (CytB) of mitochondrial electron transport complex III has been reported mutated in a large variety of human tumors as breast cancer. Mitochondrial DNA of 24 patients comprising the C14766T was analyzed by PCR-sequencing methods. The aim of this study is to summarize data on mtDNA mutation involvement in breast cancer and estimate effects of resulting amino acid changes on mitochondrial protein function. Amongst patients with breast cancer, this alteration is present in 33.3% of affected females. The polymorphism C14766T resulting in the change of Threonine to Isoleucine missense mutation in the Cytb protein was found in the large majority (33.3%) of patients. It is therefore possible that the C14766T SNP constitutes an inherited predisposition factor for the development of breast cancer.So we think this polymorphism may use as biomarker in breast cancer but more investigation needs to prove it.

J06.07

A12308G alteration in tRNA Leu(CUN) as a biomarker or usual polymorphism in Iranian breast cancer patients?

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Mitochondrial dysfunction is relevant to the genesis of many types of cancers , including breast cancer. mitochondrial tRNA genes perform several functions including processing and translation and are essential components of mitochondrial protein synthesis. Until now only few somatic mitochondrial tRNA mutations have been reported in cancer cells. In this study all 22tRNA genes in 24 Iranian females with breast cancer were investigate by PCR-Sequencing methods. A novel homoplasmic C12187T mutation located at the Tloop site for the tRNAHis and A12308G, G12192A, T15968C polymorphisms in TrnaLeu, tRNAHis and tRNAPro were found respectively. The mtDNA A12308G polymorphism is highly conserved between species during evolution and also encodes for the most represented amino acid in the oxidative phosphorylation, suggesting a key role of this tRNA in mtDNA-coded OXPHOS subunits. Inaddition, recently have been shown that breast cancer cell lines with the higher mutation frequency of A12308G are highly metastatic. Amongst our Iranian patients with breast cancer, this alteration is present in 23% of affected females. This SNP has been also reported in many types of disease as Alzheimer, Ataxia, CPEO, LHON, MERF, MELS and...

in Iranian patients. So it needs more investigate to prove the role of this alteration as a common polymorphism or an inherited predisposition factor for the development of breast cancer.

J06.08

Cisplatin and MNPs(Fe3O4) Synergistically Alter Apoptotic Genes Expression

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Breast cancer is a common cancer in women. Cisplatin is an anticancer drug. There is high rate of cisplatin resistance in breast cancers, therefore cisplatin is not the first choice in treatment of breast cancer. The mechanism of cisplatin is interaction with DNA and induction of apoptosis. Apoptosis is a common pathway that finally mediates the killing functions of cisplatin anticancer drug. In order to induce apoptosis, many gene functions must be altered. In this study, MCF-7 breast cancer cell line was used. The aim of this study was investigate the potential benefit of combination therapy with magnetic nanoparticles of Fe_3O_4 (MNPs(Fe_3O_4)) and cisplatin. Viability of cell was studied by MTT assay and gene expression was studied by RT-PCR. Analysis of viability percentage and apoptotic genes expression alteration showed that combination of cisplatin and MNPs(Fe_3O_4) have a potent cytotoxic effect on MCF-7 breast cancer cell lines. Combination of cisplatin and MNPs(Fe_3O_4) reduced IC50 of drug in 24 h from 42 µM to 10.35 µM in MCF-7 cell lines. MNPs(Fe_3O_4) and cisplatin can synergistically enhance induction of apoptosis. Cisplatin by itself can not change *BCL2* expression in MCF-7 cell line, but combination of MNPs(Fe_3O_4) and cisplatin can reduce expression of an antiapoptotic gene ,*BCL2*, after 48 h and 72 h. Thus cisplatin therapeutic dose can be reduced and consequently cisplatin side effects can be decreased. Thus our *in vitro* data strongly suggest a potential application of a combination of MNPs(Fe_3O_4) and cisplatin for treatment of breast cancer cell lines.

J06.09

Up regulation of NM23H1 a metastasis suppressor gene in the MCF-7 breast cancer cell line treated by cisplatin

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Breast cancer is one of the most common cancers in developed countries. Most of cancer deaths are due to the development of metastasis. Nm23-H1 gene is an anti metastatic factor whose expression is correlated inversely with tumour metastatic potential in breast. Nm 23-H1 encodes nucleoside diphosphate kinase A, which is responsible for the synthesis of most non-ATP nucleoside triphosphates, suggesting that this protein might be involved in a wide variety of biological phenomena in the cell. Cisplatin is the key anticancer drug in the therapy of a wide spectrum of cancers. In the present study, the expression of Nm23-H1 gene in MCF-7 cells treated with different concentrations of cisplatin at 24h was evaluated.

In this study, MCF-7 cells were treated with different concentrations of cisplatin at different times. The IC50 was determined. RNA was extracted by RNX Solution. Then cDNA was synthesized. Precise primers were designed for Nm23-H1 and TBP genes by specific software. Quantity of Nm23-H1 gene expression compare to TBP gene in different concentrations of cisplatin was analyzed using very sensitive quantitative Real-time PCR.

Nm23-H1 gene expression in MCF-7 cells treated by different concentrations of cisplatin at 24h was increased.

The results of quantitative Real-time PCR indicated that cisplatin can probably decrease metastasis, by up-expression of Nm23-H1 metastasis suppressor gene in MCF-7 cells.

J06.10

Quantitative detection of Major BCR-ABL gene transcripts by competitive RT-PCR in chronic myeloid leukemia(CML) patients

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Chronic Myeloid Leukemia (CML) is a form of leukemia with chromosomal translocation and fusion bcr/abl genes. The purpose of this study is development of a simple and low-cost technique for
quantitative detection bcr/abl transcripts in CML patients.
In this study, we used the competitive RT-PCR technique to determine the number of bcr/abl transcripts. The internal control designed from a non-human DNA source with the same flanking sequences but a smaller size than the target segment. After amplification and purification of internal control DNA, PCR products quantified based on copy numbers. In order to optimizing the reaction condition, competitive
RT-PCRs carried out separately on target and internal control DNAs each with specific copy numbers and final products of all concentrations runed on agarose gel electrophoresis.
The simultaneous reactions of target RNA with different copy numbers of internal control DNA that
showed the copy numbers of target RNA can be determined by comparison of dye density emission of DNA bands(target and control DNA) on the gel.
This study shows that competitive RT-PCR is a partially efficient and cheap method for quantitative
determination of bcr/abl transcripts, compared to other methods such as Real-time PCR.

J06.11

Antimutagenicity and Anticancer Effects of mother's milk on Acute Promyelocytic Leukemia(APL) cell line

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Currently cancer is considered as one of the main factors of mortality globally. Diet can play a vital role in cancer prevention. The purpose of this research is to examine antimutagenicity and anticancer effect of mother's milk.In this basic-applied study, the human leukemia (NB4) cell line was used as a model to evaluate the cytoxicity effects of mother's milk.in APL cells.NB4 cells were exposed to different concentrations of mother's milk (2, 5, 10 µl) and dimethylthiazol diphenyl tetrazolium bromide (MTT) assay was applied on them. The mother's milk was subsequenthy evaluated in terms of antimutagenicity and anticancer properties by a standard reverse mutation assay
(Ames Test). For Ames Test the particularity of the strain of salmonella typhimurium chosen TA100 resides in the fact that undergone a specific mutation in the Histidine operon,and for this same reason it requires histidine from a foreign supply to ensure its growth.The afore mentioned strain gives rise to reverted colonies when expose to carcinogen substance (Sodium Azide).Data obtained from this assay indicated that mother's milk significantly reduced the viability of NB4 cells and inhibited cell growth in a dose dependent manner. In Ames Test Mother's Milk prevented the reverted mutations and the hindrance percent of mother's milk was 46% in Antimutagenicity test and this value in anticancer test was 53%. Findings from the present study indicate that mother's milk is highly cytotoxic to human leukemia cells.These results have revealed antimutagenicity and anticancer effect of mother's milk.

J06.12

Limitations in management of neuroblastoma in children - a retrospective analysis

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Background: Last decades have brought great improvement in survival rate in neuroblastoma (NB) in children, due to precocious diagnosis and a multimodal approach. Still, several limitations remain in current practice. Aim: To evaluate factors that influenced survival rate in children with NB. Material and method: We proposed a retrospective analysis of cases admitted to our clinic between 1996-2011. Thirty-four children diagnosed with NB were selected. Onset age was between 11 months and 12 years, sex ratio male:female was 1:1. We performed a retrospective analysis of medical charts. Kapplar-Meier survival curve were calculated. Citostatic (protocol NBL 98) and surgical treatment were performed in all patients. Results: Statistic data regarding localization of primordial tumor, stabilization, regional and distance metastasis spread; along with paraclinical data: histological analysis, vanilmandelic acid, LDH, feritin levels are discussed. Molecular biology and DNA index cannot be assessed in our clinic. Complications related to disease were: distance metastatic spread in half of children; locoregional metastasis in 29,16% cases, intestinal occlusion in 4,1%, blindness in 4,1%, paraplegia in 4,1%. Two cases presented relapse of disease. Survival at 3 years was seen in 45,8% cases, 41.6% deceased and 12.5% were lost to follow-up. Conclusions: NB was diagnosed late and with advanced stage, in our population and this correlated with unfavorable evolution and complications related both, to disease and treatment. Genetic assessment is very important in guiding treatment differentiation, which finally prolongs survival rate in patients with NB. Improvement in this area is much needed in our department.

J06.13

IgY production against 3 epitop of the hauman DR5

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The proinflamatory cytokine, tumor necrosis factor- α (TNF-α) plays a important role in diverse cellular events such as septic shock, induction of other cytokines, cell proliferation, differentiation, necrosis, Malaria, AIDS and apoptosis. In response to TNF therapy several cell signaling pathways activated in ells that in different manner can lead to apoptosis or necrosis. However induction of witch of them is dependent on the receptors that occupied with TNF-α and subsequently activation molecules. Tumor necrosis factor- related apoptosis- inducing ligand (TRAIL) and specially the DR5 one, is generating considerable interests as a possible anticancer therapeutic agent because of is selective activation of apoptosis of this receptor as a superior affinity to ligands (TNF) show up and injected to separate hens to achieve high affinity IgY that can recognize DR5 specially and start exclusively apoptotic signaling in the cell.

J06.14

GSTP1 gene methylation profiles in Helicobacter pylori (+) and (-) antral intestinal metaplasia and distal gastric tumour patients in Turkish population

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Background /Aim: Gastric cancer (GC) is the second most common malignancy worldwide, with a high mortality rate. The incidence of GC has declined in the western countries during the last decades. The Glutathione S-transferases comprise a group of enzymes that are critical in the detoxification of carcinogens. In this study we aimed the relationship GSTP-1 methylation in patients with intestinal metaplasia with and without helicobacter pylori infection, gastric cancer and controls.

Methodology: The methylation status of GSTP1 gene between September 2009 to November 2011was analyzed by Methylation Specific PCR after bisulfate modification in H. pylori (+) (n=25) and (-) (n=25) intestinal metaplasia (IM) patients, GC (n=25), and control subjects (n=15) who underwent endoscopic examination were included into the study

Results:When we considered the GSTP1 gene methylation profile in all of the groups; 26 (28%) patients had methylated GSTP1 gene, 31 (34%) patients had unmethylated GSTP1 gene and 33 (36%) patients had heterogeneously methylated GSTP1 gene.

There was no significant difference in the methylation profile between the four groups (p>0.05).Unmethylation in the control group was more prevalent than GC and H.Pylori (+) IM (p=0.015).Unmethylation in H.Pylori (-) IM was more prevalent than H.Pylori (+) IM (p=0.00004). Heterogeneous methylation in GC and control group was more prevalent than H.Pylori (-) IM (p=0.00002, 0.025).

The relationship between H.pylori and GSTP1 methylation profile, there was no significant difference in metyhlation, unmethylation and heterogeneous methylation between the groups.

Conclusion: GSTP1 gene methylation profile is not appropriate for early diagnosis of cases with gastric cancer.

J06.15

Molecular genetic characterization of intratumoral heterogeneity in invasive ductal NOS breast carcinoma

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Aim: To characterize the different types of morphological structures, previously demonstrated in tumors of invasive ductal breast carcinoma not otherwise specified, NOS (Zavyalova et al., 2011), and their local microenvironment with cancer-associated SNPs and expression of multidrug resistance (MDR) genes.

Materials and methods: Tubular, alveolar, trabecular morphological structures and their local microenvironment were isolated by laser microdissection from 10 micron-thick sections of FFPE breast tumor. The isolated and whole amplified DNA and cDNA (RNA) were used for analysis of SNPs with the Cancer SNP Panel (Illumina) and assessment of expression of MDR genes: ABCB1, ABCC1, ABCC2, ABCC5, GSTP1, and MVP.

Results: SNP array emerged as an effective tool to detect somatic alterations. Despite of low microarray call rate, probably caused by degradation of DNA during formalin fixation, the Cancer SNP Panel demonstrated significant differences in genotypes both among the different types of morphological structures and their local microenvironment. In particular, the genotype variations affected rs6179 (GHR gene), rs3740616 (LMO2), rs717620 (ABCC2), rs4623993 (k-Ras), rs1050008 (MX1), rs569421 (GATA3), and rs529359 (PGR). MDR genes expression acts as indispensable hallmark of cancer. Similar to the SNP results, their expression was significantly differed between both the studied types of morphological structures and their microenvironment. The differences were displayed in both number (type) of expressing genes and level of expression.

Conclusion: Data obtained here show that the different types of morphological structures of breast tumor may be independent tumor clones with own specific microenvironment.

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J06.16

Origin and clonality of primary multiple superficial bladder cancers

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Primary multifocality is a common phenomenon in superficial bladder cancer (SBC). According the monoclonal theory, coexisting primary tumors arise from a single malignant transformed cell, which proliferates and spreads throughout the urothelium or might be spread during the intravesical manipulations. Another theory explains multifocality as subsequent events secondary to a field-cancerization effect, so tumors are expected to be genetically non-identical. The question weather coexisting tumors arise from the same tumor clone or develop independently has a great clinical relevance to surgery and treatment approaches. Molecular analysis of alterations patterns in each of coexisting tumors may give us an answer.

We examined tumor samples from 22 patients with primary multiple SBC (PMSBC) (2-5 tumors/patient). Genomic DNA samples were prepared from formalin-fixed, paraffin-embedded sections. Our panel included LOH analysis at 9p21 and 17p13 (microsatellite assay).

Three out of 22 (13.6%) patients had non-informative state of microsatellite markers. In nine out of 22 patients (40.9%) was shown concordant pattern of LOH in at least one of loci. In ten out of 22 (45.5%) patients we showed discordant patterns of allelic alterations. Moreover, in 5 of these 10 cases (50%) tumors of the same patient differed from each other by presence or absence of allelic misbalance, while in other 5 cases (50%) LOH of different allele in tumors of the same patient were revealed.

Our results show that coexisting multiple tumors show in almost equal proportion either concordant of discordant pattern of molecular alterations, which might mean monoclonal or oligoclonal origin correspondently.

J06.17

Investigation of microRNA expression changes in HepG2 cell line in presence of URG4/URGCP and in the absence of URG4/URGCP suppressed by RNA interference

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Hepatocellular carcinoma (HCC) originates from liver cells and is one of the most common malignant cancers in the world. MicroRNAs (miRNA), are single strand non-coding RNA molecules with the length of 18-25 nucleotides. miRNAs play an important role in the development of HCC, briefly miRNAs have a significant impact on multistep hepatocellular carcinogenesis including cellular migration and invasion. URG4/URGCP (Up-regulated gene-4/ Upregulator of cell proliferation) is up-regulated in the presence of HBxAg and has been identified and characterized by Satiroglu-Tufan et al. The full-length URG4/URGCP clone is 3.607 kb. Overexpression of URG4/URGCP in the presence of HBV X protein may function as a putative oncogene that contributes importantly to multi-step hepatocarcinogenesis. In this study, we aimed to investigate potential miRNA expression changes in HepG2 cell line model system in the presence of URG4/URGCP and in the absence of URG4/URGCP which was suppressed by RNA interference. To functionally characterize URG4/URGCP, independent cultures of HepG2 cells were stably transfected with pcDNA3 or pcDNA3-URG4/URGCP. Relative quantification of whole genome miRNAs was analyzed by Light Cycler 480 Real Time PCR using Human Whole Genome miRNA qPCR Profiling Kits. Among the 1034 human miRNAs investigated by the arrays, 77 miRNAs were up-regulated and 9 miRNAs were down-regulated in the presence of URG4/URGCP. In conclusion, we have comprehensively analyzed miRNA profiles in the HepG2 cells with the presence or absence of URG4/URGCP gene. Some of these miRNAs may play roles in the URG4/URGCP gene related disease development through the regulation of different signaling pathways.

J06.18

Differentially methylated genes in breast cancer

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Breast cancer (BC) is characterized by abnormal DNA methylation. To date the methylation status of many oncogenes and tumor suppressor genes has been investigated. However the genome-wide analysis of methylation status of many genes is necessary for identification of pathogenetic pathways of cancer development. This study was aimed to identify the functional groups of genes abnormally methylated in BC. We analyzed 16 samples with BC and 6 control histologically normal epithelium samples from women with BC using Illumina GoldenGate Cancer Panel I (Illumina, USA). Differential methylation was observed at 318 CpG-sites. Among them 252 (79.2%) were hypermethylated and 66 (20.8%) were hypomethylated. The identified differentially methylated genes were analyzed using Gene Ontology Enrichment Analysis. Hypermethylated genes belong to two functional groups: positive regulation of cell differentiation (n=8) and regulation of cell proliferation (n=12). Hypomethylated genes belong to two groups as well: cell migration (n=2) and the protein amino acid phosphorylation (n=3). Hypermethylation of genes involved in positive regulation of cell differentiation and proliferation may indicate the important role of their epigenetic inactivation in BC development. Hypomethylation of genes involved in cell migration and phosphorylation of proteins may promote invasiveness, metastasis of cancer cells, and inhibition of tumor suppressor proteins by phosphorylation. Thus, the abnormal methylation status of these functional groups of genes may play a significant role in breast cancer development.

J06.19

Elucidation of SALL4 oncogenic role in colorectal cancer; the first report

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Human cancer cells resemble stem cells in expression signatures leading to share some features notably self-renewal. A complex network of transcription factors and signaling molecules are required to continuance of this trait. SALL4 is zinc finger transcriptional activator crucial for maintenance of self-renewal in stem cells which its expression rate is not yet elucidates in colorectal tumor cells. To clarify this rate and probable clinicopathological consequences, an expressional analysis was performed. Freshly tumoral and distant tumor-free tissues of thirty eight colorectal samples were enrolled to comparatively examine the expression level of SALL4 by real-time PCR. Compare to normal tissues, greater than two-fold expression of SALL4 was interestingly detected in 89.5% of related tumors. SALL4 expression was significantly correlated with the number of involved lymph nodes especially in moderately differentiated tumor samples (P<0.05). Furthermore, higher levels of SALL4 mRNA expression was significantly associated with older age of patients whom their tumor cells were being in the lower stages (I/II) (P<0.05). These results elucidate the direct impact of SALL4 overexpression on the process of tumor cell metastasis to lymph node and consequently development of tumors into advanced stages (III/IV). Along with the promising evidence of its role in self renewal in various cancers, SALL4 is introduced as potentially interesting therapeutic targets to reverse a number of aberrations that promote colorectal tumor maintenance and development. In conclusion, we presented the first evidence for mRNA overexpression of oncogene SALL4 in cancerous colorectal samples which might enlighten new approaches for cancer therapy.

J06.20

Gene-gene interaction predicts chemotherapy response in multiple myeloma patients

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Background. Multiple myeloma (MM) is plasma cell neoplasm with low sensitivity to alkylating agent-based chemotherapy. The aim of study was to evaluate gene-gene interaction in the development of drug resistant cases.
Methods. We examined 51 newly diagnosed patients treated alkylating agent-based chemotherapy. 30 patients had clinical response and 21 patients had no response.
The deletion polymorphism of *GSTT1, GSTM1* genes and *A313G* of *GSTP1, C3435T* of *MDR1* genes polymorphism were detected using PCR and RFLP analysis. Statistical analysis was performed to investigate the influence of all analyzed genes and their combinations (MDR_2.0 Programme).
Results. The main effect was found for *GSTM1* deletion polymorphism [Table 1]. The interaction model including *GSTT1, GSTM1, MDR1* genes had higher Testing Balance Accuracy - 83,10.
Table1. Testing balanced accuracy and cross validation consistency for the best MDR models for the prediction of chemotherapy resistance

Gene	Testing Bal. Acc.	Permutation test	CV consistency
GSTM1	0,8286	0,001	10/10
GSTT1/GSTM1	0,7476	0,05	8/10
GSTT1/GSTP1/MDR1	0,8310	0,001	10/10
GSTT1/GSTM1/GSTP1/MDR1	0,7918	0,05	10/10

Conclusion: We propose to evaluate gene-gene interaction for drug resistance prediction in MM patients. Further research may develop new methods of chemotherapy resistance prevention.

J06.21

Examination of UVR-Induced DNA damage and repair and its association with apoptosis in human keratinocytes and fibroblasts

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Skin cancer has an increasing incidence in countries with large populations of white skinned individuals. UVR, by initiating the DNA damage, can lead to mutagenesis and is regarded as the prime cause of most skin cancers. As a result, UVR protection is of primary importance to prevent UVR-induced skin cancers. Cyclobutane pyrimidine dimers (CPD) are an important form of DNA damage induced by both UVA and UVB and removed by nucleotide excision repair. The persistence of CPDs, compared to other forms of DNA damage, is understood to be a major contributory factor to their mutagenicity. Using the T4endonuclease V-modified comet assay on human keratinocytes and fibroblasts, we noted that there was rapid initial repair of CPDs over the first 6h post-irradiation, following either UVA or UVB treatments, but whilst this slowed significantly in the UVB-irradiated cells, it continued to be rapid in the UVA-treated cells with levels approaching baseline within 36h. This confirmed the widely accepted slow repair of UVB-induced cyclobutane thymine dimers, but we uniquely noted far more rapid repair of UVA-induced cyclobutane thymine dimers. There were no significant differences in cell viability between the two treatments over the first 6h post-irradiation, but at 24h post-irradiation viability had decreased significantly only in the UVB-irradiated cells. These data suggest that for at least the first six hours following UVB irradiation, the majority of cells were viable and capable of repair, after that time increasing numbers of cells enter apoptosis, and therefore fail to repair the damage.

J07. Cancer cytogenetics

J07.01

Symmetric nuclei support PMN chemotaxis and reflect genomic order

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Segmentation, condensation and bilateral symmetry of the nuclei of polymorphonuclear leukocytes seem related to their function. Segmentations of the nuclei into two or more lobes and their condensation facilitate their passage (diapedesis) through the endothelial layer of blood vessels to the extravasal space and subsequent locomotion through the interstitial compartment of different tissues. Bilateral symmetry of these nuclei along with their association to the cytoskeletal fibres contribute to their efficiency in locomotion by alignment of the axis of nuclear symmetry to the axis of cellular polarity, which orients towards the direction of locomotion in response to cytokines and other stimuli. Moreover this same mechanism may maintain genomic order in spheroid nuclei of mononuclear cells all through the cell cycle and support and coordinate the allelic functions. Our observations of the cytogenetic facets of intranuclear order in blood and bone marrow cells support these assumptions.

J07.02

Complex chromosomal clones in hematological malignancies: Study on 50 cases

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Hematological malignancies are well understood with the consequences of chromosomal alterations and likelihood of treatment outcome. Employment of advanced technologies in the field facilitates better understanding of diseases with an aim in drug development. However, conventional cytogenetics plays an important role for primary diagnosis, monitoring and relapse management. During January 2012, chromosomal analysis was carried out on 50 cases with leukemia and myelodysplastic syndrome by employing conventional bone marrow culture and G-banding. Karyotypic analysis by using IKAROS had detected multiple clonal abnormalities with rare chromosomal rearrangements including numerical and structural alterations (Table). Normal karyotype and classical abnormalities were recorded in 32% and 42% cases respectively whereas 26% cases were detected with multiple complex rearrangements. Two AML cases were recorded with t(9;22) in association with monosomy 7. One case with provisional diagnosis of AL showed three double minutes in cells with varying numerical abnormalities. Case wise detail description on chromosomal rearrangements and numerical alterations will be presented with clinical and hematological expressions. Such complex structural and numerical or both rearrangements might indicate poor prognosis and interfere in treatment outcome. The study highlights the importance of conventional cytogenetics in management of hematologic malignancies and strongly recommends its application in the field.
Table: Data on chromosomal analysis on 50 leukemia cases

Provisional diagnosis (no.=50)	Case-wise chromosomal data			
AL (4)	Multiple clonal abnormalities with 31%: 48, 22%:51, 47%:46,XY,der(14)t(10p;14q32)	90%:47,XY,+8,t(10p;16q), del(13q), del(20q)	80%:complex rearrangements & 39-46 chromosomes	Multiple clones with 3 minutes with 46-54 chromosomes
ALL (2)	46,XY	100%: 46,XY,t(4;11),i(12q)		
AML (9)	3 cases: 46,XX	45,X,-Y,t(8;21)(q22;q22)	76%:45,XY,-7,t(9;22)(q34;q11) 24%:46,XY,-7,t(9;22)(q34;q11),+Ph	45,XX,-7,t(9;22)(q34;q11), breaks
	30%:Hypodiploid with 41-45 chromosomes	46,XY,t(15;17)(q22;q21)	47,XX,+11,t(16p;16q)	
Anemia (3)	1 Fanconi anemia	2 aplastic anemia		
CML (10)	46,XY	7 cases: 46,XY,t(9;22)(q34;q11)	75%:46,XX,del(3p),t(9;22)(q34;q11) 10%:48,XX,del(3p),+8,t(9;22)(q34;q11),+19 15%: 49,XX,del(3p),+8x2,t(9;22)(q34;q11),+19	
		46,XY,t(1;4), *BCR-ABL* -ve (FISH) and hyperdiploid		
MDS (20)	11 cases with normal karyotype	45,X,-Y	46,XY,+breaks	46,XX,+breaks
	46,XX,del(5q),-11, der(12) t(11q;12p),+mar	46,XY,del(5q)	90%:45,XY,-7 and 3: hyperdiploid	46,XY and 2: hyperdiploid
	12%:46,X,-Y and 2 cells with octaploidy	50%: 49,XX,+4,+6,+8		
Lymphoma (2)	30%: 46,XX,t(14;18) - DLBCL	75%: 47,XX,+21 and breaks		

J07.03

Radiation-induced modification of human somatic cells' chromosomes sensitivity to testing mutagenic exposure of bleomycin in vitro in lung cancer patients in delayed terms following Chernobyl accident

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With the help of modified „G2-bleomycin sensitivity assay" above background level of cytogenetic effect which considered as the marker of hidden chromosome instability (HCI) in 4 groups - liquidators of Chernobyl accident, liquidators of Chernobyl accident with lung cancer, unexposed healthy persons, patients with lung cancer who denied conscious contact with ionizing radiation were cytogenetically observed. In all groups significant interindividual variations of cytogenetic effects induced by bleomycin in vitro in testing concentration 5.0 mcg/ml and lack of positive correlation between background and above background frequencies of chromosome aberrations were found. It was established that group of Chernobyl liquidators with lung cancer had been the most aggravated as by expression of above background cytogenetic effects (77.3 aberrations per 100 metaphases) as by number of persons with HCI (66.6%) in comparison with 14.3, 42.4, 53.4 aberrations per 100 cells; 33.3, 46.7, 53.3% hypersensitive persons in unexposed healthy donors, unexposed patients with lung cancer and Chernobyl liquidators without cancer, accordingly. The data obtained not only confirmed possibility of radiation-induced modification of genetically determined susceptibility of human somatic cells' chromosomes to mutagenic exposure, but also permit to suggest existence of an association between radiation-induced increase of individual sensitivity to testing mutagenic exposure and realization of cancer in persons exposed to ionizing radiation. The results obtained showed acceptability of «G2-bleomycin sensitivity assay» under cytogenetic monitoring of irradiated contingents for determining HCI as one of the informative markers of human predisposition to oncopathology.

J07.04

Identification of a new rearrangement involving chromosomes 6 and 11 in a juvenile myelomonocytic leukemia with mutation in CBL gene

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Juvenile myelomonocytic leukemia (JMML) is a rare and aggressive clonal myeloproliferative disorder of infants and young children, refractory to chemotherapy and responding only to hematopoietic stem cell transplantation. Somatic or germinal mutation in *PTPN11*, *RAS*, or *NF1* and monosomy 7 are known to be the essential genetic or chromosomal abnormalities in this disease. Missense *CBL* mutations, mutually exclusive of other *RAS* activating mutations, have been recently found in JMML, arising de novo or inherited with invariable loss of the normal *CBL* allele in leukemic cells. A 3-month-old boy was referred to us for hepatosplenomegaly, fever, leucocytosis with monocytosis, presence of myeloid precursors, bilinear cytopenia, and normal level of HbF. Hypersensitivity to GM-CSF was observed in the colony assay. A diagnosis of JMML was made. Molecular studies showed no *NRAS*, *KRAS2*, or *PTPN11* mutations, but a c.1246 T>C heterozygous missense mutation in exon 9 of *CBL* gene was found, resulting in p.416C>R amino acid sostitution.

Likely, the homozygous mutation was not detected since bone marrow with low level of leukemic cells was used for analysis. The chromosome analysis on BM and peripheral blood showed 46,XY,t(11;16)(p15;q23)/46,XY karyotype. FISH analysis with specific probes for *NUP98* gene (11p15) excluded the involvement of this gene in the breakpoint. The cases of JMML characterized by chromosomal translocations as sole cytogenetic abnormality are rare and described in individual cases. The importance of *CBL* in hematopoiesis has been demonstrated, however the effect of this chromosomal rearrangement on the propensity to develop JMML has to be clarified.

J08. Statistical genetics, includes Mapping, linkage and association methods

J08.01
Association polymorphisms of IL-18 genes (137G/C, 607C/A,133C/G) in patients with allergic rhinitis in the Iranian population

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Background: Allergic rhinitis (AR) is an inflammatory disease of the nasal mucosa induced by an IgE-mediated reaction, following exposure to an allergen. Inflammatory reactions in allergic rhinitis are regulated by many cytokines.The. IL-18 is a member of the IL-1 family.It was originally described as IFN-γ -inducing factor (IGIF),and is known to influence the balance of Th1/Th2 immune response. The *IL18* gene has two promoter regions, promoter 1 and promoter 2. Three SNPs are located in promoter1, two are in exon 1, and three are in the promoter 2. This study aimed to examine the association of three different (SNPs) located in IL-18 gene (-607 C/A, -137 G/C and-133 C/G) on chromosome 11q22 with allergic rhinitis.
Methods : Genomic DNA was obtained from the blood samples of 300 AR patients and 200 healthy control volunteers. The IL-18 polymorphism was analyzed by polymerase chain reaction and restriction fragment length Polymorphism (PCR -RFLP) analysis.
Results: The frequency of the GG genotype of the IL-18/-137 gene polymorphism was greater in allergic rhinitis patients than in control. The data of the IL-18(-607C/A and -133 C/G) gene polymorphisms will be analyse to determine the distributions of alleles.
Conclusions: This study suggests that IL-18 gene variants may be participate as a risk factor in the pathogenesis of AR or in intermediary phenotypes. So Further studies are needed to reveal the associations between the IL-18 promoter polymorphism and allergic diseases in other populations.

J08.02
Study for association between the polymorphism rs10046 of the gene CYP19 A1 and the risk of premature coronary artery disease

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Objective: It is well documented that sex hormones influence the risk of developing cardiovascular disease. Several genes are involved in the synthesis of sex hormones. The CYP19A1 gene encodes the enzyme aromatase (P450aro) that is involved in the production of oestrogens from androgens. In the present study, we investigated whether the rs10046 single nucleotide polymorphism (SNP) of the CYP19A1gene is associated with developing premature coronary artery disease (CAD).
Methods: A total of 168 Caucasian CAD patients, documented by coronary angiography, aged less than 58 years and 120 healthy
controls were studied. To genotype the subjects we used the PCR-RFLP method.
Results: The frequencies of CC, CT, TT genotypes were 0.278, 0.481, 0.240, respectively, in the patient group and 0.266, 0.522, 0.212, respectively, in the control group. The frequencies of T and C alleles were 0.518 and 0.481in the patient group and 0.527 and 0.473 in the control group. Statistical analysis indicated no significant differences in genotype or in allele frequencies between the patient and the control group.
Conclusion:
The results of this study suggest that there is no association of the rs10046 polymorphism of the CYP19A1 gene with the risk of developing premature coronary artery disease. Therefore, we may conclude that this polymorphism cannot be used as genetic marker for CAD risk assessment in our Caucasian population.

J08.03
Investigation and validation of five different microsatellites in HLA-DRB1 region in the Iranian population

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Association between HLA-DRB1 and a large number of diseases such as multiple sclerosis and rheumatoid arthritis has been demonstrated. In the present study, we attempted to identify and characterize some potential microsatellites in HLA-DRB1 gene region with the aim of identification of specific markers for this gene. STR markers located next to or within HLA-DRB1 including M2_3_22, M2_2_36, D6S2878, D6S2805, D6S2879 and D6S2880 were selected from Major Histocompatibility Complex database (dbMHC). *In silico* analysis revealed that among all investigated markers, only M2_3_22 was specific for HLA-DRB1. M2_3_22 existed as single copy in all MHC haplotype sequences and located next to the HLA-DRB1. The presented primers for this STR marker at dbMHC and uniSTS were not compatible with some of the last published MHC haplotype sequences. Therefore, a new set of primer pair was designed and used to amplify this marker in 164 DNA samples obtained from Iranian unrelated individuals. M2_3_22 was successfully amplified in all DNA samples, and three different alleles were identified. The marker was found in Hardy-Weinberg equilibrium (P>0.05) in the studied population. Together, the findings suggested that M2_3_22 could be introduced as a specific locus among all the markers present in the HLA-DRB1 gene region for linkage analysis and disease association investigations.

J08.04
Genetic variation in the CYP19 gene and recurrent spontaneous abortions

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Background. The CYP19 gene encodes aromatase, a key cytochrome P450 enzyme that converts androgens to estrogens in the ovarian tissues of premenopausal women. Polymorphic variations in the CYP19 gene result in modified estrogen levels through augment aromatase activity. In the present study, we investigated the association between the C/T single nucleotide polymorphism (SNP) of the CYP19 gene and the risk of recurrent spontaneous abortions (RSA).
Methods: In this prospective case-control study 120 RSA patients and 100 healthy controls were studied. All cohors were Greeks. The PCR-RFLP method was used in order to genotype the subjects.
Results: The frequencies of CC, CT, TT genotypes were 0.275, 0.500, 0.225, respectively, in the patient group and 0.262, 0.532, 0.206, respectively, in the control group. The allele frequencies were 0.525 and 0.475 for C and T, in the patient group and 0.528 and 0.472 for C and T, respectively, in the control group. The data between the two groups were analyzed by chi-square test. The results showed that there are no significant differences in genotype or in allele frequencies between the patient and the control group.
Conclusion: The CYP19 C/T polymorphism cannot be used as genetic marker for RSA risk assessment in our Caucasian population.

J08.05
Association analysis of the Alu-element Ya5NBC51 with the level IL1RA of serum

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INTRODUCTION: Cytokine System is one of the major body systems. Alu-element Ya5NBC51 localized in the third intron of the gene interleukin-1 receptor accessory protein IL1RAP. This protein serves as a coreceptor of proinflammatory cytokines, forming, together with IL1R receptor complex.
MATERIALS AND METHODS: We studied DNA samples from 178 people aged 18-65 years living in the Republic of Bashkortostan. ELISA was performed IL1RA level in blood serum. Analysis of gene polymorphisms was performed using polymerase chain reaction (PCR). Assessing the impact of Alu-insertion in a gene on the data IL1RAP linked ELISA was performed by ANOVA.
RESULTS: Genotype IL1RAP *I/*I is associated with increased levels of serum IL1RA and allele IL1RAP*D with decreased (F = 4,68; p = 0,032). Thus, Alu-element in IL1RAP gene associated with susceptibility to inflammation.

J08.06
Association of vitamin D receptor gene BsmI polymorphisms with bone mineral density in a population of Iranian women

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OBJECTIVE: To investigate the association of vitamin D receptor (VDR) gene BsmI (rs1544410) polymorphisms with bone mineral density (BMD) in a population of Iranian women. METHODS: Blood samples were obtained from 146 pre- and/or postmenopausal Iranian women, aged 35-80 years, stratified for BMD into normal and osteoporotic groups. Anthropometric parameters including age, body height and weight were all recorded. BMD of the lumbar spine (L1-4) and femoral neck were measured using dual energy x-ray absorptiometry. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique was used to detect and analyze VDR gene BsmI polymorphisms distributions of our study groups. RESULTS: The frequency of AA and GG genotypes were significantly different in normal and osteoporotic groups ($P < 0.05$), frequency of AA was higher in patients and GG was higher in normal group. Also the GG genotype was significantly associated with increased BMD in the lumbar spine ($P < 0.05$). This association was not significant in femoral neck ($P > 0.05$). CONCLUSION: VDR gene polymorphisms have an association with the BMD in lumbar spine and may have a less effect on proximal femur BMD in women.

J09. Complex traits and polygenic disorders

J09.01
Specific risk factors predisposing to adolescent alcohol dependence: genetic pilot study in a Belarusian population.

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Adolescent alcohol abuse is the real problem of contemporary society. Belarus, according WHO, is among nine countries with extremely high alcohol consumption. About 17000 adolescents younger than 18 are included in state preventive list. The aim of the investigation: the search of molecular-genetic markers predisposing to regular alcohol consumption of adolescent. The influence of genetic factor and “family climate”: presence of relatives with alcohol abuse, parental attention and physical punishment was also taken into consideration. Four male groups were analyzed, namely, those who started regular alcohol consumption at: 12-18 years old (125 patients); 18-25 (110) ; 3) after 25 (104); controls (103) , all 442 persons from the Republican research and practice center of mental health. 74,4 % adolescents, who started regular alcohol consumption before 18, had relatives with alcohol abuse comparing to control group (42 %, p=0,0002), and two groups of elder patients (55,5% and 53,8%, p=0,001). We revealed that the proportion of DRD2 A1A2 heterozygotes was significantly increased among youngsters that suffered from frequent physical punishment comparing with the control group (p=0,009), we did not observe this effect for elder patients. Genotype A1A2 conditions the effect of poor parental attention on regular alcohol consumption for the youngest test group (p=0,018) but not for “18-25” and “after 25” groups. Alcohol dependence is a result of specific interplay between the genetic and environmental factors that differ between adolescents and adulthood.

J09.02
Screening of Alpha Galactosidase A gene mutations in patients with Hypertrophic Cardiomyopathy phenotype

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Fabry disease is an inherited storage disorder caused by a defect in the lysosomal enzyme alpha galactosidase A. GLA gene encoding alpha galactosidase A is located on the X-chromosome and more than 300 mutations have been reported in all 7 exons. This deficiency leads to accumulation of glycosphingolipids throughout the body including heart, kidney and nervous system. Cardiac involvement leads to progressive concentric, asymmetrical or apical left ventricular hypertrophy that is the most frequent cause of death in fabry disease in both genders. Fabry diseases may resemble sarcomere-gene associated hypertrophic cardiomyopathy. On routine echocardiography and electrocardiogram (ECG), there are no characteristic patterns useful for differentiation between hypertrophic cardiomyopathy (HCM) caused by sarcomere gene mutations and fabry disease. The aim of this study was to screen GLA gene mutations in patients with HCM phenotype for a disease-specific treatment with enzyme replacement therapy (ERT). 106 patients with HCM phenotype were examined. Alpha galactosidase A level was evaluated in 57 men. Patients with reduced level of alpha galactosidase A and 49 women underwent GLA gene mutation screening by PCR-SSCP method. It is, however, apparent that further expression and genetic association work is necessary to try and clarify the mixed findings that have been reported until now.

J09.03
ApoE gene polymorphism in rheumatoid arthritis patients

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Objective:Many studies indicate the importance of genetic polymorphism as factors predisposing for cardiovascular diseases and rheumatoid arthritis. Cardiovascular complications associated with atherosclerotic vessel lesions - one of the leading reason of life -time shortening in RA. ApoE allele types determine plasma lipid levels and could be the factor of relative atherosclerosis risk, also facts testified the importance ApoE alleles in Alzheimer‘s disease (AD) and immunoregulation. So possibly ApoE isoforms could influenced disease progress in RA and also could serve as predisposing marker for further atherosclerosis development.
Method: 74 rheumathoides arthritis patients and 107 healthy control were included in research. PCR-RFLP was carried out in order to check allelic frequencies investigated gene polymorphism.
Results: ApoE E3/E3 genotype was less common in RA group comparing to control - 57,5% and 72 % correspondingly (P<0,05). ApoE E2/E3 together with E2/E4 allele composed 28,7% in RA patient group, whereas in controls - 17,8% (P<0,05).
Conclusions: it should be some link between key genes in RA and cardiovascular disease, so separation of some loci could help in subdividing special patient group more sensitive for cardiovascular disease- it will be the purpose of future work.

J09.04
TNF-alpha gene promoter polymorphisms: lack of association with susceptibility to asthma in Romania

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Asthma is a complex disease characterized by chronic airway inflammation and bronchial hyper-responsiveness. Tumor necrosis factor (TNF)-alpha is a pro-inflammatory cytokine that has been implicated in many aspects of the pathology in asthma, but a clear understanding of the exact role in asthmatic patients is yet to be determined.
Objectives: The aim of this study was to investigate the association of TNF-alpha gene single nucleotide polymorphisms (SNPs) with susceptibility to asthma in Romania.
Methods: Three SNPs from TNF-alpha gene promoter (-308G/A, -238G/A and -857C/T) were examined for association and linkage disequilibrium in a sample of 106 Romanian asthmatic patients and 147 ethnically match healthy controls. The genotyping method was TaqMan Allelic Discrimination with SNP Genotyping Assays C_7514879_10, C_2215707_10 and C_11918223_10 (Applied Biosystems, USA). All the statistic tests were performed with the software package PLINK v 1.07.
Results: Patients and controls groups were in Hardy-Weinberg equilibrium for all polymorphisms. The minor allele 857*T was overrepresented in patients versus controls (23.1% vs 19%), but not statistically significant (p=0.2). Three main haplotypes were constructed based on the studied SNPs, the most frequent being 857C/308G/238G, 58% in patients versus 64% in controls. We found that neither alleles or genotypes among all three SNPs nor reconstructed haplotypes were associated with susceptibility to asthma in our population.
Conclusion: TNF-alpha gene promoter polymorphisms are not a risk factor for asthma in the Romanian studied population.

J09.05
Study of clinical significance of genetic variant c.2808G>T in CFH gene in Russian population

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Background. Atypical hemolytico-uremic syndrome (aHUS) is a rare inherited disease characterized by progressive renal failure requiring renal transplantation, thrombocytopenia, and microangiopathic hemolytic anemia. Number of genes is linked with this syndrome, but the most common genes involved into aHUS pathogenesis are CFH, CFI, and MCP genes. Mutation detection in responsible genes predisposes to higher risk of transplant dysfunction and should be taken into account in making decision related with transplantation.
Methods: Sequencing of coding and contiguous intronic areas of CFH, CFI, and MCP genes of patients with aHUS. Detection of SPNs of interest in 103 adult healthy individuals by allele-specific PCR.
Results: We did perform screening of those three genes in two patients with clinical manifestation of aHUS. We revealed the same genetic variant in both patients in 19th exon of CFH gene (rs1065489, NM_000186.3:c.2808G>T) leading to p.E936D substitution. None of genetic variants of apparent or unclear clinical significance were found in three major genes. One patient was heterozygote, and another one was homozygote carrier of this missence variant. This SNP was previously described in UniProt database as a polymorphism associated with aHUS and basal laminar drusen. To elucidate the clinical significance of this genetic variant we screened 103 healthy adult Russian individuals without any signs of renal pathology (206 chromosomes). We detected 31 heterozygous (G/T) and 3 homozygous (T/T) carriers. The frequency of minor allele (c.2808T) was 18%. We suspect that means that clinical significance of missence variant c.2808G>T in aHUS developing is low, at least in Russian population.

J09.06

Association analysis schizophrenia, Alzheimer‘s disease and alcoholism susceptibility gene polymorphisms relationship to psychodiagnostic traits in the West Siberian population

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Natural selection plays an important role in maintaining genetic variation for mental constitution. Currently, the behavior genetic variability remains poorly understood. The purpose of this study: association analysis of Alzheimer‘s disease, schizophrenia and alcoholism susceptibility genes polymorphism with mental, psychodiagnostic traits, intelligence and anxiety.
The effect of the *GAB2, CLU, PICALM, DISC1, rs13219354, ZNF804A, GABRA2, SLC6A4, ADCY3, MIR9-2, CBX7* loci polymorphisms on psychodiagnostic traits was investigated in students of Siberian State Medical University (Tomsk). All individuals (n=141) completed IQ, Cattell's, Spilberger anxiety scale, Leonhard **personality inventory** tests**.** *GABRA2* and *GAB2* gene polymorphisms were associated with „histrionic“ on the Leonhard **personality inventory**. *SLC6A4* locus revealed the relationship with the scale “B”, *GABRA2* with the scale “E”, *PICALM* and *CLU* with “Q4” and “Q3” scales and the *CBX7* gene polymorphism was associated with the “A” scale by Cattell's test. For the *ZNF804A* locus we identified associations with the „emotiveness“ (high, excessive emotionality), „histrionic“ and „disequilibrium“ accordingly to Leonhard **personality** questionnaire. For the same test the relationship of *PICALM* with the „disequilibrium” and *DISC1* polymorphisms with the „excitability” was revealed.
The greatest number of statistically significant associations were observed for *MIR9-2* polymorphism with the “C”, “F”, “H”, “F2” Cttell's scales, “hyperthymia” and “dysthymia” on Leonhard **personality inventory**, personal and situational anxiety on Spielberger test. Possibly the identified associations suggest substantial contribution of genetic variability in individual mental constitution.
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J09.07

Association of MDR1, ADRB2, IL4 and IL13 polymorphisms with therapy-resistant bronchial asthma in Russian patients

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Background. Bronchial asthma (BA) is a wide-spread polygenic disease.
Aim. To assess severity of BA and effectiveness of BA pharmacotherapy (glucocorticosteroids, beta-2-adrenergic agonists) in patients with different genetic background.
Methods. Genomic DNA was extracted from peripheral leukocytes. We investigated 5 SNPs by PCR-RFLP in 122 BA patients and in 103 healthy controls.

Gene	SNP, common designation	dbSNP	Restriction Enzyme
Multidrug resistance 1 (MDR1)	C3435T	rs1045642	MboI
Beta-2-adrenergic receptor (ADRB2)	Gly16Arg	rs1042713	NcoI
Gln27Glu	rs1042714	BseXI	
Interleukin 4 (IL4)	C-589T	rs2243250	BsmFI
Interleukin 13 (IL13)	Arg130Gln	rs20541	AluI

Results. Distribution of genotypes was similar to other European populations. We revealed numerous associations of genotypes 3435CC, 27GluGlu and alleles 16Gly, -589T, 130Gln with increased risk of BA progression and with unfavorable therapy-resistant BA course.

Allelic variant	Clinical finding	Statistics
3435CC	Increased risk of BA	OR=3.92 (95%CI 1.74-8.79)
3435CC	Increased risk of severe BA	OR=5.22 (95%CI 2.11-12.92)
3435CC	Increased risk of therapy-resistant BA	OR=6.12 (95%CI 2.42-15.48)
16Gly	Increased risk of respiratory failure	OR=17.31 (95%CI 2.01-149.28)
27GluGlu	Increased risk of therapy-resistant BA	OR=3.35 (95%CI 1.16-9.66)
-589T	High rate of BA complication	p=0.002
-589T	High dose of intravenous glucocorticosteroids	p=0.033
130Gln	Increased risk of moderate BA	OR=2.76 (95%CI 1.35-5.63)
130Gln	Increased risk of therapy-resistant BA	OR=2.09 (95%CI 1.01-4.30)

Conclusion. Analysis of MDR1, ADRB2, IL4 and IL13 polymorphisms is useful for both preventive care (revealing subjects with increased predisposition to BA) and BA treatment (pharmacotherapy optimization due to prediction of BA severity at the beginning of disease).

J09.08

Calreticulin mutations in major psychiatric disorders

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Development- and tissue-specific expression of the calreticulin (CALR) gene in the gray matter in late adolescence and early adulthood coincides with the expression of psychosis. To identify novel mutations in the regulatory regions of the CALR gene in major psychiatric disorders.We report novel low frequency mutations in the CALR promoter and intronic sequence that co-occur with the spectrum of major psychiatric disorders including schizophrenia, schizoaffective disorder and bipolar disorder type I, which did not exist in the control pool (pA increases gene expression in human and mouse neuroblastoma cell lines. This mutation is the first instance of a functional cognition-deficit mutation reversing a human gene promoter to the primitive type. We found that VPA increases gene expression in the cells with the wild-type -220C construct, whereas a dramatic decrease in gene expression was observed in the cell lines with the mutant construct (p<0.000004). A novel 1-bp insertion was also detected in intron 1 at IVSI-310, in a case of amphetamine-induced psychosis. As for the psychosis-linked CALR promoter mutations identified to-date, the IVSI mutation was not detected in the control pool. This mutation creates a RREB-1 transcription factor binding site within the first intron.We propose that major psychiatric disorders are, at least in part, a collection of low frequency mutations with possibly large effect, each contributing a small fraction of the disorders. Re-sequencing of the candidate gene regulatory regions will further clarify this model.

J09.09

Study of gene Caveolin3 in Latvian population.

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A research was carried out in order to find out more about neuromuscular diseases in Latvia. As a part of this project a certain gene - CAV3 was researched in Latvian control population of one hundred seemingly healthy people. The particular gene was never before researched in any of Baltic states. Methods of genomic sequencing were being used. Since gene is conservative, changes in the nucleotide sequence are quite rare, but can cause severe muscular diseases, like Limb Girdle Muscular Dystrophy - LGMD 1C, isolated HyperCKemia - H-CK, rippling muscle disease - RMD and Distal myopathy - DM.
The results found were quite interesting. Six polymorphisms (rs1974763,

rs1008642, rs11922879, rs57159780, rs13087941, rs139985460) and one deletion (rs116840772) already existing in databases were found. Frequencies of some of them differed from ones found in databases. The deletion had a sequence mismatch in comparison with one found in database, being shorter by two nucleotides. In addition to that five new polymorphisms were found. Two in the 5' untranslated region (1-44G>A, 1-36G>A) without any obviously possible impact on health. However three of them (220C>A, R74S, 220C>T, R74C, 257T>C, L86P) were found in protein coding region and after *in silico* testing by PolyPhen2 were acknowledged as possibly damaging.
Further work is needed to verify these results. This research is quite meaningful for our society, since the data acquired have become a basis and are being used to help treat people with neuromuscular diseases.

J09.10

The polymorphism of hemostasis system genes in newborns with neurologic abnormalities from Russia.

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Neurologic abnormalities in newborns with cerebral ischemia (CI) including intraventricular hemorrhages and thrombosis have become a focus of attention, as it is associated with neurodevelopmental disabilities and mortality. There are limited data on the predisposing risk factors of the neurologic abnormalities in newborns regarding an influence of the polymorphism of hemostasis system genes on its development.
In present work the allele and genotype frequencies of polymorphisms of six genes (*-455G/A* of *FBG*, *G20210A* of *FII*, *G1691A* of *FV*, *T1565C* of *GPIIIa*, *4G/5G* of *PAI-1* and *C677T* in *MTHFR*) were studied in newborns with CI.
The total of 50 patients with CI including 28 newborns with thrombosis and hemorrhages (group I) and 22 newborns having no hemorrhages and thrombosis (group II) and 50 healthy controls were followed. DNA was extracted from peripheral blood samples and hybridization with biochips was performed for the detection of each polymorphism.
Significant differences in the allele frequencies between the group I and controls were found for *FV Leiden* mutation (0.540 versus 0.00, respectively; p=0.019). The frequency of *MTHFR 677C* allele was higher in the group I compared to the group II (0.286 versus 0.125, respectively; p=0.045), but not significantly higher compared to the controls (p= 0.123). The distributions of genotypes and alleles for other gene polymorphisms were not significantly different between studied groups and controls.
Thus our data obtained suggest that *1691A* of *FV* and *677T* of *MTHFR* alleles may contribute to the higher risk of intraventricular hemorrhages and thrombosis development in newborns with CI.

J09.11

Influence of Dopamine D2 receptor (DRD2) gene polymorphisms on childhood obesity.

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Objectives: Feeding is associated with dopamine release in the dorsal striatum and the degree of pleasure from eating correlates with the amount of dopamine release. The number of striatal Dopamine D2 receptor **(**DRD2) receptors has also been related to obesity and increased body mass index (BMI). The purpose of the present study was to examine the association between common polymorphisms of the DRD2 gene and chilhood obesity.
Methods: Eighty six obese and 100 healthy children were included in the study. Two polymorphisms (Taq1A and Taq1B) in DRD2 gene were genotyped by PCR-RFLP method.
Results: For DRD2 Taq1A polymorphism, the distribution of A1/A1, A1/A2, and A2/A2 genotypes was 3.5%, 29.1% and 67.4% in obese children compared with 4%, 31% and 65% in controls. The allele frequency of A1 and A2 was 0.180, 0.820 in cases compared with 0.195, 0.805 in controls (p=0.4). The distribution of B1/B1, B1/B2 and B2/B2 genotypes for Taq1B polymorphism was 3.5%, 22.1% and 74.4% for cases and 3%, 27% and 70% for the controls. The allele frequency of B1 and B2 was 0.145, 0.855 in cases compared with 0.164, 0.836 in controls (p=0.2). Genotype distribution did not differ significantly from those predicted by the Hardy-Weinberg Equilibrium distribution (p>0.05).
Conclusion: According to our preliminary results, Taq1A and Taq1B polymorphisms in the DRD2 gene were not associated with Turkish chilhood obesity susceptibility. Further researches are needed to confirm the exact role of DRD2 gene polymorphism on chilhood obesity.

J09.12

Possible additional effect of rare SNP rs35995925 of the *FAS* gene on predisposition to preeclampsia

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The polymorphism of the *FAS* gene rs1800682 (-670 A>G) is a common SNP with a MAF of 0.4777. The G allele was shown to be associated with preeclampsia. Within the 100 bp region surrounding rs1800682, there are three rare SNPs - rs150130637, rs2234768 and rs34995925, which is 8 bp upstream. The aim of the study is the analysis of possible additional effect of rare variants pooled with common variant on genetic predisposition to preeclampsia. The study cohort was made up of 112 patients with preeclampsia and 90 from women with physiological pregnancy. For genotype analyses, the HRMA and dideoxysequencing were performed on DNA extracted from peripheral blood. For *FAS* expression, RNA was extracted from 19 placental tissues and the deltadeltaC method was applied. The presence of G allele was observed in 88 patients (78.6%) compared to the 63 (70%) individuals from control group. The OR for subjects carrying genotypes GG and GA was 1.5417 (95%CI 0.830-2.974). The preliminary sequencing data of 37 preeclamptic and 45 control samples confirmed the HRMA results for rs1800682. Additionally, two preeclamptic patients with rs1800682 AA alleles were heterozygous for rs34995925 and none in the control group. The relative gene expression in placental samples showed none association with the genotypes. We have detected rare alleles in preeclamptic women close to the

STAT1 binding site with possible additional effect on predisposition to preeclampsia. Following this we plane to perform allele-specific transcript analyses to study the effect of these rSNPs in placental tissue.

J09.13

Angiotensin-converting enzyme gene I/D polymorphism and fibromyalgia in a Turkish population

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Fibromyalgia (FM) is a multifactorial disease, characterized by a clinical history of generalized muscle pain for more than three months and by specific tender points. The aim of the present study was to examine the possible role of angiotensin-converting enzyme (ACE) insertion/deletion (I/D) gene polymorphism as a risk factor in the development of FM. This study comprised 150 FM patients, 137 females and 13 males, and 199 healthy controls, 127 females and 72 males. Peripheral blood samples were genotyped using polymerase chain reaction (PCR) analysis.
The frequency of D allele was 60.6% in patients and 55.5% in controls (x2=1.65, p=0.19). The distribution of ACE DD, ID, and II genotypes in FM patients were 40; 41.3 and 18.7%, respectively; the corresponding numbers for the control group were 29.1; 52.8 and 18.1%, respectively. The distribution of the ACE gene genotypes frequencies weren't statistically significant between patient and controls groups (x2=5.33, p=0.07). But the percentage of DD genotype is relatively higher in patient group than control group.
Many studies showed that the ID and DD polymorphism is strongly associated with the increased plasma or serum ACE levels. Thus the ID and DD polymorphism favors high ACE expression and activity, hence may predispose individuals to FM and its complications. In this study we have demonstrated that genotype and allele frequencies of ACE gene I/D polymorphism were not statistically association with FM. Further studies with a larger number of patients are needed.

J09.14
Association of the TPO gene in petrochemical women workers with autoimmune thyroid diseases

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Autoimmune thyroid diseases (AITD) are common, with important epidemiological data, supporting a strong genetic background on the etiology of AITD. It is known that the formation of the AIT affect toxic substances. The aim of this study was to assess the relationship between two polymorphisms of Thyroid Peroxidase gene (TPO) serum level of Anti-TPO titer and serum level of hormones TTG and T4 in petrochemical women workers. A sample of 159 participants from the "Gazprom neftekhim Salavat" was selected as the case (N=61) and control healthy women (N=98). Inclusion criteria for cases were Anti-TPO 30U/L with a history of hypothyroidism. Anti-TPO level in subjects was measured by the ELISA kit. Genomic DNA was extracted using Salting-out/Proteinase K method. Two single nucleotide polymorphisms (SNPs) (rs4927611 and rs732609) were tested in TPO. These two markers were chosen considering that the polymorphism changes the encoded amino-acid and a minor allele frequency, MAF, ≥0.3. SNP typing was carried out by means of PCR-RFLP and ARMS-PCR methods. Deviations from the HWE were not observed. D'=0.36, r2=0.11. Association of frequent variant AA polymorphic locus rs732609 C>A TPO gene with AITD occurred only at overweight women p interact=0.044). Variant CC was associated with increased levels of anti-TPO (TT 173±288.4 pmol/L; vs. CC 374.6±407.4 pmol/L; P = 0.029) and increased levels of T4 (TT 14.19±2.77 vs. CC 15.79±5.23 pmol/L; P =0.027). The selected polymorphism of exon 7 has no effect on increased levels of Anti-TPO and hormones.

J09.15
Association of a single nucleotide polymorphism in the adiponectin gene (ADIPOQ) with gestational diabetes mellitus in Bulgarian population

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Gestational diabetes mellitus (GDM) is characterized with impaired glucose tolerance with onset during pregnancy. It is a multifactorial disease, which affects between 3% and 10% of all pregnant women (data from different surveys). GDM is associated with adverse health outcomes for both mother and baby - newborns larger than normal for their gestational age, preeclampsia, higher risk of developing diabetes following the pregnancy, etc.
There is accumulating evidence that adiponectin plays a role in the pathophysiology of diabetes. In patients with type 2 and gestational diabetes lower levels of the protein have been observed. Several common single nucleotide polymorphisms (SNP) in the adiponectin gene (ADIPOQ) have been associated with predisposition to type 2 diabetes. One of those, rs266729 in the promoter of ADIPOQ, has been proposed to affect its transcription. The aim of the present study was to check if this variant was associated with GDM in Bulgarian population.
The study involved 260 pregnant women, 130 with GDM and 130 controls, recruited from local antenatal clinics in Sofia, Bulgaria. Genotyping was carried out using TaqMan assay and statistical analysis with Plink. The genotyping success rate was over 98% and there was no deviation from Hardy-Weinberg.
The rare allele (G) was found in 22% of the affected and 30% of the non affected women and the difference was statistically significant (p= 0.027, OR= 0.64).
To the best of our knowledge this is the first study identifying an association of the rs266729 polymorphism in the adiponectin gene with gestational diabetes.

J09.16
Differences in IL28B promoter region rs12979860 variants in healthy Hungarian Caucasian and healthy Hungarian Roma patients versus with Hepatitis C Virus infected ones.

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It is estimated that about 3% of the world's population is living with chronic HCV (hepatitis C virus). HCV infection may cause acute **hepatitis**, which is self-resolving in 20 to 50% of cases but does not confer permanent immunity. In 50 to 80% of cases, HCV infection becomes chronic and might result in chronic **hepatitis**, cirrhosis, and hepatocellular carcinoma. Recently rs12979860 in the *19q13* region has been shown to have impact on sustained virological response (SVR) following peginterferon alfa-2a and ribavirin (PEG-IFNalfa-2a+RBV) therapy and the wild TT genotype at *rs12979860* is a negative predictor of response to PEG-IFNalfa-2a+RBV therapy. A similarly unexpected observation is that a T allele at *rs12979860* is more common in HCV infected patients.
We analyzed total of 475 Roma, 453 healthy control and 853 HCV patients by Taqman SNP Genotyping Assay. Total of 393 HCV patients have been treated with PEG-IFNalfa-2a+RBV for at least 24 weeks. The rs12980275 *CC* genotype in HCV patients occurred with lower frequency than in healthy controls (24.6% vs. 49.2%; OR=2.56; p=0.0252). Treated patients with the CC genotype achieved SVR in higher rate, than those who have TT alleles (56.9% vs. 36.6 %; OR=2.57; p=0.0482). The Roma and Caucasian population show similar genotype distribution.
As the IL28B polymorphisms are one of the essential contributing factors for high SVR in chronic HCV patients, genetic data may be used to select the optimal treatment regimes in IFN-based therapy.

J09.17
The search for new candidate genes associated with hypertension advancement in children of Northwest Russia

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The risk of hypertension in children is associated with renin-angiotensin genetic system as was earlier demonstrated in our studies. In the present study 7 other polymorphic genetic markers were analyzed in group of children with hypertension (100 samples) and in the control group of children (100 samples). The frequencies of genotypes and alleles have been recorded for 3 genes involved in arachidonic acid turnover - biologically active substance possessing both vasodilatation & vasoconstriction properties and thus most probably involved in hypertension progression. The following SNPs of relevant genes were studied: *CYP4A11 (*rs1126742 T>C), *CYP2J2* (rs890293 G>T) and *prostaglandin-I synthase* (rs6090996 G>A). Comparative analysis did not revealed any significant differences between genotypes or alleles frequencies of these genes in the studied groups (X-squared<1.64, p>0.43). Four other SNPs (rs11191548 T>C (intergenic variant), rs17367504 A>G (*MTHFR)*, rs4977574 A>G (*CDKN2BAS)*, rs11646213 A>T (*CDH13*)), identified by GWAS in adult hypertension patients could not be confirmed in the present study (X-squared<2.44, p>0.29). Different metabolic pathway in infant and adult hypertension syndrome could be suspected.

J09.18
Prevalence and spectrum of MYBPC3 gene mutations in patients with hypertrophic cardiomyopathy from north-west of Iran

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Hypertrophic cardiomyopathy (HCM) is the most common genetic cardiovascular disease with a prevalence of 1 in 500 normal populations. The disease is the major cause of sudden cardiac death in the young and morbidity in the elderly individuals. HCM is inherited as an autosomal dominant single gene disease, characterized by unexplained ventricular myocardial hypertrophy. cMYBC is a sarcomeric thick filament protein that interacts with titin, myosin and actin to regulate sarcomeric assembly. Mutations in MYBPC3 gene are one of the most frequent genetic causes of the HCM disease. The aim of the present study was to investigate the frequency and kind of MYBPC3 gene muations in the population of north-west of Iran. DNA was extracted from 60 HCM patients by salting out method. All exons and exon-intron flanking regions of MYBPC3 gene were evaluated by PCR-SSCP assay. It is, however, apparent that further expression and genetic association work is necessary to try and clarify the mixed findings that have been reported until now.

J09.19
IL1B and IL8 polymorphisms involvement in recurrent corneal erosion in patients with hereditary stromal corneal dystrophies

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Deposits accumulation in patients with hereditary stromal corneal dystrophies (HSCD) leads to impaired attachment of epitheliocytes to basement membrane - corneal erosion. Proinflammatory microenvironment in the region of wound is crucial for its healing, inducing cell migration, neutrophils attraction etc. Two main inflammatory cytokines genes *IL1B* and *IL8* are upregulated in injured corneal epithelium. *IL1* gene -511 / and *IL8* gene -781C/T variants influence these genes expression in epithelium.

To establish possible involvement of -511 /T and -781C/T polymorphisms in corneal erosion development we investigated them in 2 groups. Case group - lattice HSCD typeI (n=46) and IIIA (n=23) patients with confirmed presence of *TGFBI* Arg124Cys or Hys626Arg mutations respectively. This group consisted of individuals with history recurrent erosion (n=56) and without it (n=13). Control group - healthy individuals (n=105) from Ukraine. Genotyping for both studied polymorphisms and *TGFBI* mutations was performed by PCR followed by RFLP analysis.

No significant differences in *IL1B* -511 /T genotype or allelic frequencies between patients with erosion and without it were found. Whereas a trend to decrease of -511TT genotype frequency in group with erosion (3,7%) comparing to control (6,7%) was observed.

Frequency of *IL8* -781TT genotype was significantly ($P<0,05$) lower in group with erosion (10,7%) comparing to patients without erosion (30,8%) and control (25%).

Our results revealed possible involvement of *IL8* -781C/T polymorphism in corneal erosion development. *IL8* -781TT genotype is associated with negative prognosis for recurrent erosion in patients with HSCD.

J09.20

Genetic Polymorphism TNF-α 308G>A and Ischemic Stroke in Northern Romania

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Background. TNF-alpha is a proinflamatory cytokine. Evidence in support for a role for TNF-alpha in this respect is emerging as evidence on de novo upregulation of TNF-alpha following ischemia is now well established.

Objectives. The aim of the present study is to evaluate the connection between TNF-alpha polymorphism and ischemic stroke in a group population from Northern Romania and to determine whether it has an influence on the risk of cerebral events .

This is a cross-sectional, randomized, case-control study for the evaluation of the frequency of TNF-α 308G>A polymorphism alleles among patients with ischemic stroke.

Material and methods. The study included 100 cases of patients diagnosed with ischemic stroke (neurological and CT scan examination), and 86 healthy unrelated controls.

TNF-α 308G>A genotyping was carried out using PCR amplification of relevant gene fragment was followed by restriction enzyme digestion. Detection of TNFα 308G>A alleles was determined through analysis of resulting restriction fragment length polymorphism (RFLP) followed by gel electrophoresis.

Results. Molecular analysis did not reveal an increased frequency of GA mutant genotype in the study group compared to the control group ($p = 0.744$, OR = 1.174, CI = 0.6183 - 2.229). The AA genotype was not present in any subjects, probably because of the smaller number of AA carriers present in the population, since the A allele is very rare.

Conclusions. We found no significant difference in distribution of the TNF-α 308G>A polymorphism between the ischemic stroke and control groups.

J09.21

Investigation of the Asporin gene polymorphism as a risk factor for knee osteoarthritis in Iran

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Osteoarthritis (OA) is a degenerative disease of the joints characterized by degradation of the hyaline articular cartilage and remodeling of the subchondral bone with sclerosis. Asporin (ASPN) gene encodes a cartilage extracellular protein that is member of the small leucine-rich proteoglycan family. Polymorphisms in the aspartic acid repeat region in the second exon of this gene, that is consist of GAT repeats, are associated with a susceptibility to osteoarthritis. The D14 allele (an allele containing 14 D repeats) is associated with increased OA susceptibility in Japanese and Han Chinese, but is not an important factor in OA etiology among Caucasians, although the D15 allele is considered a risk allele for Greek population. To apprise the possible association, that seems controversially, we explored the ASPN effect in Iranian patients with knee OA. The D repeat polymorphism was genotyped in 100 knee OA patients and 100 control subjects, and the allelic association of the D repeat was examined. Our data suggest that the D15 allele could be considered a risk allele significant only for females ($p=0.045$) of Iranian population. This association is partially similar to Greeks, that D15 allele was considered a risk allele.

J09.22

Interleukin-1 β Gene Polymorphisms in Iranian Patients With Uterine Fibroid, A Case- Control Study

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Background and abjective: Uterine leiomyoma or fibroid is currently the most common estrogen- dependent reproductive system tumor. Almost a quarter of women of reproductive age are affected with this benign tumor. These tumors are the most common reason of hysterectomy and women surgery and seriously affect women health. The aim of this study is investigation of IL-1β-511and IL-1β 3954 Polymorphisms association with uterine leiomyoma between in Iranian women of Charmahal & bakhtiari province.

Method of investigation : In this study , 276 patients with uterine leiomyoma and 157 healthy women as control are studied. The genetic polymorphisms for IL-1β-511and IL-1β 3954 were analyzed by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP). and the results analyzed with SPSS software and χ^2 test

Finding: genotypes and allelic frequencies compared are two groups. A significant difference in the allele frequencies of the IL-1 β -511 C>T polymorphism in leiomyoma groups and normal controls was found ($P < 0.05$). No difference was found in the IL-1 β -3954 polymorphism in studied cases.

Conclusion: Our findings indicated that there is a significant association between IL-1 β -511C>T promoter Polymorphism and risk increase of uterine leiomyoma on the women of our study and this polymorphism might participate in developed this disease.

Keyword: leiomyoma, polymorphism, IL-1β

J09.23

Association between MDR1 gene polymorphisms and its expression in Iranian CRC patients.

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Increase expression of multidrug resistance gene (MDR1) gene is one of the mechanisms responsible for drug resistant to chemotherapy.

There are different mechanisms such as polymorphism that result in MDR1 over expression.

It has been reported that the C1236T and C3435 polymorphisms of the MDR1 gene have substantial impact on expression or activity of P-glycoprotein (Pgp).

In this study, we investigated the possible association between MDR1 gene C3435 and C1236T polymorphisms and its expression in Iranian CRC patients.

ARMS and RFLP PCR were used for the detection of this single nucleotide polymorphism in 60 CRC patients and 60 healthy individuals.

We concluded that there was no significant association between MDR1 expression and polymorphism in patients; the results of the present study demonstrate that these polymorphisms may not play a role in inducing drug resistance by altering the expression level of MDR1 gene.

J09.24

Current experiences with newborn screening & case finding in Iran

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This review presents the current experiences with newborn screening & case finding in Iran. Iranian population is about 70 million, with high birth rate and an estimated 1 million per year. The population is characterized by a high consanguinity (25-70%) . Inherited metabolic disorders common among the population. Although research spending is rather soft in the region, there are numerous pilot studies that highlighted the high incidence of genetic defects and the need for newborn screening programmers.

1 : we searched for IEM in ill infant & children admitted in clinics or hospitals (2007-2010).

Mass screening using tandem mass spectrometry(MS/MS) and selected testing was initiated to determine IEM.

From April 2007 to March 2010, 13,500 infants and children were screened for organic, amino and fatty acid metabolism disorders and aminoacidopathies. In these group we had 2% positive result (264). Out of 264 patients , the spectrum included OA (98), aminoacidopathies (78) ,UCD (54), neurotransmitter conditions (12) and lysosomal disorders mainly MPS (14) , with a sensitivity of 97.67%, a specificity of 99.28% .

2: we searched for IEM in 5000 newborns (2009-2010), *by* Tandem mass spectrometry (MS/MS) for >20 markers of disease in a single assay. Limited information is available for setting the marker cutoffs and for the resulting positive predictive values.

We identified 22 babies with aminoacidophaties (5), OA (10) and FAO(7).

J09.25

The Future of Migraine Genetics

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Background

Migraine is a commonly occurring, neurological disorder with a substantial genetic component. With the exception of the rare monogenic forms of migraine, current technology has not proved efficient in revealing the underlying genetics of migraine. Recent genome-wide association studies (GWAS) have identified four single nucleotide polymorphisms significantly related to migraine. However, these polymorphisms explain only a small part of the heritability. The missing heritability might be hidden in rare variants, calling for a more detailed sequencing to be applied and developed. Sequencing the entire genome by exome sequencing and whole genome sequencing (WGS) are techniques undergoing an enormous development and might help to reveal the genetic mystery of migraine. We aim to explore future possibilities for migraine genetics given by the new developing technologies.

Methods

Since the technology evolves much faster than the literature, the study is predominantly based on interviews with genetics experts. Also, we evaluated studies regarding migraine genetic and next generation sequencing, which were identified using PubMed search.

Conclusion

The future research in migraine genetics will be dominated by the next generation sequencing. However, at the present time, prices and analyzing tools limit its use, and in cases with sporadic migraine, GWAS appear to be the best choice. Conversely, in cases with familial migraine, exome or whole genome sequencing will be the preeminent choice. In order to gain insight in the heritability of migraine, studies in the upcoming years will require large sample sizes and cooperation between migraine genetic consortia.

J09.26

May quantity of low functionality alleles of folic acid metabolism genes be important?

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MTHFR, MTRR and MTR genes SNPs polymorphisms have been associated with many disorders, however, findings have been inconsistent.

DNA of 274 studied cases (55 female with neural tube defect fetuses (MNTD), 35 mother children with Down syndrome (MDS), 35 mother children with cleft lip and/or palate (MCL/P), 84 women with RSA (recurrent spontaneous abortion), 35 CL/P patients, 35 chorion villi from miscarriages (CV)) and 225 control subjects was isolated using the salting out method and the analysis of SNPs polymorphisms was performed by PCR-RFLP assay.

The differences of the MTHFR 677C→T and 1298A→C, MTR 2756A→G and MTRR 66A→G allele and genotype frequencies between studied and control groups were not significant besides higher incidence of MTRR 66G allele in MDS group (p=0.009; OR=2.10; CI:1.20 - 3.70).

As all the studied conditions are polygenic we attempted to calculate the quantity of MTHFR 677T, 1298C, MTR 2756G and MTRR 66G as low functionality alleles. We speculate that accordingly to obtained results for four analyzed loci person could have from 0 to 8 low functionality alleles. Interestingly, no one case with the presence more that six low functionality alleles was detected.

The accumulation in the female genotype of 5/8 alleles: MTHFR 677T, MTHFR 1298C, MTR 2756G and MTRR 66G in homo-/heterozygous state is associated with significantly increased risk of delivering child with NTD (OR=5.90; CI:1.11 - 31.43, p=0.032); CL/P (OR=9.08; CI:1.58 - 52.2, p=0.014) and RSA (OR = 9.83; CI:2.14 - 45.20, p=0.0004). These observations were not found in MDS, CL/P and CV groups.

J09.27

Replication of the results of genome-wide association study for Parkinson's disease in patients and controls from Bashkortostan Republic of Russia

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The purpose of the investigation was to replicate the results of genome-wide analysis (GWAS) of Parkinson's disease (PD) on DNA samples of patients and controls - residents of Bashkortostan Republic. The analysis of alpha-synuclein (SNCA) gene polymorphisms at three loci (rs356219, rs356165, rs2737020) in 382 PD patients (143 - Russians, 157 - Tatars, 72 - Bashkirs, 10 - other ethnic origin) and 530 healthy individuals, matched for sex and age (133 - Russians, 307 - Tatars, 88 - Bashkirs, 2 - other ethnic origin) was performed. The association with PD development was found for loci rs356219 and rs356165. A comparison of total cohorts of patients and controls revealed genotypes and alleles associated with the disease in each locus: the genetic marker of the increased risk for PD development at rs356219 was allele *G (OR=2,02), the frequency of which was 0,45 and 0,39 in patients and controls, respectively; the protective markers for PD development were genotype * A * A (OR = 0,71) and allele * A (OR = 0,73); at rs356165 - allele * G (OR = 1,36) and genotype * G * G (OR = 1,51) increased and allele * A (OR = 0,73) decreased risk of PD development. The comparison of ethnically devided PD patients samples and controls demonstrated similar statistically significant associations (p <0,05) only in Bashkirs, while Russians and Tatars showed only a trend of such associations.

J09.28

Analysis of LRRK2 and parkin gene mutations in Slovak Parkinson disease patients

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The Parkinson's disease (PD) is the second most common progressive neurodegenerative brain disorder caused by loss of nigrostriatal dopaminergic neurons, which affect the control of body movements, with inclusion formation (Lewy bodies) in surviving neurons. It affects 1-2% of the global population above 65 years and its prevalence increases to approximately 4% in those above 85 years. Parkinson's disease is a complex neurodegenerative movement disorder characterized by bradykinesia, resting tremor, rigidity and postural instability.

To detect the most common mutations in selected exons of LRRK2 and parkin genes responsible for late and early onset form of disease, respectively, we applied a gene scanning approach using dHPLC method. In this study, we evaluated the prevalence of LRRK2 mutations in exons 31, 35, 41, 48 and of parkin (PARK2) mutation in exons 2, 6 and 7 in a cohort of 216 consecutive, unrelated Slovakian patients with familial or sporadic PD, including early and late onset patients.

We have found one exonic, eight intronic polymorphisms and a heterozygous point mutation p.Arg275Trp (c.823C>T, rs34424986) in exon 7 of parkin gene in one patient with age at onset 62 years. In the exons 31 and 41 of gene the LRKK2, and in exon 6 of the gene parkin we did not observe any variant chromatographic profile after DHPLC analyses.

J09.29

Tumor Necrosis Factor Alpha, Interleukin-2 and Interleukin-2 Receptor Beta Gene Polymorphisms in Patients with Psoriasis and Psoriatic Arthritis

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In this study, our aim is to investigate association between TNF-α, IL-2 and IL-2RB gene polymorphisms/expressions and susceptibility for psoriasis and psoriatic arthritis.

Seventy four patients with psoriasis and 74 healthy volunteers were enrolled in the study. In all study subjects, the genes analysed by PCR-RFLP method. Then, data compared between the study groups.

AA genotype in TNFα(-308) (high expression), AA genotype in TNF-α(-238) and GG, TT genotypes in IL-2(-330) are significantly increased in patients with psoriasis in comparison with control group. However, GG genotype in TNF-α(-238) and GT genotype in IL-2 are significantly decreased in patients with psoriasis in comparison with control group. Also gene polymorphisms were analyzed according to the clinical characteristics of the psoriasis patients. IL-2/GG genotype frequency was significantly decreased and the frequency of IL-2/TT genotype was significantly increased among patients with psoriatic arthritis in comparison with psoriasis patients. The frequency of IL-2/TT genotype was significantly increased in mild psoriasis pattient according to moderate-severe psoriasis patients. Also IL-2RB/CC genotype was significantly increased, among patients with late-onset psoriasis in comparison with early onset psoriasis group. IL-2RB/TC genotype frequency was significantly decreased and TT genotype frequency was significantly increased in patients with positive family history of psoriasis according to negative family history patients.

In conclusion, polymorphisms of TNF-α (-238 and -308/high expression) and IL-2 genes may contribute to susceptibility to psoriasis. Also, the IL-2/TT genotype may be a risk factor for the development of psoriatic arhritis, whereas the GG genotype may be a protective factor against psoriatic arthritis.

J09.30

The study and comparison of serotonin gene receptor (5HT3A) expression profiles in rheumatoid arthritis and systemic Lupus erythematous patients

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Background and Aims: Rheumatoid arthritis (RA) and Systemic lupus erythematous (SLE) are both autoimmune disease that their causes are still unknown. Serotonin (5HT) plays roles as a neurotransmitter and a neuromodulator with functions in brain and peripheral tissues. Recent studies reveal that in addition to the CNS, immune cells synthesis neurotransmitters such as serotonin can regulate immune functions. The aim of this study is to evaluate the serotonin receptor gene expression level on peripheral blood mononuclear cells of RA and SLE patients compared to normal individuals.

Material and Methods: In the present study, we investigated serotonin receptor gene expression in PBMCs of 40 RA and SLE patients and 20 healthy individuals using Real Time-PCR. The specificities of the obtained Real time PCR products for the respective serotonin receptors fragments were confirmed by sequenced analysis capillary system (ABI3700, Applied Bio system, USA).

Results: The results showed that 5HT3A receptors mRNA Expression significantly altered in PBMC of RA and SLE patients. Compared to normal individuals, statistics analysis shown that 5-HT3A expression level in RA patients decreased while this level in SLE patients increased.

Conclusion: It appears that both genetic and environmental factors play a role in pathogenesis of RA and SLE diseases. In this study it is suggested that serotonin and its receptors possibly can have an effective role in pathophysiology of RA and SLE diseases.

Keyword: Serotonin receptor gene expression, Rheumatoid arthritis, Systemic lupus erythematous

J09.31

Association between ACE, AGTR, Factor II and Factor V gens with Stroke Subtypes in an Persian Population

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Background and aims: Stroke is the third cause of death in the world after the cancer and cardiovascular disease. In Persian population, Ischemic stroke incidence was also considerably greater than reported in other regions. It is the first gene-association study, which determines the relationship between ACE, AGTR, Factor V Leiden, Factor II variations and stroke. A stroke Database bank was design for this project. Matched controls were randomly selected from the same geographically area. The aim of this study was to determine the association between mentioned gene ′s variations and stroke.

Method: 153 patients of stroke and 153 healthy controls were matched for age and sex. Additionally, control subjects were matched in some stroke risk factors such as diabetes mellitus, low-density lipoprotein, high-density lipoprotein, triglyceride, total Cholesterol hypertension and ischemic heart attack. The gens were determined by Multiplex ARMS- PCR, Real Time PCR, and PCR-RFLP.

RESULT: Statistically significant differences found between ACE II genotypes: 26.8% II in patients versus 15% II in controls. There were a high frequency of AA genotype and very low frequency of CC genotype in both groups for the AGTR. There was no correlation between Factor V, Factor II variations in case and control groups.

Conclusion: Statically significant different was found between ACE II genotype in both groups ($p<0.05$).Our results suggest that ACE II genotype is responsible for stroke susceptibility in Persian Population. We report that Insertion/Insertion genotype of ACE gene was an independent risk factor for Persian stroke patients in contrast to verified studies.

J09.32

Estrogen receptor α gene polymorphisms and reproductive history in women with systemic lupus erythematosus

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Background: Systemic lupus erymathosus (SLE) is an autoimmune disease that affects predominantly females. Estrogens could modulate the immune system functions through an estrogen receptor dependent mechanisms. However, the influence of the estrogen receptor alpha (ERα) gene polymorphisms on the reproductive function in autoimmune disorders (and especially in women with SLE) is not clarified.

Objectives: The aim of the study was to investigate whether two ERα-gene polymorphisms were related the reproductive history in SLE-women.

Methods: A total of 103 women with SLE were genotyped for ERα polymorphisms PvuIIT/C and XbaIA/G by RFLP analysis. The absence of PvuII and XbaI restriction sites were indicated by „P“ and „X“ and their presence by „p“ and „x“, respectively. The presence of menstrual disorders, pregnancies, miscarriages and live births as well as the age of menarche were registered in all women.

Results: XbaIA/G and PvuIIT/C polymorphisms were not significantly related to the frequency of pregnancies, miscarriages or live births in SLE patients ($p>0.05$). Menstrual disturbances were reported less frequently by women with Xx genotype than by patients with XX or xx genotypes (8% vs. 24.5%, $p=0.033$). The age of menarche was not influenced by the ERα polymorphisms ($p>0.05$).

Conclusions: XbaIA/G and PvuIIT/C polymorphisms of the ERα-gene were not related to the reproductive outcome in women with lupus. Further studies are needed to confirm and explain the relationships between the XbaIA/G polymorphism and menstrual disorders in women with lupus and other autoimmune diseases.

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J09.33

Attitude and controversial aspects in systemic lupus erythematosus onset

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Introduction: Systemic lupus erythematosus (SLE) is a multi-organic autoimmune disease with numerous immunological and clinical manifestations. A genetic predisposition in SLE disease is well documented. Material and method: We present a 12 years old girl referred to our hospital for SLE as presumptive diagnosis. Results: The patient's family history is highly significant for SLE: a maternal aunt died at age 26, due to SLE complications. Her daughter (patient's cousin) was also diagnosed with SLE. Her evolution was unfavorable, despite extra-renal epuration. She presented important cardiovascular comorbidities, but the cause of death was renal failure, at 16 years. In these circumstances, the onset of malar rash and photosensitivity demands further evaluation of other markers of SLE, in our case. Complete investigation ruled out the following diagnostic criteria: discoid rash, serositis, oral ulcers, arthritis, hematological disorders, renal involvement, antinuclear antibodies, immunologic phenomena and neurological pathology. Two out of the eleven diagnostic criteria were serially present during follow-up. Discussion: The presence of HLA-A1, B8, DR, the null complement alleles and congenital deficiencies of complement (especially C4, C2, and other early components) increase the risk of SLE in this case. If a mother has SLE, her daughter's risk of developing the disease is estimated at 1:40. For this girl, the risk is even higher, due to SLE family aggregation. Conclusions: Genetic testing should be performed to clarify the diagnosis of SLE in this case. Psychosocial aspects and genetic counseling can influence patient and family quality of life.

J09.34

The prevalence of genes *HLA-DQ* in children with diabetes type 1 of Krasnodar region

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Objective: study of the frequency of polymorphic genes alleles *HLA-DQA1* and *HLA-DQB1* in children and adolescents with type-1 diabetes (T1D) in the Russian population living of the Krasnodar region.
Methods: examination of 110 patients with T1D and 34 healthy siblings. Defining gene alleles HLA-DQA1 (8 specificities) and HLA-DQB1 (12 specificity) was performed using sets of „DNA-Technology".
Results: the data were analyzed protecting and predisposing alleles for T1D for the genes *HLA-DQA1* (*0301, *0501, *0102, *0103, *0201) and *HLA-DQB1* (*0201, *0302, *0304, *0401, *0301, *0602). Patients with T1D revealed the following relationship of diabetogenic alleles: the presence of four predisposing alleles *HLA-DQ* was observed in 39% of patients with T1D; 2 to 3 alleles was observed in 42%, and <1 allele was detected in 19%. In healthy siblings we observed the following: 4: 6%, 2-3: 50%, and <1: 44%. The most common haplotype, which includes four predisposing allele, was encountered in 72% of T1D. Haplotype DQA1*0301-DQB1*0201 was observed in 43% of patients, haplotype DQA1*0501-DQB1*0201 was observed in 51%. Preventing alleles were observed in 41% of patients with T1D, of which 8% with the observed combination of alleles DQA1*0102 and DQB1*0602. In 74% of healthy siblings we identified protecting alleles, of which haplotype DQA1*0102-DQB1*0602 was observed in 18%.
Conclusions: T1D patients in the Russian population of the Krasnodar region are characterized by a „classic" for European populations, protecting and predisposing for T1D alleles *HLA-DQ* genes.

J09.35

Association of two IL-23R gene variations with ulcerative colitis in Iran

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Background and aim: Ulcerative colitis (UC), a chronic inflammatory bowel disease, occurs in genetically susceptible individuals who mount inappropriate immune responses to endo-luminal antigens. Interleukin 23 receptor (IL23R) gene has been reported as a genetic factor associated with ulcerative colitis and other autoimmune-mediated diseases. This study was performed to evaluate two interleukin-23 receptor (IL-23R) polymorphisms association with UC in Iranian patients.
Material and methods: A number of 102 patients with ulcerative colitis and 152 sex- and age-matched healthy controls from the same origin were participated. The PCR-RFLP method was used to evaluate IL23 R SNPs, rs 10889677 (Exon-3' UTR) and rs 11209026 (Arg 381 Gln), of IL23R gene in our population.
Results: The frequency of mutant allele of rs10889677 was 46.5% in UC and 45.7% in controls. The frequency of rs11209026 mutant allele was 2.9% and 5.2% in UC and controls, respectively. None of the evaluated polymorphisms of IL23R gene were more frequent in UC patients, compared to healthy controls.
Conclusions: Our results demonstrated that rs10889677 and rs11209026 mutations of IL23R are not associated with UC in Iranian patients. Additional variants in this gene might play a role in UC disease susceptibility in Iran.

J09.36

Valuation of inherited venous thromboembolism risk within two clinically unrelated populations

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Venous thromboembolism is disease where a blood clot is formed in human venous system. This disease includes deep venous thrombosis (DVT) and pulmonary embolism (PE). Venous thromboembolism results as a combination of hereditary and acquired factors. Acquired factors include surgery, trauma, cancer etc, while common hereditary factors are F5 (coagulation factor V Leiden) 1691G-A gene mutation, F2 (coagulation factor II, prothrombin) 20210G-A gene mutation and MTHFR (methylenetetrahydrofolate reductase) thermolabile polymorphism (677C-T mutation.
In this study, 100 individuals were genotyped: 70 grouped by their clinical background (40 individuals on hemodialysis (HP) and 30 individual with breast cancer (BC)), and 30 healthy volunteers with no clinical background as control group. All samples were genotyped for MTHFR 667C-T, F2 20210G-A and F5 1691G-A mutation using in-house optimized Sybr® green method.In all three groups we found one heterozygote (GA) for F5 Leiden mutation. All other samples had wild type genotype. Only in control group we found one homozygote (AA) for F2 20210G-A mutation, and one heterozygote (GA). We also found one heterozygote in HP group and two in BC group. MTHFR genotypes in HP group are 45% of CC - wild type genotype, 37,5% of CT genotype and 17,5% of TT genotype. BC and control group had the same percentage - 60% of CC, 37% of CT and 3% of TT genotype.

J09.37

The association between VEGF -2578C/A polymorphism and amyotrophic lateral sclerosis in Russian population.

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Background. The association of -2578C/A, -1154G/A, and -634G/C SNPs with ALS was found in some European populations (Lambrechts et al., 2003). However, there was no such association in different other populations (Brockington et al., 2005; Del Bo et al., 2006; Van Vught et al., 2005; Zhang et al., 2006). We suppose the important role of VEGF in ALS pathogenesis. The aim of this study was to investigate the association between VEGF -2578C/A polymorphism and ALS in Russian population.
Methods. The peripheral blood was extracted from 197 patients with ALS and 156 controls. The ALS group included 54% (107/197) males and 46% (90/197) females aged from 20 to 83 years (51,2 ±13,4) and was comparable with controls. All of persons were the Slavs. TaqMan PCR was used for detection of -2578C/A SNP.
Results. The significant difference of the distribution of -2578A allele in ALS cases and controls was observed (p=0,014). The VEGF -2578A/A genotype increased ALS risk to an adjusted odd of 1,58 (95% CI, 0,04-0,95). There was no increased risk for males (OR=1,84, 95% CI, -0,06-1,27). The association between VEGF genotype and clinical characteristics of ALS wasn't detected. But the patients before 45 years old had a weakly increased risk of ALS (OR=1,27, 95% CI, -0,47-0,95).
Conclusions. Our data showed that VEGF genotype isn't a major cause of motor neuron degeneration in ALS, but it can modulate the risk of ALS in Russian population. Thereby VEGF genotype could be useful in choice of ALS pathogenetic therapy.

J09.38

Investigation of mitochondrial tRNA^{Thr} and tRNA^{Pro} genes mutations in autism

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Objectives: Autism as one of three recognized condition in the autism spectrum disorders (ASDs) is a neurodevelopmental, multifactorial disorder. Autism is noticeably reported to be affected by mitochondrial dysfunction which impairs energy metabolism. mtDNA encodes 22 tRNAs working as amino acid transporters for synthesis of the respiratory chain enzymes. Involvement of mutations within tRNA genes have been well documented in mitochondrial disorders. In this study, tRNA^{Pro} and tRNA^{Thr} were investigated to find mutations which are related to autism pathogenesis, as these two genes mutations have been reported to be involved in some neurological disorders.
Methods: In this study, a cohort of 24 unrelated idiopathic patients and 100 ethnically-matched Persian control individuals were obtained. PCR sequencing of mtDNA fragments was employed to investigate the involvement of mitochondrial variations in autism.
Results: A substitution , G15928A, was identified in two groups without a significant difference (P = 0.179, P> 0.05). A new homoplasmic substitusion, A15973G, was identified within the T-loop of tRNA^{Pro} gene in 1 patient as it had not been reported before. This variation is moderately conserved (75 %) among species and also, was not detected in blood sample from the patient's mother.
Conclusion: Investigation on mitochondrial varations may strengthen the role of genetics in association with autism. To reveal the relation of A15973G

and autism, more delicate molecular methods could be done for determining the percentage of heteroplasmic mtDNA in mother's sample of the patient.

J09.39

The BDKRB2 gene I/D polymorphism in Russian athletes

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Background

Bradykinin is a potent endothelium-dependent vasodilator and acts via the bradykinin B2 receptor (encoded by BDKRB2). The absence (-9), rather than the presence (+9), of a 9 bp repeat sequence in exon 1 has previously been shown to be associated with increased gene transcription, higher BDKRB2 mRNA expression and higher efficiency of muscular contraction (Williams et al. 2004). Studies suggest that insertion-deletion (I/D) polymorphism in BDKRB2 gene is associated with aerobic capacity and elite endurance athlete status. The aim of our study was to investigate the association between the BDKRB2 -9/+9 polymorphism and athlete status in Russians.

Methods

One thousand three hundred and seventy nine athletes from different sporting disciplines and 507 controls were involved in the study. The athletes were prospectively stratified into 5 groups according to the event duration and distance, covering a spectrum from the more endurance oriented to the more power oriented. Genotyping was performed by PCR.

Results. Analysis of distribution of allele frequencies revealed no significant differences between a whole group of athletes and the control group (-9 allele: 47.8 vs 45.9%; P=0.319). However, the frequency of the -9 allele was significantly higher among endurance-oriented athletes and athletes with mixed activity (endurance, power/strength): long distance running (P=0.014) and long distance swimming (P=0.022), wrestling (P=0.009) and archery (P=0.002).

In conclusion, our results are in agreement with the previous studies and indicate that the BDKRB2 -9 allele is favourable for endurance performance.

J09.40

The PPARG gene Pro12Ala polymorphism is associated with power athlete status

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Peroxisome proliferator activator receptor gamma (PPARG) gene regulates the expression of genes involved in lipid and carbohydrate metabolism, differentiation of adipocytes and myoblasts, insulin sensitivity and glucose homeostasis. The 12Ala variant of the PPARG gene Pro12Ala polymorphism is associated with decreased receptor activity [Deeb et al., 1998], leading to insulin hypersensitivity and enhanced glucose utilization [Ek et al., 2001]. As a consequence, the carriers of the 12Ala allele show better glycaemic response to exercise training [Adamo et al., 2005], higher rates of skeletal muscle glucose uptake [Vanttinen et al., 2005] and greater cross-sectional area of muscle fibers [Ahmetov et al., 2008]. In a study of Russian power-oriented athletes a higher frequency (23.8 vs. 15.1%, P < 0.0001) of the PPARG 12Ala allele compared with controls has been reported [Ahmetov et al., 2008]. The aim of the present study was to investigate the association of the PPARG Pro/Ala polymorphism and athlete status in an independent cohort of Russian athletes. Two hundred and fifty eight athletes and 1174 controls were involved in the study. The frequency of the 12Ala allele was not significantly different between a whole cohort of athletes and controls. However, in accordance with the previous study, we found statistically significant differences in genotype distribution (weightlifters: Pro/Pro - 71.2%, Pro/Ala - 22.0%, Ala/Ala - 6.8%; controls: Pro/Pro - 72.1%, Pro/Ala - 26.0%, Ala/Ala - 1.9%; P = 0.043) and Ala/Ala frequency (P = 0.013) between weightlifters and controls. Thus, the PPARG gene Pro12Ala polymorphism is associated with power athlete status.

J09.41

Apolipoprotein E polymorphisms statues in Iranian patients with Multiple Sclerosis

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Background: Multiple sclerosis (MS) is a chronic inflammatory demyelinating disorder in the central nervous system. Evidences linking Apolipoprotein E (APOE) to myelin repair, neuronal plasticity, and cerebral inflammatory processes suggest that it may be relevant in MS. The main goal of this study was to determine whether the APOE genotypes and alleles are associated with MS patients.

Materials&Methods: Totally,147 MS cases and 168 control subjects from Iranian population were genotyped for APOE gene using PCR-RFLP method. Genotype and allele frequencies for APOE gene were calculated and compared between MS cases and control subjects by Chi2 or Fisher's exact test.

Results:The frequency of APOE-ε2ε3 genotype was significantly higher in control subjects than MS patients (14.3%vs.6.1%,*P*=0.009,OR=0.39) whereas APOE-ε3ε4 genotype frequency was significantly higher in MS cases compared with control subjects (8.2%vs.3.6%,*P*=0.03,OR=2.4). APOE-ε2 allele frequency in cases was significantly lower than that of control subjects (4.4%vs.8.0%,*P*=0.03,OR=0.52). Also male controls were significantly more likely to have APOE-ε2 allele (7.8%vs.1%, *P*=0.01,OR=0.11). APOE-ε4 allele frequency in cases was significantly higher than control group (4.8%vs.2.1%,*P*=0.03,OR= 2.35).

Conclusion: The allele frequency of APOE-ε4 in our population is lower than the general population (3.5%vs.15-20%). It seems that individuals carrying APOE-ε4 allele and/or APOE-ε3ε4 genotype develop MS two times more than non-carriers. Also APOE-ε2ε3 genotype or APOE-ε2 allele may have a protective role against MS development in Iranian population. Further investigation would be warranted to understand the role of APOE alleles and genotypes and risk of MS.

J09.42

Research association of polymorphic markers of candidate genes with ischemic heart disease with the development of oxidative stress in elderly and senile patients living in the Rostov region

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The study selected patients with ischemic heart disease (n = 300). The control group (n = 280) consisted of a random sample of both sexes with no significant signs of ischemic heart disease. It was found that the development of coronary heart disease in elderly and senile patients living in the Rostov region, increases the rate of production of activated oxygen species, nitric oxide metabolite levels, the intensity of lipid peroxidation in plasma and red blood cells, reduces the activity of the enzymes superoxide dismutase, catalase in erythrocytes increases the oxidase activity of ceruloplasmin in the blood plasma and violates the stability and the structural organization of erythrocyte membranes. It was also found that residents of elderly, a manifestation of coronary artery disease is associated with polymorphic markers T174M gene AGT; L33P gene ITGB3; L28P gene APOE; C3238G APOC3 gene and the C786T gene eNOS. It is more common combination of two or more polymorphic alleles in the heterozygous and homozygous states. Identified polymorphisms in genes that regulate hemostasis and endothelial function are associated with the development of oxidative stress and impaired structural homeostasis of erythrocyte membranes.

J09.43

Haplotypes in IL-8 Gene are Associated to Age-Related Macular Degeneration

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Age-related macular degeneration (AMD) is the main cause of blindness in the developed world. The etiology of the disease is multifactorial, due to the interaction between genetic and environmental factors. Genetic and functional studies have confirmed that inflammation plays a pivotal role in the development of the disease. It's well known that interleukins mediate many of the effector phases of immune and inflammatory responses. Moreover activated macrophages secrete many proteolytic enzymes able to fragment Bruch's membrane leading to the neovascularisation. In our study, we show the association between IL-8 gene and age-related macular degeneration. We performed a case-control association study, testing for the Single Nucleotide Polymorphism rs2227306 a total of 1381 Italian subjects: 721 cases and 660 controls. On the basis of our positive preliminary results (p-value: 4.15*10-5; OR for T allele: 1.39 [1.19-1.62]), we also sequenced the entire IL-8 regulatory and coding regions of 60 patients and 30 controls stratified for their genotype at rs2227306. We identified two different haplotypes involving rs4073 (A/T), rs2227306 (C/T), rs2227346 (C/T) and rs1126647 (A/T): A-T-T-T (p-value: 2.08*10-9; OR: 1.68 [1.43-1.97]) and T-C-C-A (p-value: 7.07*10-11; OR: 0.60 [0.51-0.70]). Expression analysis on RNA extracted from whole blood of 50 donors failed to reveal evidence of correlation between genotype/haplotype and RNA expression. Our results suggest the association between IL-8 gene and the development of the disease, although further studies should be encouraged to clarify the functional effects as well as the causative variants.

J09.44

Influence of metabolic gene polymorphisms on athletic performance

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Genetic factors play important role in determination of functional physiological, biochemical characteristics and athletic performance. Regular intensive training helps elite athletes to be at the peak of human physical performance, which makes them the best objects for studying molecular mechanisms of energy supply for muscular activity. The aim of this study was to discover whether polymorphisms of genes involved in metabolic pathways (*AMPD1* C/T rs17602729, *CKMM* A/G rs8111989, *G6PC2* G/A rs560887 and *MCT1* A/T rs1049434) are associated with elite athlete status and common phenotypes related to sports performance. DNA samples of 996 Russian athletes and 1389 non-athletic Russian controls were analyzed for four gene polymorphisms by PCR-RFLP analysis. Sporting intermediate phenotypes were assessed in subgroups of athletes by measuring of aerobic capacity, strength, anthropometric parameters, muscle fiber characteristics, blood lactate and glucose concentrations. Four 'endurance alleles' were overrepresented in a group of endurance athletes compared with controls: *AMPD1* C (*P*=0.01), *CKMM* A (*P*=0.001), *G6PC2* G (*P*=0.0004), *MCT1* A (*P*<0.0001). The frequency of four 'power alleles' was higher in strength/power athletes compared with controls: *AMPD1* C (*P*<0.0001), *CKMM* G (*P*=0.007), *G6PC2* G (*P*=0.14), *MCT1* A (*P*=0.004). Associations of polymorphic variants with predisposition to certain types of physical activity (endurance and strength/power) were consistent with results of correlation analysis of these polymorphisms with measured physiological, morphometric and biochemical parameters of skeletal muscle. Thus, *AMPD1, CKMM, G6PC2* and *MCT1* genotypes are associated with sporting intermediate phenotypes as well as with athletic status.

J09.45

No major clinical impact of a common variant in Toll-like receptor 4 gene on Temporal Lobe Epilepsy.

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Evidences support the hypothesis that inflammation and neurogenesis play an important role in the pathogenesis of temporal lobe epilepsy (TLE). Coding variants in Toll-like receptor 4 (TLR4) gene have been reported to be associated with inflammatory diseases, so TLR4 may represent a reasonable functional candidate gene for TLE. The aim of this study was to determine whether a functional single nucleotide polymorphisms (SNPs), an A>G base transition at position 896 from the transcriptional start site of TLR4 (referred to as Asp299Gly), contributes to TLE. We also investigated whether this variant may influence the TLE phenotype. We used a case-control approach comparing the frequencies of Asp299Gly polymorphism between unrelated TLE patients and matched controls. In the second step, we evaluated the patient group in terms of the major clinical variables related to the epileptogenic process.
The study group included 345 patients (189 women and 156 men; mean ± SD age: 47.43±18.24) with a diagnosis of non-lesional TLE, based on comprehensive clinical, electroencephalographic, and magnetic resonance evaluations, and 370 (186 women and 184 men; mean ± SD age: 48.46±20.96) healthy controls. All individuals were genotyped for the SNP Asp299Gly in the TLR4 gene using a TaqMan 5' allele discrimination assay. Analysis of genotype and allelic frequencies between patients and controls showed no statistically significant difference. Moreover, the Asp299Gly variant did not influence age of epilepsy onset, duration of epilepsy, and response to medication. Our results illustrates that a major clinical impact of variant as a disease modifier in TLE is probably unlikely

J09.46

Extensive mutational analysis of CDK5 and CDK5R1 in patients with non-syndromic mental retardation reveals novel variants in CDK5R1 3'-UTR

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CDK5 and its activator p35, encoded by CDK5R1 gene, are highly expressed in CNS where they have a fundamental role in neuronal migration and differentiation during CNS development. Their fundamental role in CNS development and function, and their involvement in the pathogenesis of neurodegenerative disorders makes CDK5 and CDK5R1 strong candidate genes for the onset of mental retardation. We carried out the mutation screening of CDK5 and CDK5R1 coding regions, as well as of CDK5R1 3'-UTR, on a cohort of 344 patients with non-syndromic mental retardation (NS-MR). In fact, we recently demonstrated that 3'-UTR has a key role in the post-transcriptional regulation of CDK5R1 expression, through the binding of protein factors and microRNAs belonging to miR-15/107 family, and this evidence prompted us to include this region in the mutational analysis. We found one silent mutation in CDK5, and three silent and two missense conservative mutations in CDK5R1 coding region. Four novel variations in intronic regions of CDK5 were also found but never predicted to cause splicing defects. Interestingly, we found nine heterozygous variations in CDK5R1 3'-UTR: among these, six were single base substitutions and three were small deletions. None of these variations was present in 450 healthy controls. Of particular interest is the deletion of one predicted miR-15/107 family binding site, found in one patient. Luciferase constructs containing the mutations observed in CDK5R1 3'-UTR will be used to verify if these variations have an effect on CDK5R1 expression levels and therefore constitute susceptibility variants for NS-MR.

J09.47

Analysis of polygenic hypercholesterolemia candidate gene variants

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Introduction: The main factor for atherosclerosis development is high LDL cholesterol (LDLc) levels. Majority of hypercholesterolemia cases are due to complex interactions gene-environment being the most common cause the polygenic hypercholesterolemia (PH).
Aim: Our objective was to identify candidate gene variants as contributors to de genetic cause of PH.
Methodology: 378 unrelated subjects, without mutations in *LDLR* and *APOB* genes, were selected according to the following criteria: LDLc ≥ 90th percentile and Tryglyceride levels ≤ 200 mg/dl. Moreover, a control group with 525 normolipemic subjects from the *Aragon Working Health Study* (AWHS) was analysed. All subjects were genotyped using Solexa (Illumina®) technology for 18 SNPs located in 5'UTR region of the *APOE*, *APOB*, *PCSK9*, *NR5A2*, *SREBF1* and *LDLR* genes.
Results: We have observed different allelic distribution between the PH population and the normolipemic subjects within 5 genetic variants. Our results have shown a higher frequency of the minor allele in PH versus control population of the *APOB* SNPs: rs51235 (0.497 vs 0.444, p<0.0250), rs617314 (0.333 vs 0.272, p<0.0055) and rs58429712 (0.092 vs 0.027, p<0.0001); while a lower frequency was observed in the PH group for the NR5A2 polymorphism rs9427440 (0.260 vs 0.387, p<0.0001) and the *LDLR* variant rs17248720 (0.087 vs 0.164, p<0.0001), are inversely related to PH.
Conclusion: We have identified 5 SNPs associated with the phenotypic expression of PH disease. The 3 *APOB* SNPs are directly related to PH phenotype while the *LDLR* and *NR5A2* polymorphisms seem to be protective to the hypercholesterolemia development.

J10. Evolutionary and population genetics, and Genetic epidemiology

J10.01

Armenian HLA profile in the genetic context of Middle East

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Armenians are an indigenous people of the South Caucasus with strong and distinct ethnic and cultural characteristics. Despite the important geographic location of historical Armenia in terms of ancient migrations and spread of Indo-European languages and agriculture, the genetic profile of the Armenian population is not well studied yet. In this work, we aimed to answer the following questions: i) is the Armenian population geographically stratified based on HLA markers despite their ethnic unity, and ii) what is the place of the Armenians on the genetic landscape of the Middle-East? To answers these questions we analyzed the genetic structure of three large Armenian territorial groups, Karabakh, historical Western Armenia, and Iran, as well as ethnically different populations from Middle East, Europe, Africa and Far East. Initial information on HLA typings were taken from published papers and analyzed relying on low resolution genotyping results of HLA-A, HLA-B and HLA-DRB1 loci. All the three Armenian territorial groups were assessed for haplotype frequencies estimated by the EM (Expectation-Maximization) algorithm using Arlequin software package. It was shown that the three Armenian subpopulations despite their ethnic homogeneity are geographically stratified which is in compliance with the results based on the Y-chromosomal markers. The pattern of genetic relatedness between the three Armenian groups and the comparative data sets revealed that the Armenians along with Mediterranean ethnic groups form a distinct cluster on a Principal Coordinate plot and Neighbor-Joining tree based on standard genetic distances, which is supporting the Mediterranean origin of Armenians.

J10.02

Monitoring of congenital malformations in population of Republic of Moldova

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In Republic of Moldova congenital malformations (CM) occupies second place in structure of infantile mortality. Monitoring of CM in our country acts since 1991, and since 2009 they carry out work jointly with EUROCAT.

The aim of present study was evaluation of prevalence and structure of CM in Republic of Moldova based on genetic monitoring during the period 2006 - 2010.

The system of genetic monitoring in Moldova based on registration of all the range of congenital pathologies in live newborns, stillborn and children dead after birth with weight less than 500 g. on term of 22 weeks and more. CM registered in children during first year of life.

In the territory of Moldova during the period 2006 - 2010 was born 195837 children, including 3562 with CM. During this period there were 144 pregnancies terminated conform medical indications due to congenital or hereditary pathology (such as CM, chromosomal aberrations), revealed prenatal before 22 weeks of gestation. Overall prevalence of CM was 18,92 for 1000 newborns. Maximal prevalence of CM was in 2006 - 22,19 for 1000 and minimal in 2007 - 16,38 for 1000.

In structure of CM in Moldova first place occupies anomalies of musculoskeletal anomalies, cardio-vascular and multiple anomalies.

The prevalence of individual forms of CM (esophageal atresia, palate/lips clefts, omphalocele, Down syndrome) corresponds to indexes of EUROCAT. Anal atresia, spina bifida and polydactyly were more rare than in EUROCAT, and reductional anomalies were more frequent.

J10.03

A combined influence of TNFα and GSTs genetic variants in pathogenesis of COPD

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The etiology of chronic obstructive pulmonary disease (COPD) is multifactorial, including genetic and environmental factors, as well their interactions. Increased inflammation and oxidative stress are the most prominent features of COPD. Since that, variants of genes involved in inflammatory response and protection from oxidative stress, which may alter these processes, might initiate and/or progress COPD.

We performed a case-control study in order to examine the role of functional variants of tumor necrosis factor α (TNFα) and glutathione-S-transferases (GSTs) in. Total number of 86 COPD patients and 100 controls were genotyped for TNFα G-308A, GSTM1 null, GSTT1 null and GSTP1 Ile105Val variants.

Statistical significance was observed for TNFα -308GG genotype (82.5% *vs* 69%, OR=2.13, p=0.04) in COPD *vs* controls. The statistically significant results were also obtained for combination of TNFα -308GG and GSTM1 null (48.8% *vs.* 31.0%, OR=2.12, p=0.016), as well for combination of TNFα -308GG, GSTM1 null and GSTP1 105Val/ (Val) (30.2% *vs.* 15.0%, OR=2.46, p=0.014).

This is the first study in which TNFα -308GG genotype was associated with pathogenesis of COPD. In addition, TNFα -308GG genotype in combination with GSTM1 null and GSTP1 105Val/(Val) was associated with the disease, that further confirmed importance of -308GG genotype for COPD pathogenesis.

Single and complex genotypes revealed in this study indicate the importance of the analysis of larger number of candidate genes which might contribute to elucidation of COPD pathogenesis and might be valuable in prevention of the disease.

J10.04

Genetic polymorphism and haplotypes of HLA-A,-B,-DRB1 loci distribution analysis in North-West region of Russian Federation

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Umbilical cord blood contains hematopoietic stem cells and can be used as an alternative to bone marrow transplantation in certain cases. The most important factor of effective transplantation is the degree of HLA matching. There is a great genetic variety in HLA among representatives of different nationalities. The aim of given investigation is to determine the major histocompatibility complex antigens of umbilical cord blood samples from public bank, by a molecular-genetic method, to analyze loci and haplotypes HLA-A,-B,-DRB1 polymorphism of the North-West region of Russia residents. During the work 500 samples of cord blood were analyzed using polymerase chain reaction (PCR) with sequence specific primers (SSP).

It was identified that the most frequent alleles are: HLA-A*02 - 28,3%, *03 - 15,8%, *24 - 13,6%; HLA-B*07 - 12,6%, *35 - 11,4%, *44 - 8,2%, HLA-DRB1*15 - 15,8, *13 - 15,0%, *03 - 12,5%. The most frequent haplotypes are: HLA-A*01-B*08-DRB1*03 - 0.55%, HLA-A*03-B*07-DRB1*15- 0.45%, HLA-A*03-B*35-DRB1*01- 0.45%.

Obtained results of genetic polymorphism of HLA-A,-B,-DRB1 loci distribution in the North-West region of Russia inhabitants can complement existing information database on population genetics and associated diseases.

J10.05

Sequence analysis of the GJB2 gene in Romanian children with hearing impairment

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Congenital hearing loss is one of the most common sensorial impairments in human, affecting approximately one in 1000 children. DFNB1 locus, comprising GJB2 and GJB6 genes, is responsible for up 50% of cases with congenital nonsyndromic prelingual deafness in many population. More than 100 mutations have been reported in exon 2 of the GJB2 gene, whereas two mutations has been described in the donor splice site of intron 1.

In this respect, the purpose of the study was to investigate genetically children with prelingual nonsyndromic hearing loss. Here we report the first study of GJB2 gene sequencing performed in Romania.

The study was carried out on a group of 11 children with prelingual hearing loss. The children were previously found negatives for the three most common mutations involved in nonsyndromic deafness in Europeans namely 35delG GJB2 gene, del(GJB6-D13S1830) and del(GJB6-D13S1854). To amplify the entire coding exon 2 we used three primer sets. The primers for non-coding exon1 were chosen so as to include the donor splice site of intron 1.

The sequence analysis of the GJB2 gene revealed the recessive splice site IVS1+1G>A (-3172G>A) mutation. This variant was homozygous in two subjects. The mutation was also confirmed by MPLA analysis. The other deaf children were found negatives for the mutations in the GJB2 genes.

The results are promising and continuing the study will make possible the construction of a data base regarding hearing loss in Romanian population. Molecular diagnosis will also allow timely intervention at children with hearing impairment.

J10.06

Uninterrupted CCTG tracts in the myotonic dystrophy type 2 associated locus - are they really rare?

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Myotonic dystrophy represents the most common form of adult muscular dystrophies. The first genetic form of the disease, myotonic dystrophy type 1 (DM1), is caused by expansion of the (CTG)n repeat tract in the DMPK gene. The second genetic form, myotonic dystrophy type 2 (DM2), is caused by expansion of the (CCTG)n repeat tract in the ZNF9 gene. The CCTG tract is generally interrupted in healthy range alleles and is uninterrupted in pathologically expanded alleles. Our study reports the variability of the healthy range DM2 alleles found during a population study in Slovakia. In comparison with previous studies, we identified wider range and higher frequency of healthy range alleles containing uninterrupted CCTG tracts. As uninterrupted alleles were so far reported mainly on larger alleles, they were considered as possible DM2 premutations. Our findings, however, suggest that uninterrupted CCTG parts are not restricted to large alleles and can be found continuously throughout the whole range of healthy range alleles, from the smallest up to the largest ones. This emphasizes the need for further studies aimed partially to the better characterisation of boundaries between meiotically and mitotically stable alleles and those which can be considered as unstable DM2 premutation alleles.

J10.07

Development and validation of a methodology to analyze the molecular phylogeny of the family Piperaceae, Piper genus, of species found in the Western Brasilian Amazon

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Family Piperaceae, especially Piper genre, presents a fascinating array of plant species for the study of natural products in organic chemistry, pharmaceuticals and ethnobotany. The best use of these species and handling the genre can be provided with the incorporation and association studies of genetic variability. Therefore, knowing the distribution of genetic variability among and within this population is essential for the establishment of appropriate use, as well as better enforcement. Thus, this study aims to evaluate, from the DNA, the genetic structure of natural populations found in the western Brazilian Amazon, with a view to advancing knowledge and manipulation of species, favoring the development of conservation strategies and stimulating the application of a breeding program to actively participate. Preliminary data from our lab established the best methodology to be applied in this analysis. The results show that the method described by Doyle & Doyle (1990) and modified by Faleiro et al. (2003) was the most efficient in obtaining DNA of better quality and in sufficient concentrations to be used in studies of genetic diversity in plants. To molecular analysis in this population, in a comparison with different techniques, RFLP, RAPD, AFLP and SSR, the results indicates that RAPD (random-amplified polymorphic DNA) can be successfully used (Powell, 1996). This reinforces the use of this technique in studies of genetic variability. It is worth emphasizing that in this work we shall present our findings and especially focus on the recommendations that were the conclusion of this part of the investigation.

J10.08

Distribution of MEFV Gene Mutations among Turkish Patients with Crohn's Disease and Ulcerative Colitis:Preliminary Results

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Inflammatory bowel disease (IBD) and Familial Mediterranian Fever(FMF) may share some clinical and biological features; they are both inflammatory disorders characterized by the same chronic relapsing behaviour, infiltration by neutrophils at the site of injury, and abnormal regulation of apoptosis. There is a few studies about Mediterranean Fever (MEFV) gene mutations in Turkish patients with Crohn's disease(CD) and Ulcerative Colitis(UC). In this study, we aimed to investigate the frequency and distribution of MEFV mutations in Turkish adult patients with IBD. Twenty-two patients with UC and 18 patients with CD (totally 40 patients) included in the study. The most common 10 MEFV mutations detected by using microarray method after PCR amplification of DNA samples. MEFV mutations found in 14 patients. In CD patients E148Q mutation allele, in UC patients M694V mutation allele was found to be signaficantly more frequent. In conclusion, our study is important in terms of showing distribution of MEFV allele among Turkish patients with UC and CD. MEFV mutation allele frequency is higher in CD patients than UC patients.

J10.09

Prevention of mutagenic effects in human populations

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The human genome is constantly exposed to attacks by environmental mutagens. The permissible levels of exposure and the concentration of mutagens are established on the basis of preventing acute effects. It does not take account of possibility of long-term (stochastic) effects - unfavorable pregnancy outcomes (UPO) in exposed individuals. Requires a quantitative assessment of risk of stochastic effects, and measures for their prevention.
To determine the external dose doubling the incidence of UPO's in the families of exposed persons and the development of preventive measures long-term effects of radiation in human populations. Results: During the 1985-2006 years based on the data about outcomes of pregnancies and the health of the offspring in populations exposed to ionizing radiation over a wide dose range, defined as follows:1. Frequencies UPO's in 226 populations exposed to radiation after the Chernobyl accident. Additional to the natural background radiation dose were calculated for the period from 1986 to 1992 ranged from 4 to 152 mSv. 2. The radiation dose, doubling the incidence of UPO's in the 1st-generation offspring of exposed individuals. 3. The frequency of congenital anomalies among infants in the population and families of personnel of industrial enterprises are constantly contact with mutagens. 4. It is proposed package of measures for prevention UPO's in the families of personnel continually exposed to mutagens, and the population living near industrial plants - the sources mutagens.

J10.10

Index endogamy in Republic Tatarstan, Russia

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In 13 areas of Tatarstan on the basis of total sample of marriage records for 1990-2000 (N=31837 after an exception of marriages of persons of postreproductive age and inhabitants of other regions registering marriage on the historical native land) the index endogamy is counted up that appeared to be lowest in the Pestrechinsky area (0,45) like Kazan (between Pestretsy and Kazan there are 28 km), the highest (0,74) - in Aktanyshsky (to Kazan of 307 km), the most remote from Kazan from all 13 studied areas. We will noticed while endogamy index calculating for representatives of the various ethnoses occupying these areas, distinctions in migratory activity of representatives of various ethnoses are not revealed. Rural endogamy has appeared low enough - from 0,06 in the Old Drozhzhanoe to 0,25 in Menzelinsk. In cartographical extrapolation the minimum values of endogamy are found out near to big cities - Kazan and Nizhnekamsk, and maximum - in marginal areas: Drozhzhanovsky (0,70), Baltasinsky (0,69), Kukmorsky (0,67), Alkeevsky (0,70) and Aktanyshsky (0,74). These results show high degree of isolation of rural populations of Russia.

J10.11

Application of 52 Autosomal SNPs (SNP*for*ID) to Forensic Casework in Malaysia

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The analysis of degraded DNA can be problematic. Recent advances in the identification and analysis of single nucleotide polymorphisms (SNPs) have demonstrated the advantage of these markers over short tandem repeats (STRs) in that they only require small amplicons. However, before applying to casework, it is important to develop allele frequency databases from three Malaysian major ethnic groups; Malay, Chinese and Indian.
In order to genotype the population samples reliably and robustly, four sets of 13-plex SNPs were developed for 52 autosomal SNP markers (that have been identified in the SNP*for*ID project). A total of 150 DNA Malaysian samp-

les were genotyped using this multiplex assays and full, complete and clear profiles were generated. Data were collected and evaluated statistically.
Across the three ethnic groups, few significant departures from HWE were observed in Malay, Chinese and Indian ethnic groups, for example, at marker rs2107612, no heterozygosity was observed at all in Malay group (Ho=0) but the Indian group showed higher heterozygosity (above 80%).The combined mean match probabilities for the 52 SNPs of Malay, Chinese, and Indian are 2.1974^{e-18}, 6.0042^{e-18} and 1.1756^{e-18}, corresponding to a combined power of discrimination of >99.99999999%, respectively. Paired F_{st} values obtained in the study suggest that Malay group is closely related to Chinese compared to Malay-Indian or Chinese- Indian.
As for forensic casework, we have demonstrated these multiplexes with the samples that in some instances the SNPs can generate full profiles from DNA extracts that yielded no or partial STR loci.

J10.12

Prevalence and co-infection of 3 human Torque teno viruses in the Romanian population

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The TT viruses (TTVs) recently discover DNA viruses and included in the Anelloviridae family. The strains that infect humans are Torque teno virus (TTV), Torque teno midi virus (TTMDV) and Torque teno mini virus (TTMV).
The aim of the study was to determine the prevalence and distribution of the TT viruses, and the co-infection pattern in several human pathologies.
Materials and Methods: The viral DNA was studied in the blood of patients with diabetic nephropathy, breast cancer, colorectal cancer, thalassemia, healthy controls and in the saliva of healthy subjects using nested-PCR. The amplification products were analyzed by agarose gel electrophoresis. For additional genotyping, high resolution melting (HRM) was performed.
Results: The average frequency of TTV and TTMV was approximately 85% among all the subjects, while for TTMDV was 60%. The average frequency of co-infection was 55%, and the highest rate of triple co-infection (80%) was found among the thalassemic patients. The highest frequency of the viruses was also found among the patients with thalassemia. The most commonly found virus was TTV and least common was TTMDV.
Conclusions: TT viruses' prevalence is the highest among the patients with thalassemia. The co-infection pattern is not correlated to a specific pathology. The viruses can be easily detected in the subject's saliva. The estimated frequency is consistent with the values reported in other populations.
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J10.13

Clinical and genetic spectrum of single gene disorders among Saudi Arab

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The high consanguinity rate had resulted into increased incidence of rare autosomal recessive disorders in The Kingdom of Saudi Arabia. Some of these disorders had been observed to be more specific for certain tribes and families with an evidence of gene founders. This had facilitated providing a selective genetic testing for this population.
A retrospective review of 1359 patients with single gene disorders seen within our medical facility was done. We found that this population has similarities in the disease-causing molecular mutations with nearby Arabian Gulf countries*. However, novel mutations were detected for the following disorders; Arthrogryposis-Renal Dysfunction-Cholestasis syndrome, Hereditary motor and sensory neuropathy with agenesis of the corpus callosum, Aicardi-Goutieres Syndrome 3, Pelizeaus-Merzbacher-Like Disease 1, Pelizeaus-Merzbacher-Like Disease 1, spondyloconstal dysostosis type 2, h ypophosphatasia*,Gangliosidosis 1, Alstrom syndrome, IRAK4 defeicency , Abetalipoproteinemia, Fabry disease and mucopolysaccharidosis type I. We hope that this data will help to guide researchers and medical professionals within the region in molecular diagnosis of their patients.

J10.14

Characteristics of mitochondrial DNA HVS-1 sequence in patients and aborted fetuses with aneuploidies

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Background: Aneuploidy is strikingly frequent in humans: up to 20% of all zygotes have an abnormal number of chromosomes. Despite intensive research little is known about the origin of aneuploidy and its risk factors. Here we analyze mitochondrial DNA of aneuploid fetuses and patients to reveal whether certain features of mtDNA of primary oocytes make them more prone to non-disjunction.
Methods: Hypervariable segment 1 of mtDNA was sequenced in 48 patients with Down's syndrome, 22 aborted fetuses with DS and 2 aborted fetuses with Edward's syndrome - altogether 72 samples. Previously published HVS-1 sequences of 267 Byelorussians were used as controls.
Results: Attention was focused on frequencies of 16189C and its combination with 16183C as these substitutions create a polyC stretch which interferes with the replication of mitochondrial DNA and is reported to be a risk factor for metabolic syndrome and cancer. 16189C is 1.6 times more frequent in the group of aneuploids compared to the control one (p=0.07), whereas 16183C+16189C haplotype is 3.7 times overrepresented among aneuploids (p=0.005). Another allele, 16300G, shown to be a risk factor for bipolar disorder, was found in 3 cases with DS, although it is absent from the studied population of healthy Byelorussians. 16292T and the rCRS haplotype are significantly underrepresented among aneuploids (p<0.05).
Conclusions: 16189C (and its combination with 16183C) and 16300G in mother's mtDNA may be a risk factor for aneuploid conceptions. 16292T and the rCRS haplotype may be linked with some protective SNPs in the coding region of mtDNA.

J10.15

FVII R353Q and GP1a C807T polymorphisms in Ukrainian ischemic stroke patients

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Ischemic stroke is a complex pathology, with a variety of genetic and environmental risk factors. The aim of our study was to establish the possible involvement of the FVII R353Q and GP1a C807T polymorphisms into ischemic stroke development.
Case-control study including 179 patients aged 39 to 81 years with ischemic stroke and 88 patients aged 59 to 92 (control I), as well as population control (control II, n=97) without a history of stroke has been performed. Genotyping was performed by the PCR followed by RFLP analysis.
Comparative analysis of genotype distribution in ischemic stroke and control I showed a marginal association of the RR genotype of R353Q polymorphism with stroke (OR=1.79; 95% CI, 0.99-3.23; P=0.05). Also, there was a significant association between RR genotype and stroke compared to control II (OR=2.03; 95% CI, 1.15-3.59; P=0.01). Obesity as one of the stroke risk factors itself showed a strong association with stroke in our study (OR=2.35; 95% CI, 1.51-4.8; P=0.008). Moreover, while analyzing the joint effects of R353Q and obesity, interaction between these risk factors was revealed (Synergy factor=6.9, 95% CI, 1.34-36.39; P=0.02). The RR individuals with obesity had a significant susceptibility to ischemic stroke (OR=4.03; 95% CI, 1.64-9.89; P<0.01). No significant association was found between ischemic stroke and C807T polymorphism.
Our study suggests that RR genotype of R353Q polymorphism may be an additional ischemic stroke risk factor in patients with obesity, while GP1a C807T polymorphism is not associated with a risk of ischemic stroke in Ukrainian patients.

J11. Genomics, Genomic technology including bioinformatics methods, gene structure and gene product function and Epigenetics

J11.01

Epigenetic identification of stem cells: From embryonic toadult

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Stem cells, like other incredible properties they have, possess unique epigenetic structure. Growing evidence suggests that the potency of these cell to regenerate from low to complete differentiated cell lines is accurately depend on epigenetic processes occurring on DNA and chromatin. DNA methylation and histone modifications are the major contests which lead to the establishment of chromatin states in these cells. It seems that these contests have fundamental regulatory effect on the potency of different stem cells and make embryonic cells to be unique for their stemness characteristics compare to adult cells. Tracing these epigenetic change from embryonic stem cells to adult stem cells would be an appropriate approach to investigate the function of the events in reducing stemness capacity. More study in this field would help to establish new approachs to induce pluripotency in different cell types; especially in multipotent adult stem cells and it would be useful for therapeutic approaches. In this review we summarize current progress in field of embryonic and adult stem cells epigenetics and also we try to illustrate conceptual regard to epigenetic changes during embryonic development and beyond.

J11.02

Customized DNA microarray fabrication for gene expression analysis of multiple sclerosis

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Multiple Sclerosis (MS) is a complex autoimmune disease, involved many pathophysiological processes which should be considered in designing diagnostic, prognostic studies. In recent years, microarray technology becomes a valuable tool for identifying good biomarkers in diagnostic as well as prognostic processes for all diseases. Also better drug treatments may be obtained by identifying new drug targets and studying drug effects via this technique .

In this work, we designed a customized microarray for 76 susceptible genes in MS, which involved in different pathophysiological processes (e.g. inflammation, demyelination, axonal damage and repair mechanisms) based on peripheral blood analysis. Poly-l-lysine coated glass slides was used to fabricate this microarray by BioRad vers-array spotting robot. Fabricated microarray can be used to study gene expression of MS patients and healthy controls in various clinical conditions.

J11.03

Development and validation of multiplex (4-plex) PCR-based assay to assess DNA degradation

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To assess the degradation of DNA in the model organisms (pig, rabbit and human), Multiplex (4-plex) PCR- based assay was developed. Sequence data for a nuclear gene; recombination activating gene 1 (RAG-1) from rabbit, pig and human was aligned to identify conserved regions for primer design that would amplify 70 bp, 194 bp, 305 bp and 384 bp amplicons. PCR was optimised so that it worked over a wide range of template amounts (0.03 ng to 75.83 ng). Multiplex (4-plex) PCR assay was validated following the guidelines of Scientific Working Group on DNA analysis Methods (SWGDAM). The multiplex PCR was tested for its specificity, reproducibility, sensitivity and stability using ABI 310 and 3500 genetic analysers (Applied Biosystems). Samples treated with various environmental regimes and DNeasy1 were also included in this study. The multiplex (4-plex) PCR was found to work efficiently in triplicate samples of all three species until 0.3 ng of DNA template, although, partial profile is also obtained with 0.03 ng DNA template.
The result of this validation study promises that this multiplex can be used in forensic analysis to assess DNA persistence in human decomposing bodies following mass disasters and in experimental animals (rabbit and pig) and therefore is recommended for forensic purposes.

J11.04

VMD DisRg: New user-friendly implement for calculation distance and radius of gyration in VMD program

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Molecular dynamic simulation is a practical and powerful technique for analysis of biopolymers structure. Several programs have been developed to facilitate the mentioned investigation, under them the visual molecular dynamic or VMD is the most frequently used programs. One of the beneficial properties of the VMD is its ability to be extendable by designing new plugin. We introduce here a new and easy to use facility of the VMD for distance analysis and radius of gyration of biopolymers such as protein and DNA. Our plug-in is a user friendly package which calculates mentioned analysis for structure or simulation trajectories with 3D representation center of mass.

J11.05

Epigenetic aberrations in leukocytes of patients with schizophrenia: Association of global DNA methylation with antipsychotic drug treatment and disease onset

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Even though schizophrenia has a strong hereditary component, departures from simple genetic transmission are prominent. DNA methylation has emerged as an epigenetic explanatory candidate of schizophrenia's non-Mendelian characteristics. To investigate this assumption, we examined genome-wide (global) and gene-specific DNA methylation levels which are associated with genomic instability and gene-expression activity, respectively. Analyses were conducted using DNA from leukocytes of schizophrenic patients and controls. Global methylation results revealed a highly significant hypomethylation in schizophrenics ($P < 2.0*10^{-6}$) and linear regression among patients generated a model in which antipsychotic treatment and disease onset explained 11% of the global methylation variance (adjusted $R^2 = 0.11$, ANOVA $P < 0.001$). Specifically, haloperidol was associated with higher ("control-like") methylation ($P = 0.001$) and early-onset (a putative marker of schizophrenia severity) was associated with lower methylation ($P = 0.002$). With regard to the gene-specific methylation analyses, and in accordance with the dopamine hypothesis of psychosis, we found that the promoter of *S-COMT* was hypermethylated in schizophrenics ($P = 0.004$). In conclusion, these data support the notion of an aberrant epigenetic regulation in schizophrenia which may be subject to certain antipsychotic treatments. Additionally, blood DNA-methylation signatures show promise of serving as a schizophrenia biomarker in the future.

J11.06

Role of the genes encoding NMDA receptors in the development of neurologic pathologies during influenza infection

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Influenza is one of the most widespread respiratory viral diseases, infecting humans. Influenza virus may cause various neurologic complications which can lead to chronic diseases characterized by different cognitive dysfunctions. However, until recently very little was known about the molecular biological mechanisms of activity disbalance of the central nervous system in case of influenza disease. One of the dominant roles in this process probably belongs to the ionotropic glutamate receptors selectively binding N-methyl-D-aspartate (NMDAR). The main aim of this study was to analyze the expression level of the genes encoding NMDAR during influenza infection. Native NMDARs are thought to function only as heteromeric assemblies containing both NR1 and NR2 subunits. The NR1 family is encoded by a single gene. The NR2 family of subunits consists of four members arising from separate genes (GRIN2A, GRIN2B, GRIN2C, GRIN2D). The novel mRNA which can be encoded by GRIN2B gene was found. We used postmortal human lung tissues from patients who died because of complications against pandemic influenza virus disease. PCR analysis of IL-10 mRNA expression shown that the nonspecific products were irrefutable obtained. Sequence of this amplicon aligns with the intron 2 of GRIN2B. Gene-specific primers for detecting expression level of human and mouse NMDAR were designed using Primer-BLAST program. To test these primers we used total RNA isolated from mice tissues section. We are now planning to investigate the expression of NMDAR genes in cell cultures infected by pandemic and seasonal influenza virus H1N1. The work is supported by RF President's Grant.

J11.07

Cafe Variome: sharing diagnostic sequence variants with the research community

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The internet-based Cafe Variome is designed to function as an exchange portal for gene variant (mutation) data produced by diagnostics laboratories, offering users a secure environment through which to announce and discover a comprehensive listing of observed neutral and disease-causing variants in patients and unaffected individuals.

To achieve this, Cafe Variome facilitates the “publication” of data from researchers, diagnostics laboratories, and others, to any stakeholders who may wish to, for example, check for evidence of causal influence upon certain disease states, and/or incorporate the data into locus-specific databases. While data generators generally do not object to disseminating anonymised diagnostic data, they are not motivated to do so because of the effort and time involved. Cafe Variome specifically addresses these issues by:

- Enabling data analysis tools used by research and diagnostic laboratories with a “data submission” function which automatically pushes diagnostic data to Cafe Variome, which acts as a universal data reception and advertisement point
- Offering manual support to laboratories to move their variant datasets into Cafe Variome (legacy data and new data in batches or in real time).

The development of Cafe Variome (based at the University of Leicester) involves the cooperation of diagnostic software companies PhenoSystems (Gensearch) and Interactive Biosoftware (Alamut) as well as academic partners in the Bioinformatics Support Group at Leiden University Medical Centre (LUMC) whose Mutalyzer data-validation tool will allow us to feedback data-inconsistencies to submitters, and at NGRL Manchester who have extensive expertise in diagnostic databases through their development of DMuDB.

J11.08

Mutation screening of *ATP13A2* in early onset Iranian Parkinson‘s disease patients

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Parkinson’s disease (PD) is the most common neurodegenerative movement disorder whose average age of onset is 60 years. Individuals in whom symptoms first manifest before age of 40 years are classified as early onset PD (EOPD) cases.

To date, 4 genes responsible for early EOPD have been identified, *parkin*/PARK2, *PINK1*/PARK8, *DJ1*/PARK7 and *ATP13A2*/PARK9. Among these genes, mutations in *ATP13A2* are the least common. Mutations in PARK9 have been reported to cause autosomal recessive early onset Parkinson’s disease (PD). *ATP13A2* is located on chromosome 1p36, contains 29 exons, and codes a transmembrane protein of 1180 amino acids which belongs to the Group 5 P-type ATPase superfamily. The function and substrate specificity of the coded protein remains unknown. Here we present results of mutation screening in 14 Iranian EOPD patients in whom mutations in *LRRK2*, *PRKN*, *DJ1*, and *PINK1* were previously ruled out. The average age at onset of the Iranian patients was 20 years, and the range was 11-31 years. The whole *ATP13A2* coding region (29 exons) and exon-intron boundaries were sequenced from genomic DNA. A novel homozygous variation (**IVS8+19G>A**) was identified in non-coding region in one sporadic patient. To identify the effect of this variation in splicing, NNsplice software was used.The results showed, this variation isn’t effective on splice site. This patient also had a polymorphism (c.1138G>C) in heterozygous state in *PRKN*. Thus we conclude that *ATP13A2* genetic variability is unlikely to cause or influence the development of PD.

J11.09

Effects of PITX2 as a Transcription factor in ocular development on their Target genes

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The development of the eye comprises a series of inductive events. A number of genes that include transcription factors, growth factors, nuclear proteins and enzymes are involved in multiple cascades of events in ocular development and other developmental processes. These developmental regulatory networks are therefore not only important in the development of the eye but also in the development of the whole embryo as well.

The Pituitary homeobox 2 (PITX2) and Forkhead box C (FOXC1) proteins are examples of such transcription factors.PITX2 is a member of the paired-bicoid family of homeodomain (HD) transcription factors. Pituitary homeobox proteins are actively involved in a wide range of developmental processes, including formation of the pituitary gland, hind limb and anterior segment of the eye. 41 genes for PITX2 were identified by Expression profiles derived using microarray.

Usage of Bioinformatics tools for determination of promoter region, finding PITX2’s Binding sites and conservation of promoters revealed that several genes directly affected by PITX2.among these, promoter of *PLEKHG5* were cloned into PGL4-14 vector then this construct co-transfected into HeLa cells with expression vector of PITX2.

Interestingly Dual luciferase assay revealed that PITX2 transcription factor, which is known to be involve in ocular development, increase the activity of *PLEKHG5* promoter and expression of its downstream gene.

J11.10

Next generation sequencing for molecular diagnosis of neuromuscular diseases

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Introduction:

Neuromuscular diseases (NMD) are debilitating disorders with a strong impact on the individuals and society. Despite tremendous research and clinical efforts, the molecular causes of NMD are still unknown for about 40% of patients and additional genes remain to be found. In order to provide a faster and cheaper molecular diagnosis for NMD patients and to detect different types of mutations, we have validated sequence capture, DNA barcoding and next generation sequencing (NGS).

Results:

Using targeted re-sequencing of 267 genes implicated in NMD, we sequenced 16 patients (4 pools of 4 DNAs) with different types of mutations where we knew the mutations in half of them. We could successfully detect all the disease-causing variants in the 8 patients with known mutations (covering point mutations, intronic (-10nt from splice site), a small indel and a large deletion).For patients with unknown mutations, we used a ranking program and we could find the disease-causing mutations in several of them.

Conclusion:

We conclude that NGS is a powerful approach to identify potential disease-causing variants, a prerequisite for genetic counseling and better healthcare. it should allow reducing the diagnosis time and its cost.

It might represent a first screening without the need for detailed clinical criteria for inclusion that may be absent in atypical forms of the diseases or when the disease begins. In addition, the analysis might be proposed before the need of more invasive investigations such as biopsy. This emerging strategy is likely to become a standard tool for routine genetic diagnosis.

J11.11

Mitochondrial Defects in Trisomy 21 Fetuses might contribute to the Down Syndrome Neurological Phenotype

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Hsa21 trisomy has been associated to mitochondrial dysfunction in Down Syndrome (DS) mouse models and the dysregulation of genes encoding mitochondrial proteins has been demonstrated in human trisomic fetal brains by microarray analysis. Since mitochondrial function has a central role in many neurodegenerative diseases, such as Alzheimer and Parkinson, it has been hypothesized that the mitochondrial defect might contribute to the DS mental retardation.

The aim of this study was to fully characterize the mitochondrial defect in fibroblasts derived from trisomic human fetuses.

Genes mapping to chromosomes different from 21, and implicated in multiple mitochondrial functions such as respiratory chain, mitochondrial biogenesis and structure, were studied by qRT-PCR. Many of the analyzed genes, including PGC-1 α, a gene that plays a key role in mitochondrial biogenesis, were significantly down-regulated in trisomic versus euploid fibroblasts, supporting the hypothesis that Hsa21 trisomy perturbs the expression of mitochondrial genes. Mitochondria ultrastructure, assessed by electron microscopy, revealed morphological abnormalities in trisomic fibroblasts, like giant mitochondria with irregular shape, breaks of both inner and outer membranes and altered cristae pattern. Functional studies demonstrated a significant reduction of oxygen consumption rate and of respiratory chain complex I activity, a decrease of mtDNA copy number and an increased production of reactive oxygen species in trisomic fibroblasts.

These results are indicative of a widespread mitochondrial dysfunction and support the hypothesis that it might contribute to the DS neurological phenotype.

J11.12

Extended comparative analysis of high resolution array platforms for genome wide detection of CNV

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High-density oligonucleotide array CGH is now a widely available tool for the analysis of genomic copy number variation (CNV), in discovery studies, for the validation of CNVs detected with other means (i.e. sequencing) and increasingly in cytogenetic diagnostics.

Various platforms are available from several different suppliers, utilizing either array Comparative Genome Hybridization (aCGH) alone or in combination with SNP genotyping.

Earlier [Haraksingh et al., PLoS One, 2011] we carried out a quantitative comparison analysis of the performances of twelve leading genome-wide CNV detection platforms. We tested the ability of the different array types to accurately detect Gold Standard sets of CNVs in the genome of HapMap CEU sample NA12878. The Gold Standards used were a 1000 Genomes Project sequencing-based set of 3997 validated CNVs and an ultra high-resolution aCGH-based set of 756 validated CNVs.

The arrays that were originally analyzed were the NimbleGen 4.2 M, 2.1 M and 3×720 K Whole Genome and CNV focused arrays, the Agilent 1×1 M CGH and High Resolution and 2×400 K CNV and SNP+CGH arrays, the Illumina Human Omni1Quad array and the Affymetrix SNP 6.0 array. We have since then added the NimbleGen 12-plex and 6-plex platforms, respectively, to the comparative analysis.

We compared the arrays with the criteria of sensitivity, total number detected, size range and breakpoint resolution of CNV. We found that arrays with the genome-wide CNV-focused design principle generally outperformed whole-genome (evenly spaced tiling) designs. Our results should be useful when designing CNV detection strategies in both research and clinical settings.

J12. Molecular basis of Mendelian disorders

J12.01

Ataxia with oculomotor apraxia, type 2 (AOA2) in a Russian family

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AOA2 is an autosomal recessive ataxia caused by various mutations in *SETX* gene. Typical age of onset is 15-20 years (variability 3-30). Progressing ataxia due to cerebellar atrophy and polyneuropathy are main disabling features. Oculomotor apraxia (OA), inability to coordinate eye movements, is a specific sign seen in half of patients. Serum alpha-fetoprotein raise is helpful. AOA group includes AOA1 (*APRX* gene) with onset in 4-5 years and AOA3 (*PIK 3R5*) described in 2012 in a Saudi family. OA is typical also for Louis-Bar ataxia-telangiectasia. Recently an AOA1 case was confirmed in our laboratory. A first Russian AOA2 case in a 25-year-old male, an only child in the family, is presented. The disease started in 18 years, in 23 years he lost independent walking due to incoordination and weakness; ataxia in hands and dysarthria were moderate. OA was found on neurological examination but produced little symptoms. Sensorimotor axonal polyneuropathy was confirmed by electroneuromyography. MRI showed moderate cerebellar atrophy. Alpha-fetoprotein level was tenfold raised. A hereditary ataxia was considered from the disease onset, and a number of genetic tests were performed, but AOA2 was recognized only seven years later. On direct sequencing of *SETX* exons 6-8 a novel frame-shift mutation c.2623-2626 del 4 in heterozygous state was detected which is sufficient for AOA2 confirmation; the allelic mutation is in search. DNA diagnostics permits genetic counseling and prenatal testing in AOA families. OA should be purposefully searched for in children and young adults suspicious of autosomal recessive ataxias.

J12.02

High expression of IL-10 in Netherton Syndrome

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Objectives: Netherton syndrome (NS) is a rare autosomal recessive disorder, characterized by congenital ichthyosiform erythroderma, trichorrhexis invaginata and severe atopic diathesis. Considering that cytokines are involved in NS pathogenesis and that cytokine gene polymorphism may affect cytokine production, our purpose was to investigate the association between NS and IL-6, IL-10, IFN-γ, TGF-β1 and TNF-α polymorphisms.

Methods: Cytokine genotyping and haplotyping were performed in a family with NS by PCR-SSP method.

Results: We observed GCC GCC haplotypes (high expression) of IL-10 gene polymorphisms (-1082 A/G, -819 T/C, -592 C/A) in proband with NS and no association with IL-6, IFN-γ, TGF-β1 and TNF-α polymorphisms.

Conclusion: GCC GCC haplotypes which leads to high expression levels of IL-10 may be associated with etiopathogenesis of NS.

J12.03

Charcot-Marie-Tooth disease in Republic of Sakha (Yakutia)

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Background: Republic of Sakha (Yakutia) is the largest region in Russia. It is located in the North-East part. Population of republic makes 958,021 persons. The leading place in structure of a genetic pathology in Yakutia is occupied with hereditary disease of nervous system among which one of the most common is Charcot-Marie-Tooth disease (CMT). Prevalence CMT in the world makes 4,7-40 per 100,000 population, in Yakutia prevalence has made 11,8 per 100,000. Methods: the research is carried out by the materials from Republican Genetic Registry of inherited and congenital pathology of Republic of Sakha (Yakutia) in medical-genetic consultation Department of the National Medical Center. The diagnosis was established on the basis of anamnestic data, data of neurological examination, genealogic analysis, electroneuromiography and molecular-genetic analysis with the use of markers 17 dup4, 17 dup5 in gene PMP22 (locus 17 p12-p11.2). Results: we studied 113 medical records of patients with CMT in the age of 6 to 75 (average age is 33,30±1,45), male - 60 (53%), female - 53 (47%). There were 87 Sakha patients (77%), Russians - 22 (20%) and 4 other nations (3%). The CMT1A type was established in 37 out of 113 cases (33 %), the rest of patients had unknown mutations. For CMT1A is characterized by the onset in childhood, the high arch of the foot, the reduction or absence of tendon reflexes, upset sensitivity on polyneuritic type, hypotrophy of peroneal muscles, occurrence of stepping gait.

J12.04

A further case of lamellar ichtyosis having H435Y mutation in CYP4F22 gene

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Autosomal recessive congenital ichthyosis (ARCI) is a rare keratinisation disorder with an estimated prevalence 1:100.000-300.000. It is classified into two groups: Lameller Ichthyosis (LI) and congenital ichthyosiform erythroderma (CIE). The major difference between these two forms is scale property. Although plate-like, dark brown scales are common in LI, fine scales are mostly observed in CIE patients. ARCI is a genetically heterogenic disease. To date mutations in TGM1, ALOX12B, ALOXE3, NIPAL4, CYP4F22 and ABCA12 genes have been have been described in ARCI.

We report a five-months-old girl with lameller ichtyosis. Parental consanguinity in this case strongly suggested autosomal recessive inheritance. She was born with collodion membrane and had grayish scaling. There were no mutations in the molecular genetic analysis of TGM1 and NIPAL4 genes. Following this, CYP4F22 gene was sequenced and a homozygous c.1303 C >T (p.H435Y) mutation was found in the index patient. The parents were heterozygous for the mutation.

The case presented here is the first Turkish ARCI case having this particular mutation.

J12.05

Profile of Iranian Genome variation of connexin 31 gene

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Background & Objective: The connexins are a family of at least 20 homologous proteins in humans that form aqueous channels connecting the interiors of coupled cells and mediating electrical and chemical communication. Mutations in GJB3, the gene encoding the gap junction protein Connexin 31 (CX31), have been pathogenically linked to erythrokeratodermia and non-syndromic autosomal dominant (DFNA2) or recessive hereditary hearing impairment (HHI).

In the present study, we aimed to characterize the genome variation of GJB3 in Iranian patients with hearing impairment.

Methods: Twenty five non-syndromic autosomal recessive hearing loss patients who were normal for two major responsible genes of non syndromic hearing loss (GJB2 and GJB6) were tested with direct sequencing of entire coding region of the GJB3 gene.

Results: Single nucleotide sequence alteration was present in 15 out of 25 patients (60%) and 40 % of patients were normal. Five different variations that were detected are: G866A (34%), G798T (20%), C856A (6%), C357T (2%) and C93T (2%).

Conclusions: 60 percent of patients showed single polymorphism in coding and non coding part of GJB3. All patients were normal for sequencing of two well known genes for non syndromic hearing loss (connexin 26 and connexin 30). Therefore this high percentage of genetic variation in GJB3 may have pathogenic effects on Iranian population, but this needs more investigation on normal subjects

J12.06

Early-onset primary dystonia (DYT1) in Iranian patients

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Early-onset primary dystonia (DYT1) typically presents in childhood or adolescence and only on occasion in adulthood. Dystonic muscle contractions causing posturing of a foot, leg, or arm are the most common presenting findings. Dystonia is usually first apparent with specific actions; e.g., writing or walking. Over time, the contractions frequently (but not invariably) become evident with less specific actions and spread to other body regions. No other neurologic abnormalities are present, except for postural arm tremor. Disease severity varies considerably even within the same family .Isolated writer's cramp may be the only sign. DYT1 is inherited in an autosomal dominant manner with reduced penetrance. TOR1A, encoding the protein torsin-1A (torsinA), is the only gene known to be associated with this disease. DYT1 is diagnosed by molecular genetic testing of TOR1A revealing the three-base pair deletion c.907_909delGAG in most affected individuals.

Oral medications are usually tried first and include anticholinergics, baclofen, and others alone or in combination (levodopa, clonazepam and other benzodiazepines, carbamazepine, and dopamine-depleting agents).

We investigated molecularly 12 cases suspected to DYT1 using PCR and sequence analysis of TOR1A gene. C.904_906delGAG heterozygote was found in one patient. This result showed that this alteration was observed in less than %10 of our cases. The rest of cases are being investigated for the other relevant genes for familial dystonia.

J12.07

Plasmid vector encoding recombinant protein ApoB100(site B) - GFP for measuring functional disruptions of LDL-receptor

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Familial hypercholesterolemia (FH, OMIM 143890) is rather widespread autosomal dominant disease leading to increased level of low-density lipoproteins (LDL) what in turn leads to higher risk of atherosclerosis. FH is associated with disorder of LDL-receptor.

Apolipoprotein B100 (apoB) is the only protein component of LDL particles providing their binding to receptor. Sub-clinical diagnostics of FH, such as genetic analysis, is difficult due to large size of LDL-receptor gene. Study of intermolecular interactions between receptor-active domain of apoB (3359-3369) and LDL-receptor gives new opportunities of measuring functionality of LDL-receptor. For this purpose we created the plasmid vector encoding receptor-binding region known as high conservative site-B (3359-3369) of apoB fused to 5'-terminus of GFP gene. Fragment of apoB gene was amplificated through PCR. The template was human DNA. Vector was constructed by direct cloning of apoB gene fragment with SalI and HindIII endonucleases. Also we have inserted sequence encoding polyhistidine tag to 5'-terminus of *apoB* to make the metal chelate affinity chromatography of fusion protein possible. Plasmid was successfully sequenced and studied by restriction analysis. *E.coli* cells transformed with this plasmid vector and incubated in IPTG-containing medium showed presence of fluorescent protein.

Maximums of excitation and emission of fusion protein are equal to GFP characteristics. This protein has been extracted and purified on nickel agarose column. Synthesized fusion protein could become basis for diagnosticum of FH, with which functionalality of LDL-receptor could be measured. Studies of binding of fusion protein to cells receptor in cultures of hepatocytes and fibroblasts are taking place.

J12.08

To characterize and analyze the haplotypes of normal and "at risk of expansion" FMR1 CGG repeat alleles

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The mechanism of instability of FMR1 alleles during transmission from mother to offspring and the exact timing of CGG repeat expansion are still unknown and recently the investigation of instability of the FMR1 gene has been a major goal in Fragile X research.

The haplotypes which are in linkage disequilibrium with these unstable alleles provide not only potential markers of those with the potential to expand but also clues to their inherent susceptibility. This approach is important as it might contribute to understanding the mechanism for instability of CGG repeats in the initial steps of small CGG expansion.

To address this issue and make a contribution to understanding the basis of the mutational process, three flanking microsatellites markers (DXS548-FRAXAC1-FRAXAC2) and two SNPs (ATL1 and FMRb) were genotyped in a large cohort and haplotype associations compared among subgroups of normal and intermediate alleles. It was of special interest whether there were differences in the distribution of haplotypes across the normal and intermediate allele ranges in two different cohorts.

J12.09

Determination of the nucleotide variations in exon 2 of GJB2 and exan3 of GJB6 genes in central part of IRAN.

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INTRODUCTION: GJB2 & GJB6 are human genes encoding for gap junction protein. Defects in these genes lead to the most common form of congenital deafness. Some variations are reported in different countries.

METHODS: 120 individuals were evaluated by pcr-sequencing. Some of them were suffering from deafness & others were their relations that were referred for carrier detection.

RESULTS: of the 120 individuais,22 had GJB2 mutations,6 were found to be heterozygous for 35delG,4 were heterozygous for C:127 G>A (R>H),one case was heterozygous & one was homozygous for C:143 C>T (R>W),5 were heterozygous for C:153 A>G (V>I),that 3 of them were deaf & 2 of them were healthy but one case was homozygous for this mutation & he wasn't deaf. One was heterozygous for C:30 A>G & one was heterozygous for 57delT.one individual had a compound heterozygous mutation of 35delG/C:143 C>T. there wasn't any change in exon 3 of GJB6 gene. There was only one silent variant in sequencing of this gene.(G>A (T>T))

CONCLUSION: these results can help genetic counseling & molecular genetition in evaluation of GJB2 & GJB6 variation.

J12.10

Investigation of EPM2A and NHLRC1 Mutations in Turkish Patients with Lafora Disease

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Investigation of Lafora Disease Mutations in Turkish Population

The progressive myoclonic epilepsies (PMEs) are composed of a rare group of inherited neurodegenerative diseases with a prevalence of 1% of epileptic syndromes in children and adults around the world.

Among PMEs, Lafora disease (OMIM #254780) has been recognized as the most common and severe form of adult-onset progressive epilepsy with an autosomal recessive form of inheritance. Affected individuals exhibit epileptic seizures with a progressive deterioration to status epilepticus combined with progressive dementia, and starch-like polyglucosans called Lafora bodies detected in skin biopsies. The age of onset usually occurs between ages of 12 and 17 years, and patients usually die within 10 years of onset. Approximately 80% of affected individuals have displayed a mutation in *EPM2A* gene located on chromosome 6q24, which encodes laforin; a tyrosine phosphatase. *NHLRC1* (*EPM2B*) gene, recently mapped to chromosome 6p22, encodes E3 ubiquitin ligase, and was shown to carry mutations responsible for the disease.

The proposed study comprises mutational analysis of 9 patients diagnosed with the Lafora disease after all necessary clinical examinations. All exons and exon-intron boundaries of *EPM2A* and *NHLRC1* genes were PCR amplified from genomic DNA and analyzed for mutations through direct DNA sequencing.

One of the patients' revealed homozygous c.436G>A missense mutation (D146N) in *NHLRC1* gene, which was previously reported in Danish families.

This is the preliminary result of the study which is continuing with a larger group of patients.

J12.11

Novel deletion c.22-1320_633+1224del in gene CYB5R3 in patients with recessive congenital methemoglobinemia in Russia

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Recessive congenital methemoglobinemia (RCM) type I and II is a rare autosomal disease, which characterized by deficiency of soluble or soluble and membrane-bound forms of NADH-cytochrome b5 reductase (cytb5r) appropriately. The more severe type II form is characterized by cyanosis with mental retardation, the only symptom for type I is cyanosis. Mutations in gene CYB5R3 is the molecular-genetics cause of both types of disease. We have investigated patient with type II of disease and found deletion of exons 2-7 in homozygous state. Also we have investigated two sisters with type I of RCM and found in two sisters only one mutation p.Val253Met in exon 9 in heterozygosis state, which was previously described. Analysis of haplotypes for 4 markers, which flank gene CYB5R3, detected one general haplotype in patient with type II in homozygous state and in two sisters with type I in heterozygosis state. Then we developed system, which permitted us to detect exact borders of the deletion in introns 1 and 7. The size of deletion formed 12 kb and the name of deletion was c.22-1320_633+1224del. In next step we developed system, which permitted us to detect deletion c.22-1320 _633+1224del in homo- and heterozygosis state, and we revealed this deletion in two sisters. Our patients with type I and II of RCM are not relations but they live in Volga region in Russia.

J12.12

Screening DFNB59 gene mutation in non-syndromic genetic hearing loss in Iran

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Background and aim: Hearing Impairment (HI) is the most prevalent Neurosensory disorder which is heterogeneous and can also occur due to environmental causes. The majority of hearing deficiencies are of genetic origin affecting about 60% of the HI cases. A novel gene DFNB59 encods pejvakin has been recently shown to cause deafness. This study aims to determine the frequency of DFNB59 gene mutations in coding region the gene in Iran.

Method: In this descriptive experimental study, we investigated the presence of DFNB59 gene mutations in Exons (2-7) of the gene in 80 deaf subjects. DNA was extracted using standard phenol -chloroform method. The screening of gene mutations was performed by PCR-SSCP/HA procedure. Finally, the possible mutations were confirmed by direct sequencing.

Results: In all, 9 polymorphisms 793C>G were found in 80 non-syndromic, genetic hearing loss subjects studied. However no DFNB59 gene mutation was identified.

Conclusion: We conclude that the association of DFNB59 gene mutations with hearing loss is very low in samples studies.

J12.13

Mutation detection in a large Iranian family with spinocereberllar ataxia type 6 (SCA-6)

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Spinocerebellar ataxia is an autosomal dominant neurodegenerative disorder characterized by slowly progressive ataxia. 30 different loci have been reported to be responsible for cerebellar ataxia. In general SCA 1-7 are the most frequent forms and account for 50-80 % of ADCA in most studies. The mutation associated with these loci is abnormal expansion of CAG repeat. The frequency of SCA subtypes varies in different ethnic groups.

We here investigated a large Iranian pedigree with clinical presentation of ADCA consisting of 13 affected members in three generation.

Genomic DNA was extracted from blood of patients with cerebellar ataxias and unaffected individuals. To amplify a fragment of SCA6/CACNA gene containing the CAG repeat, the polymerase chain reaction (PCR) was performed. PCR products were run on 3% agarose gel, wild-type and expanded alleles were extracted from the gel and were sequenced with both forward and reverse primers. Analysis of the sequence data determined the number of repeats for the normal and expanded allele as 11 and 24 repeats respectively. The results were confirmed by capillary electrophoresis using fluorescent primer labeled with 6-FAM. The size of the normal and expanded alleles were determined as 128 bp and 169 bp respectively with capillary electrophoresis.

Investigating patients in different generation showed a stable number of repeats, with no sign of increase in the number of repeats. This finding was consistent with the clinical findings in patients because no sign of anticipation, such as earlier age of onset and more severe clinical presentation was seen.

J12.14

Clinical and molecular characterization of two large families segregating severe tooth agenesis

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Tooth agenesis is the most common abnormality affecting formation of dentition in humans. The absence of six or less teeth is usually referred as hypodontia, while oligodontia is a term used for agenesis of more than six teeth excluding third molars. Tooth agenesis can be found either in isolated form (non-syndromic) or it can be associated with systemic condition or syndrome, such as various types of ectodermal dysplasia (syndromic tooth agenesis). In addition to previously known genes (PAX9, MSX1, AXIN2, EDA, EDAR), mutations in EDARRAD and WNT10 gene were recently found to be involved in isolated forms of tooth agenesis. Here we report unusual cases of two large families of Roma origin segregating non-syndromic oligodontia with very variable phenotype (4-17 missing teeth in family I, 2-12 missing teeth in family II). Molecular analysis of seven suspected genes will be presented.

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J12.15

GJB2 caused hearing loss in patients from Belarus

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Mutations in Connexin 26 gene (*GJB2*) are responsible for more than half of all cases of prelingual nonsyndromic recessive deafness in Caucasians. The carrier frequency of the c.35delG mutation in *GJB2* gene was found to be as high as 1-4% in the European populations. Here a prevalence of *GJB2* caused cases and a mutation spectrum in 112 unrelated probands (and 8 their sibs) with prelingual nonsyndromic deafness from Belarus are shown. Among the probands there were different an ethnic groups including Belarusians (53%), Poles, Russians, Ukrainians, Azerbaijanians. In 50 probands and in all their sibs with hearing loss (45%) biallelic mutations in *GJB2* gene were detected. We revealed 8 pathological defects in the *GJB2* gene: the most common mutation c.35delG (allelic frequency: 84%), c.313_326del14 (7%), c.-23+1G>A (IVS1+1G>A) (2%), p.Met34Thr (2%), p.Glu120del (2%) and less frequent c.235delC, c.167delT and p.Ile82Met. The carrier rate in hearing individuals from Belarus for mutation c.35delG was estimated as 3% (8/234). A prevalence of hearing loss caused by mutations in *GJB2* gene was calculated to be 1/2500 in Belarus.

J12.16

The heterozygous carrier frequency of ABCA4 gene mutations in Russia

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Stargardt disease is the most common hereditary early-onset macular degeneration. It characterized by slowly progressive loss of central vision.

Autosomal-recessive Stargardt desease type 1 (STGD1) is caused by mutations in the ATP-binding cassette transporter gene (ABCA4). ABCA4 gene includes 50 exons, encodes 2273 amino-acid and mapped to 1p22. There are about 500 mutations in the ABCA4 gene have been reported.

882 unrelated control individuals and 100 unrelated STGD1 patients from Russia were screened for the prevalent European ABCA4 gene mutations: Gly863Ala, Ala1038Val and Gly1961Gln by multiplex ligation-dependent probe amplification (MLPA) and subsequent gel electrophoresis. In the control group the allele frequency of Gly863Ala, Ala1038Val and Gly1961Gln were 0.17%, 0.51% and 0.62%, respectively. In STGD1 patients the allele frequency for mutations Gly863Ala, Ala1038Val and Gly1961Gln were 1.0%, 17% and 6.0%, respectively. Heterozygous carrier frequency of mutation Gly863Ala is high in different countries of Europe (up to 1 out of 18 in Northern Europe), but it is significantly lower in Russia (1 out of 294 by our data).

Mutation Ala1038Val is the most frequent in Russian STGD1 patients, but prevailing mutation in control group is Gly1961Gln. The possible explanation is the heterozygous state of Gly1961Gln mutation provokes an age-related macular degeneration (AMD) and reduces this mutations` frequency in the group of STGD1 patient. This reseach is the first attempt to define the heterozygous carrier frequency of ABCA4 gene mutations in Russian population.

J12.17

Homozygosity mapping of a family with a mixed dystonia phenotype

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Introduction: Dystonia results from involuntary concomitant contraction of agonist and antagonist muscles, with overflow of unwanted muscle contractions into adjacent muscles. Several classifications of dystonia are based on topographic distribution, age at onset, cause, or genetics. According to the etiologic classification this syndrome includes primary dystonia, secondary dystonia, dystonia-plus syndromes, and paroxysmal dystonia. Presently, eleven genes and seven additional loci have been reported to be associated with monogenic primary dystonias. We report the mapping of a family from Iran with an autosomal recessive form of dystonia-plus syndrome with severe hearing impairment linked to the chromosome 13.

Materials & methods: Whole genome homozygosity mapping was performed in a consanguineous Iranian family with two dystonia affected children and five unaffected family members using high density single nucleotide polymorphism chips**.** Patients had childhood-onset form of dystonia, muscle atrophy, and severe hearing impairment.

***Results*:** We observed a large homozygosity region of 15 Mega bases on chromosome 13 in all affected individuals of this family but no among the non-affected individuals. Nearly 200 annotated genes exist within the linked region.

Conclusions: These findings indicate that the causative gene exists on chromosome 13. Because these patients are suffering from a mixed phenotype, we have chosen some candidate genes related to dystonia, hearing loss, and muscle atrophy in this chromosomal region. Exome sequencing and mutation screening of candidate genes within the linked region is being performed.

J12.18

Homozygousity mapping in one Iranian pedigree affected with Autosomal Recessive Congenital Ichthyosis (ARCI) reveals linkage to region 17p13 and mutation in *ALOX12B* gene

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Ichthyosis is clinically and genetically heterogeneous group of disorders characterized by abnormal skin scaling over the whole body. Autosomal Recessive Congenital Ichthyosis (ARCI) is a subgroup of ichthyosis that exhibits autosomal recessive inheritance. To date, eight genes and three additional loci have been associated with ARCI. Mutations in these genes account for disease in 70-75% of the patients.

We performed whole genome homozygosity mapping in an Iranian ARCI family using high density SNP chips.

Disease status in the family linked to a homozygous region of 2.2 Mb on chromosome 17. The ALOX12B gene associated with ARCI lies within the region and mutation screening revealed a homozygous mutation causing p.Arg442Gln. The missense mutation p.Arg442Gln (c.1325C>T) in exon 10 just was reported in one Japanese patients as compound heterozygous and p.Arg442Gln mutation was not found in his parents and was thought to be a de novo mutation. But, R442Q mutation in ALOX12B in our patients was homozygous in both patients and heterozygous in their parents. Phenotypic similarities and variations among the mutation carrying patients are detected but, two patients showed a striking palmoplantar hyperlinearity. It seems mutation in ALOX 12B gene is associated with mild form of ARCI and hyperlinearity in palms and soles.

J12.19

Molecular Genetic Diagnostics of Phenylketonuria in Kazakhstan

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The aim of our study was to determine the spectrum and frequency of the most common mutations of RAH gene in patients with phenylketonuria in Kazakhstan.

Materials and methods. Molecular genetic analysis of mutations in PAH gene (R408W, R261Q, R252W, IVS10-11, IVS12 +1, R158Q, P281L, IVS14 +5) carried out using PCR technique. We studied DNA from blood of 44 patients with phenylketonuria and their parents.

Results. The results of our study showed that in Kazakhstan the most important are the six mutations: R408W with frequency 0,455, mutation R261Q with frequency 0,136, IVS12nt1, IVS10nt546, P2812L, IVS12+1G>A with the same frequency 0,023. Besides, we had neutral polymorphism V245V in 7 exon of a PAH gene that described earlier (Dworniczak et al., 1990).

Conclusions: The examination of mutant alleles in patients and their families or investigation of alleged heterozygous carriers with pathological mutations provided good possibility for prenatal diagnosis of the fetus. This is important factor for prevention of hereditary diseases in families.

J12.20

Whole exome sequencing combined with linkage analysis identifies novel variations in a large Coronary Artery Disease family

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We sought to use exome sequencing in conjunction with linkage information to identify candidate causative mutations in a large family with CAD. Linkage analysis of this family comprising six affected and 4 unaffected individuals revealed linkage signals at 3 Loci with maximum LOD score of 2.1. We captured exomes of two affected individuals from a family and performed sequencing analysis by a second-generation sequencer with a mean coverage of 30× and sufficient depth to call variants at ~97% of each targeted exome. The shared genetic variants of these two affected individuals in the family being studied were filtered against the 1000 Genomes Project and the dbSNP131 database. After annotation and functional expectation, three variations were found to be candidates for CAD.

J12.21

Genetic study of demyelinating form of autosomal-recessive Charcot-Marie-Tooth diseases in Russian families

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Charcot-Marie-Tooth disease (CMT) is the most common inherited neuropathy. During of the scale analysis of demyelinating form of CMT in Russia the necessity was presented of research of autosomal-recessive CMT (AR-CMT). The aim was focused on molecular analysis of the selected genes associated with AR-CMT and construction of a molecular and genetic diagnostic algorithm in this group of disorders in the Russian population.

We analyzed a group of 92 unrelated patients with probably autosomal recessive inheritance. The studies covered analysis of coding regions of the GDAP1 gene using sequencing and molecular genetic analysis of eight frequent occurrence mutations using two Multiplex Ligation Probe Assay (MLPA) systems. The first MLPA-system contained six of frequent occurrence mutations in four genes: FGD4 (Met298Thr, Met298Arg), FIG4 (Ile41Thr), GDAP1 (Leu239Phe), SH3TC2 (Arg954Stop, Arg658Cys). The second MLPA-system contained two frequency Gypsies mutation in two genes: NDRG1 (Arg148Stop) and SH3TC2 (Arg1109Stop).

In result the cause of AR-CMT was determined in 26% of cases (24 patients). Mutations in GDAP1 gene were most frequent (18,5% or 17 patients). The mutation Arg148Stop of NDRG1 gene was found of three patients (3,2%). Mutations Arg954Stop of SH3TC2 gene and Ile41Thr of FIG4 gene were found of two patients (2,2%).

This is the first study focused on the autosomal recessive Charcot-Marie-Tooth disease in the Russian population, which is essential for molecular diagnostics in CMT disease.

J12.22

Abnormal Type I Collagen folding and matrix deposition in a Cyclophilin B KO mouse model of recessive Osteogenesis Imperfecta

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Recessive OI is caused by deficiency of proteins involved in collagen post-translational interactions, including the collagen prolyl 3-hydroxylation complex consisting of CRTAP, P3H1 and PPIB. The function of α1(I) Pro986 3-hydroxylation is unknown, but roles in fibril alignment and matrix cross-linking are speculated. PPIB, a prolyl cis-trans isomerase also known as cyclophilin B, is thought to catalyze the rate-limiting step in collagen helix formation. To further characterize the role of PPIB in collagen folding, Ppib-null mice were generated from a gene-trap ES cell clone with half-normal *PPIB* expression. Homozygous *Ppib*$^{-/-}$ mice were verified by real-time RT-PCR to completely lack transcripts in skin, fibroblasts, osteoblasts and femora. *Ppib*$^{-/-}$ mice weigh one-third less than WT and Het littermates. Ppib protein was absent and P3h1 reduced 50% in *Ppib*$^{-/-}$ cultured fibroblasts and osteoblasts. As expected, α1(I) P986 3-hydroxylation was reduced to 5-11% of WT. In agreement with previously described patients with decreased P986 3-hydroxylation, collagen from *Ppib*$^{-/-}$ cells had delayed electrophoretic mobility on SDS-Urea PAGE. However, thermal stability, 5-lysyl and 4-prolyl hydroxylation of fibroblast collagen were normal. Inhibiting hydroxylation of collagen with α,α'-dipyridyl resulted in Ppib$^{-/-}$ collagen alpha chains with faster elecrophoretic migration than WT chains, suggesting altered conformation due to loss of peptidyl-prolyl isomerization. In *Ppib*-/- fibroblast cultures, collagen deposition was decreased by 70% vs WT cultures, despite only moderate delay of secretion. These data suggest unique roles for Ppib in collagen post-translational processing and extracellular matrix incorporation, in addition to its chaperone function for folding.

J12.23

Kuskokwim Disease, a recessive congenital contracture disorder, is the third syndrome caused by an *FKBP10* mutation

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Kuskokwim Disease (OMIM %208200), found solely among Alaskan Eskimos along the Kuskokwim river delta, is a recessive syndrome characterized by multiple congenital joint contractures, predominantly in knees and ankles. Null mutations in *FKBP10* (Chromosome 17q21.2) have been shown to cause recessive osteogenesis imperfecta (OI), with (Bruck Syndrome 1) or without congenital contractures. Linkage mapping of Kuskokwim Disease to chromosome 17q12-q21, together with contractures as a variable feature of *FKBP10* mutations, led us to sequence this gene. We identified a homozygous 3 nt deletion in *FKBP10* exon 5 (c.875_877delACT), which causes deletion of p.Tyr293, in probands from three Kuskokwim pedigrees. This mutation occurs in the 3rd PPIase domain of FKBP65 and is highly conserved among species. *FKBP10* expression by real-time PCR ranged from 0.75-1.67 of control in five probands, suggesting that the transcripts are not degraded. FKBP65 protein was present in proband fibroblasts at 2.5-7.5% of control levels, demonstrating destabilization of the protein. Steady-state type I collagen biochemistry has near normal migration. Additionally, collagen cross-linking is decreased in probands, with 0-25% hydroxylation of the telopeptide lysine involved in cross-linking and an 80% reduction in mature cross-linked collagen deposited in culture, despite normal collagen secretion. Collagen in extracellular matrix appears more diffuse, perhaps from thinner fibrils, with a normal to decreased collagen:organics ratio shown by Raman micro-spectroscopy. In conclusion, this is the third phenotype caused by mutations in *FKBP10*, adding contractures with osteopenia to the previously described severe OI with and without contractures; the Kuskokwim mutation specifically affects collagen cross-linking.

J12.24

Identification of mutations in PANK2 in Pantothenate kinase associated neurodegeneration (PKAN) patients

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Pantothenate kinase associated neurodegeneration (PKAN) is a rare autosomal recessive genetic disease classified within the group of Neurodegeneration with Brain Iron Accumulation (NBIA) disorders. Its reported prevalence is less than 1/1,000,000. PKAN diagnosis is based on clinical features and magnetic resonance imaging(MRI) and genetic analysis. Its clinical presentations include dystonia, speech defects, dysphangia, and intellectual impairment. Dementia, severe mental retardation and severe movement disability may develop at later stages. In MRI T2 weighted images, all affected individuals exhibit hypodensity in the globus pallidus region. Only in some cases, a region of hyperdensity in the center of the region of hypodensity creates an "Eye of Tiger" sign.

Here, we report first genetic screening of Iranian PKAN patients. The subjects included eight cases. The three patients, all of them presented the Tiger Eye, harbored homozygous mutations. The mutations affected Arg>Trp and Arg>Leu and Arg>Pro . All mutations were checked in 100 control individuals. *PANK2* mutation was not observed in any of the five cases without "Eye of Tiger" sign, consistent with the proposal that there is a tight association between *PANK2* mutations and this imaging feature. The five patients without *PANK2* mutations will be pursued for identification of another causing genes.

J12.25

The results of KRT5 and KRT14 analysis in Polish patients with epidermolysis bullosa simplex

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Simplex epidermolysis bullosa (SEB) is characterized by blister formation within basal or suprabasal layers of epidermis. Twelve clinically distinct subtypes of SEB have been distinguished. The localized SEB, Dowling-Meara SEB and other generalized SEB subtypes caused by mutations in KRT5 and KRT14 are most often. Keratins 5 and 14, encoded by them, form intermediate filaments in basal keratinocytes. According to the consensus on SEB diagnosis and classification, this three subtypes are inherited in an autosomal dominant manner. The aim of present study was to investigate the spectrum of mutations in Polish SEB patients. The 34 probands (10 localized SEB; 2 Dowling-Meara SEB; 5 other generalized SEB, 17 undefined) diagnosed on the basis of clinical symptoms or immunofluorescence mapping were included. Coding regions of KRT5 and KRT14 were analyzed by direct sequencing. We found 9 distinct variants in KRT5 and 7 in KRT14, including overall 7 novel changes. In 17/34 cases full genotype was established; in remaining patients molecular analysis has not been completed yet or no mutations were identified. The most frequent mutation found in 3 distinct families was p.Glu170Lys in KRT5, including siblings with mild SEB, where it was in trans with p.Val143Ala. Both parents of these patients seem to be unaffected carriers. Only few patients with compound heterozygosity in KRT5 have been reported previously, however, to our knowledge, none of them involved p.Val143Ala. Our preliminary results broaden the knowledge about SEB epidemiology and pathogenesis. The results have also practical impact on preparing the Polish molecular diagnostics scheme.

J12.26

Limb-girdle muscular dystrophys with expressed defeats of a myocardium

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Severe lesions of the myocardium at limb-girdle muscular dystrophy (LGMD) are the serious problem, sharply reducing quality and life expectancy of patients. In most cases a myocardium is involved in the pathological process to a greater extent than the skeletal muscles, which causes the characteristic clinical picture, in which the main symptoms are different types of arrhyth-

mias and malfunctions of cardionector. It is shown that the most severe disorders of the heart are observed at the following three genetic variants of LGMD: LGMD 1B, LGMD 1D and X-linked form of Emery-Dreifuss muscular dystrophy, caused by mutations in genes LAMIN, DES, EMD, respectively. Early diagnostics of the given forms will allow to prevent heavy complications at patients and to increase duration of their life. By means of direct automatic sequencing we analyzed coding sequences and adjacent introns of the three above mentioned genes among a sample of 12 patients (6 men, 6 women), were under our supervision. At 4 from them the vertical type of inheritance of disease in a family was observed, the others were isolated. As a result, we obtained the following data: for 7 patients were identified mutations in the LAMIN gene (58,33%), for 4 in the EMD gene (33,33%) and for 1 in the DES gene (8,33%). Revealed some features of the clinical picture for patients with mutations in the genes analyzed.
The data obtained may be the basis for the algorithm of molecular genetic diagnosis of LGMD with severe cardiac conduction system.

J12.27

A novel missense mutation (p.Arg309His) in the nuclear localization signal sequence of spastin protein causes a complicated form of Hereditary Spastic Paraplegia

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Hereditary spastic paraplegia (HSP) is a clinically and genetically heterogeneous group of neurodegenerative diseases causing slowly progressive spasticity and weakness of the lower extremities. Mutations in the *SPAST* gene are responsible for approximately 40% of autosomal dominant HSP and 6-15% of sporadic cases. Here we report the case of a 53-years old man who was complaining for a progressive gait difficulty in the last 4 years. At the time of the study his neurological examination showed spastic paraparesis with mild spasticity of the legs, slight weakness of the thigh and big toe extensor muscles, hyperreflexia of arms and legs, and left extensor plantar sign. Brain MRI showed diffuse T2-hyperintensities in the posterior periventricular white matter, possibly due to chronic vascular damage, and a mild atrophy of cerebellar vermis and hemispheres. The family history was negative for neurological diseases and consanguinity was excluded. Genomic DNA was extracted from peripheral leucocytes using the salting out method, after receiving informed consent from the patient. Sequencing of all 17 coding exons of the *SPAST* gene revealed a c.926 G>A transition in the exon 6. The change substitutes the arginine 309 with an histidine (p.Arg309His) in the functional nuclear localization signal sequence (NLS) of the spastin protein. This novel mutation was not found in 200 normal chromosomes. Arg309 is conserved among species and the PolyPhen-2 modelling analysis predicts the aminoacid substitution to be “probably damaging”, with a score of 0.991. The change p.Arg309His represents the first *SPAST* mutation identified in the NLS spastin domain.

J12.28

A mutation-site approach helps predicting the phenotypic expression of MYH7 mutations

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PURPOSE. We describe 3 novel MYH7 mutations (b-myosin heavy chain) associated with Dilated Cardiomyopathy (DCM) and Left-Ventricular-Non-Compaction (LVNC), localized within the region of actomyosin interface.
METHODS. Clinical assessment of 3 children with MYH7 mutations (E525K, M528I, F540L) was performed in dedicated clinics. Genetic study included sequencing of MYH7,MYBPC3,TNNT2,TNNI3,TPM1,ACTC1,LDB3,LMNA and TAZ. Mutations occurred once in >500 samples and were predicted as pathogenic by in-silico analysis. RESULTS. E525K was found in a newborn with DCM/LVNC without familial history of cardiac disease. She underwent a heart transplant (HTx) at 5 months. The M528I variant was detected in a girl with LVNC and mitral regurgitation. The mutation was inherited by the father‘s family. F540L was identified in a 16 year-old boy with DCM/LVNC. His mother and 2 siblings were affected. The mutations lie within the 50k lower sub-domain of the globular myosin head, in a region with surface hydrophobic helical peptides that are part of the binding site actomyosin. Other mutations in the same subunit have been described (G529D, M531R, S532P, M539L, A543T, D545N and A550V): the most prevalent phenotype was DCM/LVNC or DCM. High penetrance and early onset of disease were common (52% of affected were <21 years). The evolution was severe: 3 deaths from heart failure, 4 HTxs, 1 myectomy. CONCLUSIONS. We identified 3 novel mutations in MYH7 related to early-onset DCM and LVNC. This phenotype is shared by other mutations affecting the same sub-domain of MYH7. Such a site-specific approach could help interpreting the pathogenic role of previously undetected mutations.

J12.29

Mutation analysis of the GRIN2B gene in Alzheimer’s Disease

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Various mechanisms may contribute to neurodegeneration in Alzheimer‘s disease (AD), including glutamate-mediated excitotoxicity. This excitotoxic effect appears to be mediated by the N-methyl-D-aspartate receptors (NMDARs). The NMDAR subunit 2B (NR2B) has attracted more attention due to its characteristic distribution and selective reduction in AD brain. The potential involvement of the GRIN2B gene, encoding NR2B, in the risk for AD was evaluated in an independent series of Southern Italy samples. Clinical data and blood sample were collected from 270 selected AD patients, after inform consent. All coding exons and exon-intron junctions were amplified and a mutational screening was done by DHPLC and direct sequencing. Although the six detected variants are in the coding sequence, they are silent polymorphisms: Ala5; Pro122; Ser555; Cys838; Thr888 and His1178. First, we investigated Ser555 in exon 8 and His1178 in exon 13 of the GRIN2B in our patients and 250 healthy-matched controls. No statistically significant differences were found in GRIN2B genotype and allele frequencies (Ser555 P=0.142; His1178 P=0.868) between the AD sample and controls, even when the subjects were stratified by gender, APOE and age of disease onset in AD patients. The results of the Ala5 and Cys838 polymorphisms were omitted (low frequencies detected), while the analysis of the Pro122-Thr888 variants are in progress.Systematic mutation search of the GRIN2B gene in our patients with AD failed to identify any functional changes. However, we will continue with a more comprehensive screening of GRIN2B polymorphisms that might be useful to determine the involvement of this gene in AD.

J12.30

Identification of *TNFRSF13B* (TACI) mutations in pediatric patients with Cerebellar Hypoplasia and Common Variable Immunodeficiency (CVID).

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Common variable immunodeficiency (CVID) (OMIM#240500) is the most prevalent human primary immunodeficiency, diagnosed on the basis of an impaired ability to produce specific antibodies after vaccination and markedly reduced serum levels of IgG and IgA . *TNFRSF13B*, the gene encoding transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) a member of the TNF(Tumor Necrosis Factor) receptor family , is mutated in 5-10% of patients with CVID. C104R together with A181E are the most frequent coding variants accounting for 90% of TACI mutations. The C104R mutation abolishes ligand binding and, consequently TACI signalling which plays a fundamental role in immunoglobulin class switching, production and in B-cell homeostasis regulation. We analyzed by direct sequencing all five coding exons of *TNFRSF13B* in 9 pediatric patients (5 sporadic patients and 4 siblings belonging to two families) presenting with hypogammaglobulinemia, cerebellar hypoplasia, psychomotor delay and mild to bordeline intellectual disability. We identified the recurrent C104R mutation in heterozygous state, in two affected males in one family and in one sporadic patient. In all patients with *TNFRSF13B* mutations, we also analyzed by RT-PCR, TACI expression on peripheral lymphocytes, which was not impaired. Recent evidence suggests that tumor necrosis factor receptor superfamily members (TNFRSF) are expressed in an overlapping regulated pattern during neuronal development, participating in the regulation of neuronal expansion, differentiation and development. The association between TACI mutations and cerebellar hypoplasia has not been described before and could provide interesting clues for investigating the non-immunological role of *TNFRSF13B* in brain and cerebellar development.

J12.31

Are synaptophysin (SYP) mutations causing a syndromic form of X-linked intellectual disability?

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Background

To date, about 100 genes have been identified to underlie XLID (http://xlmr.interfree.it/home.htm).

Sequencing of the coding region of X-chromosome has revealed four mutations (T92fs*45, N59_K60>K*, D277fs*59 and G217R) in the synaptophysin gene (*SYP*) at Xp11.23-p11.22 encoding an integral membrane protein of small synaptic vesicles (Tarpey et al. 2009) in families with ID.

Subjects and Methods

Exome sequencing using AgilentSureSelectHumanX-chromosome kit and single-read76nt NGS on the Illumina GAII sequencer was applied to find the causative gene in the index patient belonging to a large Finnish family with a total of nine male patients with ID in three generations. DNA of six patients and their mothers were available for the study.

Results

A novel missense mutation c.879G>A (p.Gly293Ser) in exon 6 of *SYP* was identified that perfectly co-segregates with the disease in the family. The mutations were not found in 440 Finnish anonymous blood donors respectively. In detailed clinical investigation of the three affected patients (III/5, IV/1 and IV/2) similar dysmorphic features were identified including hyperplastic supra-orbital ridges, straight eyebrows, deep set eyes, and short philtrum. The carrier mothers were normal.

Conclusions

We anticipate that mutations in the *SYP* gene cause a previously undescribed syndrome of with X-linked intellectual disability.

Grants: The Sigrid Jusélius Foundation, Helsinki, Finland

J12.32

Analysis of the *GJB2* coding region in Tuvinian deaf patients (the Tuva Republic, Southern Siberia)

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Mutations in the *GJB2* gene, encoding the gap-junction protein connexin 26 (Cx26), are the most common cause of non-syndromic deafness. To investigate the molecular basis of non-syndromic deafness in the Tuva Republic (Southern Siberia, Russia), we analysed nucleotide sequences of the *GJB2* gene entire coding region in 90 deaf patients of Tuvinian ethnicity. The Tuvinians are the indigenous population of the Tuva Republic located northwest of Mongolia and directly east of the Altai Republic. Most of the Tuvinians are descended from ancient Turkic-speaking Central Asian tribes and Mongolian-speaking groups assimilated by them. Mutations p.W172C (c.516G>C), p.L79fs (c.235delC), p.V37I (c.109G>A), p.H100fs (c.299_300delAT) in homozygous or compound heterozygous state or as a single allele were found in 24 out of 90 patients (26.7%). Besides, 18.9% of studied Tuvinian chromosomes were carrying polymorphisms p.V27I (c.79G>A), p.E114G (c.341A>G), and p.F192L (c.571T>C). Interestingly, we observed high prevalence of the p.W172C mutation (79.4% out of all mutant chromosomes): 7 patients were homozygous and 13 patients -**heterozygous for** p.W172C. The p.W172C was first reported in the Altaians (the Altai Republic) (Posukh et al., 2005) and then in one Mongolian deaf patient (Tekin et al., 2010). The p.W172C change is located in the second extracellular domain of Cx26 protein. Based on evolutionarily conservation of c.516G in the *GJB2* sequence in many species and the PolyPhen analysis, p.W172C (c.516G>C) was considered to be probably pathogenic. The study was supported by RFBR grant #11-04-01221-à and by grant of the Chairman of the Tuva Republic Government for young scientists.

J12.33

A Novel Mutation of Ceruloplasmin Gene in a Patient with Atypical Pattern of Neuroimaging

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Hereditary aceruloplasminemia (HA) is a rare autosomal recessive disease characterized by anemia, iron overload, diabetes and progressive neurodegeneration, caused by homozygous mutation of the ceruloplasmin (CP) an necessary enzyme for the normal transportation of iron by plasma transferring. Recently, some authors have demonstrated that all their patients with HA showed on T2* scans, a pattern of brain iron deposition characterized by involvement of globus pallidus, putamen, caudate, thalamus, dentates and substantia nigra. Here, we report a case of patient affected by HA with an atypical pattern of neuroimaging. The patient was a 47-years-old man who presented progressive psychiatric symptoms, neurological symptoms and type I diabetes. A patient's cousin with a biochemical diagnosis of aceruloplasminemia was asymptomatic. Laboratory findings showed mild anemia, low serum iron concentration and high ferritin concentration value. CP was undetectable. Liver function test were normal. Genetics screening for hemochromatosis, HFE and CP genes was performed and demonstrated in CP gene a novel mutation (IVS6+1G/A) into a splice donor site on the intron 6. The same mutation was found in the asymptomatic cousin, confirming the biochemical diagnosis of HA. The T2*-MRI scans, showed the brain iron deposition as a marked hypointensity in all the typical cerebral structures. No abnormalities were found in the globus pallidus as typically occurs in a patients with HA. In conclusion, this is the first evidence of a patient affected by HA with the brain iron deposition which does not involve the globus pallidus.

J12.34

Sacsin-related ataxia caused by the novel missense mutation Arg272His in a patient from Southern-Italy

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Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is an early-onset cerebellar ataxia with spasticity, amyotrophy, nystagmus, dysarthria, and peripheral neuropathy. SACS is the only gene known to be associated with the ARSACS phenotype. The gene was initially reported to be encoded by a single gigantic exon. Recently eight additional exons were identified upstream of the giant exon. The SACS protein product, sacsin, is believed to integrate the ubiquitin-proteasome system and Hsp70 chaperone machimery. Recent studies indicates a role for sacsin in regulation of mitochondrial dynamics. To date, more than 70 different mutations, predominantly located in the giant exon, have been identified worldwide; of these only seven, exclusively located in the giant exon, were identified in Italian patients. The authors identified a novel homozygous variation c.815G>A, which results in the missense mutation p.Arg272His (R272H) in a patient from Southern-Italy. This mutation was present in heterozygosis in both unaffected parents as in one unaffected sibling and in several unaffected relatives. The phenotype of our patient closely resembled the classic phenotype. R272H missense mutation falls in the seventh coding exon of the SACS gene, hence not in the carboxyterminal giant coding exon which contains most reported sacsin mutations. R272H represents the first described Italian mutation located upstream of the giant exon of the SACS gene. The residue is completely conserved in evolution. The missense mutation R272H was not detected among 200 control chromosomes. Although it is also in a well-conserved region of the protein, no functional domain is defined for this region.

J12.35

Lack of the VPS35 Asp620Asn mutation in southern Italian patients with familial Parkinson's disease

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Parkinson's disease (PD) is a common neurodegenerative disorder, affecting 2% of those over the age of 75 years. Although generally considered a sporadic disease, Mendelians forms of the disease are described (SNCA and LRRK2 for causing autosomal dominant PD and 3 genes causing autosomal recessive, juvenile PINK1, Parkin, DJ1). Recently using an exome sequencing based approach, 2 indipendent groups have identified a missense mutation in vacuolar protein sorting 35 homolog (VPS35 c.1858G>A; p.Asp620Asn) as the probable cause of late onset PD in a number of kindreds. To estimate the frequency of the Asp620Asn mutation in VPS35 in familial PD, we screened for this variant in a southern Italy PD cohort. Our population included 114 patients with familial PD, having at least 1 relative among their first degree, second degree, in third degree family members with a formal diagnosis of PD (major PD genes had been analyzed and positive cases (9 LRRK2) were not excluded. This variant was not detected in any of the 150 analyzed familial PD cases, thus indicating that this mutation is rare among familial PD cases in the southern Italy.

J13. Metabolic disorders

J13.01

The diagnosis and the clinical features of a rare disease; Alpha-mannosidosis

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Alpha-mannosidosis is a rare autosomal recessive lysosomal storage disease of glycoprotein catabolism caused by a deficiency of lysosomal **alpha-**mannosidase activity. The incidence is approximately 1 of 500,000 live births. Characteristic clinical features include mental retardation, coarse facial features, ataxia, hearing loss, dysostosis multiplex, and immunodeficiency. Three clinical subtypes include a mild form recognized after age ten years with absence of skeletal abnormalities, myopathy, and slow progression (type 1); a moderate form recognized before age ten years with presence of skeletal abnormalities, myopathy, and slow progression (type 2); and a severe form manifested as prenatal loss or early death from progressive central nervous system involvement (type 3). Assay of acid alpha-mannosidase enzyme activity in leukocytes or other nucleated cells is the confirmatory diagnostic test. We presented a 8-year-old girl with psychomotor development delay. She was an adapted child. She attended a special education. On her physical examination was consisted with **alpha-**mannosidosis. The urine tests for mucopolysaccharides showed small amount of dermatan sulfate. The alpha-mannosidase enzyme activity was 1.6 umol/g/h (normal range: 100-800 umol/g/h), 3.2 umol/l/h (normal range: 20-100 umol/g/h) in white cells and plasma, respectively. Although **alpha-**mannosidosis is a rare disorder, it is important to consider the **alpha-**mannosidosis in the differential diagnosis of young patients with neurodevelopmental disabilities. We could not forget the importance of the screening for oligosaccharides in children with neurodevelopmental delay with mild phenotypic signs and symptoms. Early diagnosis allows more effective medical management and genetic counseling.

J13.02

Inflammatory cytokine gene expression profile in patients with coronary artery disease

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Objective. Both adaptive and innate immune systems are involved in coronary artery disease (CAD). The aim of this study was to evaluate cytokine expression profiles in un-stimulated peripheral blood lymphocytes (PBMCs) of patients with coronary artery disease.

Methods. Expression profiles of IL-4, IL-6, IL-10, IL-17, IL-23, INF-γ, TNF-α and TGF-β1 were determined in individuals with and without CAD using Real-time PCR.

Results. IL-4, and IL-10 gene expression were decreased in un-stimulated PBMCs of patients with CAD, while IFN-γ gene expression as a prototype of Th1 immune response and IL-6, were increased in patients with CAD compared to individuals without CAD. However, the differences were not significant. Nevertheless, a significant decrease in IL-23 gene expression in un-stimulated PBMCs of patients with CAD compared to those without CAD was found ($p<0.001$, 95% CI: 0.29-0.80).

Conclusion. Our data reinforce the potential role of the IL-23-IL-17 axis as a critical regulatory system that bridges the innate and adaptive arms of the immune system in the complex mechanisms associated with the development of atherosclerosis. Since IL-23 is main cytokine in Th17 pathway, future studies focusing on the role of Th17 immune response in atherosclerosis will be important to clarify the regulatory mechanisms involved in pathogenesis of CAD.

J13.03

A Case of Persistent Neonatal Hypoglycemia

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Introduction: Hyperinsulinism is the most common cause of persistent hypoglycaemia in early infancy. Hyperinsulinemic infants are usually macrosomic, reflecting the anabolic effects of insulin during pregnancy; also, in most cases there is no history of maternal diabetes. Common symptoms of hypoglycaemia are non-specific and include increasing demands for feeding, hypotonia, irritability, jitteriness, and frank seizures.

Case report: Two days old female newborn from a mother with no history of diabetes was admitted to our clinic presenting with macrosomia and severe hypoglycaemia for further management. Glucose level upon arrival was extremely low, 0.72 mg/dL (0.04 mmoL/L) and despite vigorous therapy the severe hypoglycaemia persisted throughout the first two months of life. Further investigations revealed inappropriately elevated insulin levels at the time of documented hypoglycaemia, as well as lack of acidosis or ketonuria. The presence of organomegaly, structural abnormality, and tumours was assessed by abdominal echography and CT scan

Conclusions: Blood glucose levels should be closely monitored in infants with hyperinsulinism even if the baby does not present with jitteriness, frank seizures or other signs of hypoglycaemia. An increase in glucose level of at least 40 mg/dL proves that glucose mobilization has been inhibited by insulin and that the mechanisms of glycogenolysis are intact.

J13.04

Does inherited thrombophilia influence the course of Wilson disease?

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Wilson disease (WD) is an autosomal recessive disorder of copper balance leading to hepatic damage and neurological disturbance. Clinical manifestations include hepatic disease ranging from acute liver failure to chronic liver conditions more frequently. Mechanisms that influence the course and clinical presentation of WD are not well understood. Here we describe two young (25 and 20 years old) female patients whose WD manifested as acute liver failure first. Patient 1 was pregnant woman developed signs of preeclampsia and acute liver failure at 30 weeks gestation. Patient 2 had thromboembolic complications after the liver transplantation performed for acute liver failure. Inherited thrombophilia testing was performed in both patients before the diagnosis of WD was made and both were heterozygous for Leiden mutation. Later WD was confirmed and they were found to be compound heterozygotes for p.H1069Q and unknown mutation of ATP7B gene. The incidence of WD is 1 per 11000 and the frequency of heterozygous Leiden mutation is 2,9% in Belarus. The probability of random coincidence of these conditions is $2{,}6x10^{-6}$. We conclude that more studies are necessary to investigate the influence of inherited thrombophilia on course of WD. Inherited thrombophilia testing can be useful for patients with acute liver failure.

J13.05

Preliminary Results of Early Diagnostics of Lysosomal Storage Diseases by Tandem Mass-Specrometry

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To measure the enzyme activity of dried blood spots by tandem mass spectrometry had used standard operation protocols (SOP) for the diagnosis of lysosomal storage diseases. Had examined the enzyme concentration β-glucocerebrosidase (ABG), galactocerebrosidase (GALC), α-galactosidase (GLA), α-iduronidase (IDUA) from 46th of newborns in dry blood spots by tandem mass spectrometry.

The substrate molecule had subjected to degradation products under the action of enzymes in extracts of dry blood spot. The appearance of products is directly proportional to enzyme activity.

The results of research had showed that the concentration of metabolites did not differ from the control of standard indicators. The values of enzyme activity were in the range of 95 percent and are consistent with low, medium and high levels of standards.

J13.06

A novel IDUA gene mutation in an Iranian family affected by mucopolysaccharidosis type I

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Mucopolysaccharidosis type I (MPS I) arises from a deficiency in the α-L-iduronidase (IDUA) enzyme. MPS-I is an autosomal recessive disease. Although the clinical spectrum in MPS I patients is continuous, it was possible to recognize 3 phenotypes reflecting the severity of symptoms, viz., the Hurler, Scheie and Hurler/Scheie syndromes. In this study, One Iranian MPS I family was investigated. The proband was a three years old girl who had severe symptoms of MPS-I including gibbus deformity of the lower spine, progressive skeletal dysplasia and linear growth ceases. After clinical investigation, DNA extracted from proband was sequenced for whole IDUA gene. In sequencing results 1 novel mutation was identified. It was a 32bp homozygote deletion located in IVS 5 and c.590-607 (exon6) deletion codon: 197-202. For more investigation and confirmation of the mentioned deletion we examined parents. Result approved heterozygote deletion for parents.
Key words: mucopolysaccharidosis type I, α-L-iduronidase, novel mutation.

J13.07

Molecular Study of Multiple Endocrine Neoplasia type 2 in Iranian Patients

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Multiple endocrine neoplasia type 2 (MEN 2) is classified into three subtypes: MEN 2A, FMTC (familial medullary thyroid carcinoma), and MEN 2B. All three subtypes involve high risk for development of medullary carcinoma of the thyroid (MTC). MTC typically occurs in early childhood in MEN 2B, early adulthood in MEN 2A, and middle age in FMTC.
RET is the only gene known to be associated with MEN type 2. Molecular genetic testing of the RET gene identifies disease-causing mutations in 98% of individuals with MEN 2A, more than 98% of individuals with MEN 2B, and in about 95% of families with FMTC.All MEN 2 subtypes are inherited in an autosomal dominant manner. The probability of a de novo gene mutation is 5% or less in index cases with MEN 2A and 50% in index cases with MEN 2B. Offspring of affected individuals have a 50% chance of inheriting the mutant gene. Approximately 98% of families with MEN 2A have a RET mutation in exon 10 or 11 Prenatal testing is possible.
Testing for known common and rarer mutations is performed in our laboratory. PCR and Sequence analysis of exons 10, 11, 13, 14, 15, and 16 (hot spotes exons) was applied to detect these mutations.
Up to now, we investigated molecularly 7 cases suspected to MEN type 2 syndrome (majority with thyroid papillary carcinoma), each of these heterozygote mutations C 630 R (exon 11) , C634 F(exon11) , G691 S(exon11), L790 F(exon 13) were found in one patient , respectively.

J13.08

New frameshift mutation in the *SMPD1* gene causesNiemann pick disease type A in a child from southwest Iran: A case report

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Niemann pick disease type A (NPA: OMIM #607608) is a variants of a group of lipid storage disorders that is inherited in an autosomal recessive manner. The *SMPD1* gene encoding the enzyme sphingomyelinase is disrupted by pathogenic mutations, which leads to accumulation of sphingomyelin in different organs.
We report firstly a 2.5 years old boy with NPA in southwest Iran. Initially, the diagnosis resulted on the basis of consultation and clinical symptoms. The suspected individual was subjected to the molecular genetics diagnostics.
A novel mutation was observed at codon 247 in the *SMPD1* gene that might be causative for the formation of the disease.
The present report is the first molecular genetics diagnosis of the NPD type A in south west Iran.
The detected deletion in the *SMPD1* gene is remarkable because of its novelty.

J13.09

Two New Mutations in Iranian Niemman-Pick patients

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Types A and B Niemann-Pick disease both result from the deficient activity of the lysosomal hydrolase, acid sphingomyelinase. Type A Niemann-Pick disease is a severe neurodegenerative disorder of infancy which leads to death by three years of age, whereas Type B disease has a later age at onset, little or no neurologic involvement, and most patients survive into adulthood.
The first symptom in NPD-A is hepatosplenomegaly, usually noted by age three months; over time the liver and spleen become massive. Psychomotor development progresses no further than the 12-month level, after which neurologic deterioration is relentless. Acid sphingomyelinase (ASM) deficiency is inherited in an autosomal recessive manner. SMPD1 is the only gene known to be associated with acid sphingomyelinase deficiency.
The SMPD1 gene is composed of six exons and is located on chromosome 11p15.1-11p15.4.
This study included 20 patients suffering from Niemann-Pick disease. The initial diagnosis was based on clinical and biochemical findings.
For genetic diagnosis ,all exons amplify and sequence to find defective mutations in this gene. The result showed Gly 508 Arg was found in 6 patients . This mutation was reported previously .Val 36 Ala was found in 3 patients And del CT in codon 473 was found in one patients. These mutations were not reported.

J13.10

Association of the angiotensin converting enzyme (ACE) gene I/D polymorphism with sarcoidosis in Turkish patients

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Sarcoidosis is a chronic inflammatory disease of complex pathogenesis and unknown etiology characterized by noncaseating epithelioid granuloma that invades the lung, eye, liver and other organs. Angiotensin converting enzyme (ACE) gene insertion (I)/deletion (D) polymorphism has been investigated for a genetic predisposition to sarcoidosis in different populations, but results have been inconsistent and inconclusive. This study was carried out to detect the frequencies of I/D polymorphism genotypes and allele of ACE gene in Turkish patients with sarcoidosis. Genomic DNA obtained from 154 persons (70 patients with sarcoidosis and 84 healthy controls) was used in the study. DNA was amplified by polymerase chain reaction using allele-specific primers. Amplified products were assessed with UV transilluminator by being exposed to 2% agarose gel electrophoresis. The allele frequencies and genotype distribution of the groups were analyzed with the chi-square test. There were no statistically significant differences between controls and sarcoidosis cases with respect to genotype distribution (x^2=4.202, p=0.122) and allele frequencies (x^2=1.358, p=0.244). Our results suggest that there is no genetic predisposition to sarcoidosis in Turkish population.

ACE genotype distrubution and allel frequencies
in Turkish patients with sarcoidosis and controls.

Groups	N	Male	Female	Genotype II (%)	Genotype DD (%)	Genotype ID (%)	D allele	I allele
Control	84	41	43	18 (21.4)	14 (16.7)	52 (61.9)	0.524	0.476
Sarcoidosis	70	18	52	15 (21.4)	21 (30)	34 (48.6)	0.457	0.543

J13.11

VEGF gene mRNA expression in un-stimulated PBMCs of patients with coronary artery disease and its association with -2578*C/A polymorphism

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Objective: Based on previous reports on association between VEGF and pathogenesis of vascular disorders the aim of this study was to verify the expression of VEGF mRNA in unstimulated peripheral blood mononuclear cells (PBMCs) of patients with and without coronary artery disease (CAD) and comparing the expression of VEGF expression in patients carrying va-

rious genotypes for VEGF -2578*C/A polymorphism.
Methods: The study was performed patients who underwent coronary artery angiography and patients with >50% stenosis in vessels considered as case groups (CAD+) and normal vessels group as control (CAD-). VEGF mRNA expression was examined using quantitative real-time PCR and genotyping for VEGF -2578*C/A was performed using ARMS-PCR technique.
Results: VEGF mRNA expression was significantly decreased in CAD+ patients compared to CAD- patients (p=0.01, 95%CI=-4.2- -0.4). Also in patients carrying AA genotype VEGF mRNA expression was increased compared to patients carrying CC and CA genotype.
Conclusion: Increased expression of VEGF in patients without CAD is indicating an important role for VEGF in CAD development. More studies on larger number of samples are required to further confirm the results obtained in our study.

J13.12

Prevalence of signs of Hurler and mild Hunter syndrome in infants of the first year of life

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Background: The prevalence of mucopolysaccharidosis (MPS) is 1 case in 16,000-30,000 births, and MPS II accounts for 80% of all cases. Clinical presentations depend on the type of enzyme defect. Usually patients with MPS initially have normal development and the signs of pathology are seen later in childhood, which causes late diagnosis and makes lower their quality of life.
Objectives: To evaluate the prevalence of pathologic signs during the first year of life in patients with MPS I and MPS II in order to diagnose MPS early.
Materials and methods: 7 children with MPS I (Hurler) and 5 children with MPS II (mild Hunter) were examined and their history was analyzed.
Results: Only 29% of MPS I and 20% of MPS II cases were diagnosed during the first year of life. During the first/second half of the first year of life MPS I manifested with hydrocephalus (71% / 86%), mental and neurologic retardation (43% / 71%), hearing impairment (29% / 43%), corneal clouding (29% / 43%), hepatomegaly (14% / 14%) while MPS II manifested with hydrocephalus (80% / 80%), umbilical or/and inguinal hernia (40% /
60%). In addition in the second half of the first year coarse facial features (43%) and umbilical hernia (14%) were revealed in MPS I and kyphoscoliosis (40%), coarse facial features (20%), hepatomegaly (20%) in MPS II infants.
Conclusions: The revealed prevalence of clinical signs can help a pediatrician to diagnose MPS I and mild MPS II during the first year of life.

J13.13

Analysis of side effects due to valproic acid in patients with epilepsy respective of SNPs polymorphisms in *CYP2C9, CYP2C19* and *MDR1*

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Epilepsy is a chronic disorder caused by brain lesion and characterized by repeated convulsive and/or other seizures. The valproic acid (VPA medication is the basic approach to the therapy of epilepsy. Detoxification of VPA and its major metabolites is known to be carried out by cytochrome P450 enzyme system with special emphasis on CYP2C9 and CYP2C19 as well as on ATP-binding cassette (ABC) transporter, P-glycoprotein-MDR-1. Analysis of VPA treatment efficiency respective of polymorphisms in SNPs in *CYP2C9* (430C>T and 1075A>C), *CYP2C19* (681G>A) and *MDR1* (3435C>T) was a main goal of the present study. PCR-RFLP analysis was carried out in 76 epilepsy patients and in 210 individuals of the control group. The genotype and allele frequencies of the relevant genes were different in the patient compared to the control groups (P>0.05, χ2<5.99). Chronic adverse complications were associated with *CYP2C9* allele (**2* and/or **3*), which correlates with reduced activity of this enzyme (P=0,048 F=0,049). The multivariate logistic regression analysis also supported that carriers of particular SNPs alleles have higher risk of chronic adverse events. Analysis of SNPs polymorphism in cytochrome P450 genes allows more precise control of the necessary dosage of VPA medication, thus making epilepsy treatment more efficient, personalized and less toxic.

J13.14

Chinese Hamster Ovarian (CHO) cell lines expressing mutant ATP7B

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Wilson disease (WD) is a rare autosomal recessive disorder of copper (Cu) homeostasis that is caused by mutations of gene *ATP7B.* WD patients mostly present hepatic and/or neurological manifestations, however, severity and age of **onset** are variable. We explored whether Cu resistance of tissue culture cell lines that express specific mutations of *ATP7B* can predict severity and course of disease. Chinese Hamster Ovary cells that lack intrinsic *ATP7B* expression were used for stable expression of *ATP7B* mutants by retroviral vectors. 12 mutations were chosen for analysis from reports of homozygotic patients. To determine Cu resistance MTT assay was established. *ATP7B* protein expression was determined by Western-blot analysis. Cu resistance of the cell lines appeared to be highly specific and could be classified into three groups. The first group showed high copper resistance similar to that of wild-type *ATP7B*. The second group showed low copper resistance similar to native CHO cells, the third group displayed intermediate resistance. Magnitude of *ATP7B* protein expression correlated with level of Cu resistance in most but not all cases. *ATP7B* mutants found in patients having a mild clinical presentations were highly resistant to Cu. In contrast, an early onset and severe disease was found in the group of mutants that showed low Cu resistance. The third group with intermediate Cu resistance was heterogenous with respect to clinical manifestation of disease. Our observations indicate that functional characterization of *ATP7B* mutants can give further insights into the understanding of individual mutations and for prediction of WD.

J13.15

The mutational spectrum of Phenylketonuria in Egypt : A unique pattern of mutations including four common mutations and five novel mutant PAH alleles.

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Phenylketonuria (PKU) is one of the most common inborn errors caused by deficiency of the hepatic phenylalanine hydroxylase gene (PAH). Extensive studies identifying mutational spectrum of PKU have been published during the last two decades. The frequencies of the different mutations vary in different locations of the world. On the basis of phenotype/genotype correlations, determination of phenylketonuric genotype is crucial for better classification of the clinical phenotype and treatment, including tetrahydrobiopterin therapy. We report here on the mutational spectrum of *PAH* gene in 150 Egyptian families using the following systems: (1) PCR-RFLP analysis for common Mediterranean mutations; (2) single stranded conformational polymorphism; (3) direct sequencing; and, (4) Long range PCR for detection of large deletions. Of the 300 mutant alleles, 288 (96%) were genotyped and a total of 25 distinct mutations were identified including 5 novel mutations. R176X, R243X, Y198fs and IVS10-11G>A were prevalent mutations in our population. The mutational data obtained reflects a unique pattern of mutations in Egyptian patients with PKU which will permit precise carrier detection, prenatal diagnosis and genetic counseling for these families.

J14. Therapy for genetic disorders

J14.01

Strong association between ABCB1 gene (MDR1) 3435 C>T polymorphism and multiple drug nonresponder patients of chronic hepatitis C

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The curing viral infection of chronic hepatitis C (CHC) is a main strategy to prevent progression of liver disease and cancer. Some CHC patients are failed to respond to common antiviral theray in some populations. In the current study it was aimed to find the possible role of multidrug ressistance gene 1 polymorphism(MDR1) in nonresponder patients with CHC. Peripheral blood samples were used for total genomic DNA isolation. In a total of 55 HCV positive patients [31 male (56.4%), 24 female (53.6%) and mean age-min-max; 56.9±9.66 (39-71)]; 19 responder (34.5%) 21 non-responder(38.2%) and 15 recurrence (27.3%) were included, genotyped for functional MDR1 gene polymorphism. Target gene were genotyped by multiplex PCR-based reverse-hybridization StripAssay method. The current results indicate that codon 3435 C>T polymorphism in exon 26 of MDR1 gene is associated with colchicine resistance in nonresponder CHC patients.

J14.02

Creation of the carriers containing gene encoding site of apolipoprotein B100 and green fluorescent protein (GFP) gene for transfection into eukaryotic cells.

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The purpose of this work is creation of new carrier for transfection the genetic information into eukaryotic cells. Apolipoprotein B100 (apoB) provides binding of low-density lipoprotein (LDL) particles to the receptor. Such cells as hepatocytes, fibroblasts and lymphocytes have LDL-receptor. We have created fusion protein consisting GFP and high conservative receptor-binding region named site B (3359-3369) of apoB. Site B is strongly requied for interaction between carrier and cells LDL-receptor, and its entering into the cells. Presence of GFP in the recombinant polypeptide will help to determine the localization of the protein by fluorescence microscopy. We used plasmid pTRC99a-p7 with two unique restriction sites for SalI (after sfGFP) and HindIII (downstream). We have created plasmid with GFP gene, sequence encoding site of apoB, five histidine codons for chromatography purification of recombinant and stop codon. The resulting plasmid was successfully sequenced. As a result we synthesized protein, which peaks of excitation and emission were equal to the GFP peaks. It had high affinity to nickel agarose. This fusion protein is basis of new construction for transfection the genetic information into eukaryotic cells. It contains GFP and site B of apoB. Amino-acid sequence of siteB could be used as an alternative way of delivery of different therapeutic substances into eukaryotic cells. Studies on cell cultures are expected.

J14.03

Complexity of rehabilitation treatment in a case with limb-girdle muscular dystrophy

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Limb-girdle muscular dystrophy comprises a heterogeneous group of disorders, with progressive evolution, affecting especially proximal limb musculature. Here we report the case of a 39-year-old man with autosomal recessive limb-girdle muscular dystrophy, with negative family history, diagnosed 7 years ago. The clinical examination revealed major motor deficit, severe muscle hypotrophy in the scapulohumeral girdle and arms, impossibility to perform active movements of the scapulohumeral joint, regarding passive movements abduction and flexion limited to 90 degrees. Walking and standing were impossible, requiring wheelchair, person-assisted transfers from clinostatism to sitting position. He can maintain a sitting position without hand support, but otherwise requires constant help of another person. Assessment with functional ambulation classification showed that the patient needed firm continuous support from one person, who helps carrying weight and with balance. Particularity of the case is represented by the rapid evolution, with impaired self-care capacity and occurrence of osteoporosis due to immobilization. In 2010 he fell when transferring from wheelchair to bed, with the fracture of left body of tibia, right humeral head, resulting into the complex regional pain syndrome. Rehabilitation program objectives were maintaining mobility, transfers re-education, gain maximum independence. Methods were medication and physical-kinetic treatment for basic disorder and complications, with hydrokinetotherapy, massage, electrotherapy, laser. The patient should have done daily kinetic program, but plaster immobilization forced him to interrupt exercises, with repercussions on the achieved performances. Early diagnosis and improvements in management of patients with this disorder by a multidisciplinary team will improve their prognosis and quality of life.

J14.04

Intrathecal Enzyme Replacement Therapy for Neurological Impairment in Mucopolysaccharidosis 1(4 Iranian cases)

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MPS1 is an autosomal recessive disorder caused by deficient activity of the lysosomal enzyme alpha-L iduronidase, which leads to accumulation of heparan sulfate and dermatan sulfate, resulting in a progressive multisystem disease with respiratory, skeletal, and neurologic manifestations. Treatment for MPS1 consists of supportive care, and enzyme - replacement therapy with Laronidase. Bone marrow and hematopoietic stem cell transplantation is the treatment of choice for patients suffering from MPS1 with no or minimal central nervous system manifestation.

Case Report: We report 4 Iranian cases of MPS type 1, 3boys who are 60 months-old, 34 months-old, & 30months -old; and 1 girl who is 41 months-old .They have phenotype of MPS1, coarse facial features, prominent forehead, corneal cloudy, sleep disturbance, hepatosplenomegaly, inguinal hernia, joint stiffness, and dysostosis multiplex congenital. They diagnosed MPS1 on the basis of clinical findings, an elevated urinary glycosaminoglycan level and low alpha- L- iduronidase activity in leukocytes. For 3 of the cases mutation analysis revealed homozygous mutation in the IDUA gene, and for one of them reveals novel mutation . They have been started injection of aldurozyme intravenously every week from 26 months - old, 18 months-old, 12 months-old, and 19months-old. To prevent neurological impairment before bone marrow transplantation, they receive intrathecal enzyme replacement of aldurozyme monthly from 29 months-old, 21months-old, 21months-old, and 31 months-old. They tolerate intrathecal ERT with no adverse events. One of them when he was 50 months - old, he received bone marrow transplantation, but others are now receiving aldurozyme intravenously and intrathecally.

J14.05

The Spinal Muscular Atrophy Therapeutics: Progress and Promise

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Spinal muscular atrophy (SMA) is an inherited neuromuscular disorder that causes degeneration of α-motor neurons. Frequently, muscle weakness is very severe causing affected infants to die before reaching two years of age, but mild forms of the disease can be characterized by relatively static muscle weakness for many years. SMA is caused by recessive mutations of the SMN1 gene, but all patients retain at least one copy of SMN2, a similar gene capable of producing low levels of full-length SMN protein. No treatments currently exist for SMA patients, but the identification of therapeutic targets as Hydroxyurea ,Quinazoline derivatives, Salbutamol, Small molecules , Antisense oligonucleotides, aminoglycosides and proteasome inhibition , Riluzole and Ceftriaxone, Olesoxime and Embryonic stem (ES) cells also the development of suitable animal models for preclinical testing have resulted in increased drug development efforts in the past ten years. Here, we review the current status of many of these programs, including those designed to activate SMN2 gene expression, modulate splicing of SMN2 preRNAs, stabilize SMN protein, replace SMN1, provide neuroprotective support, and transplant neural cells. The knowledge of SMA pathogenesis and the development of clinical candidates have increased considerably since the discovery of the disease-causing gene. Drug development in SMA has been characterized by robust collaborative efforts between academic, government, pharmaceutical, and non-profit organizations. As the promise of a treatment for this devastating disease continues to grow, we are hopeful that progression over the next 15 years will be even more rapid than the last.

J14.06

The effect of age and strain on screening, proliferation, and differentiation of chicken bone marrow mesenchymal stem cells

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Background: Noticing the practical significance of stem cells, this study was conducted to culture and screen bone marrow mesenchymal stem cells derived from Raf and Hilline chicken strains and investigate the effect of age and race on the morphology and differentiation of the generated cells.

Materials and Methods: In this fundamental study, bone marrow cells from 3 to 25 day-old Raf and Hiline chicken strains were cultured in low glucose DMEM, 10% BFS. Then third passage bone marrow cells of the two strains were compared in terms of morphology, differentiation to bone, cartilage, and adiposity. Data were analyzed through SPSS software.

Results: In culturing Raf chicken derived bone marrow cells, in contrast to Hiline chicken strain, colonization took place and they almost had a better fibroblastic morphology. The results indicated higher yields of differentiation to bone, cartilage, and adipose tissues in Raf chicken derived bone marrow cells than Hiline chicken. These differences were statistically significant. Also, 15 days was the most suitable age for screening the mesenchymal stem cells of chicken.

Conclusion: Screening and proliferation of mesenchymal stem cells from 15-day old Raf chicken bone marrow cells are good resources for differentiation and purification of chicken bone marrow mesenchymal stem cells.

J14.07

Treatment with bisphosphonates in osteogenesis imperfecta

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Introduction. Osteogenesis imperfecta, a genetic disease of bone formation, with autosomal recessive trait, is characterized by bone fragility and reduced bone mass due to mutations in genes coding for type I collagen. Different severity of clinical signs is due to imperfect genotype-phenotype correlation. Because the treatment with bisphosphonates (antiresorptive agents that inhibit osteoclast function and stimulates osteoblastic bone formation) was recently introduced, the authors have proposed to evaluate the effects of pamidronate in patients with osteogenesis imperfecta.

Patients and method. The study group consisted of 11 patients (3 girls and 8 boys), aged between 4 ys 1 mo - 18 ys 3 mo, who were registered in the Centre of Genetic Disease of the First Pediatric Clinic Cluj with osteogenesis imperfecta. The method consisted in: clinical and bio-humoral assessment regarding the parameters of bone mineralization, radiological examinations, osteodensitometry; assessing occupational score.

Results. Treatment with bisphosphonates (commercial preparations Aredia) was administered in 2 - 14 cycles, at a dose of 0.5 - 1 mg/kg/day IV infusion, 3 consecutive days at 4 months interval. The drug was well tolerated, the only side effect being fever recorded in the first cycle of treatment in 4 patients. Bio-humoral parameters of phospho-calcic metabolism remained within normal limits, except for reduced alkaline phosphatase values in a girl who associated hipophosphatasia tarda. Bone pain has resolved, fractures have not occurred (except in one patient), occupational score and Z score were improved.

Conclusions. Under bisphosphonates treatment the clinical symptoms, occupational score and Z score were improved.

J14.08

A cell model system to monitor the translocation of Ataxin-3 in SCA3

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Spinocerebellar ataxia type 3 (SCA3), also known as Machado-Joseph disease (MJD), is an autosomal dominantly inherited neurodegenerative disorder caused by the expansion of a CAG repeat in the MJD1 gene encoding a expanded polyglutamine repeat in the Ataxin-3 protein. Ataxin-3, the affected protein in SCA3, is mostly a cytoplasmatic protein, however, it distributes to different subcellular regions i.e the cytoplasma and the nucleus in different cell types. The characteristic hallmark of this disease is the formation of Neuronal intranuclear inclusions (NII) including expanded and misfolded causative protein. Whether NII are toxic or protective, their presence indicated that expanded ataxin-3 plays a role in the nucleus. In addition, transcriptional regulation of ataxin-3 occurs in the nucleus and nuclear localization of ataxin-3 indeed aggravate the symptoms in SCA3 animal model. Therefore, if ataxin-3 localizes in the nucleus, this is a negative event. Prevention of ataxin-3 nuclear translocation maybe ameliorate this disease progression. To gain insight into how to inhibit the translocation of Ataxin-3, we will undertake a small molecular library screen to explore which small molecules influence the location of this protein in a SCA3 cell model, analyze the mechanisms regulating Ataxin-3 transport and attempt to develop a therapeutic strategy for this disease. For this, a high-throughput screening method is established in this project.

J15. Laboratory and quality management

J15.01

Cloning and transient expression of cytoprotective factor, Nrf2, in mesenchymal stem cells using the adenoviral expression system through Gateway technology

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Background and Objectives: Nuclear factor erythroid-2 related factor 2 (NRF2) is one of the potent cytoprotective factor. The goal of this study was cloning and transient over expression of the human Nrf2 gene in MSCs using the adenoviral expression system based on gateway technology.

Material and Methods: In order to induce expression of Nrf2, MSCs were exposed to UV for 1 hour. Full length cDNA of Nrf2 was isolated and cloned into pENTR TOPO/D vector by TOPO cloning reaction. To construct the expression clone, a LR recombination reaction was carried out between the entry clone and destination vector, pAd/CMV/V5-DEST. The Recombinant virus was produced in appropriate mammalian cell line. MSCs were infected by the recombinant virus expressing Nrf2.

Results: The results showed that human recombinant Nrf2 was successfully cloned and the accuracy of the gene and its frame in the vector were confirmed by DNA sequencing. Expression of Nrf2 in MSCs was confirmed by RT-PCR and western blot analysis. The results indicated that the expression of Nrf2 is transient.

Conclusions: The adenoviral expression system through Gateway technology was successfully used to clone and over-express cytoprotective factor, Nrf2, in MSCs.

J15.02

Italian National External Quality Assessment in molecular genetic testing - VII round (2010-2011)

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The Italian External Quality Assessment for molecular genetic testing, coordinated by the Istituto Superiore di Sanità, started in 2001; it cover four pathologies: Cystic Fibrosis (CF), Beta Thalassemia (BT), Fragile X-Syndrome (FX) and Familial Adenomatous Polyposis Coli (APC). A web utility dedicated to this activity has been developed and, since 2010, participation is open both to public and private Italian laboratories.

In 2010 the number of participants was 53; 43, 17, 15 and 5 laboratories participated for CF, BT, FX and APC schemes respectively.

In each scheme four DNA samples were validated and sent to participants together with clinical and technical information. Laboratories were asked to use their routine procedures and protocols to analyse samples. A panel of assessors reviewed the final returns to assess the quality of genotyping, interpretation and reporting.

In 2010 assessors reviewed 320 genetic testing analyses. Genotyping results showed complete and correct data in 98.5%, 95.9%, 100%, 100% of CF, BT, FX, APC samples analyzed respectively; satisfactory interpretation of data was recorded only in 9.9%, 32%, 20%, 40% of CF, BT, FX, APC reports respectively; lack of information/inaccuracy in reports was detected in all schemes.This work will show in detail all 2010 results comparing, when possible, our data with those of other European schemes.

J15.03

The Italian External Quality Assessment in classical cytogenetics: results of the 2010/2011 round

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The Italian External Quality Assessment in classical cytogenetics, coordinated by the Istituto Superiore di Sanità, started in 2001 and covers prenatal, postnatal and oncological diagnosis. The scheme is retrospective and each part of the scheme stands alone; a web-utility dedicated to the CEQ has been developed.

Since 2010 the EQA has been recognised as istitutional activity; laboratories pay a fee for each scheme as published in an official document issued by the Italian government (GU n.199-28th August 2009). Participation is open either to public and private laboratories.

Assessment takes into account technical, analytical and interpretative performance; an assessment system with scores was developed. Assessors are selected in collaboration with the Italian Society of Human Genetics and, at the end of the round, participants receive a report with marks and comments to improve the analysis.

The total number of laboratories participating in 2010-VII round was 69, 17 of them were private (i.e. 40%); in particular 55, 60 and 22 laboratories participated in the prenatal, postnatal and oncological scheme respectively. A banding quality not adequate for the analysis was observed in about 6% and 10% of 108 prenatal and 120 postnatal cases respectively; an analytical error was identified in one case, out of 44, in oncological diagnosis. An use of the nomenclature ISCN not appropriate was detected in about 15%, 13% and 75% of reports in prenatal, postnatal and oncological diagnosis respectively. Not completeness and/or inadequacy of information in reports was the most frequent analytical inaccuracy recorded in all schemes.

EMPAG Plenary Lectures

EPL1.1

Priority setting in genetic testing: a pilot discrete choice experiment

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Given the increasing number of genetic tests available for clinical practice, decisions have to be made on how to allocate limited health care resources to them. Many criteria have been proposed to guide the process of priority setting. However, their relative importance is still unclear. This paper explores the feasibility of discrete choice experiments to identify and weight various criteria in a form that allows prioritisation through a rank ordering of different testing options. Therefore a pilot discrete choice experiment was carried out, using face-to-face interviews, among 22 genetic professionals. Respondents chose between two generic scenarios (dual choice options) that described testing options, represented by medical and non-medical attributes. Choice data was used to rank order a set of seven testing options on the basis these attributes and their relative weights. A series of follow up questions were asked to learn about participants' understanding of the choice format and potential improvements in the discrete choice instrument. The criteria "Prevalence", "Severity", "Clinical Utility" and "Alternatives available" were significant, all including "Infrastructure available" and "Urgency" had positive signs. A preliminary rank order of tests could be established. Findings from this pilot demonstrate the discrete choice methodology to be a feasible approach to use stated preference techniques in priority setting of genetic tests. We believe that the DCE framework is an important step towards the development of a rational approach to priority setting that meets the needs of decision makers.

EPL1.2

First Trimester Screening, it's not a routine test An education module for General Practitioners to help women to make an informed choice

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In NSW, Australia, health care professionals should inform all pregnant women about first trimester screening (FTS) and give appropriate risk information to enable an informed choice. This requirement usually applies to general practitioners (GPs), with increased risk results usually referred to genetic counsellors (GCs) or obstetricians. Challenges for GPs include addressing patients' different perceptions and interpretations of risk; presenting the pros and cons of screening; the possible implications of an increased risk result; the potential for coercion in guiding decision making; and the inconsistencies in the availability of FTS and genetic counselling services. To support GPs, and at the request of providers of professional development, an education module, First Trimester Screening- it's not a routine test, was developed to be used in group educational settings or stand alone. Content included a slide presentation of information and three video case studies of GP consultations that addressed issues anecdotally reported to commonly occur. Process evaluation consisted of piloting the module in GP education sessions (1 hr 52 GPs) and GCs (5). The module was rated highly relevant, useful and reflected questions that arise in GP practice (96%) and comments included prompting future more thorough discussion of FTS, having discuss over more than one session, adopting ideas from the way the GP in the videos phrased her comments and be more confident. 13% of GPs requested a further video case discussing increased risk results be included. The next process evaluation results following incorporation of recommendations will be presented.

EPL1.3

What do pregnant women and their partner know about Down Syndrome when they decide to undergo prenatal diagnosis?

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Information to expecting parents about prenatal diagnosis (PND) for chromosomal aberrations focus on the risk of bearing children with chromosomal abnormalities, i.e. Down syndrome (DS), and the risks associated with amniocentesis and chorionic villus sampling. Routinely, no information is given about the symptoms of DS and the consequences for the child and the family. This study examined what prospective parents know about medical, cognitive and social aspects of DS. A questionnaire was answered by 208 parents taking a CUB test (combined ultrasound and biochemistry) at Uppsala university hospital during 2011.

Only 25% of the parents had received information about DS and more than 60% would like more information. The reason for taking a CUB test was, for 70% of the parents to get a warranty of a healthy child. Half of the parents had not taken a position on what to do if the CUB test showed an increased risk of DS. Almost all knew that DS is caused by a chromosomal abnormality. Still there was a vast lack of knowledge about medical and cognitive complications in children with DS, as well as social consequences for the children and the families.

This study shows that a high proportion of parents who undergo a CUB test have little knowledge of DS and might consider invasive PND procedures, and perhaps also pregnancy termination, without knowing what Downs syndrome implies. Improved information to expecting parents about medical, cognitive and social consequences of DS, could help the parents to make informed decisions regarding PND.

EPL1.4

How do women make decisions about genetic carrier screening for fragile X syndrome? A qualitative study

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Background:

As population-based genetic carrier screening becomes more common there is increasing need to evaluate decision-making, ensuring participants make free and informed choices. Carrier screening for fragile X syndrome (FXS), the leading cause of inherited intellectual disability, provides individuals with information about their health and risk of having children with FXS. It is important individuals participating understand the personal and familial implications of such screening. This study aimed to explore perceptions and decision-making styles of non-pregnant women from the general population offered FXS carrier screening in Australia.

Methods: Purposive sampling was used to select equal proportions of participants who declined and accepted screening. 37 qualitative, semi-structured telephone interviews were conducted after women accepted or declined screening, but prior to receiving test results (if tested). Interviews were transcribed with data coded into themes.

Results: A range of decision-making styles emerged from the data, including: relying on initial gut reaction, being influenced by previous experiences and in-depth deliberation. Most participants felt they made an informed decision, although there was substantial variation in perceptions of an informed decision and a 'good decision'. Preliminary analysis suggests differences in how informed decisions are defined in the literature and perceived women considering screening.

Implications: This research provides valuable insight into women's decision-making processes and how they view an 'informed decision'. These data will be instrumental in informing the development of tools for evaluation of informed decision-making in genetic screening programs and in determining how best to support women through the testing process in the future.

EPL1.5

Development and validation of a short family history screening tool for chronic disease prevention in primary care

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Introduction: Family history (FH) is an important risk factor for many common diseases, yet no simple, validated tools exist in primary care for accurate FH management. We aimed to develop and validate a short FH screening tool for systematic FH assessment in primary care. Diabetes (DM), ischaemic heart disease (HD), breast (BC) and colon cancer (CC) were selected as marker conditions as they fulfil screening criteria, and effective interventions and lifestyle strategies exist for their primary and secondary prevention.

Methods: Participants were identified via randomised electronic searches in 10 Eastern England general practices. During a practice-based consultation participants completed a FH Questionnaire (FHQ), then had a 3-generational ‚gold standard' pedigree taken. In stage 1 the FHQ comprised 12 items; in stage 2 the shorter FHQ was validated against the same 'gold standard'

pedigree, and the psychological impact of FH screening was examined using questionnaires containing validated measures at baseline and 4 weeks.
Results: 1,147 participants were recruited (stage 1: 618; stage 2: 529). Among stage 1 participants, 32% were at increased risk of one or more marker conditions (DM 18%, HD 13%, BC 5%, CC 2%). Determination of the sensitivity, specificity, and predictive values of each item allowed the refinement of the FHQ to 6 items for identification of any of the four conditions (sensitivity 91%, specificity 61%). Results of stage 2 validation of the FHQ-6 will be presented, with psychological findings indicating that FH knowledge, including awareness of being at increased risk, does not increase anxiety.

EPL1.6

Measuring patient benefits from interventions in clinical genetics and genetic counselling services: Are we finally able to do this?

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An enduring challenge in evaluating interventions in clinical genetics and genetic counselling is how best to measure patient benefit. Patient Reported Outcome Measures (PROMs) offer a possible solution. PROMs are self-completion questionnaires used in research to evaluate patient benefits from new interventions e.g. in randomised controlled trials, and for service evaluation in routine clinical practice. PROMs differ from satisfaction questionnaires because they measure change over time in outcomes valued by patients, rather than simply reporting satisfaction with service received. Current UK health policy encourages use of PROMs data. But in clinical genetics, there is no consensus about the best PROMs to use. A research programme involving ~450 patients and 130 clinicians tackled the issue of PROMs use in clinical genetics (Manchester 2003-2011). Mixed methods were used to identify patient benefits: (a) a systematic review of validated outcome measures used (b) a Delphi survey to identify consensus amongst clinicians and patients about appropriate outcome domains and (c) qualitative interviews and focus groups. The next stage involved validating the Genetic Counselling Outcome Scale (GCOS-24), a new PROM developed from the qualitative data, and the existing Perceived Personal Control (PPC) scale. Both PROMs were proven to (i) have high internal consistency (PPC: Cronbachs α=0.83, GCOS-24: α=0.87) (ii) have concurrent validity with health locus of control, satisfaction with life, depression, and authenticity and (iii) capture statistically significant patient benefit following clinic attendance (PPC: Cohens d=0.4, GCOS-24: d=0.7). These properties suggest that both GCOS-24 and PPC are appropriate validated PROMs to evaluate clinical genetics services.

EPL2.1

Breast cancer risk communication by health care providers' in 4 European countries

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Cancer genetic clinics are focused on communication of familial/genetic risks but other factors intervene in breast cancer (BC). Our objective was to investigate the options chosen by European Breast Surgeons (BS) and General Practitioners (GP) to present BC risk factors according to the « state of the art » of a better risk communication.
Questionnaires were sent to 3999 GPs and 3293 BS in 4 countries (UK, France, the Netherlands, Germany). Assessment of family risks and communication about risk factors including alcohol, obesity, oral contraception, hormonal replacement therapy (HRT), physical exercise, early pregnancy, breast-feeding were investigated as well as the formats preferred to present risks (numbered, verbal, framing …).
The overall answer rates were 30% and 37% for GPs and BS, respectively. Risk family history and HRT were the most frequently risk factors explained by GP's and BS. Countries and providers differed significantly ($p<0.001$) for the kind of factors discussed particularly for those other than family history. Event frequency was the most frequently used presentation (47% GPs; 52% BS), particularly in UK. Absolute risks were more frequently presented than relative risks. Only 11% of GPs' and 17% of BS would present risk communication including absolute and relative risks with both negative and positive framing and no verbal presentation. Preferences and declared behaviours will be presented according to specialty and country after multivariate adjustment on personal characteristics.
In order to optimise cancer risk communication in medicine, initial and vocational training could be reinforced by published guidelines issued from multidisciplinary task forces.

EPL2.2

International variation in physicians' attitudes towards prophylactic mastectomy - comparison between the UK, France, Germany, and the Netherlands

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Introduction

Prophylactic mastectomy (PM) has proven to be the most effective method to reduce the risk of breast cancer in high-risk women. The present study aimed to present and compare the attitudes towards PM among physicians in France, Germany, the Netherlands and the UK.

Methods

An international sample of 1196 general practitioners (GP) and 927 breast surgeons (BS) were surveyed using a mailed questionnaire.

Results

Both GP's as well as BS's opinions towards PM significantly differed from one country to another. Only 30% of the French and 27% of the German GPs were of opinion that PM should be an option for an unaffected female BRCA1/2 mutation carrier, as compared to 85% and 92% of the GPs in the Netherlands and UK, respectively. Similarly, 78% of the French and 66% of the German BS reported a positive attitude towards PM, as compared to 100% and 97% of the BS in the Netherlands and UK, respectively.
In the whole sample of GPs, a positive attitude towards PM was associated with country of residence, being female, and having more knowledge of breast/ovarian cancer genetics, while among BS there was a positive association with country of residence and having more knowledge of breast/ovarian cancer genetics as well, and, in addition, with a higher number of newly diagnosed breast cancer patients last year.

Conclusion

These results demonstrated the international variations in the attitude towards PM among physicians. This might reflect that different policies are adopted to prevent breast cancer in women at-risk.

EPL2.3

"Life at risk": Negotiating identities on healthy carriers of a genetic alteration predisposing to cancer.

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Cancer survivorship often involves identity reconstruction and integration of the experience into one's self-concept, and may even lead to radical transformations in one's identity. The increasing availability of genetic testing enables other family members who have not yet developed cancer to determine if they have inherited the risk; those with a mutation are known as previvors. Once a previvor is identified, they are faced with complicated and often difficult decisions and this new situation may have important psychological and social impacts. To date, little is known about the formation of previvor identities and the extent to which genetic diagnosis is central to one's identity. The main objective of this study was to conduct a qualitative investigation based on a phenomenological framework in order to understand the lived experience of cancer previvors and to examine the centrality of the genetic diagnosis and the associated life changes. Eighteen previvors were studied using a qualitative semi-structured interview. All interviews were subjected to interpretative phenomenological analysis. We have identified at least four different basic strategies of identity negotiation in these previvor individuals showing that adjustment to a pre-symptomatic genetic diagnosis is an active psychosocial process of negotiating identity strategies resulting in both positive and negative life changes. Genetic diagnosis centrality was fairly low in our studied population. According with our results, adoption of a specific previvor identity may impact well-being and health behavior changes and warrant further research.

EPL2.4

Women's experiences of familial ovarian cancer screening: A qualitative study

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Until recently, the main risk management options for women at increased risk of familial ovarian cancer have been risk-reducing surgery to remove the ovaries or ovarian cancer screening (OCS) as part of a research study. The psychological evaluation of familial OCS (PsyFOCS) was conducted to examine the psychological impact of taking part in OCS on women who are at increased genetic risk. The aims of the qualitative component of PsyFOCS were to understand 1) women's experiences of OCS and 2) the catalysts for surgery to remove the ovaries and reactions to subsequent withdrawal from OCS. Semi-structured interviews were conducted with 48 women who were or had been taking part in OCS. Women were chosen on a number of demographic and clinical criteria to endeavour to capture a range of OCS experiences. Interview topics included: family history, cancer risk, the screening experience, risk management decisions and information provision. Results suggested that OCS provides reassurance for women and they feel privileged to take part in OCS. A number of catalysts, including OCS test results, and secondary considerations were found to prompt surgery. The emotional impact of discontinuing OCS following surgery varied between relief, acceptance and loss. In conclusion, OCS appears to be an acceptable risk management strategy for women at increased risk of ovarian cancer. However, OCS results may prompt women to reconsider their risk management options. These findings highlight the benefit women feel they receive from OCS as well as the importance of the timing of decision-making about risk management options.

EPL2.5

What counts as successful in genetic counselling for presymptomatic testing in late onset disorders? The consultands' perspective.

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Genetic counselling must be offered in the context of presymptomatic testing (PST) for severe late-onset diseases; however, what is effective genetic counselling is not well-defined, and measurement tools that allow a systematic evaluation of genetic practice are still not available. The aims of this qualitative study were to (1) recognize relevant aspects across the whole process of genetic counselling in PST for late onset neurodegenerative disorders that might indicate effective practice from the consultand's perspective; and (2) analyse aspects of current protocols of counselling that might be relevant for a successful practice. We interviewed 24 consultands undergoing PST for late-onset neurological disorders (Huntington disease, spinocerebellar ataxias and familial amyloid neuropathy ATTRV30M) in the three major counselling services for these diseases in Portugal. Main themes emerging from the analysis of content were (1) consultand's general assessment of the PST process in genetic services; (2) appropriateness and adaptation of the protocol to the consultand's personal expectations and needs; and (3) consultand's experience of the decision-making process and the role of engagement and counselling skills of the counsellor. Participants provided also a set of recommendations and constructive criticisms relating to the length of the protocol, the time gap between consultations and the way results were delivered. These issues and the construction of the relationship between counsellor and counselee should be further investigated and used for the improvement of current protocols.

EPL2.6

Impact of predictive genetic testing for Huntington's disease (HD), Familial Cardiomyopathy (FCM) and Hereditary Breast and Ovarian Cancer (HBOC) in young people

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Introduction

Whilst debate has focused on testing of minors for late onset genetic disorders, less is known about the impact on young people (<25years) who have had predictive testing often many years before the likely onset of symptoms.

Method

36/61 individuals who had a predictive test for HBOC, HD or FCM, age 15-25, in our Centre, at least 3 months previously, agreed to participate. Telephone interviews with the 36 participants (10 HD, 16 HBOC and 10 FCM) were audiotaped, transcribed and analysed using Interpretative Phenomenological Analysis.

Results

None of the participants expressed regret at having the test at a young age. Participants saw the value of pre test counselling not in facilitating a decision, but rather as a source of information and support. Several reflected it had been difficult to emotionally rehearse the potential outcomes prior to testing and thought this was related to their youth. Differences emerged amongst the three groups in parent/family involvement in the decision to be tested. Parents in HBOC and FCM families were a strong influence in favour of testing, whereas in HD the decision was more autonomous and sometimes went against the opinions of parents/grandparents. Participants from all three groups proposed more tailoring of predictive test counselling to the needs of young people.

Conclusion

Some individuals will benefit from knowing their genetic status in young adulthood. The challenge for professionals is adapting the counselling process to the needs of the young person, with possible emphasis on post test support.

EPL3.1

The impact of NIPD on clinical practice: what do prenatal care providers need to know?

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New genetic technologies impact on the practice of a wide range of health professionals who may be called upon to offer these tests to their patient groups. One such development is non-invasive prenatal diagnoses (NIPD) which has the capacity to transform prenatal diagnosis. To understand how health professionals in the UK see NIPD impacting on clinical care, interviews and focus groups with professionals from fetal medicine (n=7), clinical genetics (n=12) and midwifery (n=46) were conducted, audio taped and transcribed. Thematic analysis was employed to elicit common views regarding the integration of NIPD into clinical practice and the education needs of the workforce who will deliver this service. All three groups recognised that NIPD will impact on the service they provide, however they believed this new technology should be viewed as supplementing current roles as opposed to changing practice. As such, the identified educational needs focused primarily on the procedural issues associated with NIPD, such as the laboratory process and appreciating the implications of test results. Participants stated that any educational package developed needed to reflect the service model for delivering NIPD to ensure education was relevant to the health professional's role. These findings have informed the development of a national competence framework outlining the clinical activities and underpinning knowledge required by health professionals who offer NIPD to women. This framework forms the basis of an online educational package which has been developed by the National Genetics Education and Development Centre to support the implementation of NIPD into clinical practice.

EPL3.2

Non-invasive prenatal diagnosis for fetal sex determination - benefits and disadvantages from the service users' perspective

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Prenatal fetal sex determination is clinically indicated for women who are at risk of having a child with a serious genetic disorder affecting a particular sex. Ultrasound has been the traditional method used, but early fetal sex determination using non-invasive prenatal diagnosis (NIPD) can now be performed using cell free fetal DNA in maternal plasma. The study aim was to assess the views and experiences of service users who had used NIPD for fetal sex determination. A qualitative approach using semi-structured interviews was used. Forty four participants (38 women and 6 partners of participating women) were recruited. Participants' views and experiences of NIPD were overwhelmingly positive. Concerning benefits over traditional methods, three themes emerged: (1) technical aspects of technology; (2) timing; and (3) enhanced decision-making. Practical advantages of NIPD included avoiding miscarriage, and there were a number of psychological advantages associated with timing such as perceived control, early re-engagement with pregnancy, normalisation and peace of mind. NIPD facilitated a stepwise approach to decision-making whereby women divided time into manageable 'chunks' and focused on the immediate future. This is likely to have been a coping mechanism. A number of disadvantages were discussed including concerns about social sexing and increased bonding at a time in pregnancy when miscarriage risk is high. However, participants felt these were minor in comparison to the advantages. Until definitive genetic diagnosis using NIPD is available, NIPD for fetal sex determination is perceived as a good interim measure with a number of notable advantages over traditional methods.

EPL3.3

Using discrete choice experiments to explore stakeholder preferences for non-invasive prenatal diagnosis compared to current invasive testing

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Non-invasive prenatal diagnosis (NIPD) using cell-free fetal DNA has the potential to bring many positive improvements for prenatal diagnosis. There are, however, many challenges ahead for successful implementation and it is critical that stakeholder preferences and opinions are considered. Discrete choice experiments (DCE) have been widely utilised in healthcare research to examine stakeholder preferences. Participants choose between a series of healthcare options and in doing so reveal their preferences and trade-offs in a way that reflects the complex nature of real-life decisions. Here we describe the use of DCE's to compare patient and health professional preferences for key attributes of NIPD relative to invasive testing for Down syndrome and for single gene disorders such as sickle cell anaemia and beta-thalassemia. Participants include; 1. pregnant women and partners attending maternity services for routine procedures; 2. members of relevant patient support groups; and 3. health professionals involved in prenatal care. Women (n=335) and health professional's (n=181) were found to have differing preferences when considering diagnostic tests for Down syndrome. The key attribute for women's decisions regarding testing was no risk of miscarriage, while for health professional's accuracy and timing were most important. Studies exploring preferences for diagnostic tests for single gene disorders are ongoing. Our results are important for the implementation of NIPD. In particular, women's strong preference for tests with no risk of miscarriage indicates that test safety has the potential to outweigh other factors in decision making and highlights the need for effective pre-test counselling and informed consent processes.

EPL3.4

Ethical issues in the use of pronuclear and maternal spindle transfer to prevent mitochondrial disorders

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Mutations in mitochondrial DNA are passed from mother to child and can cause diseases such as Leber hereditary optic neuropathy, MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes) and maternally-inherited Leigh syndrome. However, assessing the risk of a mother passing on mitochondrial mutations and whether, and how severely, a child will be affected is complex. Prenatal diagnosis (PND) and preimplantation genetic diagnosis (PGD) are possible but predicting the outcome for a child who inherits the mutation is difficult. The new techniques of pronuclear transfer (PNT) and maternal spindle transfer (MST) may enable women to avoid transmitting mitochondrial disease to their children. PNT and MST are currently permitted in research in the UK; the Human Fertilisation and Embryology Authority is undertaking a consultation on whether it would be acceptable to use these techniques in treatment. Both PNT and MST involve the creation of embryos containing nuclear DNA from a man and a woman, plus mitochondrial DNA from another woman. There are therefore some ethical considerations additional to those raised by PND and PGD. These include that the child would have a genetic connection to three people, and that PNT and MST alter the germline of the child i.e. the alteration would in turn be passed on to future generations. An explanation of the techniques and a review of the ethical issues will be presented to help professionals providing clinical services and those involved in psychosocial and other research to engage with the debate about using PNT and MST in treatment.

EPL3.5

The impact of carrier results generated by newborn screening on the family

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Newborn screening for sickle cell disorders and cystic fibrosis identifies carriers in addition to children with the diseases. Carrier results must be conveyed to parents, yet there is a lack of evidence to inform best practice. This study examined the impact on families of newborn screening carrier results, and subsequent familial communication through semi-structured interviews with 67 purposively sampled family members with experience of receiving carrier results communicated by a range of different methods. Data were analysed thematically by constant comparison, and validated via member checking. Parents valued carrier results and sought to share this information with their extended families and to inform their children in the future. Parental anxiety or distress was attributed to how information was communicated during the screening process rather than the result itself; appeared more common in those with less prior awareness of the disease or possibility of a carrier result, resulting in concerns their child had a serious problem, particularly while awaiting results, before seeing a professional, or when left in an "information vacuum"; or could be triggered by family communication and responses to carrier results. However, family communication and support provided great benefits to some respondents. Practice suggestions developed from respondent suggestions included: timely access to an informed health professional, improvement of community awareness and information, greater support after communication of results in considering and accessing cascade testing, and negotiating further communication within their families.

EPL3.6

Divergent views on newborn and infant genetic screening: perspectives of parents of healthy children and relatives of children with genetic conditions causing developmental delay

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Debate exists about introducing genetic conditions for which there are no curative treatments into newborn screening, although this information can be valuable for future reproductive decision-making and early interventions may improve quality of life. One important consideration for policy makers is the views of consumers about acceptability of such programs. We conducted semi-structured in-depth interviews with 14 parents of 26 healthy children (10wks- 13yrs), as well as 15 parents of children with Duchenne muscular dystrophy (DMD) and 10 relatives of individuals with spinal muscular atrophy types 2 and 3 (SMA2/3). Interviews were digitally-recorded, transcribed and thematic analysis performed, with independent coding by at least 2 researchers.

Almost all participants supported screening for such conditions provided it is voluntary. However, parents of healthy children generally thought that newborn screening should be offered, perceiving this as a convenient time, while parents of children with DMD or SMA2/3 were overall much more supportive of screening at a later stage (~1 year old), as they valued having a period of 'normality'. They perceived that receiving a diagnosis from newborn screening would have impacted negatively on the relationship with their child. Those favouring infant screening suggested it could be targeted to children whose parents are concerned about their child's development, while parents of healthy children often worried that targeted screening might reduce equity of access. These divergent views probably reflect the contrasting experiences participants had with regard to their children's development. These findings highlight the ethical complexities of offering screening for conditions causing developmental delay.

EPL4.1
Evaluation of the efficacy of two models of delivering information about treatment-focused genetic testing among young women newly diagnosed with breast cancer

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Background: Increasingly, women newly diagnosed with breast cancer with a relevant cancer family history or other high risk features are being offered genetic testing to guide their treatment (Treatment-Focused Genetic Testing 'TFGT'). In this randomised controlled trial, we evaluate two ways of offering information about genetic testing to young women at diagnosis.
Methods: Women (<50 years) at diagnosis before definitive breast cancer surgery, with either suggestive cancer family history or other high risk features, are invited to participate by their surgeon. After completion of a baseline questionnaire, participants are randomised to receive information about TFGT either: a) in educational materials (Intervention) or, b) at a genetics service (Control). Free rapid genetic testing is offered; results are disclosed at a genetics service. Self-report questionnaires assess demographic information, decisional uncertainty about TFGT, surgical and psychosocial outcomes. The second questionnaire is administered after the intervention; the third and fourth questionnaires are completed 2 weeks after results disclosure, and at 12 months, respectively.
Results: Preliminary results for change in decisional conflict are reported for 62 women who completed the first and second questionnaires, all of whom opted for TFGT. Decisional conflict (DC) decreased following receipt of information about TFGT, with no difference in mean change between the two groups (Intervention N=33, M = -13.8, SD = 20.7; Control N=29, M = -17.6, SD = 25.8), t60 = 0.642, p = .523.
Conclusions: These early data suggest that both modes of delivering information about genetic testing to women at breast cancer diagnosis are equally effective.

EPL4.2
Psychological outcomes of familial ovarian cancer screening: No evidence of long-term harm

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Background: Ovarian cancer screening for women at increased genetic risk in a large UK study involved 4-monthly CA125 tests and annual ultrasound, with further tests prompted by an abnormal result. The longer-term psychological and behavioural effects of frequent ovarian cancer screening are unknown. *Methods:* Prior to their first routine 4-monthly CA125 test, 1999 (62%) of N=3224 women completed baseline psychological measures. One week follow-up questionnaires were completed by 1384 participants (86%): 1217 (89%) women who received a normal screening result and 167 (69%) women with abnormal results. Of these, 141 (86%) completed a further questionnaire one week after being returned to routine 4-monthly screening (primary end-point). A total 912 (78%) women completed nine month follow-up questionnaires. Measures included cancer distress, general anxiety/depression, reassurance, and rate of withdrawal from screening. *Results:* Compared to women with normal results, women with abnormal results reported moderate cancer distress (F=27.47, $p \leq .001, \eta^2=0.02$) and were significantly more likely to withdraw from screening for surgery ($\chi^2_{(1)}=18.92$, p<.001) after their abnormal result. These effects were not apparent after return to routine screening or at longer-term follow-up. No differences were found in general anxiety or depression. Regardless of screening result, women reported high overall reassurance gained from screening (p=0.15). *Conclusions:* Women participating in frequent ovarian screening who are recalled for an abnormal result may experience transient negative effects, which can prompt reconsideration of risk management options. Health professionals and policy makers may be reassured that familial ovarian screening does not cause sustained psychological harm.

EPL4.3
Are illness perceptions a useful predictor of emotional distress over time in individuals undergoing cancer genetic risk assessment?

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Background: It is well recognised that objective risk status rarely predicts psychological responses to risk information. The predictive utility of Leventhal's self-regulation model in explaining psychological responses to undergoing cancer genetic risk assessment was explored.
Methods: Questionnaire data from 331 individuals undergoing cancer genetic risk assessment was analysed to explore associations between baseline psychological variables (taken upon referral) and psychological distress and emotional outcomes one month later whilst waiting for risk assessment results (Time 2) and following the provision of risk information (Time 3).
Findings: Illness perception variables (measured by the Illness Perceptions Questionnaire-Revised) explained 20.3% of the variance in cancer-specific distress at Time 2 and 11.8% of the variance at Time 3, with strong beliefs about the consequences of being at risk of cancer uniquely contributing to psychological distress. The same variables explained 7% of the variance in positive affect at Time 2 with beliefs about greater consequences of being at risk (p=<0.01) and stronger beliefs in screening/surgery being able to control their chances of getting cancer (p=<0.01) making a unique contribution. Following risk provision, those at high risk reported significantly greater understanding of their risk than those at average risk (p=<0.01).
Discussion: Understanding cognitive representations of being at risk may have some use in explaining psychological responses to cancer genetic risk assessment. Specifically, these findings suggest that those receiving a high risk result have a greater understanding of its implications: this may reflect differences in the mechanisms by which individuals at different levels of risk are informed.

EPL4.4
The underestimated impact of prophylactic mastectomy

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Purpose The decision for bilateral prophylactic mastectomy with immediate breast reconstruction (BPM) in BRCA1/2 mutation carriers is a radical decision. The impact on cancer distress, general mental and physical health, and satisfaction with body image, sexuality and the partner relationship was investigated.
Methods Fifty women opting for BPM completed psychological questionnaires at baseline, 6 and 21 months after surgery. With repeated measures ANOVA the quality of life in time was explored with a prospective design.
Results Cancer distress significantly decreased after BPM. Physical health and satisfaction with body image diminished shortly after BPM, but increased on the long-term. General mental health improved on the short-term, but decreased thereafter. Satisfaction with sexuality tended to decrease up to 21 months. In the end, 30% was not satisfied with the aesthetic result of their breasts and 30% indicated they had less frequent sex with their partner than they used too.
Conclusion Although cancer distress significantly declined, the psychosocial impact of BPM including immediate breast reconstruction should not be underestimated. Particularly, the intimate relationship can be adversely affected. Adaption to the new body image and the impact on femininity and identity may take a long time. Psychological consults should be provided preoperatively as well as postoperatively to catch the patients and partners in need of psychological counseling.

EPL4.5
The impact of risk-reducing hysterectomy and/or oophorectomy in premenopausal women at high risk of endometrial and ovarian cancer due to Lynch syndrome

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Lynch syndrome is an inherited predisposition to cancer caused by mutations in mismatch repair genes. The lifetime risk of endometrial cancer for women who carry mutations is 40-60%, and the lifetime risk of ovarian cancer is 7-12%. There is no proven efficacy of screening for the early detection and treatment of either cancer. Another option for women is risk-reducing hysterectomy and/or bilateral salpingo-oophorectomy. We used a combined methods study to explore women's experience of such surgery and the impact it had on their cancer worry, general health and menopause-specific quality of life. We sent validated questionnaires to and conducted semi-structured interviews with 15 of the 24 women invited to take part (response rate 62.5%). The results suggest that risk reducing surgery does not lead to significant psychological distress. Women tend not to think or worry much about developing cancer. Women tend to be distressed about the physical and somatic symptoms associated with menopause; their social well-being is somewhat affected, but sexual difficulties are minimal. The 5 major themes identified from the interviews were: motivating factors; taking control; benefits of surgery; physical and emotional costs of surgery; and, experiences of HRT and the menopause. Recommendations from the study include that professionals discuss the menopause, its side effects and HRT in detail prior to surgery.

EPL4.6

The use of an electronic genealogy database in cancer genetic counseling in Iceland

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Background: Pedigrees are key tools in cancer genetic counseling where accurate and comprehensive information is needed for risk assessment. Counselees often have imcomplete information. We have adopted the use of an electronic population-based genealogy database to generate a full 3 generation pedigrees.

Materials and methods: From January 2007 to January 2012, over 600 counselees have been seen in the cancer genetic counseling clinic, for 3 or 4 visits each. During the intake the counselee signs a consent for tracing her family through the database of the Genetical Committee of the University of Iceland (GCU) and the Icelandic Cancer Registry. The GCU holds accurate information on at least all Icelanders born after 1840. As cancer diagnosis recording is mandatory, the Cancer Registry provides very accurate information.

Families with pedigrees in the clinic are 265. Pedigrees made during intake, include10-25 individuals and the electronic pedigrees 40 - 2000 individuals, most commonly 3-500. Families with the BRCA2 founder mutation are 42 and BRCA1 families 5. Tested individuals are 541 resulting in over 107 BRCA2 and 14 BRCA1 carriers.

Conclusion: In our experience this method adds considerable information. No disapprovement on the behalf of the families have been noticed. This is especially inportant in the light of the policy of many cancer registries to only release individual information based on informed consent. We argue, in part based on our experience, that persumed consent should suffice. Such policy would be consistent with other sharing of individual´s genetic pedigrees, between health care facilities.

EPL5.1

Self- and Other-Oriented Reassurance in Telegenetic Counselling

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This paper focuses on telephone-mediated genetic counselling in Hong Kong where nurses contact mothers whose newborns have been diagnosed with a mild hereditary disorder (G6PD deficiency, commonly known as favism). Since this condition is preventable through avoidance of certain food and medication, it becomes imperative that when mothers are given the 'affected' status of their child, reassurance of the manageability of the condition ensues.

Our data is drawn from 50 transcripts of audio-recorded telephone counselling encounters as part of a funded study. We use thematic discourse analysis (Roberts and Sarangi, 2005) to demonstrate that the 'affected' status of the child is always delivered first, which is immediately and briefly mitigated before explanations about causes and consequences of the condition are offered. The delivery of 'good news' in the form of reassurance follows a particular structural pattern: typically advice is offered about life-style practices that the mothers must adhere to as a way of avoiding the inherent risks associated with the condition. We argue that this 'positive' framing of advice allows nurses not only to reassure mothers that they are able to manage their child's condition, but also to become self-reassured that there is manifest uptake of the advice by the mothers (e.g. via confirmation check questions; recycling of information). We examine how the nurses orient themselves to the mothers' existing knowledge of the condition vis-à-vis identifiable reassurance trajectories. In conclusion, we discuss the ways in which the reassurance trajectories are a specific feature of the telephone mode of counselling.

EPL5.2

Profile of genetic counsellor and genetic nurse practice in Europe

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The opportunities for genetic nurses and genetic counsellors to work professionally in Europe have varied according to the country of practice, the health service structure and educational opportunities. The European Society of Human Genetics is supporting development in these two professions through the promotion of a new European registration system. To inform the design of a European Master level curriculum and registration process, we undertook an online survey of 216 practitioners working in 19 European countries to ascertain current areas of practice, legal regulation, collaborative working and clinical responsibilities. Of the respondents, 82.7% were genetic counsellors and 9.9% were genetic nurses. It was a legal requirement to work with a medical colleague for 40.8%, while another 32.1% always did so, however many of the remaining respondents were unsure about the legal obligation in this regard. The majority of respondents stated that they alone or with a medical colleague took responsibility for making the first contact with the family (87.9%) drawing the pedigree (85.2%), explaining a genetic test to the patient (79.5%) and providing psychological support through the testing process. Over 81% managed some cases without the input of a medical doctor. These findings indicate that genetic nurses and counsellors in Europe are working autonomously and are making a substantial contribution to the care of patients. However, with a specific registration system operating in only four countries (United Kingdom, Netherlands, France, Israel), a unified European registration system is required to ensure comparable standards of education and competent practice operate across countries.

EPL5.3

Rational and reasonable: requests for genetic testing of children

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Professional guidelines state that predictive genetic testing of children for adult onset conditions should generally be delayed unless the result would affect medical management of the child or until the child can make a decision about testing for themselves. Based on 50 semi-structured interviews with UK genetic service professionals and families who have spoken with genetic services about childhood predictive genetic testing, this paper will explore the reasons given by HCP and parents for testing against the guidelines. These include parental anxiety about the unknown status of their child; the parent's right to know and decide what and when to tell their children; and the need to maintain a positive relationship with parents. In examining these accounts we explore how the 'reasonable parent' is constructed to argue for or against testing outside of the guidance. The paper concludes by exploring the implications for genetic service current practices.

EPL5.4

Assessing the effects of genetic counseling for people with serious mental illness: findings of the first randomized controlled trial

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Serious mental illnesses (schizophrenia, bipolar and schizoaffective disorder) cumulatively affect ≥3% of the population. They are complex disorders (typically arising as a result of the combined effects of genetic and environmental factors) for which no genetic testing is clinically available. Previous work shows that people with serious mental illness want genetic counseling but that few have had it, and no studies had examined its effects in this population. We conducted the first randomized controlled trial to test the effects of genetic counseling among people with serious mental illness. We hypothesized that as compared to a control intervention or a waitlist group, genetic counseling would: decrease internalized stigma, increase perceived control and knowledge about mental illness, and facilitate more accurate risk perception when measured 4 weeks later. Between 2008 and 2011, psychiatric diagnosis (and therefore eligibility) was confirmed by Structured Clinical Interview for 120 individuals (44=male, 66=female, mean age=42) with serious mental illness, who were then randomized to one of three groups, each of n=40: genetic counseling, a control intervention (involving an educational booklet about the causes of mental illness), or a waitlist group. Before, immediately after, and one month after the interventions, participants completed validated measures of internalized stigma and perceived control, and a purpose designed measure of knowledge and risk perception. The waitlist group completed the same measures at baseline, and one month later. Those randomized to the waitlist or control interventions were offered genetic counseling at the end of the study period. Results will be presented.

EPL5.5

Assessing wellbeing in women caring for children with Duchenne or Becker muscular dystrophy

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Background: Caring for children with progressive disorders places significant demands on mothers. This study assesses perceived needs, motivations, and wellbeing of mothers of offspring with Duchenne or Becker muscular dystrophy (DBMD). We examine the effects of mothers' carrier status, self-concept, worry, caregiver burden, perceived control, and coping efficacy on adaptation.

Methods: Mothers were recruited through advocacy organizations, a DBMD registry, and from clinic populations to complete one online survey each year for five years. This abstract includes data from the first 124 participants in the first survey. We anticipate reaching the target sample (over 200) by May 2012.

Results: Preliminary results are reported for 124 respondents. 55.6% of affected children were ambulatory. Mothers endorsed highest needs for ways to deal with uncertainty about their child's future (60% med/high); specific ways to cope with being a mother of a child with DBMD (57% med/high); specific ways to manage fears (55% med/high); and better ways to get needed support (55% med/high). The predictor variables dispositional optimism (r=.427, p<.001), self concept (r=-.384, p<.001), coping efficacy (r=.531, p<.001), perceived control (r=.286, p<.001), perceived burden (-.273, p<.001), and self-efficacy (r=.309, p<.001) were significantly correlated with mothers' adaptation. Backwards elimination regression was used to assess the ability of the variables to predict adaptation. The preliminary model showed that dispositional optimism (β=.191, p=.041) and coping efficacy (β=.424, p<.001) explained 30.7% of the variance in adaptation (F=26.11, p<.001).

Conclusions: These early data suggest that interventions targeting coping efficacy may improve the adaptation of mothers of individuals with DBMD.

EPL5.6

Interventions and Outcomes for Inherited Retinal Dystrophy: A Qualitative Examination

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The needs and outcomes of Inherited Retinal Dystrophy patients are not fully understood. Furthermore, there is a disparity between the way genetic ophthalmology services are delivered in the UK. This research used thematic qualitative analysis to identify and describe the needs and outcomes desired by a set of UK patients and their families (n=20). Extensive prior qualitative research has identified five outcome domains in clinical genetics: Behavioural Control, Cognitive Control, Decisional Control, Emotional Regulation and Hope, referred to as Empowerment. Yet, the relevance of these five domains to inherited eye disease is unknown. The data were analysed through the Empowerment theoretical framework to determine what attributes, if any, differentiate retinal dystrophy patients from other clinical genetics patients. The research found that patients' desired outcomes relate to medical, psychosocial and practical categories of need, most of which line up closely to the Empowerment domains. However, three themes discovered in the data do not have a corresponding Empowerment outcome measure: Information about benefits, Adaptations and Mobility. Thus, a new outcome domain, Independence, defined as "The ability to participate fully in social, family, economic, educational and/or public life", is proposed to reflect the desired practical outcomes. Independence is a logical outcome for coping with a potentially disabling condition. It may not apply to genetic conditions that are non-disabling, which could account for its absence in previous studies. This data will be used to design and evaluate an optimal care model for genetic retinal services.

EPL6.1

Providing written information as an aid in Genetic Counseling - dispensable or helpful?

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Providing written information as an aid in Genetic Counseling - dispensable or helpful?

Written information can complement and aid genetic counseling . This information allows patients to re-read the information presented during counseling thus strenghtening and deepening it. An appendix with additional addresses and consultation possibillities helps to increase the options for becoming fully informed. This can be of great importance, in particular when the genetic counseling entails difficult decisions , .

Since 1996 the Association for Psychosocial Aspects of Human Genetics (VPAH eV) has been publishing the brochure „Bad news after prenatal diagnosis - A companion brochure for women and couples who consider termination of pregnancy". The brochure enjoys ever-increasing demand, and theVPAH has now published the 13th updated edition. In addition, the brochure has also been available online for several years.

The poster shows the positive reception history that the booklet has seen since its inception, and which has reached a preliminary high with the enactment of the Gene Diagnostics Law in Germany. In addition, the poster shows the recipients of the brochure by professional category, as well as the regional distribution of purchasers in Germany.

Finally, an outlook is given on further patient brochures that the VPAH e.V. publishes orissues or plans to publish. These (will) treat the use of predictive tests in the context of HNPCC, breast cancer or neurological diseases.

EPL6.2

Communicating familial breast cancer risks: risk presentation formats and women's preferences

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Background: Besides the effectiveness of risk communication formats, it is also important to consider patient preferences to improve patient-centred care. This study assessed women's preferences and satisfaction with different risk presentation formats.

Methods: 279 unaffected women with a breast cancer family history were allocated to receive one of five additional risk consultations after standard genetic counseling, in which breast cancer risks were presented as: 1) percentages (X%); 2) frequencies (X out of 100); 3) frequencies and graphical format (10x10 human icons); 4) lifetime risk and age-related risk in numerical format; 5) lifetime risk and age-related in both numerical and graphical format. Preferences and satisfaction were assessed 2-weeks follow-up.

Results: Both numbers and words (37%) and numbers only (26%) were preferred most. Of the numerical formats, 55% preferred percentages. Women who had received graphical displays favored graphical displays more than other women (p<.001). This preference was lower for women who were lower educated compared to those higher educated (5%vs.41%). The majority (73%) preferred to hear both lifetime risk and 10 year age-related risk. This preference was not affected by experience with the format. However, women >40 years preferred the age-related format (without lifetime risk) more than younger women (20%vs.2%). There was no difference in satisfaction between groups.

Conclusions: Lifetime cancer risk information in addition to age-related risk in a numerical format (percentages) was most

preferred. The results suggest that in counseling, women's preference for a risk communication format may be influence by previous experiences and women's age and education.

EPL6.3

The Signal-Trial: Evaluation of a Checklist to Improve Communication about Psychosocial Problems in Cancer Genetics

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Introduction

Approximately 20% of individuals undergoing oncogenetic counseling with/without DNA-testing experience clinically relevant levels of distress. Within the counseling session, information provided is mostly biomedical and provider-driven. The use of a checklist, completed by counselees prior to the counseling session, might facilitate discussion of psychosocial issues. The aim of this trial is to evaluate the use of a checklist as an aid in 1) facilitating communication, 2) increasing counselors' awareness, and 3) improving the management of psychosocial problems.

Methods

In total, 260 individuals undergoing oncogenetic counseling at the family cancer clinics of the NKI-AVL (Amsterdam) or the UMCU (Utrecht) will be randomised to either a group whereby the results of the checklist, completed prior to the counselling session, are shared with the genetic counselor (intervention), or a group where the results of the checklist are not used within the counseling (control). The counseling sessions are audiotaped for purposes of content analysis.

Results

Preliminary data on the first 98 participants indicate that counselees experience problems in the following psychosocial domains: living with cancer (95% of the counselees), genetics (67%), children (51%), family and social issues (30%), emotions (24%), and practical problems (14%). Whether these issues are more frequently addressed in the intervention group as compared to the control group will be evaluated when the study matures.

Discussion

If proven effective, the use of a oncogenetics-specific psychosocial problem checklist can be recommended as an aid in facilitating communication, increasing counselors' awareness, and improving the management of psychosocial problems within cancer genetics.

EPL6.4

Disclosure of information within french families with BRCA mutation

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Disclosure of genetic information to first-degree relatives in families with a genetic predisposition was studied in two French regions. Data for 64 women were collected (24 index cases, 40 relatives with the BRCA mutation).
Concerning disclosure to children over 18, 98% of these had been informed (100% of daughters and 96% of sons) and 46% underwent genetic screening.
For siblings, all of sisters and 98% of brothers had been informed and 44% underwent genetic screening.
85% of parents had been informed; 59% of mothers and 44% of fathers had undergone genetic screening.
Concerning age of the communicant, incomplete information to first-degree relatives was observed in 14% of those above 60 years of age, 11% in the 50/60 age group, 5.5% in the 40-50 years, and 0% in those between 30 and 40 years.
Complete information of the relatives was also more observed when the communicant was index case (96% versus 92,5% for relatives), and was symptomatic (97% versus 85% for asymptomatic women)
The reasons for non-disclosure of the information to relatives included family dispute (33%), wish not to worry others (50%), or no particular reason (17%).
This study showed that disclosure of the information is well done for children and siblings, whatever the sex, and less constant for parents. Disclosure of the information was better in young and/or symptomatic women. The reasons for not providing the information were not related to poor understanding or difficulties reiterating the information to members of the family.

EPL6.5

The impact on self and family of receiving genetic test results following participation in the Australian Ovarian Cancer Study (AOCS)

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The generation of clinically significant genetic data during research studies raises a number of ethical issues about the disclosure of this information to research participants and their family. Little is known about individuals' experiences of receiving research results . This qualitative interview study investigated research participants' (n=10) or their nominated next of kin's (n=15) experiences of receiving notification that (genetically) significant information is available following the proband's participation in the Australian Ovarian Cancer Study (AOCS). This paper describes the emotional impact of receiving a notification letter, interviewees' views about the personal/familial relevance of receiving this type of feedback and their subsequent/intended disclosure practices. In general, interviewees had a mixed response to receiving feedback. AOCS participants' tended to be more positive, acknowledging that genetic information may be useful (primarily) for their kin. Next of kin, in contrast, described themselves as distressed at receiving feedback, particularly those who were unaware of their mother's participation in AOCS and who were not expecting to be contacted about her results. All interviewees described the different implications of receiving genetic information for self and others. Both AOCS participants and next of kin expressed an intention to disseminate the information to relatives following confirmation of the result in an accredited laboratory. We discuss the practical and ethical issues raised by the adoption of different strategies to avoid causing distress in next of kin, for example, seeking next of kin's consent to feedback at the outset versus only disclosing research findings to the participant.

EPL6.6

Enhancing family communication about genetics: ethical and professional challenges

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Genetic counsellors describe a sense of responsibility towards the at-risk family members of a person newly diagnosed with a genetic condition, wishing to ensure that they are appropriately informed about their status and options. While a small number of probands directly state their intention not to inform their relatives, many who do intend to communicate this information appear to be unsuccessful for a wide range of reasons. Some evidence suggests that successful communication of genetic information may be enhanced by follow up support from a genetic counsellor. Nonetheless, when we began to develop a genetic counselling intervention to optimise the number of informed family members for evaluation in a funded randomised control trial, we were faced with a number of ethical and professional challenges. We will share those challenges, drawing on the Reciprocal-Engagement model of genetic counselling and concepts of autonomy, to describe a framework for their resolution. While the resulting intervention aims to facilitate decision-making and is theoretically grounded in genetic counselling, little is known of practice in this area. Strategies used in practice by genetic counsellors when addressing difficulties in family communication with clients were identified while training genetic counsellors to deliver the intervention. These strategies will be compared and contrasted with the intervention.
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EPL7.1

Intentions to receive individual results from whole-genome sequencing among participants in the ClinSeqTM study

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The volume of data generated for a single individual and the wide range of findings from whole genome sequencing raise critical questions about the return of results and their potential value for end-users. We conducted a mixed-methods study of 311 participants in the ClinSeqTM study to assess attitudes toward learning results, perceived opinions of valued others, and how these variables predict intentions to receive results within four categories of findings ranging from medically actionable to variants of unknown significance. 294 participants indicated a preference to learn their genome sequencing results and six were unsure. Most often participants cited disease prevention as their reason, including intention to change their lifestyle behaviors. A third expressed a general desire to know, reflecting those who generally valued information and others who sought to understand the personal implications of findings. Participants had positive attitudes, strong perceived social norms and strong intentions to learn results overall, although there were significant mean differences among categories of findings. Attitudes and social norms for medically actionable and carrier results were most highly rated. Strong intentions were motivated by confidence to use the information to prevent future disease and belief in the value of information. It behooves investigators to facilitate participants' desire to learn a range of information from genomic sequencing, while promoting realistic expectations for its clinical utility and perceived personal utility.

EPL7.2

Introducing high-throughput seqencing in the clinical setting - but what will patients think ?

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Developments in genetics, in particular the advent of high-throughput sequencing technologies, are expected to have a profound impact on health and healthcare, yet much remains to be learned about how people - present or potential recipients of such care- perceive and frame these expectations. What do laypeople anticipate ? How are expected changes understood and portrayed ? Are these expectations grounded in their previous experiences of clinical care or do they reflect principles and values attached to medicine ? In order to explore these questions we carried out a series of 8 focus groups (a total of 64 participants) in three categories : laypeople, research participants and members of patient organisations. This work was done in the context of the European Techgene project, which aimed to develop high-throughput tests for clinical use. The results of these focus groups gave insights into the types of results that people are willing to receive ; the desire for a new kind of physician patient relationship and the differences between the clinician's and the layperson 's perspectives on the distinction between clinic and research. This research provided an opportunity to confront ethical theories about these new technologies with empirical data to test ethical reasoning in context and to allow it to be grounded in the values and concerns of service users. Such empirical ethical analysis is crucial for the development of guidance that is applicable to the experiences and expectations of those who will use these emerging genetic technologies.

EPL7.3

Rapid genetic testing for BRCA1/2 genes: How could oncogenetic counseling deal with an urgent choice?

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The life-time risk to developing breast cancer in BRCA1/2 mutation carriers is 43% and 46%, respectively, and ten years risk of developing a contralateral breast cancer is 25%. Risk reducing bilateral mastectomy (RRBM) can reduce breast cancer risk in BRCA1/2 mutation carriers up to 95%. Rapid genetic testing is now available for newly diagnosed breast cancer before having surgical treatment. To guarantee autonomous and informed decision we elaborated a new model of oncogenetic counseling focused mostly on psychological aspects and surgical counseling. Since 2008, at the Modena Centre for Hereditary Breast and Ovarian Cancer (Italy) has been performed a rapid genetic testing for women with hereditary profile at the time of breast cancer diagnosis. The entire oncogenetic counselling path is completed within three weeks. So BRCA1/2 carriers have the opportunity to choose for RRBM. About 71 mutational analyses were performed and 25 (35%) patients were mutated. Among the 25 patients with a positive result, 13 (52%) had a RRBM at the surgery time for the breast cancer. A psychological follow-up was performed in all patients undergone RRBM. After 12 months from the intervention, patients showed an overall good emotional adjustment and satisfaction with their decision whereas only in a minor case sexual and psychological problems arose. Our study shows that a rapid genetic testing in patients with a hereditary profile significantly improved the choice of RRBM and that is it fundamental to guarantee both multidisciplinary counseling and psychological follow-up to these women.

EPL7.4

The impact of diagnostic developments in prenatal diagnosis; a psychological challenge

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The introduction of new techniques for screening of genetic anomalies such as whole genome microarray analysis (WGA) into the clinic of prenatal diagnosis ensues a number of psychological challenges for pregnant couples. WGA generates more information about the current and future health of the unborn child than conventional karyotyping (CK), the gold standard for genetic chromosome analysis in prenatal diagnosis. One the one hand couples need to determine the extent of information they wish to receive about the (future) health of their unborn child. On the other, professionals may withhold or disclose information in the interest of the (health/future autonomy of the) unborn child. Our experience thus far is that WGA generates more probabilistic results (e.g. increased risk of learning disabilities) than CK. Probabilistic information is more difficult to grasp than information about actual presence of an anomaly. Moreover, probabilities represent an uncertainty that impedes decision-making. A best possible decision about the course of a desired pregnancy needs to reflect consistency with personal values and considerations in order to be processed adequately emotionally. Thus, the broadening of knowledge about the current and future health of the unborn child implies that pregnant couples will have to anticipate a variety of outcomes, determine the extent of information they wish to receive, interpret the meaning of probabilistic results and assimilate the results with personal values and considerations into their decision about the course of their desired pregnancy. Health care professionals must attend each of these themes in the pre- and post test counselling.

EPL7.5

Close parental relatedness identified by SNP microarray - challenges for genetic counselling

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Introduction

Molecular karyotyping by microarray is recommended as 'first tier' testing in evaluation of individuals with intellectual disability, developmental delay, multiple congenital abnormalities, and autism spectrum disorders. SNP microarrays extend diagnostic scope beyond detection of submicroscopic pathogenic copy number changes by also revealing uniparental disomy, chimerism, chromosomal mosaicism and long continuous stretches of homozygosity (LCSH) creating the potential to determine previously undisclosed incestuous relationships, posing legal and ethical challenges for genetic services.

Methods & Results

SNP microarray was performed on 11000 consecutive samples. LCSH was detected in 1156 samples (10.5%). Close parental relatedness (greater than 6.25% LCSH) was detected in 322 samples (3%), with 4 samples showing homozygosity levels >20%, consistent with a first-degree relationship between parents. Two known cases of first-degree unions were detected with homozygosity levels of 17.3% and 19%.

Discussion

Identification of close parental relatedness poses unique ethical and legal challenges for genetic services. Can we reliably differentiate between extensive LCSH arising from incestuous relationships and consanguineous unions over several generations? How these findings should be reported to avoid inappropriate or insensitive disclosure whilst fulfilling legal and ethical obligations, protect the child's right to privacy, whilst ensuring medically relevant information is available is complex. We will present the approach our service has developed to deal with results indicating close parental relatedness. Next generation microarrays are likely to combine both the CGH and SNP platforms. These issues will arise with greater frequency, necessitating the development of specific guidelines.

EPL7.6

Criteria for responsible introduction of genome-based-technologies and information into public health care

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With the incredible advances in genome sequencing over the last decade, there has been a shift towards studying more common complex disorders. The integration of this information into the health care setting has proved to be much more problematic than for monogenic disorders. Furthermore, with the 1000$ genome just around the corner, there is a strong push for the uptake of additional genomic testing. Although laudable, these advances also bring with them a slew of ethical and social issues that challenge the normative frameworks used in clinical genetics until now. With this in mind and considering a previous report from the Dutch Health Council, we outline herein five principles that should be considered in order to introduce genome-based-technologies and information (GBTI) into public health.

1) Their introduction should be based on a solid scientific foundation.
2) GBTI introduced into the health care system and financed by public funds should be focused on significant health problems.
3) The advantages of introducing and offering GBTI should outweigh the disadvantages.
4) The autonomy of patients, and individuals in general, must be respected.
5) The offer of GBTI funded from public sources should be justified in the context of the overall healthcare budget.

EMPAG Workshops

EW1

Parents & Children in Families with Huntington's Disease

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In families with Huntington's Disease (HD), family dynamics are often unfavorable for the healthy psychological development of children. In a recent study, we found that persons who grew up with a parent with HD had been exposed to more adverse experiences in childhood than the general population. This may have life-long negative consequences; we found that adults with a parent with HD have poorer mental health and more fear of intimacy and abandonment than the general population.

Recently, in the Netherlands, a meeting with professionals (psychologists, social workers) was held, to explore the nature and extent of problems that professionals encounter in their work with HD families. It was agreed that HD families present with specific difficulties concerning parents and children. Adequate support can not always be given, because couples may not be open for support with child rearing, and because existing support is not tailored to the needs of HD families.

Professionals working in Clinical Genetics, where persons apply for predictive testing and receive counseling on reproductive options, may have an opportunity to address parenting issues in an early stage, before neurological diagnosis of HD and before problems with parent-child interactions occur.

In this workshop, we will exchange experiences with parent-child issues in HD families, and with existing forms of support. Hopefully, we can cooperate in a search for more adequate, specialized programs for prevention and intervention, so that children who are growing up in HD families now and in the future have optimal chances of becoming stable adults.

EW2

Methodological guidelines for empirical validation of genetic counselling interventions

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Actual constraints and better awareness of critical factors underlying psychosocial based interventions had increased needs, and doubts, about genetic counselling efficacy and effectiveness. Further from the ethical foundations of genetic counseling importance and simple experience-based evidences, there is still a huge gap on empirical validation of genetic counseling interventions and still low number on meta-analytical results are conclusive. We had advocated on several contexts the need for substantial evidences about efficacy and efficiency of genetic counseling interventions and about better understanding of interview skills and programs structure specific impacts. Based on this point of view, it is proposed a model for future research on genetic counseling clinical research based on known guidelines adapted to this context.

Aspects related to frequent difficulties on randomized controlled trials as: 1. Manualization of interventions respecting broad literature reviews and clinical opinions; 2. Sampling aspects envisaging minimum bias; 3. Aspects concerned with proper control groups; 4. Sophistication of methodological design; 5. Problems with outcome measures; 6. Statistical aspects to quantify counselling effects; 7. Concerns will long term effects, acceptability and robustness of interventions, are addressed on this workshop and specific research contingencies are reflected.

Guidelines are operationalized and explained on two levels of discussion: past relevant research examples and based on ongoing or future participant's projects. Participants will be invited to share their clinical research dilemmas in order to enable solutions for future research.

EMPAG Posters

EP1 Psychosocial issues in Neurodegenerative diseases

EP01.01

The challenge of diagnosis and counseling for intermediate alleles in Huntington's disease: a clinical example

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In presymptomatic testing for Huntington's disease (HD), counselees expect to receive a clear cut result about their carrier status. However, we are increasingly confronted with counseling dilemmas related to the presence of intermediate (IA) or reduced penetrance (RP)alleles, of which the individual clinical outcome is more difficult to predict than for full penetrance alleles. Moreover, these alleles can expand into full penetrance alleles upon transmission , which has specific disease risk implications for the offspring.

We present a family in which the index patient, a 45-year old man, was clinically diagnosed with HD and shown to carry a HD allele with 43 CAG repeats. Remarkably, he was the youngest in a sibship of nine, with no other affected individuals in the family. His father, who died at age 78, reportedly showed aggressive behavior throughout his life but the diagnosis of HD had never been suspected in him. His mother died at age 77 without signs of HD.

Subsequently, six siblings requested presymptomatic testing: three showed a normal molecular test result, although, in two of them, the pre-test neurologic evaluation was inconclusive regarding the possibility of early signs of HD; three other sibs had an IA of 34 CAG. The clinical history of the father suggested that, most likely, he also a carried the 34 CAG allele and that expansion occurred in the index patient.

This case illustrates the unusual segregation and counseling issues that may arise in families segregating an intermediate HD allele.

EP01.02

Psychological effects of presymptomatic DNA testing for Huntington's Disease. An Italian research.

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Aim: The aim of the present study is to investigate the psychological well-being of presymptomatic Huntington's disease (HD) gene carriers and non-carriers 6-12 months after the genetic testing.

Methods: From May 2009 to October 2011 we evaluated 18 presymthomatic subjects resulted carriers of HD gene and 18 noncarriers. The instrument used is the Psychological General Well-being Index (PWBGI).

Results: Both the 18 subjects resulted carriers (N:18, M:5, F:13; mean age: 47,35, range age: 26-75) and the 18 subjects noncarriers (N:18, M:7, F:11; mean age: 45,93, range age: 26-69) don't show any significant difference from normative data, but the carriers mean scores are lower than the normative scores; moreover the Global Index Score indicate a moderate level of distress (71,37). The Student T-test comparison between carriers and non-carriers shows statistically significant differences in Vitality scale (11,78 versus 14,56; p<.01) and in the Global Index Score (71,37 versus 82,33; p<.05).

Conclusions: The results of the present study indicate that the presymptomatic HD gene carriers in comparison with noncarriers 6-12 months after genetic testing present a significant lower vitality and a significant worse level of the global index of well-being. The research, despite the limited sample, highlights a psychological critical situation in presymptomatic HD gene carriers and suggests the importance of specific psychosocial interventions. Further studies are needed in order to explore in a longitudinal perspective how the communication of HD gene carrier condition impacts on the psychological well-being and the quality of life of the individuals involved in genetic counseling process.

EP01.03

Genetic conditions: a challenge for couple/ family therapy

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As a psychologist working and learning with genetics patients in a central hospital, I have become aware of the extreme importance of the family therapy in addition to the individual. During my clinical practice, I have realized that genetic conditions can put family dynamics at risk.

For instance, the termination of pregnancy after prenatal diagnosis. As Klass (1988) pointed out, this experience can have an effect of estrangement between the couple.We know by experience that women often complain about their partners who seem to have no grief feelings. Peppers and Knaps (1980) called out our attention to the tremendous differences between women and men mourning processes and named it *Incongruent Grief.* If we take this knowledge to our practice we will be able to help the couple to recognize these differences in a good instead of a scary way.

What about the cons of a diagnosis/predictive testing kept in secret? We will discuss a case of a woman who decided not to confess her diagnosis of Familial Amyloidotic Polyneuropathy to her husband, and how that decision put the couple bonds at risk. We will compare this case with another woman who asked for family support the diagnosis of CADASIL. Accepting that the secret was a bigger risk, than the disclosure, for her family' bonds, she decided to share the diagnosis with her partner and son.

We will discuss the pros of couple/family therapy in addition to the individual. Examples will illustrate the impact of genetic conditions over the family dynamics.

EP2 Communicating Genetic information

EP02.01

The Process of parental disclosure in Duchenne Muscular dystrophy (DMD)

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The communication process between parents and their affected children regarding a genetic condition and its prognosis is rarely explored. The aim of this study was to investigate this complex communication process about the diagnosis and prognosis of Duchenne muscular dystrophy. To explore this the researcher conducted 1) 6 interviews with parents of affected individuals and 2) 4 interviews with Health professionals involved in the care of the affected boys. This data was transcribed and coded for thematic analysis. We identified the appropriate method of communication, the role of family culture, key issues raised by such communication needs and appropriate source. The most appropriate method of communication was indicated as a 'drip feed' approach using language suited to the child's development. The family culture towards communication played an enormous part is how comfortable they felt discussing difficult issues. The key issues raised during this process were mutual protection, a tension between autonomy and protection and parental responsibility.

EP02.02

Genetic counselor's report to patients in clinical genetics: A survey to establish a quality management

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Genetic counseling is defined as a communication process dealing with genetic diseases, (recurrence) risks, diagnostic possibilities, and choices in individual and family life. An important part is the report for counselees. It is supposed to enable them to reflect on the counseling session, receive help in decision making, and inform their physicians and family members adequately.

In order to get an overview on the current practice and with regard to future quality management of genetic counseling we asked genetic institutions in Germany to send us anonymously and via internet such reports concerning six common diagnostic conditions of genetic counseling, one typical letter for each condition. Between 17 and 40 institutions responded - 40 to the issue "recurrent abortion", 37 to the issue "hereditary cancer", 32 to the issue "prenatal trisomy 21", and 17 to the issues "fragile X syndrome", "infertility pairs", and "unknown syndrome", respectively.

Reflecting the gathered reports with special regard to already existing guidelines we developed a catalogue of criteria, which adequate reports should fulfill. We classified the criteria in general, formal and technical criteria, criteria of content and counseling specific criteria. Additionally, we established a check list for writing a "successful" genetic report, well structured according to topics like general requests, necessary information, anamnesis, medical reports, results of investigations, contents of the counseling, conclusion.

Both, the check list and the catalogue of criteria, could be useful tools for writing adequate for-client reports. Moreover, they might be helpful in establishing a reasonable quality management of genetic counseling.

EP02.03

Using conversation analysis to improve genetic counseling: An example regarding counseling of fellow healthcare personnel

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Background and aim: Conversation Analysis (CA) is a qualitative method where recurring speech-patterns are noted and broken patterns identified. For instance if the patient change turn-design from minimal responses to long responses, and thereby change the semi-preallocated turn-taking system. The analysis of the transcription is data-driven, and therefore inductive. CA of recorded consultation might improve provider awareness of mechanisms of communication.

Method: In 4 of 10 recorded counseling sessions the patient had a profession or an education within the health sciences. In one of these sessions excerpts were transcribed and sequentially analyzed. The CA examines how the genetic counselor and the patient linguistically cast the patient"s identity.

Result: Analysis showed that there was an ongoing negotiation of the patient's identity as patient and health care professional respectively. The role of the patient expected by the counselor may therefore be less evident to the patient. This observation was then communicated to the counselor along with suggestions of how to deal with such negotiations.

Discussion: This analysis illustrates, that CA might be a useful tool to analyze the interaction in genetic counseling, because of the data-driven approach. Concurrent interviews with the counselees might be a good supplement to the CA. Genetic counseling is best when provided form a multidisciplinary team. Inclusion of professionals with knowledge of CA might further increase the capacity of the team.

EP02.04

Communication in paediatric clinical genetics: a case study illustrating a useful analytic technique

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Various techniques have been employed to explore interactions in genetic consultations ranging from quantitative methods which have assessed over one hundred consultations, to in-depth qualitative analyses of single transcripts. Overwhelmingly research has demonstrated that dialogue is dominated by the clinician, with content being mostly educational or scientific in rather than psychosocial.

This presentation will illustrate how an interactional sociolinguistic framework, in particular, one making use of Mishler's 'voices' in medical consultations, can enable researchers and clinicians to gain a greater understanding of the apparent medical dominance illustrated in these studies (Mishler, 1984).

Mishler describes two different 'voices' present during medical interactions. Talk relating to the knowledge and experience of the client is described as the 'voice of the lifeworld'. The 'voice of medicine' refers to the technical, scientific talk of the medical world. Mishler argues that the typical or 'unremarkable interview' is dominated by the voice of medicine, while the voice of the lifeworld remains mostly suppressed. Barry et al. support Mishler's theory that allowing more space for the lifeworld can result in more efficient medical consultations (Barry et al. 2001).

In analysing two contrasting paediatric clinical genetic consultations we track the voice of medicine and the voice of the lifeworld through the lens of interactional sociolinguistics. It will be demonstrated how clinicians can effectively engage with the voice of the lifeworld, while preserving other essential elements of the consultation.

We believe that this framework could be useful for both researchers and clinicians to enhance a client-centred model of care.

EP02.05

'I realised it was coming, it was just a question of time': living with the knowledge of increased familial risk

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Introduction

The use of family health screening tools is increasing in primary care settings. However, little is known about the psychosocial/behavioural impact of familial risk assessment for common chronic diseases . This qualitative study explored people's response to receiving information about their risk of developing one of four marker conditions: diabetes, heart disease, breast and colon cancer. These conditions fulfil screening criteria, and effective interventions and lifestyle strategies exist for their primary and secondary prevention.

Methods

Thirty participants (aged 24-50, 22 females), recently informed of their personal risk, were recruited via the FAST study, set in 10 East of England general practices. Purposeful sampling led to a cohort of population (N=12) and increased (N=18) risk participants. Data were collected using semi-structured interviews, transcribed verbatim and interpreted using framework analysis.

Results

Receiving information about increased personal risk did not appear to cause psychological distress. Participants used mental models of health to assess salience of risk to themselves and kin. Four personalising processes were identified: 1) actively making lifestyle changes and seeking further health advice; 2) acknowledging risk but not enacting lifestyle changes or seeking further screening;3) not presently perceiving personal risk as high but acknowledging possible changes in the future; 4) being at population risk was generally perceived as a validation of current lifestyle.

Discussion

Participants were influenced by their knowledge of marker condition risk, but reported that being at higher risk did not always lead to preventive behaviours.

Furthermore, being at population risk could lead to fewer preventive behaviours.

EP02.06

Non-syndromic neurossensorial prelingual deafness: the importance of genetic counseling in demystifying parents' beliefs about the cause of their children's deafness

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Introduction: Recent advances in molecular genetics have allowed the determination of the genetic cause of some childhood non-syndromic hearing loss. Nevertheless, only a small proportion of families are referred to a clinical genetics service for proper genetic counseling. In Portugal, there are no published studies about the prior beliefs of parents about the causes of hereditary hearing loss of their children and their genetic knowledge after the genetic counseling offered by professionals with specific training. The aim of this study was to assess beliefs about possible causes of non-syndromic neurossensorial prelingual deafness in order to improve the quality of communication.

Methods: Forty-four parents (24 mothers, 20 fathers) of twenty-four children with the diagnosis of non-syndromic neurossensorial prelingual deafness due to mutations in the connexin 26 gene (*GJB2*) answered a questionnaire about genetic knowledge before and after the genetic counseling.

Results: Before counseling 15.9% of the parents knew the cause of deafness, while at a post-counseling setting this percentage was significantly higher. No differences were found between the answers of mothers and fathers before and after genetic counseling. Parents' level of education was a significant factor in pre-test knowledge. After genetic counseling 95.5% of the parents stated that the clinical genetics consultation had met their expectations; 70.5% remembered the inherited pattern, 93.2% recalled the recurrence risk of deafness.

Conclusion: It is important genetic counselors to take into account parents' beliefs and assess their genetic knowledge, in order to increase the knowledge and demystifying parents' beliefs.

EP02.07
Young adults‘ attitudes towards newborn screening and carrier identification

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Research examining parents’ experiences of receiving carrier result information from newborn screening suggests that anxiety and distress are caused by unpreparedness for results and how these results are communicated, rather than the results per se. Questions have been raised about how feasible it is to convey newborn screening information adequately during pregnancy, a time when parents often experience information overload. To understand how these newborn screening processes can be improved for future generations and to investigate the feasibility of providing newborn screening prior to pregnancy, young adults’ understanding of and attitudes towards newborn screening were investigated. Thirty-four young adults, with no experience of screening, took part in one of seven focus groups. Thematic analysis suggested that respondents recognised the benefits of carrier knowledge on altering future reproductive decisions, and despite concern regarding the stigmatisation of carriers, typically expressed a desire to have access to personal carrier status. After viewing key information from Cystic Fibrosis and Sickle Cell Disease websites, adults became preoccupied by the personal threat of being a Cystic Fibrosis carrier, yet did not acknowledge the risk of being a Sickle Cell Disease carrier, despite re-evaluating its perceived severity. Some adults were unable to accurately interpret inheritance diagrams. Young adults were not interested in receiving screening information prior to pregnancy; rationales varied from worry associated with screening information (avoidance) to the inability to conceptualise its relevance (denial). Incremental impartation of information aimed at increasing adult’s interest in screening to enable them to appreciate the personal relevance is suggested.

EP3 Reproductive Decision making

EP03.01
Impact of genetic counselling on reproductive planning of couples in families with myotonic dystrophy type 1

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Myotonic dystrophy type 1 (DM1) is a neuromuscular, multisystem, progressive disease, with autosomal dominant inheritance. Genetic counselling is a delicate process in this disease, as reproductive decisions are difficult due to the variable clinic presentation and the phenomenon of anticipation of age-at-onset. We planned (1) to assess the impact of genetic counselling in families with DM1, their reproductive choices and the factors that influenced it, (2) to consider the results of prenatal diagnosis, and (3) to assess the influence of psychosocial elements in their reproductive planning. A retrospective study of 10 years used a questionnaire aimed at couples with a family history of DM1, followed by genetic counselling for reproductive planning. The main reproductive choice was not to have children (55.6%), followed by pregnancy and prenatal diagnosis (33.3%). In 60% of those couples who had prenatal diagnosis, the foetus was a carrier and the option was for termination of pregnancy. The main factors that have influenced reproductive decisions were (1) the risk of having an affected child, and (2) the existence of other children prior to genetic counselling; (3) the psychosocial impact of the disease also contributed to the reproductive choices. Genetic counselling has a strong impact on reproductive choices in families with DM1. A multidisciplinary team should always be involved in the counselling process, to meet the expectations and the needs of counselees and their families, and to provide the support they need at all stages of their decision-making process about reproductive options.

EP03.02
As the old cock crows, the young cock learns. An intergenerational approach in the perspective of family planning in case of a gene mutation carrier

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A BRCA gene mutation carrier has an increased risk of 60-80 % to develop breast cancer and 5-60% to develop ovarian cancer. Its diagnosis may lead to an increased psychosocial disturbance. Female gene mutation carriers are faced with the choice to decide for screening or risk-reducing surgery. Since BRCA carriers may develop breast cancer at an age before reproduction, and the fact that they have 50% probability to pass the gene defect to offspring, this may influence their decisions as how to fulfill the wish to start or complete their family. There is a range of options in the context of reproductive choices: to have children regardless the risk, to remain childless, or adoption, or sperm- or egg donation, or to consider assisted reproduction techniques such as PND or PGD. The moral struggle and concerns that these couples have to face can be driven by various factors: their own desires, desires of the partner, the responsibility to future offspring, prevailing values with respect to the hereditary disorder in the family of origin, opinions in their wider social network and in society in general.
To prevent an unnecessary burden for the prospective parents, it is desirable that themes regarding reproduction are scheduled in the pre- and posttest counseling. In a situation where the decision-making process of reproduction stagnates, it can be beneficial to analyze the underlying reasons from the perspective of an intergenerational approach. This will be illustrated on the basis of several case reports.

EP4 Living with genetic disease

EP04.01
Psychological impact of diagnosis of thrombophilia on women with childbearing potential

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Background: Thrombophilia is a inappropriate tendency to thrombus formation. In recent years numerous studies were conducted in the field of thrombophilia, in an attempt to prevent the consequences of thrombotic disease.However, there are few studies to evaluate impact of diagnosis on quality of life for patients with childbearing potential.
Methods: Between 02.2010-01.2012 in Oncomed Timisoara we evaluated 94 patients with thrombophilia, all women. A questionnaire was given to every women, to asses quality of life.
Results: Only 70 patiens accepted to answer the questionnaire. 70% were hopeful about the future, hope generated by the fact that they found the source of miscarriage, but they were frustrated 85% of the patients, stressed about being unable to conceive. Only 27% of them were mentally exausted by the number of attemps to become pregnant and all of them were decided to try again. All patients answered that they where able to get organized and take care of daily activities; still, 25% had difficulties to resolve conflicts and 40% were not able to provide emotional support for others. All patients were able to mantain a normal sexual life. 90% of them experienced sudden mood changes, 75% felt overly sensitive to others comments, but only 37% felt at least once lost.
Conclusion: The answers showed that despite the depression associated with the miscarriages, all of them were still hoping to become mothers. This result shows that a close collaboration between hematologist and psychologist is the key to a good quality of life for these patients.

EP04.02
Quality of life and subjective health complaints in acute intermittent porphyria

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Acute intermittent porphyria (AIP) is a metabolic disease inherited in an autosomal dominant way with reduced penetrance and variable expressivity. Symptoms presents as attacks of abdominal pain, vomiting, muscle aches, muscle weakness, and in extreme cases, respiratory paralysis. Triggering factors can be medications; hormones, alcohol, physical and psychological stress, hunger and fast. There exists little formal information about the subjective experiences of persons suffering from this condition, but previous research indicates that AIP can cause serious life style consequences and reduce quality of life in those affected.
The aim of this study was to describe self-reported quality of life and subjective health complaints in persons with latent, manifest and active AIP respectively, and to investigate the relationship between quality of life and disease related variables, demographic variables and coping strategies.
A written postal survey was distributed to all persons registered with AIP older than 18 years in Norway. Response rate was 55% (n= 140). The instruments WHOQOL-Bref, SHC, IES and MHLC were used, in addition to demo-

graphic data and information on disease severity in the family.
Quality of life in AIP patients was significantly reduced in regards to physical challenges. An overall trend in the material was that disease activity increased subjective health complaints and decreased quality of life. Results indicate that manifest and active AIP has an important impact on the lives of those afflicted.

EP04.03
Features of hospital adolescent with hereditary disease

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We recently began offering specialized psychological help to patients with hereditary diseases. One of the first patients in this project is D - a twelve years old boy with primary oxsalosis. D is waiting for the kidneys and liver transplantation and has been carried daily hemodialysis for almost year and a half. Also he has a tracheostomy which significantly reduced his ability to talk. He spent in a hospital two years. D has two younger healthy siblings. His parents are both alive and also haven't any genetic disorders.
Metods used:
- observation
- conversation
- Dembo-Rubinshtein's method for self-esteem studying
- Drawning of nonexistent animal (projective method for studying attitudes and emotional states)

Also D's mother was asked to make a test APE (analysis of family education).
D looks below his age. He doesn't eager to communicate either with other people. D's defensive strategy has been withdrawal from situation through games and movies. He refuses to work with psychologist because he "tired of talking".
D's self-esteem is lowered, but level of claims is high. He praised his skills and intellect at low rate and self-confidence at high at the same time.
D has increased anxiety and high level of verbal aggression, which has been effectively suppressed.
His mother is placing increased responsibilities on D, especially in social sphere.
To help D to effectively cope his situation it is necessary to lower demands which are applied to him and help him to express his aggression and grief.

EP5 Psychosocial issues in cancer genetics

EP05.01
Sharing genetic cancer information with relatives: development and validation of an instrument to assess counselees' knowledge, motivation and self perceived competence.

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Introduction
Despite the use of genetic services, counselees do not always share genetic cancer information with their at-risk relatives. Reasons for not informing relatives may be categorized as a lack of: 1) knowledge, 2) motivation and/or 3) self perceived competence. The aim of this cross-sectional study was to develop and assess the psychometric properties of an instrument that measures counselees' knowledge, motivation and self perceived competence.
Methods
Consecutive counselees who visited the department of Genetics with questions regarding the possibility of hereditary breast and/or ovarian cancer or colon cancer (including Lynch and FAP/MAP) were asked to complete a home-sent questionnaire after receiving a summary letter from the department of Genetics. This letter included information from the last counselling session. Knowledge, motivation and self perceived competence were assessed with a study-specific questionnaire. Analyses will address the acceptability of the instrument, its dimensionality (i.e. whether knowledge, motivation and self perceived competence items constitute separate scales), the reliability of separate scales and the instruments validity.
Results
Overall, 214 of 343 questionnaires were included in the analyses (response rate 62%); 108 breast and/ovarian cancer and 106 colon cancer. We will report on the instruments' acceptability and its properties to reliably and validly assess counselee characteristics that might hamper sharing genetic information with relatives. Such instrument will allow the evaluation of interventions aimed at enhancing counselees' ability to be a competent, motivated and confident informant of their at-risk relatives, which in turn may lead to more relatives taking up genetic services.

EP05.02
Impact of genetic counselling in women with a family history of breast cancer

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A semi-structured telephone interview was designed to assess the impact of genetic counselling in a series of asymptomatic women with a family history of breast cancer (BC). To date, 58 women who underwent genetic counselling for BC between 2003 and 2011 have been interviewed, and the data of 42 were available for a preliminary analysis. Age ranged from 24-71 years. The majority of women considered the information received during counselling as clear (quite clear: 31.0%; very clear: 33.3%; extremely clear: 21.4%) and helpful (quite helpful: 33.3%; very helpful: 33.3%; extremely helpful: 7.1%). Twenty-five (59.5%) stated that their perceived risk of BC had changed after the counselling: for 20 (80%) it had decreased, for 3 (12%) increased, while 2 did not specify. Twelve (28.6%) declared to have made useful decision for their health after the counselling: most appropriate breast surveillance (75.0%, n=9), healthier lifestyle (8.3%, n=1) or intensification of surveillance for fear (8.3%, n=1). Nevertheless 27 women (64.3%) stated they had not followed the surveillance recommended by the counsellor. The majority of women (88.0%, n=37) had shared the information received with their family: parents (37.8%, n=14), sisters and brothers (32.4%, n=12), daughters (10.8%, n=4) or other relatives (10.8%, n=4). The family reaction was reported as positive (i.e. listening, support) by 29 of these women (78.4%).
These preliminary data suggest that genetic counselling has a significant impact on awareness, risk perception, and communication within the family, but not on surveillance. The reasons for such a low compliance to surveillance will be investigated.

EP05.03
Counselling and clinical implications of an unclassified variant in MLH1 in a family with a history suggestive of Lynch Syndrome.

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Unclassified variants (UVs or variants of unknown significance) are now a relatively frequent occurrence in genetic tests. Differentiating between a benign polymorphism and a pathogenic mutation can be technically difficult, particularly for missense changes and intronic variants. A number of strategies exist to help this process, but it is not always possible to confirm/exclude pathogenicity because of insufficient data, lack of resources and/or samples from other affected and unaffected individuals within the family. In this case a clinical diagnosis may be tentative and/or predictive testing is unavailable. Genetic professionals face challenges when communicating this information to patients and families. The lack of certainty can cause confusion and/or frustration. We present a case study that highlights some of the counselling implications. A 45 year old woman was referred because of a family history of colon, endometrial and pancreatic cancer. This was suggestive of Lynch syndrome, and the patient was considered to be at 50% risk. She was keen to pursue predictive genetic test to clarify the risk for her and her children. Immunohistochemistry in her cousin's bowel cancer and her mother's endometrial cancer showed loss of MLH1 protein, and molecular testing in the cousin identified a UV in the MLH1 gene. *In-silico* analysis suggested the variant was pathogenic, but no confirmatory tests (e.g. functional studies) were available. Therefore, predictive genetic testing to refine the patient's risk was not possible. She found this frustrating, particularly given the invasive nature of bowel and endometrial screening.

EP05.04
New strategies needed to improve familial colorectal cancer prevention

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Background: Currently, only 12-49% of individuals with an increased familial colorectal cancer (CRC) risk are referred for highly effective cancer prevention. This study was performed to improve referral rates for genetic counselling and surveillance colonoscopies for high-risk and moderate-risk families, respectively.

Methods: Eighteen hospitals participated in a clustered RCT. Nine intervention hospitals received a website and brochures about familial CRC risk for patients and clinicians, and education and guideline pocket cards for clinicians. Patients in nine control hospitals received usual care. Data were collected from patients and clinicians using questionnaires and medical records.
Results: Fifty-five percent of patients (n=478/862) and 34% of clinicians (n=47/137) participated. In the intervention group, 110/161 patients (68%) and 7/20 clinicians (35%) visited the website; 34/161 patients (21%) read the brochure. Patients valued clinicians' information as most useful.
Clinicians rated the education and guideline pocket cards as most useful.
In the intervention group, 1/10 high-risk patients (10%) was referred for genetic counselling, versus 5/34 (15%) in the control group (*p*=.705). In the intervention group, relatives of 6/21 (29%) moderate-risk patients had received surveillance colonoscopies, versus 23/43 (53%) in the control group (*p*=.065).

Conclusions: Implementation of tailored digital and printed information did not improve referral rates for genetic counselling or surveillance colonoscopies of individuals at an increased familial CRC risk. Although patients and clinicians appreciated the materials, patients preferred clinicians' advice regarding their familial risk; clinicians preferred more traditional materials. Therefore, new strategies aimed both at patients and clinicians are needed to improve familial colorectal cancer prevention.

EP05.05

Unclassified variants in *BRCA1 and BRCA2*; Assessment of *in silico* analysis and proposal for communication in genetic counseling

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Background: In nearly 15% of *BRCA1/2* tests an unclassified variant (UV) is identified. In the Netherlands the four-group classification system of Bell is in use. In the current practice, class III UVs are communicated with the counselees and class II are not. Aim was to investigate whether UVs in classes II and III showed significant differences in their *in silico* characteristics and would this classification justify differences in counseling protocols regarding communication.
Methods: 88 missense UVs in *BRCA1/2* were analyzed. *In silico* analysis of UVs was performed using SIFT-analysis, Grantham score and AGVGD for the predicted severity of amino acid substitutions.
Results: 60% (n=53) of the UVs were predicted to be tolerated by SIFT-analysis and scored as neutral (C0) by AGVGD. Of the remaining 35 UVs, sixteen were scored as C0, eight were scored C15-C25 (intermediate) and eleven were scored C35-C65 (likely to be deleterious). Class III UVs more frequently show *in silico* parameter outcomes suspicious for a deleterious effect. The observed differences, however, are not absolute. Four UVs classified in class II had similar *in silico* profiles to five UVs in class III.
Conclusion: This study showed that in general *in silico* analysis is consistently applied and is able to discriminate between different classes of UVs. Aditional analyses will be required to classify UVs with more certainty. To reduce psychological distress in UV families we propose that communication of an UV should not primarily depend on its class, but on the possibility to perform additional research in the family.

EP05.06

Breast cancer patients prefer BRCA-testing without prior face-to-face genetic counseling: Preliminary results

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Background: Currently, all breast cancer (BC) patients receive face-to-face genetic counseling prior to *BRCA1/2* mutation testing (DNA-intake). We are evaluating a different format for such an intake: telephone, written and digital information sent to BC patients' homes, and face-to-face contact following *BRCA*-testing (DNA-direct).
Patients/Methods: From August 1st 2011 to February 1st 2012, 160 BC patients referred for *BRCA*-testing could choose between DNA-intake and DNA-direct. Participants received questionnaires on psychological distress and satisfaction, just after inclusion and following face-to-face *BRCA*-result disclosure.
Results: 59% preferred DNA-direct over DNA-intake (p=0.03). Median age at inclusion was 51±11 [23-74] and age at first BC diagnosis 48±10 [23-74]. Indications for *BRCA*-testing included: positive family history (78%), age at BC diagnosis below 40 (25%) or both breast and ovarian cancer (4%). Comparing baseline characteristics, the DNA-direct group showed a lower age of youngest BC-affected relative (p<0.03), higher use of BC-related websites (p<0.01) and more referring physicians having discussed personal consequences of hereditary BC (p<0.01). At baseline the number of cases with psychological distress did not differ. Preliminary analysis of known (incomplete) DNA-results showed *BRCA*-mutations in 11% of DNA-intake and 9% of DNA-direct (n.s.). All detected *BRCA*-mutation carriers fulfilled current *BRCA*-testing criteria. Days between inclusion and result disclosure was 100±30 [22-140] versus 70±25 [23-202] in DNA-intake and DNA-direct, respectively (p<0.001).
Conclusion: More BC patients preferred the DNA-direct procedure, considering it acceptable. Processing time in DNA-direct was reduced by one month. Whether DNA-direct entails more psychological distress in mutation carriers will be evaluated later in the project.

EP05.07

Occurrence of endometrial cancer at young age in a Lynch syndrome family: implications for genetic counseling.

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Lynch syndrome is a genetic predisposition to colorectal, endometrial and other types of cancers. This case illustrates that, although endometrial cancer is a well-known component of Lynch syndrome, early referral for genetic counseling based on family history of this tumor is still not systematic. We report a family in which Lynch syndrome was indentified through a patient who developed colorectal cancer at 46 years of age and one year later two sebaceous skin tumors. He was referred for genetic counseling after his dermatologist suspected Muir-Torre syndrome. The family history revealed that his sister died of endometrial cancer at 41, his mother died of a gynecological cancer at 61 and a paternal cousin developed uterine cancer at 50. Immunohistochemistry analysis of the colorectal cancer showed extinction of MSH-6 and MSH-2 proteins. Molecular analysis identified a mutation in the *MSH2* gene. By the time the result was available, his 27 year old niece had been diagnosed with endometrial cancer. Genetic counseling was offered to the family including the patient's 16 year old daughter. They did not follow up on this offer. We contacted the patient a year later and learned that his niece had died of metastatic endometrial cancer. He was very concerned about his daughter's cancer risk. The daughter and her parents received genetic counseling and requested genetic testing. Although rare, endometrial cancer at young age may occur in Lynch syndrome. This case raised questions about when uterine surveillance should begin and at what age predictive genetic testing should be offered.

EP05.08

Consent to tissue testing: Other-orientation and responsibility in cancer genetics

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Although consent theory fundamentally focuses on the 'autonomous' choices of patients involved in clinical testing, in recent years there have been more novel conceptualisations. Within medical genetics there has been increasing recognition of the uncertainties and shifting responsibilities involved in predictive genetic testing.
In this study we take consent to tumour testing for evidence of mis-match repair defects, indicative of Lynch Syndrome, as an example of the interplay between individual autonomy, population health screening, familial involvement and beneficence. This work is part of a broader PhD study exploring the complexities of consent in the context of novel genetic testing technology. We use data (transcripts of audio-recordings) from 11 semi-structured interviews with clinicians and 13 observations of clinic sessions in the UK and Australia where consent is sought. Adopting discourse analysis, we explore the rhetoric of consent as manifest in these interviews and encounters. We relate our findings to the concept of responsibility (in relation to self, relatives and unrelated others).
We demonstrate how testing is framed as beneficial, altruistic and of minimal burden. Using observational data we demonstrate how clinicians reassure and minimise the risk due to the preliminary nature of the testing,

which enables those attending clinic (or their relatives) to be available to consent. The familial dimensions of testing and consent, in terms of communication and burden, are played down in professional interviews and yet are foregrounded in observations. This analysis provides an alternative frame to explore the dynamics of the consent process within cancer genetics.

EP05.09

A qualitative study of provider and patient experiences with decisions about risk-reducing surgery in women at increased familial risk of ovarian cancer

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Women at increased familial risk of ovarian/breast cancer are offered risk-reducing bilateral salpingo-oophorectomy (RRBSO) to reduce their risk of ovarian cancer. As there is no evidence for effectiveness of ovarian screening, surgery is the only active management option available to these women. However, this decision is complex and depends on patient values and preferences.

We explored the views of healthcare professionals through semi-structured interviews and the views of patients through focus groups to obtain a detailed picture of the process of decision-making about RRBSO.

Eleven interviews with professionals, including genetic counsellors and gynae-oncologists, were conducted. They felt that women's questions mainly related to surgical menopause and hormone replacement therapy (HRT), although a number of other factors, such as body-image and risks of surgery, were also discussed.

Five focus groups with women at increased risk of ovarian/breast cancer were held. In agreement with professionals, women were especially concerned about surgical menopause and HRT. Additionally they felt it was important for them to understand their personal risk and the effects of surgery on that risk. Many women felt that elective surgery was an extreme step and said they needed a catalyst, such as a confirmed genetic mutation, to sway them towards surgery.

Both professionals and women felt that standardised, evidence-based information to facilitate deliberation about RRBSO was currently not available and that this would be helpful to support women's decision making. The results of this study will be used to develop decision support for women considering RRBSO.

EP05.10

Factors which influence participants to follow up genetic test results as a result of taking part in a population based ovarian cancer research study?

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Background: The Australian Ovarian Cancer Study (AOCS), a population based study, recruited women with invasive ovarian cancer between 2002 and 2006. BRCA1 or BRCA2 mutation testing has been undertaken. Women in whom a mutation has been identified, or their next of kin in the case where the women is deceased, have been told of a mutation in writing and the availability of obtaining mutation results through a family cancer clinic. The AOCS Psychosocial project has interviewed individuals who received these letters.

Aims of AOCS Psychosocial study:

1. explore individuals' understanding of information contained in the letter they received from the researchers
2. determine factors that inform individuals' decisions about whether or not to contact a Familial Cancer clinic and take up genetic testing information

Results: a total of 25 in depth interviews have been undertaken. Factors influencing follow up acted as both barriers and enablers.

Personal
Emotions, experience and readiness
Social
Familial obligations and genetic responsibility
External
Life demands and participation in psychosocial research study

Although AOCS participants were not primarily recruited to a familial cancer study as such, uptake rates were similar to previously reported familial cancer genetics studies. The results from AOCS provide a new insight into some of the barriers which prevent research participants, and their next of kin, accessing clinical genetic services and assist in understanding why there are low numbers of individuals who make contact with a clinical genetics service following participation in a genetic research project.

EP05.11

New Model of Support and Information for Women and Men with a BRCA 1 / 2 gene fault

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Introduction
Individuals with BRCA 1 & 2 gene faults are a unique group. They may not have had cancer and are referred to by one support organization as "previvors" (FORCE).
In 2001, The Association of Genetic Support of Australasia (AGSA) was approached to provide support for BRCA 1 / 2 gene fault carriers describing anxiety around cancer risk, decision making related to risk management and grief due to lack of support.
Aims
Emotional support, accurate information / management options and analysis of participant evaluations ensuring applicability of format.
Method
From 2001 - 2004 invitations were sent to past attendees and individuals selected by Cancer Genetic Counsellors. In 2005, invitations were mailed to all families within NSW with a known BRCA 1 / 2 gene fault.
The program is devised by AGSA and a committee of consumer advocates and genetic counselors. Presentations are determined by evaluations.
Results
In 2001, 15 attended, in 2009, 102 attended; of these 70% were first time visitors. Evaluations reflect larger numbers did not affect access to information and support. Participants attend after diagnosis, to assist with decision making and others come each year. Men and women attend and discussion groups for men have focused on needs of men as partners, fathers, brothers and carriers.
This event is a non clinical approach providing emotional support. Presentations by cancer specialists ensure validity of information. Evaluations completed by 73 % of attendees show a positive response to attending. AGSA seeks funding to run the program nationally.

EP05.12

Teachable moments and missed opportunities in cancer genetic counseling.

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The label 'teachable moment' (TM) has been used to describe naturally occurring life transitions or health events thought to motivate individuals to spontaneously adopt risk-reducing health behaviors. Three key constructs underlie whether a cueing event is significant enough to be a TM: the extent to which the event (1) increases perceptions of personal risk and outcome expectancies, (2) prompts strong affective or emotional responses, and (3) redefines self-concept or social role. Teachable moments have been used in preventive care to promote changes in life styles (i.e. smoking cessation) and to improve cancer screening adherence. In cancer genetic counseling, teachable moments could be a form of opportunistic counseling that could take advantages of health concerns and events in patients' lives to increase willingness and commitment to change behavior and to improve adherence to cancer screening and prophylactic measures. To do this effectively, genetic counselors need to recognize and explore the salience of patient concerns and identify opportunities to link them with unhealthy behaviors. The purpose of this study is to review and discuss the concept of TM and to explore the discourse between genetic counselors and patients identifying potential teachable moments for health behavior changes during the cancer genetic counseling process.

EP6 Education and professional development

EP06.01
The Manchester MSc Genetic Counselling Programme: 20 years experience

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We present 20 years experience of running the first master's genetic counselling programme in Europe. The course provides a vocational and academic training for genetic counsellors, and the career and professional outcomes provide evidence of its success. 115 students have graduated from 17 intakes. After graduating, 103 (90%) went on to practice as genetic counsellors (mostly in the UK but also elsewhere in Europe and internationally), and when and where eligible, have become registered or certified genetic counsellors. Graduates have also made a significant contribution to the academic field of genetic counselling through research and education.

The programme's curriculum and experience has helped to inform the establishment of additional masters and other training programmes for genetic counsellors in Europe, as well as the professional competencies required for UK genetic counsellor registration.

Since its inception the programme has prioritised 3 key elements, academic modules, clinical placements and research; however the content has evolved to ensure students are prepared for current and future practice given changes in clinical genetics including referral patterns and professional roles. This reflects a greater emphasis on autonomous practice, and a shift from primarily paediatric and reproductive genetics to encompass adult onset conditions including cancer, neuropsychiatric and cardiac genetics. A recent practical change has been to move from annual to biennial intake of a larger group which has been positive for both staff and students. Advantages have included efficiencies of scale for the teaching team, and for students realistic job prospects within current NHS constraints.

EP06.02
Global engagement with an online genetics education resource: using Google Analytics to evaluate visitor activity and behaviour in countries developing genetics-genomics within nursing practice

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The rapid increase in gene-disease discoveries offers real promise of clinical applications for people and families affected by genetic conditions but for which health professionals are unprepared because of lack of training. The availability of clinically relevant education resources is critical to enabling nurses and other health professionals to develop the appropriate genetics-genomics knowledge and skills to provide optimum care for individuals and families. Online education resources play an essential role in this but such resources can be personnel-intensive and developed over an extended period. Optimising such resources, particularly in economically challenging times, is essential.

Telling Stories, understanding real life genetics (www.tellingstories.nhs.uk) is a web-based education resource using real life stories to promote understanding of the impact of genetics-genomics in healthcare. Google Analytics provides time series data for analysing web usage to optimise website effectiveness. We present data of visitor activity and behaviour from 123 countries from 2009-2011 and consider how the application of the web analytics:

- informs approaches to enhancing visibility of the website;
- provides an indicator of engagement with genetics-genomics both nationally and globally;
- informs future expansion of the site as a global resource.

Telling Stories is an accessible, broad-reaching resource that is of global relevance for health professionals, attracting over 33,500 visitors between 2009-2011, with a steady increase in numbers of returning visitors. The majority of visitors come from the UK, USA, Netherlands, Canada and Australia. More needs to be done now to enhance its accessibility for people of other languages and cultures.

EP06.03
Flexible online learning in genetics: meeting the needs of the medical specialty trainee

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Widespread inclusion of genetics into medical specialty training in the UK is placing demands on genetics specialists to provide education. A review of the Royal College of Physicians' (RCP) specialty curricula showed over 80% of the 27 medical specialties (excluding Clinical Genetics) include genetics training. Training directors for these specialties have expressed concern about how to provide equitable and appropriate training in genetics to their trainees. One approach is to provide e-learning opportunities. The National Genetics Education and Development Centre has collaborated with the cardiology curriculum committee of the RCP to develop a genetics e-learning package for cardiology trainees. This online package covers the genetics learning outcomes within the curriculum using modules based on clinical encounters that cover a range of conditions and contextualise the genetic concepts. Modules can be accessed at any stage during training, allowing trainees to individualise their learning by completing them during relevant clinical rotations. The core genetic concepts are presented separately, allowing learners to access information as needed. The electronic format provides a record of progress and completion for inclusion into the electronic training portfolios. These materials are currently being piloted to inform a wider evaluation strategy which will assess the acceptability of this mode of delivery and the perceived ability of UK trainees to apply the knowledge learnt to their clinical practice. E-learning provides a viable option to deliver genetics education to medical specialty trainees that complements face-to-face teaching and clinic observation while offering flexibility in meeting the individual needs of specific training programmes.

EP06.04
A collaborative evaluation of a dual model of consultative supervision combining individual and team supervision in genetic counselling practice

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We outline key components of the design, delivery and evaluation of a model of consultative clinical supervision for a team of genetic counsellors at Liverpool Women's NHS Foundation Trust, England, incorporating the criteria and recommendations of the Association of Genetic Nurses and Counsellors (AGNC). This integrative, multitheoretical, dual model, combining individual and team based supervision provided by consultative supervisors, contributed to local awards for excellence in patient care, team working and partnerships. The project was undertaken as a collaborative endeavour between the team and the team supervisor, the Head of Psychosocial Services at Alder Hey Children's NHS Foundation Trust, Liverpool. Normative, formative and summative evaluation was designed-in at the start of the programme by the supervisor. The Lead and Deputy Lead Genetic Counsellors, in their capacity as supervisees and senior practitioners with leadership and management responsibilities for the team, contributed principles from real-world, action-research and summative evaluation. Whilst this is specific to a particular team of UK NHS professionals, as a flexible and pragmatic model of reflective practice designed to support and develop healthcare professionals, the principles, framework and structural components which informed the model, and the data it has produced, are likely to be of wider interest in the genetic counselling community and beyond. There is particular significance for the wellbeing and continuing professional development of staff and the enhancement of the of quality patient care.

EP06.05
Challenges for cancer genetic counselling: findings from Portuguese oncogenetic services

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Cancer risk counselling has grown rapidly in recent years to become a major area of specialisation within genetic counselling. In some countries, oncogenetic services are still not provided by specifically trained professionals. Psychosocial support is only partially made available for individuals and families throughout genetic counselling protocols. We examined the current practice of oncogenetic counselling through the professionals' views and ascertained the needs for the provision of psychosocial support in cancer genetics services. A qualitative study was designed; semi-structured focus groups and individual interviews were performed involving 30 professionals from Portuguese healthcare institutions where oncogenetic counselling is offered (geneticists, gynaecologists, oncologists, nurses, psychologists and genetic counsellor trainees). Current practice, unmet service needs and issues for improving practice were the major themes identified in participants' perceptions. Findings suggest: professionals' practice is aligned with the teaching model; the genetic counselling agenda is predominantly informative-based with a nondirective focus; a scarce workforce of adequately trained psychosocial professionals, aggravated by other structural and organizational constraints are serious drawbacks to consistent psychosocial delivery; multidisciplinary teams working in genetics were stated as priority, along with genetics education for healthcare professionals in primary care. Cancer genetics healthcare needs an adequate training and organization towards collaborative standards of care and functional forms of access for patients. Portuguese genetic counsellors have recently completed their training and may therefore ease some of the needs. This study may contribute for envisioning paths for the integration of a psychosocial-oriented stance in oncogenetic services.

EP06.06

Knowledge and attitudes of Italian nurses toward Genetics

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In order to explore the understanding and attitudes of Italian nurses and midwives toward genetic healthcare, a cross-sectional survey using a self-administered questionnaire was carried out in Bologna, Italy, in 2010. In 2011 the survey was extended to a larger population of Italian nurses by using a questionnaire uploaded to the Survey MonkeyTM website. Specialist genetic nurses currently work in many countries; however there are very few genetic nurses in Italy and the knowledge of Italian nurses to provide care for people with or at risk for genetic conditions in unclear.

Out of 102 (85%) nurses and midwives responding to first study, 61% believed genetic counselling was only an informative and advisory process, while 53.9% did not specify; 62% (n=63) did not identify nurses' role in genetic healthcare, but 28% (n=26) believed nurses could provide information and support.

The second study was completed by 385 nurses, the majority (40.4%, n=131) correctly answered four of five questions on knowledge of genetics. Knowledge scores did not change by age, but was positively correlated with academic qualification. Only a minority (26.8%, n=103) of respondents believed genetics was very relevant to the nursing role.

The findings of these studies indicate that although nurses have the basic knowledge of genetics, they need more education not only on genetics topics, but also on the transferability of those into nursing care.

EP06.07

The Home Coming of Genetic Counsellors: The Cyprus Experience

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The impact of culture in genetic counselling and cultural competence of genetic counsellors are important topics masters level training programs and academic research. They are also crucial in clinical settings. Genetic counsellors are being taught to become self-aware of other ethnic/cultural groups, to acknowledge similarities and differences between their own and other cultures and to understand how such similarities/differences impact socially and/or institutionally the care of diverse patients.

Although, masters-level programs are steadily emerging in several countries, most genetic counsellors are still or have been trained in countries other than their own home-country, such as United States (US) and United Kingdom (UK), where these programs are well-established. These counsellors adopt a professional-culture that is shaped by the healthcare and social culture of their host-country. It has been noted that not enough attention has been given to how such genetic counsellors "readapt" to their own culture, both socially and professionally, after returning to their home country.

Our Clinical Genetics Clinic (CGC) gives emphasis to cultural competence, especially as our patient load culturally so diverse. Among the team are two masters-level genetic counsellors; a Greek-Cypriot and a Turkish-Cypriot genetic counsellor who have been trained in the US and UK. Both genetic counsellors have similar and different experiences about training overseas and returning to their own country. The poster addresses on these experiences and challenges faced in work-place and small community due to the clashes of their cultural identity specific to their own country/community and professional culture they acquired through their overseas training.

EP06.08

E-learning in Genetics - Multimedia Educational Training Program

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Telemedicine is born with the Internet.Tele-education is one of its application. An on-line learning infrastructure is considered essential for the delivery of educational programs in medical genetics.The use of a performing animation system allows the geneticists to explain the essence of the fundamental genetic phenomena. The flash technology was used to access animation on the Internet, as it already represents a standard in creating animation that has Internet impact. The navigation system of the FLASH animation allows each scene to be accessed separately using representative images of a scene as buttons. Respecting the scientific truth by taking into account the limits imposed by the technical possibilities may represent a true challenge for the people involved. For didactic purposes, the phenomena (mitosis, chromosome and chromatin structure, DNA replication and DNA repair) were divided into several stages. The number of the scenes is directly proportional with the complexity of the phenomenon. As learning in the traditional manner involves transmitting the information under the form of a text simultaneous with the scrolling image, a short explanation of the scene is inserted. The text is available in English and Romanian. The use of the multimedia (graphics and 3D animation) programes enhances teaching process of any fundamental genetic phenomenon and contributes to the computer supported training in the field of medical genetics.

EP7 Access to genetic services

EP07.01

Systematic review of research related to barriers to access to genetic services

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The genetic components of disease are being identified through rapid progress in genomic research and new scientific knowledge is quickly being translated into clinical practice. Demands on genetic services are increasing in parallel to these advances. There is a global challenge for healthcaresystems in different countries to efficiently integrate genetic services into their healthcare infrastructure, to utilise scientific advances and, most importantly, provide equitable access for citizens while minimising healthcare costs. To achieve this, it is important to identify which factors can prevent or influence patients' access to genetic services. As part of a doctoral study on the provision of genetic services for the Turkish Cypriot community in Cyprus, a systematic review of empirical evidence on barriers to accessing genetic services was conducted. Five electronic databases were searched for studies published in English in peer-reviewed journals between 2000 and 2010; 27 articles were included in the review. The majority of studies were undertaken in the United States (n=17), focused on cancer genetic services/counselling (n=17) and used quantitative methodology (n=19). Identified barriers were related to individuals (n=17), to institutional/healthcare professionals/systems (n=14) and to the community (n=5). There is diversity in how access to genetic services is researched and the majority of barriers we identified derived from studies that were not directly investigating barriers to access. More specific research on this topic is urgently needed to inform development of "targeted interventions" to enable equitable access to genetic services for individuals in a range of populations.

EP07.02

Patient Expectations and Attitudes towards a Specialist Genetic Eye Service

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BACKGROUND: Little research has explored the views of patients referred to specialist genetic eye clinics. Future service development must be informed by the perspectives of patients to ensure that services are accessible and meet their needs.

METHOD: Semi-structured telephone interviews were undertaken with patients referred to the Genetic Eye Clinic in Manchester, UK. Participants were interviewed before their first appointment. The interview transcripts were analysed using Interpretative Phenomenological Analysis (IPA).

RESULTS: Forty six people were invited to participate and 9 agreed to be

interviewed (response rate 20%): 5 participants were patients with a visual impairment; 4 were the parent/carer of a child patient. The major themes identified were: lack of preparation and restricted expectations due to unfamiliarity with the service; psychological adjustment to the diagnosis of an eye condition impacting on needs and expectations; concern about the future; hope for future treatments; and, positive attitudes towards genetics.
CONCLUSIONS: The participants had consistently positive attitudes towards genetic eye services, genetic testing and genetic research. However lack of preparation and knowledge of available services, particularly genetic counselling, meant families may not get the most out of their appointment. A leaflet, available in a range of formats, has been devised to improve patient experience. The results from this study improves our understanding of the counselling needs, expectations and attitudes towards genetics, including hopes for treatment, in families with inherited eye conditions.

EP07.03

The role of the Cardiac Genetics Nurse in Inherited Cardiac Conditions Services: impact on the quality of the patient and family experience

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In 2008 the British Heart Foundation launched an initiative to fund nine Cardiac Genetic Nurse (CGN) posts across England (n=8) and Wales (n=1) for three years, in order to improve the quality of service provision to patients and families affected by inherited cardiac conditions. A single case study approach was taken to evaluate the initiative, collecting data from multiple sources. Themed semi-structured interviews were held with all CGNs, Cardiology and Genetics Clinical Leads from each of the nine sites, Genetic Counsellors and a patient representative, at key points during the project. A patient focus group was also held. Detailed thematic analysis of the qualitative data was conducted using NVivo 8.0. Three broad categories emerge from the data pertaining to the quality of patient/family experience:
1. 'Continuity' relates to the CGN role in providing support and continuity along the patient journey. Time, Giving clarity, and Managing anxiety and uncertainty, are important sub-themes.
2. 'Coordinating care for the patient and family' highlights the CGN role in providing patient and family centred care, with sub-themes of Preparing; Having the bigger picture; and Knowing the patient and family.
3. 'Coordinating care across the pathway' concerns the CGN role in coordinating the multidisciplinary team across the two specialties and linking with primary and secondary care and others.
The CGNs enhance the quality of the patient/family experience through an holistic approach to care, offering clarity and continuity along a potentially complex pathway, often at a time when patients and families will be feeling particularly vulnerable.

EP8 Ethical issues

EP08.01

Parental views on confidentiality when adolescents are seen alone in the metabolic clinic

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Clinical guidelines for health practitioners suggest that adolescents should be seen on their own for part of the clinic consultation. However, little is known of parental attitudes on this issue. We explored the views of parents of adolescents in our Metabolic Clinic, in which this is standard practice.
Of 40 eligible parents, 33 completed a questionnaire handed to them in clinic, seeking parental understanding of confidentiality, their views about when information should be shared with them and their perception of benefits/concerns of consulting with young people alone.
The main advantages parents identified in adolescents seeing clinicians alone were: practicing talking to the doctor alone; taking responsibility for their own health; helping them become more mature.
Concerns identified included the possibility of not being informed about important issues (including eating disorders, cigarette smoking, alcohol and illicit drug use, pregnancy, and problems with parents); not being informed of the treatment plan, and concerns about their child possibly not understanding the issues or not remembering the treatment plan. Parental understanding of a confidential consultation with young people alone was remarkably similar to that in a general adolescent clinic (N=86), but they were more likely to see the advantages of confidential discussions in promoting self management skills. However, they seemed more protective of their children, as evidenced by their expectation that medical consultations where their children are seen alone, should begin at a later age.

EP08.02

When to tell? - Disclosing Genetic Information

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Little research exists on when and how parents should go about disclosing genetic information to their affected children and siblings, as well as its impact. This presentation will detail quantitative and qualitative research amalgamated from reports sourced directly from the Association of Genetic Support of Australasia (AGSA) - a peak umbrella organisation established in 1988 for rare genetic conditions, and its extensive Rare Disease Database representing over 950 genetic conditions in 2,200 families.
This presentation will outline the dilemmas faced by parents upon receiving a genetic diagnosis for their child. Negative effects of withholding information from all family members will also be discussed. Recommendations for when to disclose genetic information, as well as strategies employed for information sharing will be discussed. Finally, implications for the health professional are diagnosis delivery, and the importance of securing access to an appropriate support group will be explored.

EP08.03

Sharing data from whole genome studies: empirical study of ethical implications

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Elements of a person's past, present and future medical health can now be revealed in a matter weeks via whole exome sequencing of a saliva/ blood sample. Such technology is frequently used in research to understand the genomic basis of disease and will very soon be used within clinical health services.
It has been considered good practice for many years to conduct genomic research anonymously and not share any individual results with research participants. However, there is mounting pressure to change this approach and begin to share individual results with research participants. There is, however, no clear guidance or evidence to suggest what sort of data research participants want - should it only relate to clinically actionable conditions or would participants be interested in receiving results with broader implications or even their raw sequence data? There is an urgent need for large-scale empirical research to gather evidence on what is reasonable, responsible and indeed ethical to share.
As a contribution to this process we have designed a mixed-methods study and have launched an innovative questionnaire (see www.genomethics.org) that uses film to explore the ethical implications of whole genome research. The questionnaire is accessed online. We are inviting genetic counsellors, genomic researchers, health professionals and lay members of the public worldwide to complete the questionnaire and are aiming for 20,000+ responses. This poster will introduce the study design, explain why the research into genome ethics is important and discuss the relevance of this to both the research and clinical genetics community.

EP08.04

A New Initiative for Genomics and Society Research

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Genomic science is advancing rapidly and is increasingly international, proactive and coordinated. In contrast, research into the ethical, legal and social implications (ELSI) of genomic science, a lynchpin of appropriate policy development, so far remains largely regional, reactive and fragmented. Given the achievements in terms of scholarship, policy information and public engagement that have emerged from ELSI's conventional research methodology so far, important progress can legitimately be expected if ELSI research adopts strategic, large-scale and collaborative approaches analogous to those that have characterised genomic science. This presentation will present an international initiative, which is developing an infrastructure and a research culture aimed at making large-scale ELSI research more efficient, effective and economical. The aims of this initiative are to:-

- facilitate new collaborations;
- increase output;
- provide tools to help coordinate endeavours;
- reflect the international character of genomic science appropriately; and
- avoid unnecessary research redundancy.

This will facilitate networking, critical reflection and the development of proactive strategies for international ELSI research in genomics and derived fundamental or translational sciences. In doing so, it will not only provide maps of the international ELSI landscape to inform and coordinate future research, but will also foster a new way of thinking and doing international ELSI research and fuel the rapid advance of ELSI research in the short, medium and long term.

EP08.05

Who's to blame? A reflection work about identification of genetic potential in high-performance athletes

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The sport in general is an activity that is gaining more importance and more people around the world. In order, growing, developing high-performance athletes, many companies invest in the preparation of these athletes for international competitions like the Olympics Games. In this sense, innovative studies both in the field of sports as in studies of health, seek to identify and recognize these genetic patterns in athletes in the various methods for indicating potential physical abilities required for different sports or genetic predisposition to diseases that may likely to affect its performance. Although innovative, and hold large financial interest, the ethical issues involved in this process should be discussed. A key role has to be devoted for assessing quality of laboratories and delivering information regarding such tests. Many other aspects have been discussed in the preparatory debates to the revision, such as consent issues or biobanks and are absent from the proposal. However these issues are among the most problematic in research and practice. Thus these work alerts discuss the consequences of this lack of regulation. Unless the delineation of responsibilities is clarified result may no longer be acceptable. How to delimit this seemingly, open-ended avenue of potential professional responsibility and possibly, liability? What are the mechanisms available? Moreover these without these assignments the results can cause irreversible damage to the candidates analyzed.

EP08.06

"A morass of considerations": Exploring attitudes towards primary care ethnicity-based haemoglobinopathy screening.

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Background: Although advised by the WHO, the Netherlands does not have a national haemoglobinopathy carrier screening program. HbP carrier testing for those at risk is at best offered on the basis of anaemia without facilitating reproductive choice. Registration of ethnicity has been shown to be controversial and may complicate the introduction of a screening programme. However, other factors may also play a role.

Aim: To explore perceived barriers and attitudes amongst general practitioners (GPs) and midwives regarding ethnicity-based haemoglobinopathy carrier screening.

Method: Six focus groups with a total of 37 GPs and midwives were conducted, transcribed and content analysed using Atlas-ti.

Results: Both GPs and midwives struggled with correctly identifying ethnicities at risk leading to several complex considerations. Ethical concerns regarding privacy seem to originate from World War II memories when ethnic and religious registration facilitated deportation of Jewish citizens, coupled with the current political climate. Some midwives thought the ethnicity question might undermine the relationship with their clients. Despite this, both groups seemed positive and are familiar with identifying ethnicity and use this in individual patient care. Software programs prevent GPs from registering ethnicity of patients at risk. Financial implications for patients were also a concern.

Conclusion: Although health professionals are generally positive, ethical, financial and practical issues surrounding ethnicity-based HbP carrier screening need to be clarified before introducing such a programme. Primary care professionals can be targeted through professional organisations but need national policy support.

α-globin variant: P05.28
α-L-iduronidase: J13.06
β –Thalassemia: P14.23, J05.06
β-myosin: P02.126
β-thalassemia: J05.07
γ-radiation exposure: J03.19
µ-opioid receptor: J02.01

1000 Genomes: P08.24
10q25 deletion: P02.002
10q25 microdeletion: P02.011
11p13 deletion: P03.083
11p15 region: P02.041
13q: P02.099
13q12.3: P02.003
13q14 deletion: P07.08
15q13.3 microdeletion: J03.18
15q25.2 microdeletion: P02.004
16 q24.3 microdeletion syndrome: P02.142
16p duplication: J03.16
16p11.2-p12.2 deletion: P03.002
16p11.2-p12.2 duplication: P03.002
16p11.2v: P05.21
17q21.31 microdeletion region: P02.158
17q21.31 microdeletion: P03.004
17q21.31: C01.1
17q22: J02.14
18q: P06.068
19p13.3 deletion: P02.248
1p36: P03.043
1q24q25: P03.062
1q43-q44: P03.018
2/0 carrier: P12.265
20p13 deletion: P02.005
20q11: P02.170
20q13.33 region: P03.020
21 hydroxylase deficiency: P12.037
21q deletion: P03.005
22 tRNA: P13.29
22q11 deletion syndrome: J05.08
22q11: P03.095
22q11.2 Deletion Syndrome: P02.007
22q11.2 deletion: P02.006
2nd generation sequencing: P11.001
2p14-p15: P02.008
2p21 deletion syndrome: P03.006
2p24 region: J02.52
2q24.2-q24.3 deletion: P03.007
-308 polymorphism: J09.20
3D animation: EP06.08
3D-FISH: C04.3, P03.036
3p deletion/duplication: P02.009
3p26.3: P03.018
3q29 deletion: P02.010
3'UTR: P11.008
400 K array CGH: P11.003
46, XY DSD: J04.16, P02.011
46,XY ovotesticular DSD: P04.02
47,XYY: P10.35
4p deletion: P03.140
4q: P03.070
5-fluorouracil: P06.001
5-HT3: P09.069
5-HTT: J11.05
5-hydroxymethylcytosine: P06.002
5p deletion: P03.008
5q13.2 duplication: P03.009
5q21 deletion: P02.238
5q31: P02.012
6q14.1 duplication: P03.010
6q22: P09.036
7p22.1 microduplication: P02.013
8p23.1 duplication: P03.140
8q11.1-q11.23: P02.014
8q21.11: J02.55
8q24: C14.4
9p deletion syndrome: P02.058

A
a surrogate covariance matrix: P02.017
a: P05.66
A90G polymorphism: P05.63
AA amyloidosis: P15.01
Aagenaes syndrome: J02.51
Aarskog-Scott: P02.017
ABCA1: P12.229
ABCA4: J12.16, P12.001, P12.235
ABCB1 gene: J14.01, P02.094
ABCB4: P12.002
ABCC8: P13.19
ABCG2: P09.071
abdominal aortic aneurysm: P08.01, P09.001, P09.002
abdominal defects: P02.018
aberrant splicing: C10.2
ABHD12: P12.252
abnormal movement: P02.007
abortion: P05.06, P05.17
access to genetics services: EP06.05
accessing services: P01.01
accreditation: P15.02, P15.10
ACE gene: J09.13, P09.003
ACE I/D: P08.25
ACE: P10.01
aCGH: P02.097, P03.011, P03.012, P03.013, P03.048, P03.128, P05.33, P11.081, P12.056
A-cgh: P05.29
achalasia: P09.004
Acrodysostosis: C09.2, P12.003
Acroosteolysis: P12.004
Acute GVHD: P08.10
acute intermittent porphyria: EP04.02
Acute Leukemia: J06.01, P06.003, P06.089
acute liver failure: J13.04
acute lymphoblastic leukemia: P06.004, P06.005, P07.01
acute myeloid leukemia (AML): P07.33
acute myeloid leukemia: C02.3, P06.006, P06.120, P07.02
Acute myocardial infarction: P08.36
Acute Promyelocytic Leukemia: J06.03
ACVR1: J02.16
ADAM33: P09.041
ADAMTSL4: P02.086
add chromosome: P03.126
adiponectin: J09.15, P10.53
adipophilin: P09.005
adMD: P12.022
Adnroctonus crasicauda,: J03.01
adolescent: EP04.03, J09.01
ADPKD: P12.005
ADRB2: J09.07
adult myeloid leukemia: P06.007
advanced maternal age: P04.29
AFF2 gene: P12.097
Affymetrix SNP arrays: P11.078
AFQ056/Mavoglurant: P14.06
African patients: P12.076
age and gender: J03.10
age of pregnant women: P05.02
Age related macular degeneration: J09.43, P09.007
age: J04.01
Age-at-onset: P10.20, P10.21, P10.23
ageing: P09.035, P09.130
agenesis of corpus callosum: P02.172, P03.046
age-related hearing loss: P08.31
Age-related macular degeneration: C14.2, P09.008
Aging: C04.4, P06.072, P09.057
AGL gene: P13.13
Agnathia-Otocephaly: P12.006
AHR pathway: P06.016
Aicardi-Goutières syndrome: P02.019
AIMP1/P43: P12.122
AKT1: P02.210
Alas2: P12.084
Albinism: J02.38
Albright's Hereditary Osteodystrophy: J02.49, P02.212
albumin: P06.106
ALC1/CHD1L: P06.136
Alcohol dependence: P09.009
alcoholism: J09.01, P08.02, P13.39
Aldurozyme: J14.04
alfa-synuclein (SNCA): J09.27
alignment: P11.095
alkaline phosphatase: P13.20
All activities with a Presentation Number are included.
ALL: P06.008
allele frequencies: P10.42
allele specific expression: C08.2
Allele-bases N test: P08.27
allelic effects: P09.064
allelic variants: P08.16
allergic rhinitis: J08.01
Alopecia areata: P09.010
alopecia: P02.106
ALOX12B: J12.18
Alpers syndrome: J02.02
Alpha Galactosidase A: J09.02
Alpha Globin: P10.02
Alpha thalassemia: P02.119, P10.02
Alpha-mannosidosis: J13.01
ALPL gene: P13.20
Alport syndrome: P12.007, P12.008, P12.009
Alternative splicing: P06.216, P11.018
Alu repeats: P12.123
Alu: P12.236
ALX4: P02.020
Alzheimer's Disease: C11.1, J12.29, P08.03, P09.011, P03.014, P09.012, P09.013
AMD: C14.2
amelogenesis imperfecta: P02.021
Amerindians: P10.03
AMH gene: P12.197
Amino acids: C17.1
aminoglycoside-induced hearing impairment: PL2.4
AML: PL2.1
amniocentesis: J05.01, P05.02, P05.19
Amplicon sequencing: P06.212
amplicon: P02.217
Amplicon-Based Ultra-Deep Next-Generation Sequenci: P06.190
amyloidosis: J02.44
Amyotrophic Lateral Sclerosis: P10.04, P12.011, P12.012
Anaplastic Astrocytomas: P06.108
ancestral centromere: P10.05
ancestral haplotype 8.1: P06.195
Ancestry: P10.03, P10.06
androgen insensitivity syndrome: P02.022
androgen receptor gene (AR): P09.129
androgen receptor gene polymorphisms: P06.034
androgen receptor: P09.058
androgenetic alopecia: P09.078
androgenetic biparental mosaicism: P05.70
aneuploidies: P05.30
aneuploidy screening: P04.03
aneuploidy: J05.04, J10.14, P03.022, P05.03, P05.04, P05.53
aneuploydia: J03.02
Aneurysms-Osteoarthritis Syndrome: P02.023, P12.226
Angelman syndrome: P03.137
Angelman: P02.024, P02.025
angiotensin 2 type II receptor gene: P04.39
angiotensin-converting enzyme: J13.10, P12.209
Angiotensinogen: P08.04
aniridia: P12.013
ANKRD11: P02.142
annexin A5: P04.41
anomalies: P12.144
Anorectal malformation (ARM): P02.026
anti VEGF treatment: P09.091
Anticancer: J06.11
anticipation: P11.005
Anticoagulant therapy: P14.22
anti-leukotriene therapy: P09.017
Anti-mutagenicity: J06.02, P06.009
Antineoplastic drugs: P03.015
anti-neuronal antibodies: P02.103
antioxidants: P09.130, P14.08
antisense oligonucleotides therapy: P12.147
antisocial behavior: P01.15
ANXA5: P04.04, P04.41
AOA2: J12.01
Aortic aneurysm: C01.3, P14.14
Aortic Diseases: P12.086
aortic valve stenosis: J01.05
AP4 syndrome: C15.2
APC gene: P06.010, P06.011, P06.096, P06.162
APC: C10.2
Apigenin: P06.009
aplastic anemia: P03.129
APO E: P08.42, P09.013
APOA1: P09.108
APOE–ε4 allele: J09.41
apolipoprotein B100: J14.02
apolipoprotein E: J09.03, J09.41
apoptosis: P06.104
apparently balanced rearrangements: P03.016
aprosencephaly: J02.36
arab: P12.205
ARBs: P03.025
ARCI: P02.027
AR-CMT: J12.21
ARCMT: P10.07
arginine vasopressin: P09.014
ARID1B: P12.051
Armenians: J10.01
ARMS PCR: J05.06
ARMS2: P09.110
Arm-to-leg transformation: C03.1
ARNTL: P09.084
aromatase: P04.11
ARPKD: P12.015
array analysis: P03.078
array CGH: J11.12, P02.002, P02.028, P02.029, P02.030, P02.034, P02.047, P02.053, P02.078, P02.203, P02.221, P02.255, P03.009, P03.017, P03.044, P03.046, P03.062, P03.074, P03.082, P03.083, P03.097, P03.106, P03.111, P03.112, P03.115, P05.15, P06.169
array comparative genomic hybridization: P02.258, P05.05, P05.12, P05.67, P09.015
Array painting: P10.08
Array: P02.149, P02.155, P03.023
array-CGH: P02.014, P02.161, P02.230, P02.261, P03.016, P03.019, P03.020, P03.065, P03.084, P04.02, P04.24,

P04.37, P05.07, P05.48, P07.06, P07.27, P09.015, P09.026, P11.059, P11.061, P11.070, P13.01, P02.079, P02.228, P02.247, P03.018, P03.051, P05.06, S06.1, S06.2
Arrhythmia: P12.016
arrhythmogenic right ventricular dysplasia: P12.017
Arsenic trioxide: J06.03
Arthropathy: P02.051
ARVC: P09.016
ASB10: C14.1
ASCL1: P06.013
ASD: P09.044
ASDs/MR: P02.213
ASLX1: P02.170
Asphyxiating thoracic dysplasia: P12.018
ASPM: P12.145
asporin: J09.21
assay validation: P15.11
assay: P09.042
assessment scheme: P15.15
assessment: P01.25
assignments: EP08.05
Assisted Reproductive Technology (ART): P04.09, P03.094
association study: J08.02, J09.45, P06.016, P09.045, P09.116, P13.27
Association testing: P08.39
association with decreasing risk: P09.053
association: C17.3, J09.06, J09.31, P08.04, P09.007, P09.073, P09.076, P09.080, P09.081, P09.097
asthma: J09.04, P09.017, P09.018, P09.019, P09.020, P09.021
ASXL1: P02.043, P02.044
asymmetry: P02.031
ataxia: J12.34
Ataxin 3: P09.092, J14.08
atherogenesis: P09.022
atherosclerosis: P09.001
athlete status: J09.39
athletes: J09.40, P09.068, P09.083
athletic performance: J09.44
ATM: P12.019
atopic dermatitis: J12.02, P09.023
ATP13A2: J11.08
ATP2A2: P02.074
ATP5β: P09.087
ATP6V0A2: P02.032
ATP7B: J13.14, P13.02, P13.49
atrial septal defect and patent foramen ovale: P09.024
Atrichia with papular lesions: P12.020
Atrioventricular canal defect: P02.184
atrophoderma vermiculatum: P02.033
ATR-X syndrome: P03.036
ATRX: P10.09
Attention-deficit/hyperactivity disorder: P09.025
attitudes: EP08.06
Atypical hemolytic uremic syndrome: J09.05
audiological analysis: P02.109
Audiological examination: P02.257
auditory neuropathy: P12.184
AURKA NEK6: P06.012
Autism Spectrum Disorder: P09.030, P12.021
autism spectrum disorders: S10.3
AUTISM: J02.03, J09.38, P02.034, P03.002, P03.020, P03.021, P03.022, P03.098, P05.21, P09.026, P09.027, P09.028, P09.029, P11.003, P11.117, P13.25
autistic features: P02.103
autoimmune: P09.117
autosomal dominant intellectual disability: P12.167
autosomal dominant macular dystrophies: P12.022
Autosomal dominant spinocerebellar ataxia (AD SCA): P02.035
autosomal dominant: P02.106
autosomal markers: P10.10
autosomal recessive congenital ichthyosis: C18.2, J12.18
Autosomal recessive cutis laxa type I: C09.5
Autosomal recessive deafness: P10.11
Autosomal recessive diseases: P12.125
autosomal recessive inheritance: P02.120
autosomal recessive long QT syndrome: P02.036
Autosomal recessive nonsyndromic hearing loss: P08.05, P12.023, P12.024, P12.025
Autosomal Recessive Primary microcephaly: C15.3
autosomal recessive: P02.132, P02.225, P11.060, P12.054, P12.231
autozygosity mapping: C15.2, P08.37
AUTS2: P08.02, P09.027
Axenfeld: P02.037
AZF factor: J04.08
AZF: P04.05
azoospermia: J04.11, J04.17, P04.50
B3GALTL gene: P12.194
BAC Based High Throughput FISH Analysis: P06.007, P06.056
BAC Genome Array-CGH: P03.042
backward design: P01.25
BACs on Beads: P05.52
balanced rearrangement: P03.023, P03.133
balanced translocation: P03.021, P03.024, P03.078, P07.28
Balkan endemic nephropathy: P09.031
Bannayan-Riley-Ruvalcaba Syndrome: P02.038
Barcode: P15.12
Bardet–Biedl syndrome: P02.039
Bardet-Biedl: P12.026
barriers to access: EP07.01
Bartsocas-Papas syndrome: P12.027
basement membrane: P12.154
batch effects: P11.015
Bathing suit ichthyosis: P02.027
Bayash: P10.50
Bayesian: P08.40
BBS: P12.026
B-cell activating factor: P06.004
B-cell lymphoma: P07.03
BCL11A: P11.037
BCL-2 prompter: J06.04
bcl-2: P06.035
B-CLL: P06.013, P06.014
bcr/abl transcripts: J06.10
BCR-ABL transcript: P06.015
BCR-ABL: P06.058
BDKRB2: J09.39, P10.01
BDNF: P11.004
Becker: EPL5.5
Beckwith-Wiedemann syndrome: P02.041, P02.091, P02.231, P11.005
BECKWITH-WIEDEMANN: J02.04, P02.040, P02.129
beedi workers: J03.22
behavior genetics: J09.06
Behavior: S03.3
Behçet's disease: P08.06
Belarus: J12.15
beliefs: EP02.06
Benign familial infantile epilepsy (BFIE): P12.028
Beta Globin LCR: P12.243, P12.029
Beta thalassemia intermedia: P12.029
beta toxin,: J03.01
beta-thalassemia: J05.02
bezafibrate: P14.19
Bicuspid aortic valve: P14.13
Bidirectional Mendelian Randomization: P10.12
bilharzial bladder cancer: P06.209
biliary atresia: J02.05
BIM: C10.3
Binning: P08.39
biobank for research: P11.063
biobank: P01.02, P01.03, P10.36
biochip: P09.012, P09.123, P09.127
bioenergetics: P13.31
bioinformatic validation: P12.196
biomarker analysis: P08.03
biomarker: P06.069, P06.070, P06.087, S07.1
Bipolar affective disorder: P09.032
bipolar disorder: C06.4
Bipolar: P09.069
birth defects: P02.220
bisphosphonates: J14.07
bladder cancer: J06.16, P06.016, P06.018, P06.019, P06.020, P11.006
bladder exstrophy epispadias complex(BEEC): P08.08, P09.033
blepharophimosis: P03.088
BLM: P06.036
Bloch- Sulzberger syndrome: P02.042
blood coagulation: P02.107
Blood: P11.105
Bloom syndrome: P06.036
Blue Rubber Bleb Nevus Syndrome: C01.4
BMI: P09.066
BMP1: C03.3
BMP2-Smad-RUNX2: P12.207
BMPR2: P02.215
BN-PAGE: P13.32
BOADICEA: P06.032
BOC cancer: P06.021
Bohring-Opitz syndrome: P02.043
bone marrow failure: P06.022
Bone marrow transplantation: P07.15, P08.10
Bone mineral density: J08.06, P09.103
bone mineralization: P02.108
bone: S15.3
boric acid: P07.04
BOS: P02.044
Boston-type craniosynostosis: P02.045
brachydactyly: P02.046, P12.030, P12.031
BRAF gene: P02.052
BRAF: P06.023
brain malformation: P02.139
brain: P02.062, P03.014, P03.022
Brainstem malformation - Neurodevelopmental: P02.082
Branched-chain α-keto acid dehydrogenase complex: P12.032
branchio-oculo-facial syndrome: P02.047
Brazilian Population: P10.03
BRCA 1 / 2: EP05.11
BRCA: EP03.02, EP05.06, EPL6.4, P01.04, P06.021, P06.024, P06.025, P06.026, P06.027, P06.214
BRCA1 and BRCA2: P06.117
BRCA1: EP05.10, P06.028, P06.029, P06.101, P06.156, P06.161, P12.033
BRCA1, BRCA2: EPL4.6
BRCA1/2 mutation: EPL4.4, P05.59
BRCA1/2: C10.4, EP05.05, EPL6.5, P06.030, P06.031
BRCA2: EP05.10, P06.161, P06.196, P07.05
BRCAPRO: P06.032
breakpoints: P02.089
breast & ovarian cancer: P06.024, P06.033
Breast and ovary surveillance: P01.04
Breast cancer: EP05.02, EP05.06, EPL2.1, EPL4.1, EPL4.4, EPL6.2, J06.04, J06.05, J06.06, J06.07, J06.08, J06.09, J06.15, J06.18, P01.05, P02.178, P06.002, P06.029, P06.034, P06.035, P06.036, P06.037, P06.038, P06.040, P06.041, P06.042, P06.043, P06.044, P06.045, P06.046, P06.047, P06.052, P06.112, P06.141, P06.149, P06.150, P08.07, P11.064, P11.072
breast/ovarian cancer: P06.030
BRESEK/BRESHECK syndrome: P02.048
bronchial asthma: J09.07, P09.062
Bronchial mucosa: P06.137
Bronchopulmonary dysplasia: P09.034
Bronze and Age populations: P10.39
Brugada Syndrome: P12.034, P12.035
Bulgarians: P10.58
Burkitt lymphoma: C10.5
BWS: P02.049
bystander-type effect: J03.17
c.35delG mutation: P10.29
c.859G>C mutation: P12.230
C15orf2: P12.036
C1orf24: P06.208
C8orf37: C11.5
C9orf72 gene: P10.04
C9ORF72: P12.011, P12.012
CA: P03.025
CACNA1A: P12.083
CAD: J13.02, J13.11
Cadasil: P02.050, P12.173
cafe-au-lait: J02.30
CAG repeat disorders: P12.121
CAG repeats: P02.124
CAH: P04.07, P12.037, P12.263, P13.05
CAKUT: P11.007
calcified external ear auricles: P02.209
CALCR: P09.101
Caldesmon: C02.5
CALHM1: P09.011
Calreticulin: J09.08, P09.112
campomelic dysplasia: P11.123
Camptodactyly: P02.051
CAMTA1 gene: P12.167
cancer development: P06.217
cancer early stage: S02.3
Cancer genetic counseling: EPL4.6
cancer genetics: EP05.12
cancer penetrance: C10.4
cancer predisposition: P06.030
Cancer susceptibilty: P01.34
cancer testis antigens: P06.069, P11.057
Cancer: C02.6, EP05.08, EPL1.5, EPL4.3, P06.048, P06.049, P06.066, P06.067, P06.090, P06.128, P06.148, P06.171, P06.202, P07.14, S08.3, S09.1
Cancer-Testes Antigen: P06.050
candidate gene approach: P12.091
candidate gene capture: P13.07
candidate gene: P02.221, P03.021
cannabis: P09.113
Cantú syndrome: C18.1
Capillary electrophoresis: P11.075, P11.115
capillary malformation: P02.167
CAPN3: P11.065
Carbonic anhydrase: P06.187

cardiac abnormalities: P02.197, P03.069
Cardiac Channelopathies: C16.4
Cardiac desmosomes: J02.46
cardiac fibrosis: P11.053
cardiac genetics: EP07.03
cardiac hypertrophy: P11.053
cardio vascular fitness: P09.035
Cardio-facio-cutaneous syndrome: P02.052
cardiogenetics: P02.245
Cardiology specialist training: EP06.03
cardiomyopathy: C12.5, J02.44, P01.06, P12.038, P13.04
Cardiovascular DIsease: C14.6
cardiovascular: C05.1
Carney-Stratakis syndrome: P06.051
cartilage: PL2.5
Cartilage-hair hypoplasia: P12.039
case family: P06.005
CASK gene: P03.017
caspase 8, caspase 9: P12.189
Caspase-8: P06.052
CASR: P09.101
CAT gene C1167T: P13.10
cat-eye syndrome: P02.057
Cathepsin C Gene: P12.186
causative genes: S01.3
CAV3: P12.040
Caveolinopathies: J09.09, P12.040
CBL gene: J07.04
CBMN assay: J03.21
CBX8: P06.053
CCM genes: P12.041
CCR: P03.026
CCR5: P09.002
CCT7: C05.5
CD2: J05.07
CD40LG: J05.10
CD73: P13.03
CDG: P13.04
CDG1a: P12.198
CDH1: P06.063, P06.100
CDK5: J09.46
CDK5R1: J09.46, P11.008
CDKL5: P12.042
CDLS: P12.043
CDMP1: P12.030
CEBPA: C02.3
Celiac Disease: C17.1, P09.036, P09.037, P09.038, P09.039, P09.040, P11.126
Cell free fetal DNA: P05.09
cell free plasma DNA: P06.212
cell line: P06.129
Cell model: J14.08
cell-based reporter assay: P13.12
cell-free DNA: P09.054
central nervous system: J02.52, P02.053
centriole: P10.13
centromeric probe: P05.32
CEP135 gene: C13.4
cerebellar hypoplasia: J12.30
cerebral cavernous malformations: P12.041
cerebral cortex: P09.047
cerebral ischemia: J09.10
cerebro-oculo-nasal syndrome: P02.054
cerebrovascular lesions: P12.041
Cerkl: P09.109
ceruloplasmin: J12.33
Cervical cancer: P06.054, P06.055, P06.135, P06.151, P07.06
cervical carcinoma: P07.04
CES: P02.057
CFH gene: J09.05
CFH: P09.110
CFL2: P02.055
CFTR Mutation: P12.063
CFTR: P01.09, P15.03, P15.04
CGC referral: P06.031
CGG stretch shrinkage: P02.104
CGH array 44K: P03.085
CGH Array: P02.171
CGH: P03.122, P05.10
CGH-array: P02.248, P05.58
channelopathy: P12.038
Charcot-Marie-Tooth disease: P10.14
Charcot-Marie-Tooth: P02.056, P12.219
CHARGE syndrome: P02.006, P12.045
CHD2: P03.092
CHD7: P12.045
chemotaxis: J07.01
chemotherapy: J06.20
Chernobyl accident liquidators: J03.17
childhood ALL: P06.056
childhood cancer: P02.172
childhood obesity: J09.11, P09.082, P12.132
childhood T-NHL: P06.083
Children and Adolescents: J02.42
Children: EW1, J14.07, P13.08
chimeric gene CYP21A2/CYP21A1P: P13.05
ChIP: P11.009
ChIP-Seq: C06.5
CHK2 gene: P06.204
CHO cell: P11.010
Choice of spouse: P10.15
Choices about results from WGS: EPL7.1
cholestasis: J02.51
cholesterol synthesis: P13.43
chordoma: P06.057
chorionic villi: P03.027
chorionic villus: P05.17
chromatin structure: P11.011
chromatin: P11.042
chromosomal abberation: J02.06, J03.19, J03.21, P02.206, P03.030, P03.075, P03.085
chromosomal abnormalities: J01.03, J02.07, J03.02, J03.22, P03.094, P04.09, P04.31, P05.29, P05.37, P05.65, P07.12
Chromosomal alterations: P06.054
chromosomal breakpoints: P03.061
chromosomal disorder: P03.032
Chromosomal inversions: P03.033
chromosomal mosaic: P05.11
Chromosomal mosaicisim: P03.077
chromosomal translocation: J07.04, P07.16
chromosomal: P03.038, P04.08
Chromosome 1; trisomy 1q: P03.034
chromosome 10q: P02.244
Chromosome 14: P03.054
chromosome 15: P02.034
chromosome 20: P03.035, P03.124
chromosome 21: P04.14
chromosome 22: P02.057
chromosome 2p14-p15: P03.089
chromosome 4: P05.32
chromosome 5: P03.024, P03.072
Chromosome 6: J02.07
Chromosome 9p deletion: P02.058
chromosome disorders: P03.113
chromosome evolution: P03.105
chromosome microarray analysis: P05.05, P05.12
chromosome pathology: J05.04
chromosome rearrangement: P04.47
chromosome territories: P03.036, P11.011
chromosome translocation: P06.198
CHROMOSOME: EP04.01, J03.08, J03.09, J04.07, P03.053, P05.53
chromothripsis: PL2.1
Chronic hepatitis C: J14.01
chronic lymphocytic leukaemia: P07.07, P07.08
Chronic lymphoid leukaemia: P07.09
Chronic myeloid leukemia: P06.015, P06.058, P06.059, P07.10, P07.11, P07.18
chronic obstructive pulmonary disease: P09.041
CHST3: P02.059
CIC: P06.167
CIITA gene polymorphisms: P09.139
cilia: P12.199
Ciliogenesis: P12.220
ciliopathies: C15.5, P02.136, P02.201, P11.012, P12.046, P12.126
ciliopathy: P02.039, P12.018, P12.047
circadian rhytm genes: P09.084
circulating nucleic acids: P05.13, P06.060
cisplatin and apoptosis: J06.08
Cisplatin: J06.09, P06.043, P06.104
cis-regulation: C08.4
cis-regulatory element: P02.224
CIZ: P07.01
Classic bladder exstrophy (CBE): P08.08
Classic Ehlers-Danlos Syndrome: P12.048
classical cytogenetics: J15.03
classical Hodgkin lymphoma: P06.061
classification: J02.10
CLCN1 gene: P12.152
CLCN1, DMPK, SCN4A.: P02.176
clear cell RCC: P06.062
cleft lip: P12.244
cleft lip/palate: P02.028, P09.073
cleft: P06.063, P09.125
cleidocraneal dysplasia: C03.2, J02.08
clinic: P11.046
clinical and genetic heterogeneity: C15.5
clinical application: P15.13
clinical database: P01.28
clinical diagnostic testing: P11.086
Clinical feature: J13.0, P13.34
Clinical Genetics: EP02.04
Clinical management: P06.218
Clinical practice: EPL3.1
clinical prediction model: P08.07
Clinical use of NGS: P01.27
clinical utility: P01.07
Clinical: J02.22
Clinically actionable mutations: C02.6
CLL: P07.12, P07.17
clonal evolution: P06.098, P07.08, P07.12
Clonal Sequencing: C07.3
clonality: J06.16
clopidogrel: P11.108
CMDH: P12.087
c-MET: P06.113, P06.137
CML: C10.3, J07.01, P07.13
CMML: P06.064
CMT 4A: P10.07
CMT: J12.03
CMT2C: P12.049
CNTNAP2: P09.029
CNV: C03.1, C09.4, J11.12, P02.203, P03.037, P03.123, P09.026, P09.032, P11.013, P11.070, P13.01, S06.2
cobalamin defects: P13.06
Cockayne Syndrome: P12.050
Cockayne: J02.50
Codon 12 and 13: P06.127
Coeliac disease: P09.042
Coenzyme Q10: P13.44
Coffin-Siris syndrome: P12.052
Coffin-Siris: P12.051
cognitive ability: P10.37
COH1: P02.060
Cohen syndrome: P02.060
coinheritance: P08.44
COL1A 2: P02.181
COL1A1 gene: P12.180
COL1A1: P12.053
COL1A2 gene: P12.180
COL1A2: P02.195
COL2A1: P02.115
COL4A5 gene: P12.009
COL4A5: P12.007
COL4A5, COL4A4, COL4A3: P12.008
COL5A1: P12.048
COL5A2: P12.048
COL7a1: P02.085
COL9A3: P12.054
Colchicine resistance: P14.05
Cold induced sweating syndrome type I: P12.060
collagen: P12.183
colon cancer: P06.065, P06.092
colon: P06.146, P07.14
Colorectal cancer: C10.6, J06.19, J09.23, P06.010, P06.068, P06.069, P06.070, P06.071, P06.072, P06.073, P06.074, P06.075, P06.076, P06.077, P06.078, P06.079, P06.080, P06.082, P06.091, P06.119, P06.203, S09.3
colorectal carcinoma: P06.131
Colorectal neoplasms: P01.35
Colorectal: P06.066, P06.067
combined CGH+SNP Array: P05.14
comet assay: P06.086
common haplotype: P12.166
common mutation: P02.019
Common Trisomies: P05.22
communication: EP02.01, EPL6.3
community website: J01.08
Comparative Genomics: P10.51
Comparative: P03.076
Competence: P01.08
Competitive RT-PCR: J06.10
complex abnormality: P03.038
Complex chromosomal rearrangement: J07.02, P03.039, P03.040, P03.071
complex disease: P09.043, P09.059
complex I deficiency: C12.4
Complex rearrangements: C04.1
complex translocation: P05.31
complex: P11.051
compound heterozygozity: P02.159
Comprehensive cancer gene sequencing: P11.014
computer-supported training: EP06.08
COMT: J11.05, P08.09, P09.093
Cone-Rod Dystrophy: C11.5
Congenital adrenal hyperplasia: P04.07
Congenital anomalies: J10.02, P02.030, P03.019
Congenital diaphragmatic hernia: P03.034, P05.15
Congenital disorders of glycosylation: P13.07
Congenital fibrosis of extraocular muscles: P02.062
congenital heart defect: J02.32, P08.11, P09.015
Congenital heart diseases: J02.08
congenital hypogonadotropic hypogonadism: P04.10
congenital ichthyosis: J12.04
congenital limb malformation: PL2.3
congenital malformation: J03.20, P02.053, P02.063, P02.216
congenital myasthenic syndromes: P02.064
congenital myopathy: P02.055
congenital: P02.061, P10.47

Congenitaladrenal hyperplasia: P05.16
connective tissue disorder: P02.023
Connective Tissue: P02.065
Connexin 40: P09.131
Connexin31: J12.05
Consanguinity: P03.050, P03.119, P12.055
Consent: EP05.08, EPL6.5
Constitutional mismatch repair-deficiency syndrome: P06.083
constitutional: C04.5
constitutive heterochromatin: P03.027
Contactin genes: P09.044
contigous gene syndrome: P02.029, P12.056
contractures: J12.23
Conventional cytogenetics: J07.02
COPD: J10.03
copy number alterations: P12.213
copy number change: P06.131, P11.061, P11.140
copy number variant: P03.041, P03.120, P09.111, P09.118, P11.015
Copy number variation (CNV): P02.026
copy number variation: P04.24, P05.15, P09.044, P09.126, P11.068
copy number variations (CNVs): P09.033, P09.129
copy number variations: C04.2, P09.120, P13.30, PL2.3
Cord Blood: P04.09
core-duplicon: P10.16
corneal dystrophy: J02.09
Cornelia de Lange syndrome: P02.066, P12.058
Cornelia de Lange: P12.057
Coronary Artery Disease: J08.02, J12.20, P09.045, P09.046
coronary heart disease: P09.106
Coronary rates: P10.28
corpus callosum agenesis/hypoplasia: P05.48
corpus callosum: P02.165
cortical thickness: P09.047
Costello: P02.067
Counseling: EP05.02, EP06.04, EPL7.5, J02.31, P01.05
COX deficiency: P13.47
COX15: P13.23
CpG oligonucleotides, IL-2: P07.17
CpG-island hypermethylation: P06.099, P06.100
Craniofacial anomaly: P02.123
craniofrontonasal dysplasia: P02.068
craniofrontonasal syndrome: P02.069, P02.246
craniostenosis: P02.068
craniosynostosis: P02.070, P09.048, P12.264
CREBBP: P02.222, P12.213
CreERT2: P11.016
criminal violence: P09.049
criminality: P10.35
Crisponi syndrome: P12.060
CRLF1: P12.060
Croatian population: P06.200
Crohn´s disease: J10.08, P09.050
cross cultural counselling: EP06.07
Crouzon Syndrome: P02.071
CRPS: P09.051
cryptic chromosomal abnormalities: P03.111
cryptic chromosome rearrangements: P03.042
cryptic rearrangements: P02.261
cryptical genomic imbalance: P03.016
CSF1R: P02.072
CTC: C10.6
CTCF protein: P11.040
Ctnnb1: P06.111
CTSC: P02.198
Currarino: P12.148
Cutaneous Human T cell Lymphoma: J06.02
cutis laxa: C09.6, P02.032, P12.061
CVD risk factors: P08.26
CVID: J12.30
CYB5R3: J12.11
cyclin D2: P06.213
cynomolgus: P03.011
CYP19 gene: J08.04
Cyp19A1: J08.02, P04.11
CYP1B1: P11.017
CYP21A2: J04.04, P05.16, P12.037, P12.263, P13.05
CYP2C19: P10.56
CYP2C9: P02.199
CYP2C9*2*3: P14.22
CYP2C9, CYP2C19: P06.205
CYP2D6: P06.205, P14.05
CYP3A4*1G: J06.01
CYP3A5: P10.56
CYP4F22 gene: J12.04
CYP51A1 lanosterol 14α-demethylase: P13.43
Cystic fibrosis (CF): P09.052
cystic fibrosis: EPL3.5, P01.09, P01.10, P01.11, P04.12, P11.018, P12.063, P13.08, P13.35, P15.03
Cystic fibrosis-like: P12.076
Cytochrome P-450 Enzyme System: P10.17
cytochrome P450: J13.13
Cytogenetic abnormality: J03.09
cytogenetic analysis: P05.38
Cytogenetic: J02.38, J03.03, P03.110, P05.17, P05.54, P07.25
cytogenetically balanced t(1;9)(q43;q32): P02.247
Cytogenomic: P03.107
Cytokine gene polymorphism: P08.10
Cytokine: J13.02, P04.48, P08.29, P09.124
Cytokinesis-block micronucleus assay: P07.31
D4Z4: P11.042, P12.098
D835 Point mutation: P06.003
Dandy-Walker malformation: P02.105
Danon: P02.073
Darier: P02.074
database: C07.6, C09.1, P12.196
DCM: P12.064
De novo mutation: C06.3, C13.2, P09.118, P09.119, P11.035
de novo rearrangements: P04.38
de novo translocation: J03.04
de novo: C07.5, P02.095, P03.124, P11.019
deafness: J12.32, P02.036, P02.075, P10.18, P10.19, P12.066, P12.067, P12.253
decision making: S08.1
decision: EP05.09
decrement of CMAP: P02.176
deep-intronic mutation: P11.018
DEFICIENCY: P13.42
dehydrated hereditary stomatocytosis: P05.18
Del 17q21.31: C01.2
del20p12.3: P03.141
Del22q11.2: J01.01
del9q34.11-q34.13: P02.076
Deletion 10q11.21-q11.22: P03.044
deletion 1p36: P03.045
deletion 1q43q44: P03.046
Deletion 20q: P03.047
deletion 3p: P02.079
deletion 3p22.1-p24.1: P02.077
deletion 4q13.2-q22.3: P03.048
deletion 8pter: P03.049
deletion: J12.11, P03.023, P03.038, P03.043
deletion/duplication: P04.05
delivery of health care: P01.23
Delphi study: P01.22
demography: J04.01, P10.18
dental faculty: J01.02
dental students: P01.12
dentatorubropallidoluysian atrophy (DRPLA): P02.035
description: P11.051
Desmoplakin: J02.46
desmosome: P09.016
determine the major histocompatibility complex: J10.04
Development Delay: P03.050
Development: P11.124
developmental delay: EPL3.6, J03.20, P02.078, P02.079, P02.087, P02.169, P02.225, P03.007, P03.044, P03.051, P03.055, P03.057, P12.218
developmental disorder: P03.143, P09.126
DFNB: P12.106
DFNB1: J10.05
DFNB9: P12.184
DHCR7: P02.236, P12.229
DHFR: P04.13
Diabetes risk in Cystic Fibrosis: P09.053
diabetes: P13.08, P13.09
Diabetic ketoacidosis: P13.06
diabetic nephropathy: J10.12, P09.054, P13.10
Diagnosis: EP08.02, P02.001
diagnostic algorithm: P12.130
Diagnostic Genetic Testing: C07.3
diagnostic test: P06.033
diagnostic: J02.10, P02.056, P05.52, P12.046, P12.082
Diamond-Blackfan anemia: P02.029, P02.080, P02.081
diaphragm agenesis: P02.002
diaphragmatic hernia: P03.058
dicentric: P03.052
Diencephalon- Mesencephalon: P02.082
differential allelic expression: P12.058
differential expression: P11.113
differentially methylated genes: P04.32
Differentially Methylated Regions: C16.2
differentiated thyroid cancer: P06.084
digital clubbing: C09.3, P12.068
Dilated cardiomyopathy: C07.4, J02.24, J12.28, J02.47, P02.250, P08.04, P12.064
Dilated: P02.126
dioxidine: P06.086
direct-to-consumer genetic testing: P01.14
Direct-to-consumer: P01.13
disclosure: P01.26
Discourse Analysis: EP02.04
dislipidemia: P13.03
disorder of sex development: P02.039
disorder-centred use of NGS: P01.27
disorders of sex development: P02.154
distal 15q duplication syndrome: P02.083
distal hereditary motor-neuropathy type VII: C11.4
distance: J11.04
Distress: P01.34
distribution with spike: P08.35
DLG2: P09.032
DM2 mini expansions: P12.069
DMD: EP02.01, EPL3.6, P11.020, P11.021, P11.081, P12.070, P12.124
DMRT1: P04.02
DMuDB: C07.6
DNA concentracion: P06.060
DNA damage: P04.30, P06.085
DNA fragmentation: P04.49, P04.54, P06.060
DNA methylation: J11.01, P06.070, P06.071, P06.072, P06.073, P06.086, P06.087, P09.057, P11.022, P11.023, P11.024, P11.025, P11.026, P11.027, P11.028, P11.058, P14.07, PL2.6
DNA microarrays: P06.093, P06.164
DNA persistence: J11.03
DNA Q-PCR: P06.059
DNA repair: J06.21, P06.088, P06.089
DNA: P02.084
DNA-Methylation: P04.25, P11.029
DNMT3A mutations: P06.006
DNMT3B: P06.090
dolichol kinase: P13.04
Donor Cell Leukemia: P07.15
Dopamin receptor gene: J09.11
dopamine transporter: P09.009
Dopamine: J09.11
dosage-sensitive network: P02.200
DOT1L: PL2.5
double heterozygous variations: P11.077
double trisomy: P05.19
down- and up-regulation: P09.099
Down syndrome: C04.6, EPL1.3, J05.12, J11.11, P03.053, P04.14, P05.20, P05.63, P08.11, P09.056, P11.030
DPD enzyme: P06.001
DPPA2: P06.091
DPYD gene: P06.001
Dravet syndrome /GEFS+: P12.217
Dravet syndrome: P12.078
DRB1*1501: P09.085
Drosophila melanogaster: P12.102
drug discovery: P14.09
drug resistance: P06.092
drug response: S07.2
drug-to-drug interaction: P11.107
DSP30+IL2: P07.22
Duchenne muscular dystrophy: P11.020, P12.071
Duchenne: EPL5.5
ductal breast cancer: P06.093
dup(16): J03.05
Duplication 10q: P03.055, P03.056
duplication 14q32: P03.057
duplication 17q25.1: P02.078
duplication 2p: P03.058
duplication 5q35.3: P02.189
duplication breakpoint: P03.143
duplication Xp11.23-p11.3: P03.059
Duplication: P03.035, P03.054
duplication/deletion: J03.06
Dutch population: P11.134
DYNC2H1: P12.018, P12.126
Dynein Assembly: P12.200
DYSF: P11.065
dysmorphia: P03.110
dysmorphic syndromes: P03.114
dysmorphism: J02.39, P02.008, P02.067
Dysmorphology features: P02.264
dysmorphology: P02.096
dystonia: J12.06, J12.17, P12.245
Dystrophic Epidermolysis Bullosa(DEB): P02.085
dystrophin: P12.124
Dystrophinopathy: EPL5.5
DYT1: J12.06

early infantile epileptic encephalopathy: P12.190
early onset dementia: P02.072
Early onset nephropathy: P12.072
early pregnancy loss: P11.032
early spontaneous abortion: P05.19
Early stress: S03.3
early-onset Alzheimer disease: P12.073
Early-Onset Autosomal Recessive Retinitis Pigmento: P12.074
Eastern Siberia: P10.11
EB: P12.075
EBV-immortalized cells: P12.225
E-cadherin: P06.099, P06.142
ECO syndrome: P02.090
Ectodermal dysplasia: J02.53
Ectopia lentis: P02.086
ectopic mineralization: P12.207
EDN1: P09.051
Education: EPL1.2, J01.02, P01.12, P01.20, P01.33
efficacy of drug therapy: P11.107
EFNB1 gene: P02.068
EFNB1: P02.069
EGFR expression: P06.114
EGFR gene: P06.165
EGFR negative: P06.123
EGFR: P06.094, P06.105, P06.126
EG-VEGF: P04.45
Egyptian: P12.132
Ehlers Danlos syndrome: C18.3, J02.11, P02.065, P02.157
Ehlers-Danlos: P12.240
EIF3a: P02.087
elderly and senile: J09.42
ELISA: P06.019
eln: P02.088
Emanuel syndrome: P02.089
Emery-Dreifuss muscular dystrophy: C11.6
Empowerment: EPL5.6
EMQN: P15.15
EMX2: P02.011
ENaC mutations: P12.076
Enamel hypoplasia: C18.5
endemic region: P12.109
endocannabinoid system: P13.27
endogamy: J10.10, P10.15
Endolymphatic sac tumors: P02.257
endometrial cancer: EP05.07
endothelial cell: P07.03
Endrocrine-cerebro-osteodysplasia: P02.090
energy metabolism: P12.120
ENG gene: P12.112
ENU-derived mouse models: C12.6
enzyme activity: J13.05
Enzyme replacement therapy: P13.14, P14.10
EOP: J11.08
Eosinophilia: P06.064
EP300: P12.214
EPG5: P12.254
epidemiology: P02.127, P02.129, P02.161, P10.48
epidermal growth factor receptor: P06.074
epidermolysis bullosa simplex: J12.25
epigenetcs: P11.072
epigenetic modification: P11.040
epigenetic regulator: P11.121
Epigenetics: J02.04, J11.01, P02.091, P02.092, P06.163, P06.179, P09.009, P11.022, P11.058, P11.076, P11.118, P13.26, S03.3
Epigenome: P06.199, P11.089
Epigenome-wide association study: P09.057
epigenomics: P11.011
epigenotype: P06.071
Epilepsy Panel: P12.082
Epilepsy: C18.5, J02.12, J02.17, J03.18, J13.13, P02.093, P02.094, P12.078, P12.079, P12.080, P12.081
epileptic encephalopathy: P02.093, P02.095
epimutations: P11.032, P11.033
Episodic ataxia: P12.083
epistasis: P08.28
epistatic loci: P08.12
epithelial-mesenchymal transition (EMT): S09.2
EPM2A: J12.10
Epstein-Barr virus: P06.061
eQTL mapping: P08.13
eQTL: C08.4, P09.085, S01.3
ERCC2 gene: P06.084
ERCC6: P12.050
ERCC8: J02.50, P12.050
Erosive esophagitis: P06.012
erythrocyte glucose uptake: P02.110
Erythropoietic Protoporphyria: P12.084
ERα: J04.12
eSNPs: C17.5
Esophageal adenocarcinomas: P06.012
Esophageal Cancer: P06.050, P06.095, P06.174
estimations of age: P10.29
estrogen receptor: J09.32
estrogen: P11.009
ethical implications: P01.15
ethical: EPL7.6
ethics: EP08.03, EP08.04, EPL3.4, P01.16
ethnic minorities: P01.01
ethnicity: EP08.06
ethnic-specific diseases: P10.34
etiology: P02.127, P12.066
euchromatic variants: P05.21
European comparison: EPL2.2
European population: P10.28
evaluation: EP06.04, EPL1.6
EVI1 deletion: P03.129
Evolution: P10.09, P10.13
evolutionary models: P11.043
Ewings Sarcoma: J02.13, P07.16
exome sequencing: C07.2, C11.3, C12.3, C12.4, C15.4, J12.20, P02.033, P02.080, P09.043, P11.035, P11.059, P12.055, P12.085, P12.086, P12.087, P12.114, P12.125, P12.191, P12.193, P12.227, PL2.2
exome: C11.1, C18.4, P06.202, P11.034, P11.046, P11.088, P11.090, P11.091, P11.119, P12.052, P12.131
exomics: P12.179
exon 1: P02.022
exonization: P11.052
exons first nucleotides: P12.233
exon-specific array: P11.142
exon-specific microarray: P11.140
exosome: P11.105
exostoses: P02.179
expansion mechanisms: P12.069
expansion: J10.06
expectations & facts: P11.091
Expressed Sequence Tag(EST): P11.057
expression gene: P04.40
expression genetics: S07.1
expression microarray: P09.099
expression profile: P09.050
expression profiling: P12.134
expression quantitative loci: C08.5
expression: P06.092, P06.130, P06.171
Expressional analysis: J06.19
External quality assessment: J15.02, J15.03
Eye anterior segment dysgenesis: P12.088
F10: P02.107
F8: P12.103
FAAP100: P08.14
FABP9: J04.06
Fabry disease: J09.02, P14.03
face analysis: P02.096
face genotype-phenotype: P02.096
facial dysmorphism: P02.097
Facio-audio-symphalangism: J02.14
factor VIII: P12.103
FAM124B: P12.045
Familial adenomatous polyposis: C10.2, P06.011
Familial Amyloid Polyneuropathy: P10.20, P10.21
familial cancer: P01.01
Familial Cardiomyopathy (FCM): EPL2.6
familial cases: P12.057
Familial colorectal cancer: EP05.04
Familial diarrhea syndrome: C12.2
Familial Dysautonomia: P14.04
Familial hemophagocytic lymphohistiocytosis: P12.089
Familial hypercholesterolemia: J12.07, P11.049
familial hyperlipoproteinemia: P13.11
familial information: EPL6.4
Familial Mediterranean Fever: J02.27, P02.098, P02.164, P10.22, P12.090
Familial risk: EP02.05
Familial translocation: P03.026
family aggregation: J09.33
Family communication: EPL6.6
family dynamics: EP01.03
Family history of cancer: P06.211, P06.215
family history: C10.4, EPL1.5
family Piperaceae: J10.07
family therapy: EP01.03
FANCA: P11.036
FANCD1: P11.036
Fanconi anemia: P08.14, P11.036, P11.136, P12.091
FAP: P01.17, P06.096
FAPATTRV30M: P10.23
FAS and FASL: P06.057
FAS: J09.12
FAVISM: EPL5.1
FBLN5: C09.5
FBN1- fibrillin-1 gene: P12.239
FBN1 gene: P02.160
FBN1: P02.086
FBN1, TGFBR1, TGFBR2, SMAD3: P12.137
FcγR: P08.15
Feingold: P02.099
female pattern hair loss: P09.058
Fertility genes: P10.24
FERTILITY: P04.15
fetal autosomal aneuploidies: C16.1
Fetal central nervous system anomalies: P05.07
Fetal Chromosomal Abnormalities: P05.36
fetal hemoglobin: P11.037
Fetal Karyotyping: P05.22
fetal malformations: P05.61
Fetal programming: C16.5, P11.038
fetal sex determination: P05.23
fetus: J02.15, J05.02
FGF23: P13.12
FGFR2: P02.070, P02.071
fibroblast reprogramming: P11.063
fibrochondrogenesis: J02.15
Fibrodysplasia Ossificans Progressiva: J02.16, P02.100
Fibromyalgia: J09.13
Filamin A: P02.101
Filipin staining: P13.37
Fine-mapping: P09.059
FIP1L1-PDGFRA: P06.097
first degree relatives: P09.135
first time researched: J09.09
first trimester screening: P05.24
first-degree relatives: EPL6.4, P02.130
FISH method: P05.11
FISH: J05.09, P03.040, P03.055, P03.057, P03.060, P03.066, P03.079, P03.080, P03.084, P03.135, P07.06, P07.19
FKBP10: J12.23
FKBP14: C18.3
flexibility: P09.114
FLNA: J02.17
FLT3 gene: P06.003, P06.120
fluorescence activated cell sorting: P07.26
fluorescence in situ hybridization (FISH): P03.061
fluorescence in situ hybridization: P07.01, P07.09, P07.10, P07.31
fluorescent quantitative PCR: P05.03
FMF: P12.143, P14.05, P15.01
FMF, BTAL, Thrombosis, Pyrosequencing: P15.06
FMR1 Gene: J12.08
FMR1: P12.093, P12.094, P12.095, PL3.3
FMR2 gene deletion: P02.102
Focal Segmental Glomerulosclerosis: P12.156
Foensic casework: J10.11
folate metabolism: P04.13
folate: P05.20, P10.25
Follicular Lymphoma: P06.098
follow-up: J02.28
football players: P08.17
Forbes-Cori disease: P13.13
Formation Mechanisms of CCR: P03.026
FOXC2 mutation: P12.203
FOXL2: P03.088
FOXP1: P11.039
fracture: J14.03, P09.102
fragile sites: J03.07, P03.025
fragile X syndrome: EPL1.4, J05.03, P02.103, P11.040, P12.095, P14.06, P14.07, P14.08, PL3.3
Fragile X: J01.01
Fragile-X Syndrome: P12.097
fragile-x: P02.104
frameshift: P06.140
FRAS/FREM pathway: C18.6
FRAS1: P12.096
Fraser Syndrome: C18.6, P02.105, P02.227, P12.096
FRAXE Mental retardation: P12.097
free fetal DNA: P05.13
frequency: P12.069
frequent mutations: P12.166
Friedreich Ataxia: P11.041, P14.09
Frizzled 6: P12.153
FRM1: P04.15
frontonasal dysplasia: P02.020, P02.106
frontotemporal dementia: P12.012
Frontotemporal lobar degeneration: P12.163
Fryns-like phenotype: P03.034
FSHD: P11.042, P12.098
FTO gene: P09.061

FTO: P09.060, P09.097, P09.098, P09.136, P13.09
Functional assay: P07.05
functional X disomy: P03.059
fusion gene: P02.133, P06.184
fusion protein: J14.02
FUT2: P09.037
FV Leiden mutation: J02.45
FVII R353Q: J10.15
FX deficiency: P02.107
FXPOI: P12.250
FXS: J12.08
FXTAS: P12.250
G2/M cell cycle chekpoint: P12.019
G2-bleomycin sensitivity assay: J07.03
G6PD Deficiency: EPL5.1
GABA receptors: P09.080
GABRA2: P08.02
GABRB2 gene: P09.025
gain 2p: P07.17
Galactaceamia: EP08.01
Gastric Cancer: J06.14, P06.053, P06.063, P06.099, P06.100
gastroschisis: P02.018
GATA2: C02.3
Gaucher disease: P12.099, P13.14, P14.10
G-banding: J05.11
GCK: P13.33
GCM syndrome: P02.114
GDAP1 gene: P12.113
GDAP1: J12.21, P10.07
GDF5 gene: P02.046
gene conversion: P06.157, P06.158
gene deletion: P06.101, P12.164
gene dosage at 10q11.22: P09.030
gene expression profiling: J05.12, P06.014, P06.037, P06.175, P11.044
gene expression: J11.02, J11.06, J13.02, J13.11, P03.011, P04.44, P06.124, P06.182, P09.039, P09.040, P11.030, P11.043, P11.047, P11.093, P11.114, PL2.6
gene F5: J04.02
Gene Frequency: P12.063
gene function: C08.6
gene HLA-DQA1: J09.34
gene IKZF1 (Ikaros): P07.18
gene knock down: P06.192
gene mutation: P12.100
gene network and canonical pathway analysis: P06.037, P06.175
gene network: C08.6
gene polymorphism: J05.14, J06.20, P05.64, P08.01, P08.17, P08.22, P08.30
gene polymorphisms and expressions: P06.132
gene regulation: C03.2
gene SETX: J12.01
gene TREX1: P02.019
Gene x Environment: P10.26
gene: J05.13, P06.038, P08.16, P12.052, P12.085
Genealogy database: EPL4.6
gene-covariate interaction: P08.43
gene-environmental interaction: P09.113, P09.062
gene-gene interaction: P08.43, P08.45
general population: P03.030
generalized linear mixed model: P08.40
genes HLA-DQB1: J09.34
genes of predisposition: P09.121, P09.122
genes: P09.049, P09.128
Genetic association: J09.14, P10.45
genetic background: P10.18
genetic burden: P09.086
Genetic carriers: J01.09
genetic conditions: EP01.03
genetic coounselor's report: EP02.02
Genetic counseling: EP02.06, EPL5.4, EPL6.1, EPL6.2, J01.03, J09.33, P01.18, P01.19, P01.35
genetic counselling awareness: J01.08
genetic counselling education: EP06.07
genetic counselling: EP02.01, EP07.01, EPL1.6, EPL6.6, EW2, P02.178, P02.196, P03.032, S08.1
Genetic counsellor training: EP06.01
Genetic counsellors: EP06.01, P01.20, EPL5.2
Genetic Diagnosis: P15.07
genetic differentiation between populations: P10.27
Genetic diseases: P01.24
genetic disorders: P02.108
genetic distances between populations: P10.44
genetic diversity: P10.34
Genetic Education: P01.21, P01.22
Genetic Engineering: J15.01, P11.016, P11.045
genetic epidemiological study: P10.27
Genetic eye services: EP07.02
Genetic heterogeneity: P12.193
genetic knowledge: EP02.06
genetic knowledge, skills, attitutes: J01.02, P01.12
genetic modifier: P12.135
Genetic monitoring: J10.02
Genetic nurse: EP06.06, EPL5.2
genetic polymorphism: J10.04, P04.39, P05.20, P06.115, P09.019
genetic potential: EP08.05
genetic predictor CFH genotype: P09.091
genetic predisposition: P09.064
genetic profile: P09.072
genetic replication analysis: P06.200
genetic risk modelling: P09.008
genetic risk score: P09.008, P10.28
genetic screening: P09.132
genetic services: EP07.01, P01.23
genetic skin disorders: J12.02
genetic susceptibility: P08.09, P08.18, P09.012
genetic test: P13.35
Genetic Testing and Counselling: J01.04
Genetic testing: EPL1.1, EPL2.5, J02.13, J02.24, P01.07, P01.10, P01.24, P06.028
genetic variability: J10.07
genetic variation: P10.17, PL2.6
genetic: C17.3, P09.063
genetic-epigenetic: P06.098
genetics curriculum: P01.25
genetics education: EP06.02, EP06.03, EPL3.1
genetics orientation: P01.33
genetics variant: J12.29
genetics: J06.15, P01.06, P02.031, P09.074
genodermatoses: C18.2, P12.127
genome research: P01.26
Genome Sequencing: P10.51, P11.074, S10.1
Genome wide association studies: P09.046
genome wide association study (GWAS): P09.065
genome wide association: P11.126, P11.143
genome wide linkage analysis: P12.232
genome wide: P06.207
genome: P11.046
genome-based-technology: EPL7.6
Genome-Wide Association Studies (GWAS): J09.25
genome-wide association study (GWAS): P08.08, P10.33
genome-wide association study: C14.5, P09.047, P09.111, P12.264
Genome-wide association: P13.41
genome-wide DNA methylation: J06.18, P11.049
genome-wide linkage analysis: P12.114
genome-wide: C08.5, P11.047
Genomic and exomic investigation: P12.088
genomic and transcriptomic profiling: P11.055
genomic control: P10.46
Genomic imbalances: P03.009, P03.082
Genomic Instability: C10.1, P03.014, S02.3
genomic order: J07.01
genomic profiling: P07.27
genomic rearrangements: P06.101
genomic technology: P09.024
genomic testing: P01.02
genomics: EP08.03, EP08.04, P11.079
genotoxic risks: P03.015
genotoxicity: P07.04, P07.20
genotype calling: P08.19
Genotype concordance: P08.24
genotype phenotype correlation: P03.062
Genotype: P02.040
genotype-phenotype correlation: P02.180, P06.168, P08.20
genotype–phenotype delineation: P02.004
genotypes: P10.10
genotyping: C07.5, C16.3, P04.12, P05.43, P11.013, P11.136, P12.242
gentic assessment: J06.12
germ line mutations: P06.197
germline mosaicism: P12.006
germline mutation: P12.175
geroderma osteodysplastica: C09.6
Gestational Diabetes: C16.5, J09.15, P11.038
GFP: J12.07
GH treatment: J02.40
Gibbon: P10.08
GIST: P06.102, P06.103
GJA1 mutation: P02.193
GJA5: P09.131
GJB1: J02.18
GJB2 gene: P02.118, P05.50, P12.100
GJB2 mutation: J12.15
GJB2: J02.25, J10.05, J12.09, J12.32, P02.109, P02.198, P10.11, P10.29, P12.067, P12.107, P12.224
GJB2, GJB6: P12.023
GJB6: J10.05, J12.09
glandular epithelia: P11.135
glaucoma: C14.1, J11.09
GLCCI1: P10.30
GLHS: P02.111
GLI3: P02.202
GLIDE: P11.122
Glioblastoma multiform: P06.108
Glioblastomas: P06.104, P06.105, P06.106, P06.107, P06.109, P07.19
Glioma: C02.1, P06.048, P06.144
Gliomas: P06.145
GLOMERULAR BASEMENT MEMBRANE: P12.008
Glucocerebrosidase Gene: P12.099
glucocorticoid receptor function: P08.20
Glucocorticoid receptor: P09.034
glucocorticoid: P10.30
Glucose tolerance: P11.023
Glut1 Deficiency Syndrome: P02.110
Glut1 deficiency: P12.101
GLUT9: P13.17
glutamate receptor: P02.133
glutaric aciduria type I: P13.15
Glutathion S-transferase: P04.16
Glutathione S-transferase: P10.31
glutathione S-transferases polymorphisms: P09.022
glutatione: P06.115
Glycogen storage disease type III: P13.13
GNAS gene: P02.212
GNAS, IGF2, SNURF imprinting defect: J02.49
Gnetic linkage analysis: P12.106
Golgi: P11.048
Gomez-Lopez-Hernandez syndrome: P02.111, P02.112
gonadal dysgenesis: P03.125
gonadal tumours: P06.210
gonadoblastoma: P02.227
Google Analytics: EP06.02
Gordon: P12.206
Gorlin syndrome: P02.113, P06.110
Gorlin-Chaudhry-Moss syndrome: P02.114
GP1a C807T: J10.15
GPD1: C12.1
GPI pathway disease: C13.1
GPM6A: P12.102
GPRIN2 PPYR1 genes: P09.030
gr/gr microdeletion: J04.11
granulosa cell tumour: P06.111
great impact on health: J09.09
greece: P01.30
GRIA1: P09.018
GRIN2B: J12.29
GRIP1 mutation: C18.6
group counseling: P01.06
growth hormone deficiency: P03.070
growth retardation: P02.116, P03.058
Growth: P02.115
GS Junior: P12.159
GST: J10.03
GSTP1: J06.14, P06.112
GUCY1A3: C05.5
GUCY2C: C12.2
guideline: P01.07, P01.26, P11.110, P15.07
GWA: P10.32
GWAS: C05.1, C08.4, C14.3, J09.27, P08.11, P08.23, P09.066, P09.104, P09.115, P10.38, S05.2

H
h: P05.66
H19: P11.005
haematopoietic stem cell transplantation: P05.51
Haemocyanin: P06.018
haemoglobinopathies: EP08.06
haemolytic: P08.21
haemophilia A: P12.103, P04.36
hairless gene: P12.020
hallmark chromosomal translocation: P07.03
hallux duplication: P02.069
Haloplex: P11.054
haplogroup: P10.58
haplotyp: J12.08
haplotype: C17.2, P04.56, P08.26
haplotypes: P09.014, P10.39
Harboyan syndrome: J02.09
Hb Q-Iran: P05.28
Hb variants: J05.06
HBB: P12.242
HBOC: P06.025

HBV: P06.116
HCM: J02.19, P12.104
hcv: J09.16
HDAC inhibitors: P06.113
HDAC: P06.146
HDAC9: P09.078
HDL: P11.122
HDL-C: P11.049
Head and neck cancer: P06.114, P06.115
Head and neck squamous cell carcinoma: P06.216, P10.46
health behavior changes: EP05.12
health care: P01.02
healthy participants: P01.03
healthy women: J03.11
hearing genes: P12.105
Hearing Impairment: J12.12, J12.17, P02.117, P11.050
hearing loss: J02.09, J12.09, J12.15, P02.118, P02.156, P12.066, P12.106, P12.107, P12.108
heart defect: P02.162
heart disease: EPL1.5
HeLa: P07.34
helminth invasion: P09.019, P09.062
Hematological malignancies: J07.02
hematological trait: P09.065
hematopoietic cells: J04.03
hemifacial microsomia: J02.20
hemodialysis: P09.054
Hemoglobinopathies: P02.119
hemophilia: P02.174
hemostasis system genes: J09.10
hepatic fibrosis: J02.15
hepatitis B virus: P07.20
hepatobiliary diseases: P12.002
Hepatocellular carcinoma: J06.17, P06.116
hepatocytes: P11.121
Hep-G2: P11.116
HER-1: P06.128
HER2/neu gene: P06.040
hereditary amyloidosis: P12.109
hereditary angioedema: P12.110
Hereditary Breast and Ovarian Cancer (HBOC): EPL2.6, P06.117
hereditary breast/ovarian cancer: P06.118
hereditary cardiomyopathy: P12.111
hereditary chronic pancreatitis: P02.211
hereditary diffuse leukencephalopathy with spheroi: P02.072
hereditary diseases: P02.061
Hereditary Haemochromatosis: J01.04, P13.16
Hereditary hemorrhagic telangiectasia: P12.112
hereditary motor and sensory neuropathies: J02.18
hereditary motor and sensory neuropathy: P12.113
hereditary multiple exostoses: J02.21
Hereditary recurrent fevers: P15.07
hereditary recurrent neuropathy: P12.114
hereditary renal hypouricemia: P13.17
Hereditary Spastic Paraplegia: P02.120, P12.115, P12.116, P12.117
hereditary: EP05.06, J09.36
heritability: P09.066, P09.067
heterochromatic regions: P03.063
heterochromatin: C06.1, J03.08, P03.063
heteromorphism: P04.08
heteroplasmy: P11.073, P13.22
heterotopias: J02.17
heterotopic ossification: J02.16
HFE: P13.16
HGVS: P11.051
HHI: J12.05
HIF: P06.188
HIF1A: P09.068
High Resolution Melting: P06.029, P06.197
higher order structure: P11.068
high-grade brain tumors: P06.083
high-IQ: P10.47
high-performance athletes: EP08.05
High-resolution melting: P15.04
high-throughput seqencing: EPL7.2, P01.27, P02.056
HIGM1: J05.10
HIP1 huntingtin interacting protein-1: P02.121
hirsutism: P02.122
histone deacetylation: P12.259
histone modifications: P11.038
history and future: S08.3
HIWI: P06.119
HLA Class II genes: P09.139
HLA genotyping: P09.135
HLA region: P09.004
HLA typing: P09.042
HLA: J10.01
HLA-A3: P13.16
HLA-C: J04.05
HLA-DRB1: J08.03
HLA-G 14 bp insertion/deletion polymorphism: P04.46
HLA-matching: P05.51
HLA-system: J10.04
HMBS: P12.118, P13.42
HMGB1: P02.003
HMR: P06.024
HMSN: P05.41, P10.14
HNF1A: P13.18, P13.33
HNF1B: P05.35
HNPCC: EP05.03
hnRNP C: P11.052
HOGA1: P12.201
holoprosencephaly: J02.22, J02.23, P02.123, P02.222, P02.228, P05.29
homocysteine: P02.117, P06.151
Homologous recombination: P12.091
Homology modelling: P12.152
Homozygosity Mapping: C03.6, J12.17, J12.18, P08.05, P11.060, P12.117
Homozygosity: P10.57
homozygous mutation: P02.036, P12.208
hormone receptor genes: P09.058
hospitalism: EP04.03
HOX C: P02.168
HOXD13: P12.031
hPEPP1: P06.192
HPV: P06.054, P06.095
HRAS gene: P02.067
HRM: P12.212
HRMCA: P12.035
Hs 683: P11.116
HSAN1: C01.5
HSD17B3 deficiency: J04.16
HSP: J12.27
HSP110: S09.3
HSPA4 deficient mice: P11.053
HTR3: P09.069
Human brain wiring: P02.082
Human chronic Lymphocytic Leukemia: P06.009
human defensin-1 gene: P06.120
human leukemia: J06.11
human leukocyte antigen allele: P08.18
human longevity: P08.22
Human Papilloma Virus: P06.121, P06.133, P06.041, P06.134
human populations: P10.17
human spermatogenic cells: P11.024
HumanMethylation450k: P06.073
Humero-spinal dysostosis: P02.059
Hungarian: P10.25
Hunter syndrom: J13.12
Huntington Disease: P02.124, P12.121, EP01.02, P02.124, P12.120, P14.11, EPL2.6, EP01.01, EW1, P12.121
Hurler syndrom: J13.12
Hutchinson–Gilford progeria syndrome: P12.134
HVS-1: J10.14
hydrocephalus: P02.076, P02.145, P02.229
hydrops: P05.18
hydroxymethylation: P11.025
hyper eosinophilic syndrome: P06.097
hyperandrogenism: J04.04
Hypercholesterolaemia: P11.054
hypercholesterolemia: C05.2, J09.47, P12.085
hyperhomocysteinemia: P02.125
Hyperinsulinism: J13.03, P13.19
hypermethylation: P06.122, P06.144
Hyperphosphatasia with Mental Retardation: C13.1
Hyperspectral Imaging: P03.064
hypertension: J09.17, P08.42
hypertriglyceridemia: C12.1
hypertrophic cardiomyopathy: J09.02, J09.18, P02.073, P11.055, P12.237, J02.48, J02.24
Hypertrophic: P02.126
hyperuricemia: P09.071
Hypodontia: J12.14
Hypoglicemia: J13.03
hypomethylation ICR1: P02.233
hypomethylation: P02.232
Hypomyelinating leukodystrophy: P12.122
hypophosphatasia: P13.20
hypoplasia: P02.122
hypospadias: P02.127
hypotonic infants: P02.128
Hypotrichosis: P12.020, P12.123
hypoxia: P09.001

I
IBD mapping: P12.191
ICAM-1: P09.002
ICCA syndrome: P12.028
ID3: C10.5
identification molecular cause: C15.1
Identity by descent: P12.074
Identity: EPL2.3
idiopathic dilated cardiomyopathy: P08.18
IFAP syndrome: P02.048
IGF2: P02.049, P04.18
IGF2R: P11.120
IgG synthesis: P10.33
IgH locus: P06.013
IL-10: P04.46
IL12RB1 gene: P09.140
IL13 and KIAA0350: P09.010
IL-18: J08.01
IL1B: J09.19
IL-1β: J09.22, P04.44
IL-23: S09.1
IL23R gene: J09.35
IL28B: J09.16
IL8: J09.19, J09.43
ILDR1: P12.024
Illumina: P03.032
IMAGe syndrome: P02.173
imagistic: J02.22
imatinib: P06.058, P07.13
immune system: C08.3
Immune-related diseases: P08.15
immunhistochemical loss of MMR
proteins: P06.154
Immunochip (Illumina): P09.004
immunohistochemistry: J02.13
immunotherapy: P06.178
Impact: EP08.02
imprinting genes: P11.032
imprinting: P02.092, P11.026, P11.056, P11.111
Imputation: P08.23
Impute: P08.24
IMSI: P11.033
in silico analysis: EP05.05
in silico: P11.057
inborn errors of metabolism: P13.21
Inbreeding: P10.57
Incest: EPL7.5
Incidence: P02.129
indel mutation: P12.030
Individual intentions to receive results from WGS: EPL7.1
individualized patient care: P06.139
induced pluripotent stem cells: C04.6
infant: J02.05
infantile cortical hyperostosis: P12.053
infertility: J03.03, J03.08, J03.12, P03.033, P03.039, P03.077, P03.119, P04.08, P04.19, P04.28, P04.38, P04.56, P11.033
Inflammation: P06.095, P06.123, P09.045
Inflammatory bowel disease: P09.072, P11.058, S01.3
influenza virus: J11.06
information: EPL1.3
Informative: P10.06
informed choice: EPL1.3
Informed consent: S16.3
informed decision making: EPL1.4
informed-consent procedure: S16.1
infrarenale aneurysma: P02.160
Inheritance: P02.130
Inherited cancer predisposition: P04.20
inherited cancer syndromes: C02.4
inherited metabolic disease: P13.01
inherited thrombophilia: J13.04
INPP5E: P02.131
insertion/deletion polymorphism: J13.10
insulin resistance: P13.03, P13.41
Insulin-like growth factor-I receptor: P06.074
integrative approach: P11.079
intellectual deficiency: P01.28, S10.3
Intellectual disabilities: P03.041
Intellectual disability (ID): P02.026, C01.1, C13.2, C13.3, C13.5, C13.6, C15.1, C15.3, J02.55, J03.18, P02.009, P02.076, P02.093, P02.097, P02.102, P02.132, P02.133, P02.134, P02.135, P02.140, P02.158, P02.162, P02.237, P03.012, P03.065, P03.066, P03.067, P03.068, P03.069, P03.076, P03.089, P09.028, P11.019, P11.039, P11.059, P11.060, P11.061, P11.069, P12.042, P12.124, P12.125, P13.25
interaction variables: P02.017
interaction: J05.13, P08.12
interchromosomal effect: P03.133
interethnic difference: P10.52
interferon-g: P09.106
intergenerational: EP03.02
interleukin 6: P04.21
Interleukin polymorphisms: P06.019
Interleukin-17A and IL17F: P08.41
interleukin-18: J08.01
interleukins: P09.017
Intermediate alleles: P12.094
Internal control,: J06.10
Internet-based epidemiological survey:

P10.32
interphase FISH: P03.131
interphase fluorescence in situ hybridization: P07.26
interstitial 2p13.3p15 deletion: P03.069
Interstitial Cajal Cell: P06.124
Interstitial deletion: J02.07, P03.070, P03.121
Interstitial Duplication in 5p15.31-5p15.2: P02.264
interstitial duplication: P02.116
interstitial fibrosis: P12.154
intervention: EPL6.6
Interview: EP05.02
Intestinal cell kinase: P02.090
Intestinal Metaplasia: J06.14
intra familial phenotypic variation: P06.011
Intra/interethnic variability: P10.31
Intraflagellar transport (IFT): P02.136
Intrathecal Enzyme Replacement Therapy: J14.04
intratumoral heterogeneity: J06.15
Intrauterine fetal demise: C16.4
Inv Dup 15: P03.066
inv dup del: P03.071
invading pathogen response: P11.044
invasive procedures: P05.24, P05.69
Inverse PCR: P11.062
inversion: P03.024, P03.072
Inversions: P11.062
inverted duplication 9p: P03.117
inverted duplication: P02.205
Ion Ampliseq: P11.014
ion channels: P12.034
Ion Torrent: P11.106
Ion Transport: PL2.2
ionizing radiation: P06.014
iPS: P12.245
iPSC: P12.246
iPSCs: P11.063
Iran: P02.252, P04.36, P05.30, P06.125, P06.174, P10.22, P10.40, P12.024, P12.025, P12.090, P12.168
Iranian Azeri Turks: P02.164
Iranian hereditary hearing loss: J12.05
Iranian patients with SCCE: P06.142
Iranian population: J08.03
IRF6 gene: P09.073
IRF6: P08.32
IRGM: P10.54
iron: P04.22
Irp2: P04.22
irritable bowel syndrome: P09.074
ischemic heart disease: J09.42
ischemic stroke: J09.20
Isl1: P12.016
ISO 15189: P15.02
isochromosome 18p: P03.073
isoforms: P13.18
isolate: P09.086
isolated cleft: P03.074
isolated populations: P10.34
isolated: P02.253
Israeli Arab community: P01.18
ITGB3: P04.33
ITPA gene: P14.12
IVF: P04.04
IVS1+1G>A: P02.109
IVSII-666: J05.07

J
J-131: P03.075
Jacobsen syndrome: P02.137, P02.138
JAK kinases: C02.2
JAK-STAT signaling pathway: P08.22
Janus kinase 2: P06.125
jaundice: J02.05
Jeune Asphyxiating Thoracic Dystrophy: P12.126
joint contractures: J02.25
Joubert Syndrome: P02.131, P02.139, P12.178
JSRD: P02.131
junctional epidermolysis bullosa: P12.127
juvenile myelomonocytic leukemia: J07.04, P06.166
Juvenile polyposis syndrome: P12.228

K
Kabuki syndrome: P02.141, P02.140
Kalirin-7: P12.187
KANSL1: C01.1
KaryoArray: P03.076
Karyotype evolution: P07.23
karyotype: J02.40, P03.053, P03.144
karyotyping: P04.31
KBG: P02.142
KBTBD8: P11.048
KCNA5: P09.075
KCNJ11: P13.19
KCNQ1: P12.128
Kearns-Sayre-Syndrom: P02.143
KELL genotype: P05.09
Keratosis follicularis: P02.074
KIAA0196: P02.243
KIAA0442: P09.027
KIAA1267: C01.2
KIAA1328: P05.31
KIAA1632: P12.254
KIAA1797: C02.1
KID syndrome: J02.25
kidney: P02.141, P11.007
kidney-diseases: P12.130
KIF11: S12.1
killer-immunoglobulin-like receptor: J04.05
Kinase: P12.032
Kit gene: P06.124
Kit ligand: P09.034
KIT: P06.102
Klinefelter syndrome: J04.14, P02.144, P03.077, P10.35, P03.130
knee osteoarthritis: J09.21, P08.25
knockout mouse: C13.5
Knowledge: EP06.06, P01.05
K-ras mutation: P06.076
KRAS: P06.075, P06.079, P06.126, P06.127
K-ras: P06.121
KRT14: J12.25, P12.075
KRT5: J12.25, P02.235
KTDC7: P12.179
Kuskokwim Disease: J12.23
kyphoscoliosis: J02.11

L
L1 syndrome: P02.145
L1CAM: P02.145
Label Free Karyotyping: P03.064
Laboratory: P15.10
lactation: P09.005
Lafora: J12.10
LAMA2: P02.146
LAMB3: P12.127
Lameller Ichthyosis: J12.04
LAMIN, DES, EMD: J12.26
laminin: P11.064
Laminopathies: P02.147
LAMP2 gene: P02.073
Langer-Giedion-syndrome: J02.52
Language disorder: P11.039
LARGE gene: P02.149
Large vestibular aqueduct syndorme: P02.150
Laryngeal cancer: P06.129
Laryngeal: P06.128
Late Onset Disease: P04.20
Latvian population: P10.59
law: EP08.04
LDLR: J09.47
LDL-receptor: J12.07, J14.02
learning disability: P02.063, P12.102
learning: P01.37
Leber amaurosis: P12.211
Left Ventricular Filling Pressure: C14.6
Left Ventricular Non Compaction: J12.28
leg ulcers: P09.107
legal abortion: J01.05
legal aspects: P01.29
Leigh syndrome: P13.22, P13.23, P13.31
leiomyoma: J09.22
Lenz microphthalmia: P12.131
LEOPARD syndrome: P02.151
LEP: P13.02
LEPR: P08.26
LEPRE-1 gene: P12.181
Leptin: P12.132
Leri Weill Dyschondrosteosis: P02.152
Leri's pleonosteosis: P12.241
LETM1: P03.139
leukemia: P02.226, P06.130
leukocyte telomere length: P09.035
leukodystrophy: P12.232
Leupaxin: C02.5
Leydig cell hypoplasia: P02.153, P02.154
LGMD: P11.065, P11.066
LHCGR: P02.153
LHR: P02.153, P02.154
Li-Fraumeni Syndrome: C02.4
Limb deficiency: P02.216
Limb-girdle muscular dystrophies: P11.066
limb-girdle muscular dystrophy type 2A: P12.071, J14.03, J12.26
limk1: P02.088
LIMS: P15.12
LINC-complex: C11.6
Lingual plexiform neurofibroma: J02.26
linkage analysis: J12.20, P08.27, P12.202
linkage disequilibrium: P06.189
linkage study: P09.043
lipid genes: C05.3
lipid genotype scores: C05.2
lipid meabolism genes: J04.15
lipid metabolism: C11.2, C12.3
lipid profile: P08.16
lipidomics: P09.076
lipids: P10.37
lipoproteins: C05.3
Lissencephaly: P02.171
Lithuania: P10.41
Lithuanian-Latvian study sample: P09.138
Liver Cancer: P06.116
liver disease: P13.21
liver fibrosis: C12.1
liver metastases: P06.131
LMNA gene: P02.147, P13.24
LMNA: C07.4, P12.134, P12.204
LMX1B: P02.177
Loeys-Dietz: C01.3
LOH: P02.155, P06.068, P06.145
LOI: P04.18
long QT syndrome: P12.128, P12.135
long QT: P12.237
longevity: P09.077
long-term: J02.28
loose anagen hair: P02.187
Losartan: P14.13, P14.14
Low level mosaicism: P03.067
low-penetrance polymorphisms: P06.042
LPL: P11.023
LRP: P06.149
LRRK2: J09.28
LRRTM2: P02.012
LSDB: P11.067, P11.084
LTBP4: C09.5
lung adenocarcinoma: P06.123
lung arteriovenous malformation: P12.112
lung cancer: J07.03, P06.087, P06.122, P06.132, P06.133
lung carcinoma: P06.134
Lung squamous cell carcinoma: P06.121
Lymph node metastasis: P06.045
Lymphoblastic leukemia: P06.015
lymphocytes: P12.189
lymphoedema: J02.51
lymphoid blast crisis: P07.18
lymphoma: P07.22
Lynch syndrome: EP05.04, EP05.07, EPL4.5, P06.135, P06.176, P06.214
lysosomal disorder: P13.36
lysosomal storage diseases: J13.05
LZTS1: P06.002

M
m.3337G>A: P02.260
m.A1555G: P02.156
M374: P11.133
Maat-Kievit-Brunner type: P02.192
Machado Joseph Disease: P09.092, P12.136
macrocephaly: P02.165, P02.213
macrosatellite repeats: P11.068
MACS syndrome: P02.157
Malaysian population: J10.11
Male infertility: J03.09, J04.06, J04.10, J04.15, P03.078, P04.16, P04.24, P04.25, P04.27, P04.52, P04.55
male-pattern baldness: P09.078
malformation: P02.122, P02.061
malfunctions of cardionector: J12.26
Malignancy: P06.152
malignant glioma: P06.136
Malignant pleural mesothelioma: P06.137
malignant transformation: P06.022
malnutrition: P09.003
Malta: P10.36
Management: P02.065
Mandibuloacral Dysplasia type A: P02.147
manifesting carrier: P12.070
Mannose-binding lectin (MBL): P09.052
mapping complex traits: P08.20
MAPT: P02.158
Marfan and Loeys-Dietz Syndromes: P12.137
Marfan syndrome: P02.159, P02.160, P12.086, P14.14
Marker chromosome 19: P05.33, P03.073, P03.079, P03.080, P04.28, P05.32, P03.081
Maroteaux type of acromesomelic dysplasia: P12.223
Marshall-Smith syndrome: P12.138
Marveld 2: P12.139
massive parallel sequencing: P06.033, C16.1
Mathematics: C10.1
Matrix Metalloproteinase genes: P09.114, P06.132
mature lymphoid malignancy: P07.22
Maturity onset diabetes of the young: P02.001
mBAND: P07.07
MBTPS2: P02.048
MC1R: P06.138
MC4R: P09.098

MCA: P03.082
MCA/MR syndrome: P02.168
MCKD: P12.140
MCPH: P08.37
Mcph1: P12.141
MDM2,CDK4,RB1: P07.19
MDR1 3435 C>T: P06.206
(MDR1) 3435 C>T polymorphism: J14.01
MDR1: J09.07, J09.23
MDR3: P12.002
MDS: P07.23
Mechanotransduction: P14.16
MECP2 CDKL5 FOXG1: P10.49
MECP2 duplication: P02.161
MECP2: C04.1, P12.142, P13.46
MED12: P02.192
MED13L: P02.162
Medical Communication: EP02.04
Medical Genetics for the public: P01.21
medical students: J01.06
medullary cystic kidney disease: P02.163
Medulloblastoma: P07.24, P07.25, PL2.1
MEFV gene mutations: P02.098
MEFV gene: J02.27, J10.08, P02.164, P08.38, P12.143, P08.06, P10.22
MEG3: P11.069
meiotic errors: P04.29
meiotic segregation pattern: J03.14
meiotic sex chromosome inactivation: C16.6
MEIS1: P09.103
melanoma: P06.023, P06.138
MEN 1: P06.139
MEN 2A, FMTC ,MEN 2B: J13.07
MEN I: P06.140
Mendel 190: P01.21
mendelian disorder: P12.202
Mendelian randomization: P10.37
Meningococcal disease: C14.3
mental disorders susceptibility gene polymorphism: J09.06
mental disorders: P09.119
mental illness: EPL5.4
Mental retardation: C04.1, C15.2, J05.03, J11.11, P02.014, P02.030, P02.137, P02.165, P03.017, P03.019, P03.051, P03.083, P03.084, P03.085, P03.121, P03.126, P03.127, P03.145, P05.31, P11.070, P11.071
Mental retardation, autism, epilepsy: P03.042
mental: P12.144
merosin deficient: P02.146
Mesenchymal Stem Cell: J15.01, S09.2, P14.15
Meta-analysis: C14.3, C14.4, P08.13, P10.38, P11.094
metabolic bone disease: C12.6
metabolic diseases: P13.34
metabolic disorder: P12.061, P13.25, J13.01
metabolic gene polymorphisms: J09.44
metabolic syndrom: P13.26, P09.067, P13.27, P13.28
metabolic trait loci: C05.4
metabolic: J09.24
Metabolites: P11.143
metabolomics: C05.3
Metabotropic Glutamate Receptor Theory: P14.06
Metastasis: P06.091, P06.150
METFORMIN TREATMENT: P13.28
methemoglobinemia: J12.11
method validation: C16.3
method: C17.3
methotrexate: P06.129
Methylated DNA Immunoprecipitation: C16.2
methylation change: P11.069
methylation index: P11.006
Methylation: C16.5, P06.109, P06.112, P06.141, P06.142, P06.143, P09.031, P11.064, P11.072, P12.093
Methylenetetrahydrofolate reductase: P05.49
methylmalonicacidemia: P13.06
methylome: P06.207
Methylparathion: J03.23
M-FISH: J03.06
mFISH: P03.063
MFRP: P02.166
MFS-Marfan syndrome: P12.239
mGluR5 inhibitors: P14.07
mGluR5: PL3.3
MGMT methylation: P06.106
MGMT: P06.109, P06.144, P06.145
MGP gene: P09.116
MHC Fine-mapping: C17.1
MHC: P06.195
mice expression study: C09.4
mice: J03.23
Micro metastasis: P06.187
microarray technology: P04.40, P11.043
microarray: J03.20, J11.02, P02.234, P03.056, P05.34, P05.54, P08.19, P09.100, P11.093, P11.118, S06.1
microbiomics: P09.074
microbiota: P13.26
microcephaly: P02.167, P02.169, P03.088, P12.145, P12.146
microcytic hypochromic anemia: P08.44
microdeletion 12q13.13: P02.168
microdeletion 15q26.1: P03.092
microdeletion 17q12: P05.35
microdeletion 3q26: P03.093
microdeletion 9q33.3-q34.1: P02.169
microdeletion sindrome: P03.096
microdeletion syndrome: J02.55
microdeletion: P02.008, P02.012, P02.134, P02.180, P02.263, P03.089, P03.090, P03.091, P03.098, P05.10, J04.08
microdissection: P10.08
Microduplication syndrome: P02.170
microduplication: P03.090, P03.095
micronuclei frequency: J03.10, P03.075
micronuclei: P07.20
micronucleus: J03.23
microphthalmia: P02.204, P03.056
microRNA: P02.099, P06.077, P06.078, P06.208, P11.030, P11.094
Microsatellite instability: P06.078
microsatellites: J08.03
Middle East societies: P01.19
Middle East: J10.01
Midwife: P01.08
mielodisplastic syndrome: P06.088
Migraine: J09.25, P09.080, P09.081
Miller-Dieker syndrome: P02.171
minigene: P06.196
minimal deleted region: P07.07
minimal residual disease: P06.059
miR-302: P14.18
miR-449: P06.113
miRNA Biogenesis: P11.137
miRNA: J04.10, J06.17, P02.118, P06.146, P06.201, P09.038
mirror: P11.141
Misalignment: P11.138
miscarriage: P03.094, P05.34
mismatch repair gene: P02.172
Mismatch repair: P04.30
misscarriage: P05.06
missed abortion: P05.14, P04.31
missense mutation: P02.022
mitochondial disease: P13.29
Mitochondria respitoray chain: P09.087
mitochondria: C12.5, J11.11, P09.082, P13.30, P13.31
Mitochondrial complex III genes: J06.05, J06.06
Mitochondrial deletion: P02.260
mitochondrial disorder: P11.073, P12.061, C12.4, EPL3.4, P13.32, P13.45
mitochondrial DNA: P10.39, P10.40, P11.074
mitochondrial tRNA genes: J06.07
Mitochondrial variant detection: P11.075
mitochondrial: P02.143, P13.22
mitotic and meiotic chromosomes: P11.024
mitotic spindle: P09.048
Mixed Gonadal Dysgenesis: P04.58
MKS/JBTS: P11.012
MLC1: P12.147
MLH1 gene: P02.077
MLH1: EP05.03, P11.076
MLL: P07.02
MLPA: J02.03, J02.23, P02.236, P02.252, P03.095, P03.096, P03.127, P05.36, P05.37, P06.025, P06.160, P06.169, P12.013, P12.148
MLPA, array-CGH: P03.114
MLPA-system: J12.21
MMP3: P09.041
MMP-8 promoter gene: P09.090
MMP-9: P06.148
MMR mutations: P06.135
MNPs(Fe3O4): J06.08
MNX1: P12.148
model assumptions: P08.28
Model Validation: P11.016
Model-Based Multifactor Dimensionality Reduction: P08.28
modifier genes: P12.057
Modifiers: P10.20
MODY diabetes: P13.18
MODY: P13.33
MODY/Type 2 Diabetes: P11.077
Molecular analysis: P02.128
Molecular and Histopathological methods: J04.13
Molecular Combing: P12.098
molecular diagnosis: J11.10, P11.050, P11.102, P12.096
molecular diagnostics: P12.071
Molecular genetic testing: J15.02
Molecular karyotype: P02.025
Molecular Karyotyping: C04.2, P02.009, P02.077, P03.067, P03.068, P03.120
molecular profile: P06.177
molecular screening: P12.130
monoclonal gammopathy of undetermined significance: P07.26
Monosomy 11q: P03.107
Monosomy 21: P09.056
monosomy 9p: P03.108
monosomy chromosome 7: P02.173
Monozygotic twins: C04.4, P03.004, P09.120, P11.027
mood disorders: P11.004
MOPD type 2: P02.208
MOR gene: J02.01
morbid obesity: P03.010
Morin: J06.02
Morocco: P10.15
mosaic 22q11 microdeletion: P05.39
mosaic Fanconi's anaemia: P12.174
Mosaic Homozygosity Reporter: P11.078
Mosaic Turner syndrome: P02.066
mosaic: C04.5, P03.054, P05.38
Mosaicism: J02.26, P02.255, P03.134, P03.141, P04.32, P05.55, P05.57, P11.132, P12.095
mother's milk: J06.11
motivation to enroll: P01.03
Mouse: S04.2
mov10L1: J04.17
Movement disorders: P12.079
Moyamoya: P02.174
MPS II: J02.28
MPS type 1: J14.04
MRP1: P06.149
MSc Genetic Counselling: EP06.01
MS-MLPA: J02.04, P02.041
MSX2: C03.2, P02.045
mtDNA alteration: P06.150
mtDNA mutation: J06.05, J06.06, J06.07, P10.19
mtDNA: J10.14, P06.020, P10.41
MTHFR 677T: P02.117
MTHFR: P06.151, P09.083, P11.029
MTHFR, Factor II, Factor V: P04.53
MTHFR, MTR, MTRR: J09.26
MTHFR-CLCN6-NPPA-NPPB: C14.6
MTHR C677T: P06.152
mTOR inhibition: P14.20
mTOR: PL3.2
MTR: P04.33
MTRNR1 gene: P10.19
MTT: J13.14
mucopolysaccharidosis type I: J13.06
mucopolysaccharidosis: J13.12, P13.34
multi drug resistance: P06.049
multi-ethnic: P09.059
Multifactorial diseases: J09.43
multiFISH: P04.28
multifocal: P06.107
multiple congenital anomalies: P02.028, P03.047
Multiple endocrine neoplasia type 2 (MEN 2): J13.07
multiple myeloma: J06.20, P07.27
multiple sclerosis (MS): P10.33
Multiple Sclerosis: J09.41, J11.02, P09.084, P09.085, P09.086, P09.087, P09.088, P09.089, P09.139, P11.079
Multiples chromosome alterations: J03.11
Multiplex Ligation-dependent Probe Amplification: P07.09
Multiplex PCR: P11.129
Multiplex: J11.03
Multisomatoform Disorder: P08.29
mUPD7: P02.233
muscle fibers: P08.30
muscular dystrophy: P12.087, P12.150
Mutagens: J10.09
mutant mouse model: P11.045
mutation database: P02.175
mutation detection: P02.064, P06.201, P12.023
Mutation distribution: P12.099
mutation frequency: P13.47
mutation panel: P12.170
Mutation prediction: P11.017
mutation rate: S10.1
Mutation Screening: P06.096, P12.212
MUTATION: J04.07, J05.10, J12.24, J12.33, J12.34, J12.35, J13.15, P02.211, P05.16, P06.038, P06.065, P06.103, P06.140, P08.06, P08.38, P11.067, P11.084, P11.133, P12.067, P12.113, P12.233, P12.261, P13.15, P13.35
mutation,: P12.197
mutation; IDH2: P06.048
mutations analysis: C14.1
mutations of RAH gene: J12.19
mutations: J02.18, P02.135, P06.062,

P06.094, P12.040, P12.083
Mutations: J02.27
MUTYH: P06.065, P06.154
MYBPC3 MYH7 TNNT2: J02.48
MYBPC3: J09.18, P12.104
Mycoplasma sp. DNA: P06.180
myelodysplasia: P02.173
Myelodysplastic Syndromes: P07.28
Myeloproliferative disorders: P06.125
MYF6: P08.30
MYH7: J12.28, P12.104
Myhre syndrome: C03.5, P12.227
myocardial infarction: C05.5, P09.046, P09.090, P09.137, PL1.3
myopia: P02.044
Myotonia congenita: P12.152
myotonic distrophy: J03.04
myotonic dystrophy type 1: EP03.01
myotonic dystrophy type 2: J10.06
myotonic syndromes: P02.176
Myriad: P06.032

N
NADH dehydrogenase IV (ND5) gene: P11.041
nail dysplasia: P12.153
Nail-Patella Syndrome: P02.177
NANOG: P14.18
Nanoparticles: P06.043
NanoString: P06.181
Nasal polyposis: P06.097
nasopharyngeal carcinoma: P06.155, P09.142
National Register: J10.02
natural killer cell deficiency: P12.174
NB4 cells: J06.03
NBN: P06.156
NDUFAF2: J02.50
NDUFS4 gene: P13.32
Necrospermia: P04.49
necrotizing enterocolitis: J05.13
Negotiation: EPL2.3
NEK1: P12.220
nemaline myopathy: P02.055
neonate: P13.11
neonates: J05.14
neovascular AMD: P09.091
nephrotic syndrome: P12.154, P12.155, P12.156
nerve regeneration: P14.15
Nested-PCR: P05.23
Network: C17.5
Neu4: P11.080
Neural tube defect: P11.029, P12.157
neurite outgrowth: P09.105
neuroblastoma (NBL): P07.33, J06.12
Neurocognitive phenotype: P02.144
Neurodegeneration: P12.122, P12.231
neurodegenerative disease: P10.04, P09.092
neurodevelopmental disease: P12.246
neurodevelopmental disorder: P02.004, P03.097
Neurofibromas: P12.164
Neurofibromatosis 1: P12.160, P14.16
Neurofibromatosis type 1: J02.29, P02.180, P06.157, P06.158, P06.168, P12.161, J02.26, P06.159, P06.160
Neurofibromatosis: J02.30, P02.178, P02.179, P12.158, P12.159
Neurofibromin: P12.158, P12.162
neurogenesis impairment: C04.6
Neurogenesis: C13.4
Neurogenic Inflammation: P09.081
neuroleptic: P02.024
Neuromuscular disorders: J11.10, P11.081
neuromuscular transmission: P02.064
Neuromuscular: P01.30
Neuropathies: P05.41
neuropathology: P08.03
Neuropathy: C01.5, P10.14, P12.223
neurotransmitter systems: P09.105
neurotrophic hypothesis: P11.004
Neurotrophine 3: P14.17
neutral variant: P02.087
new generation sequencing: P11.083
New Mutations: J13.09
new syndrome: P03.093
Newborn screening: J09.24, P14.09
Newborn: J13.03
Next Gen sequencing technology: P11.025
Next generation sequencing (NGS): P11.130, C02.6, C03.6, C07.2, C07.3, C10.5, C11.2, C15.3, J09.25, J11.10, P02.250, P06.161, P06.178, P11.012, P11.034, P11.037, P11.050, P11.073, P11.084, P11.085, P11.086, P11.087, P11.112, P11.132, P12.082, P12.105, P12.137, P12.163, P13.07, P15.03, P11.129, C15.5, P02.201, P11.102, P11.125, C13.2, P06.028, P11.028, P11.088, P12.080, P12.159, P12.211, P12.251, P12.253, S16.1, P02.143, P12.160
NF1 haploinsufficiency: P14.16
NF1: J02.29, J02.30, J02.31, P06.157, P06.158, P06.159, P06.160, P12.033, P12.158, P12.160, P12.162, P12.164
NFIX: P12.138
NF-KappaB: C06.5
NFkB pathway: P09.039
NF-Y: P11.089
NGS data-sets: S16.1
NGS panel: P02.136
NGS: C12.6, P02.060, P02.217, P05.41, P11.054, P11.085, P11.090, P11.091, P12.038
NHLRC1: J12.10
Nicolaides-Baraitser syndrome: P02.237
nicotine dependence: P09.093
NID2: P06.143
Niemann pick disease: J13.08, P12.165, J13.09
Niemann-Pick type C disease: P13.36
Nijmegen Breakage Syndrome: P07.32
NIPBL: P02.066, P12.043
NIPD: P05.42
niRNA: P06.162
NIS: P06.163
NLRP7: P11.026
NLS: J12.27
NM23H1 gene: J06.09
NMDA receptor: J11.06
NMDAR1 gene: P09.063
NMR spectroscopy: P12.120
NOG: J02.14
Non- invasive: P05.23, P05.43, C16.2
Non- syndromic cleft lip with/without palate: P09.132
non-coding RNA: P11.093, P11.109
non-distrophic myotonias: P12.166
Nonfracture Osteogenesis imperfecta: P02.181
non-invasive prenatal diagnosis: C16.1, EPL3.1, EPL3.2, S16.3, P05.13
Non-Obstructive Azoospermia: J04.13
nonophthalmos: P02.166
non-progressive congenital ataxia: P12.167
Non-Responder: C01.6
nonsense mutation: P11.142
Nonsense-mediated mRNA decay: C13.3
Non-Small Cell Lung Cancer: P06.079, P06.164, P11.094
Non-Small Cell Lung Carcinoma: P06.165
Non-syndromic Hearing Loss: P12.168
non-syndromic mental retardation: J09.46
Nonsyndromic sensorineural hearing loss: P12.169
Non-syndromic: P12.107
Noonan syndrome with multiple giant cell lesions: P02.188
Noonan syndrome: P02.185, P02.186, P02.187, P06.166, P12.171, P12.172, P12.222
Noonan: J02.54, P02.175, P02.182, P02.184, P12.170
Noonan/CFC-syndrome: P12.175
NOP56: P12.216
normal hearing function: P08.31
Notch1: P06.061
notch3: P02.050, P12.173
novel expression profile: P10.16
novel insertion: P02.251
novel mutation: J12.01, J13.06, P02.052, P02.085, P02.235, P02.243, P12.090, P12.171, J13.08, P02.163, P12.029
novel syndrome: P12.174
NPC1: P13.36, P13.37
NPC2: P13.37
NPHS1: P12.155
NPHS2: P12.155
NPS: P02.177
NQO1*2: P13.48
NRAS p.G12S: P12.175
Nrf2: J15.01
NRXN1: P03.097, P03.098
NSCL/P: C14.4, P08.32
NSCLC: C10.3
NSD1: P02.189, P02.240
NSDHL: P12.047
NSHL: P12.139, P12.169
nsSNPs: P11.095
NTD: J09.26
NTERA2: P04.11, P11.124
NTM: P02.138
Nuclear localization: P06.203
nuclear organization: C04.3, P03.027
nuclear pore complex: P12.036
nucleic acid purification: P11.131
numerical abnormalities of human chromosomes: P05.03
NUP98: P07.02
nursing: EP07.03
Nutrition: P11.076

O
obesity: P02.214, P06.211, P06.215, P09.061, P09.097, P09.098, P10.12, P10.55
observance: P01.04
OCA2: P12.177
Occupational exposure to Formaldehyde: P07.31
OCT4: P14.18
OCTN2: J02.37
Ocular: P02.182
oculo-auriculo-vertebral: J02.32
Oculocutanous Albinism: P12.177
oculodentodigital dysplasia: P02.193
ODDS: P02.193
oesophageal atresia: P09.099
of hereditary, prelingual, nonsyndromic hearing lo: P12.100
OFD1: J02.33, P12.178
Ohdo syndrome: P02.192
olesoxime: P14.11
oligo microarrays: P03.122
Oligodendroglioma: P06.167
Oligodontia: J12.14
oligozoospermia: P04.50, P04.57
oman: P12.205
-omics approaches: S07.2
OMIM#114000: P12.053
omphalocelle: P02.018
oncogenes and tumor-suppressor genes: P06.093, P06.164
oncogenetic services: EP06.05
Oncogenetics: EPL6.3, C02.4
Online communities: J01.09
online education: EP06.08
Online learning: EP06.03
online resources: EP06.02
oocytes: P04.03
oophorectomy: EP05.09
OPCML: P02.138
ophthalmic: P02.042
opioid requirement: P09.050
OPMD: C06.6
Opsoclonus-Myoclonus-Ataxia: P12.179
optic atrophy: P02.010
optic nerve glioma: P06.168
oral cancer: P06.169, P06.170
oro-dental abnormalities: P02.207
orofacial clefts: P01.33
orthodontic treatment: J02.20
orthologues: P11.095
osteoarthritis: P09.100, PL2.5
osteocyte: P12.162
osteofibrous dysplasia: P02.194
Osteogenesis Imperfecta: C03.3, J12.22, J14.07, P02.195, P02.196, P11.102, P12.180, P12.181, P12.183, S15.3
osteoporosis: P09.101, P09.102, P09.103
OTOF: P12.168
OTOFERLIN: P12.184
ototoxic aminoglycosides: P02.156
outcome: P05.55
outcomes of pregnancies: J10.09
Outcomes: EPL5.6
ovarian cancer: EP05.09, EP05.10, EPL2.4, EPL4.2
ovarian tumors: P06.110
ovarian: P06.171
over-expression: P06.035
Overgrowth: P02.197
oxidative stress: J04.10, J09.42, P09.022, P14.08
oxphos complex I deficiency: P14.19
OXPHOS deficiency: P13.23
oxsalosis: EP04.03
oxytocin and vasopressin systems: P09.049

P
p16/INK4a: P06.122
P2RY12: P10.43
P2RY5: P12.123
P450 oxidoreductase: P10.42
p53 gene mutation: P06.174
p53 gene: P11.086
p53: P06.020, P06.172, P06.173, P10.24, P11.103
P86L: P09.011
PABPN1: C06.6
Paget’s Disease of Bone: P09.104
PAH gene: P12.195
Pain: P02.239, P09.051
PAK7: C06.4
Pallister-Killian syndrome: J05.09
palmitoyl transferase: P02.265
Pancreatic cancer: P01.34
panic disorder: P11.104
Pantothenate kinase associated neurodegeneration: J12.24
Pantothenate kinase: J12.24

papillary thyroid cancer: P06.175
Papillion-Lefevre syndrome: P02.198, P12.186
paracentric inversion: P02.179, P03.105
Paracrine: S09.2
Paraganglioma, GIST: P06.051
parametric linkage: P08.35
parental origin: P03.037, P05.68
parent-of-origin: P06.191
Parents: EW1
parial trisomy 7q: P03.106
parietal foramina: P02.020
parkin: J09.28
parkinson disease: P02.007, P12.187, P12.188, J11.08, J09.27, J09.28, P12.189
partial lipodystrophy Dunnigan-type: P13.24
Partial microdeletions: P04.27
Partial monosomies: J03.15
partial monosomy 10q: P03.109
partial monosomy 13q: P03.106
partial monosomy 9p, partial trisomy 9p: P03.117
Partial Trisomy 1q: P03.107
partial trisomy 2p: P03.108
partial trisomy 3p: P03.109
partial trisomy 7q: P03.110
partial trisomy: J03.05
Patau syndrome: P04.47
Pathogenesis prediction: P11.041
pathogenic mutation: P13.29
pathological biomarkers: P11.055
pathway-based association study: P09.105
patient expectation: EPL7.2, EP07.02
patient experience: EP07.03
patient perception: EPL7.2
patient perspective: EPL2.5
Patient preferences: P01.35
Patient Reported Outcome Measures: EPL1.6
Patient story: P01.08
PAX6: P12.013
PCDH19: P12.081, P12.190
PCG: P12.191
PCNT: P02.208
PCOS: J04.12
PCR technique: J05.03
PCR: J03.21, P05.47, P06.180
PCR-RFLP: J06.01
PCR-SSCP: J09.18
PD: J12.35
PDE4D: C09.2
PDE6A: P11.112
Pde6c: P09.109
PDGFRA: P06.102
PDGFRB: P06.064
pediatric patients: P02.108
pediatric: P06.080
pedigree segregation analysis: J03.14
pegnancy: J03.11
PEHO syndrome: P12.192
pejvakin gene: J12.12
Pelizaeus–Merzbacher disease: J02.34
Pendred syndrome: P02.150
penetrance: P12.128
Peptidylariginine deiminase: P09.117
peptidyl-prolyl cis-trans isomerase: C18.3
pericentric inversion: J03.05, J03.12
periodontitis: P09.106, P11.127
Periventricular nodular heterotopia: P02.101
peroxisomal disorder: J02.35
Perrault syndrome: P12.193
Persian Population: J09.31
personality: P09.014
Peters plus syndrome: P12.194
Peutz Jeghers syndrome: P12.236
Peutz-Jeghers syndrome: P12.228
PEX7 gene: P12.208
PF22 (DNAAF3): P12.200
PGD: P04.36
PGM: C08.2, P06.126, P11.009, P11.010, P11.105, P11.106
PGS: P01.16
PHA2: PL2.2
PHAII: P12.206
pharamacogenetics: P09.021
PHARC: P12.252
pharmacogenetic: P02.199
pharmacogenetics: P06.008, P06.066, P09.121, P09.122, P10.42, P11.107
Pharmacogenomics: J01.10, P11.108, S07.1, S07.2
phasing: C17.2
Phenocopy: P02.001
Phenomics: C09.1
phenotype: P02.040, P03.111
Phenotypic map: P03.116
phenotypic variability: J02.02, P03.008, P06.218, P12.217
phenotyping: P11.045
phenylalanine hydroxylase: P12.195, J13.15, P13.39
Phenylketonuria: EP08.01, J12.19, J13.15, P12.195, P13.39
Phf13: C16.6
Philadelphia chromosome: P07.15
Phosphatase: P12.032
phosphate homeostasis: P13.12
phosphatidylserine: P14.04
phospholamban gene (PLN): J02.47
physical performance: P08.17
physician's attitudes: EPL2.2
Pierre Robin sequence: P11.123
Pierre Robin: P09.125
PIGV: P03.043
PIK3CA: P06.108
Piper genre: J10.07
Pitt-Hopkins: P05.48
PITX1: C03.1, P12.031
PITX2: J11.09, P02.037
PIWIL2: P04.25
PJ34: P06.163
PJVK: P12.025
PKD1: P12.005
PKD2: P12.005
PKHD1: P12.015, P12.196
PKP2: P12.017
Plasma DNA: C10.6
plasminogen activator inhibitor: P05.64
Platelet transcriptome: P11.109
PLCE1: P12.156
pleiotropic effects: C05.4
PlEKHG5: J11.09
pleuropericardial manifestations: P02.098
PLS3: P12.225
plug-in: J11.04
Pluripotency: P11.089
PMD syndrome: P12.197
PMD: J02.34
PMM2: P12.198
PMP22 gene: J05.05
PMS2: P06.176
PNPLA1 mutations: C18.2
podocyte: P11.125
POF: P04.15
POI: P12.094
Point mutation: P02.119
Pol II: C06.5
polar bodies: P04.03
polar body diagnosis: P04.29
Polar body: P04.37
POLG1: J02.02
POLR1D: P12.248
polycaprolacton nanofibre: P14.15
Polycystic kidney disease (ADPKD): P02.200, P02.201, P02.163
Polycystic liver disease (ADPLD): P02.200
Polycystic ovary: P13.41
polydactyly: P02.202
Polyglutamine disease: P14.21
polyglutamine: P12.136
polymicrogyria: P02.203, P02.204, P12.199
polymorphic DNA loci: P10.44
polymorphism and expression: P04.48
polymorphism: J02.19, J04.17, J06.04, J08.06, J09.03, J09.10, J09.13, J09.21, J09.22, J09.23, J09.29, J09.35, J09.39, J12.12, P04.21, P04.51, P06.010, P06.045, P06.052, P06.206, P08.25, P08.36, P08.41, P09.003, P09.007, P09.025, P09.102, P09.123, P09.127, P13.28, P04.16, P06.183, P09.107
polymorphisms in TCF7L2 gene: P09.053
polymorphisms:
polyposis of colon: P06.162
PON1: P09.108, P10.43
Popliteal pterygium syndrome: P12.027
population genetic survey: P10.44
Population genetics: C06.3, P11.074
population isolate: P10.45
Population screening: J01.04, P11.003
Population stratification: P10.46
population: J01.10, J10.10, P09.077, P10.40, P10.41
porencephaly: P02.204
PORPHYRIA: P13.42
postmenopausal women: P09.061
postmortem genetic testing: P02.245
Potocki-Lupski syndrome: P02.205
POU3F4: P12.258
power: J09.40
PPAR/PGC-1-alpha: P14.19
PPARG: C06.2, J09.40
Ppib: J12.22
PPP2R1A: P06.177
Prader Willi Syndrome: P02.128, P12.036, P02.206, P02.207
Prader-Willi Angelman Critical Region: P03.128
PRDM9: P04.38
preaxial polydactyly: P12.256
prediction: C08.6, P11.110
predictive diagnosis: P03.090
predictive marker: P06.172
predictive testing: P01.17
predisposition: C02.2, J09.01
preeclampsia: J09.12, P04.39, P04.40, P04.41, P05.49
pregnancy loss: J03.13, P04.04
pregnancy rate: P04.42
pregnancy: EP04.01, P02.104, P04.33, P05.47
pregnant women disorders: J01.05
preimplantation diagnosis: P05.50
pre-implantation Genetic Diagnosis (PGD): P03.039, C16.3, P04.42, P05.51, P05.59
preimplantation genetic screening: P04.43
preimplantation: P04.30
pre-malignancy: P07.32
pre-mortem hippocampus: C08.5
prenatal ascitis: P05.18
Prenatal BoBs®: P05.57
Prenatal care: P02.196
prenatal diagnosis: EP03.01, EPL7.4, J01.03, J05.01, J05.08, J05.11, P01.10, P03.072, P03.112, P03.113, P03.134, P05.05, P05.11, P05.12, P05.33, P05.37, P05.39, P05.58, P05.59, P05.60, P05.68, S06.1, EPL6.1, J01.01, J05.04, P05.61
prenatal megacystis: P03.047
Prenatal screening programmes: S16.3
prenatal test: J05.12
prenatal tumour: P05.70
Prenatal: EPL1.2, J05.02, J05.09, P05.10, P05.52, P05.53, P05.54, P05.55, P05.56, S06.2
PREPL: P03.006
Presymptomatic testing: EP01.02
preterm delivery (PTD): P13.43
pre-test counseling: P05.60
prevalence rates of hereditary disorders: P10.27
prevention: P01.15
Previvor: EPL2.3
PRG4 gene: P02.051
Primary care: P01.22
Primary Ciliary Dyskinesia (PCD): P12.200
primary cilium: P09.048
Primary coenzyme Q10 deficiency: P13.44
Primary congenital glaucoma: P11.017
primary hyperoxaluria: P12.201
primary hypertrophic osteoarthropathy (PHO): P12.068, C09.3
Primary Immunodeficiency Disorders: C15.4
primary liver cancers: P06.178
primary lymphedema: P12.202, P12.203, S12.1
Primary microcephaly: C13.4, P08.37
primate evolution: P10.05
primate gene family: P10.16
Primordial dwarfism: P02.208
Primrose syndrome: P02.209
Priority setting: EPL1.1
PRKAR1A: P12.003
PRKCA: P09.088
problems: J02.10
Profession: P01.20
professional practice: EPL5.2, P01.23
progeroid syndrome: P12.204
PROGINS: P06.034
Prognostic Biomarkers: P06.181
prognostic marker: P06.172, P06.006
prognostic value: P06.046
Progressive myoclonic epilepsy: P12.205
Progressive Osseous Heteroplasia: P02.100
Prokineticin receptor: P04.45
prolin synthesis: P13.45
Prolyl 3-Hydroxylation: J12.22
prominent cardiac hypertrophy: P02.151
promoter hypermethylation: P06.155
promoter: J09.08, J09.47
pronuclear and maternal spindle transfer: EPL3.4
prophylactic mastectomy: EPL2.2, EPL4.4
propofol: P13.48
prosopagnosia: P10.47
prostaglandin transporter SLCO2A1: P12.068
prostaglandin: C09.3
prostate cancer: C02.5, P06.094, P06.127, P06.179, P06.180, P06.181, P06.182, P06.183, P06.184, P06.193, P06.213
prostate tumour: P06.185
protamine gene: P04.51
protein modeling: P12.186
Proteus syndrome: P02.210

Proteus syndrome, Proteus-like syndrome: P14.20
protrombine mutation: J02.45
Proxy-SNP: P10.38
Prpf31: P09.109
PRPS1: P12.169
PRRT2: P12.028, P12.079
PRRX1: P12.006
PRSS1: P02.211
pseudoarthosis: P02.194
pseudogene integration: P11.111
pseudogene: P04.13
Pseudogenes: P11.138
Pseudohypoaldosteronism: P12.206
Pseudohypoparathyroidism type 1a (PHP-1a): P02.212
Pseudohypoparathyroidism: P11.056, P12.003, J02.49
pseudoxanthoma elasticum: P12.207
Psoriasis: J09.29, P09.110, P09.124, P12.262
psoriatic arthritis: C14.5, P09.111
Psychiatric disorders: P03.041, P09.112
psychiatric symptoms: P02.144
psychological aspect: EPL7.3
psychological challenges: EPL7.4
psychological impact: S08.1
Psychological well-being: EP01.02
psycho-onco-genetics: S08.3
psychosis: J09.08, P09.113
psychosocial impact: EPL4.5
Psychosocial problems: EPL6.3
psychosocial support: EP06.05
PTCH: P02.113
PTCH1: P06.110
PTCHD1: P09.028
PTEN: P02.038, P02.213, P06.105, P06.107, P06.217
ptosis: P02.214
PTPN11 gene: P02.151
PTPN11: J02.54, P02.182, P02.184, P02.185, P02.188, P06.166, P12.170, P12.171, P12.172
PTPN11, SOS1: P02.186
public health: J01.10
public view: P01.14
pulmonar hypertension: J03.16
pulmonary arterial hypertension: P02.215, P09.075
pure subtelomeric microduplications: P03.114
putative pathogenic mutations: P12.111
putative telomerase suppressor: P06.055
PYCR1 defect: P13.45
PYCR1: C09.6
pyrosequencing: P06.084, P06.204

Q
Q318X, R356W: P12.263
qatar: P12.105
QF-PCR: P03.113, P05.04, P05.30, P05.38, P05.57, P05.62
qPCR: P09.020, P11.013, P11.022, P12.093
QTL mapping: C08.3
Qualitative analysis: P05.24, P05.69
qualitative methods: EPL3.5
quality assessment: EPL2.5
Quality assurance: P15.10, P15.11
Quality Control: P11.119, P15.14
quality management: EP02.02
quality of life: EP04.02
Quantitative fluorescence-PCR: P05.65
quantitative real-time PCR: J05.05
quantitative trait locus: P09.024
quantitative traits: P08.35, P09.077
Questionnaire: EP06.06, EP08.03

R
r(7): P07.23
R14Del: J02.47
RA: P08.38
RAD51C: P06.186, P07.05
RAD51D: P06.118
Radial ray deficiency: P02.216
radiation exposure: J03.17, J07.03
Radiation induced mucositis: P09.142
radiosensitivity: P12.019
radiotherapy: P06.173
RAG-1: J11.03
randomized controlled trial: EPL5.4, EW2
Range of Motion: P09.114
rapid BRCA1/2 genetic testing: EPL7.3
rare disease: P10.48, P01.37
rare genetic disorder: P02.139
rare SNP: J09.12
Rare variants: P08.23, P08.39, P10.45, P11.117
RAS/MAPK pathway: P02.188
Ras-MAPK: P02.186
rasopathy: P02.175, P02.217
RASSF1A gene: P06.040
rat model: P14.11
RB1 gene: P06.190
RB1: P11.111
RBPs: P11.008
RCAD: P05.35
RCDII: P09.115
RCDP disease: P12.208
RCDP: J02.35
Real Time PCR: P06.056, P06.007, P06.053, P06.193
real-time quantitative polymerase chain reaction: P07.10
Receptor activator for NFκB ligand: P09.089
recessive disorders: P12.055
recessive inheritance: P12.218
Recessive mutations: P12.181
recipient: P02.084
reciprocal translocation t(1;11) (p36.22;q12.2): J03.14
Recombination: P04.14, P06.176
rectal cancer: P06.173
Recurrence: P11.019
Recurrent abortion: P03.033, J03.12
recurrent deletion: P12.138
recurrent erosion: J09.19
recurrent infections: P12.004
recurrent miscarriage: J03.07, J04.04, J04.05, P04.44
recurrent mutation: P13.24
recurrent pregnancy loss: P04.21, P04.45, P04.46
recurrent spontaneous abortion: P04.19
recurrent spontanues abortions: J08.04
recurrent translocations: P07.28
reduced folate carrier-1: P05.63
Reduced-folate carrier-1: P05.49
REEP1: P02.242
Reference gene: P09.020
refractory celiac disease: P09.115
registry: P01.30, P10.48
regulation: P01.29
rehabilitation: J14.03, P02.125
Relative: P06.152
renal and vesicoureteral abnormalities: P02.070
Renal cell carcinoma: P06.187, P06.188, P06.189
renal defects: P02.140
Renal Stone: P09.116
renal transplantation: J09.05
renal tubular dysgenesis: P12.209
renal: P06.191
Renin: P12.140
repeated measurements: P08.40
Repeated pregnancy loss: P04.53
repeated sequence: J04.09, P03.037
Replication study: P08.32, P09.010
reported mutations: P13.46
reproduction: EP03.02
reproductive history: J09.32
reproductive loss: J04.01, J04.02
reproductive options: EP03.01
reproductive patterns: P10.24
research participation: EPL6.5
Research: EP08.02
Resequencing: P11.090
Resource allocation: EPL1.1
respiratory distress: J05.14
Response to chemotherapy: S09.3
RET gene: J13.07
retardation: P12.144
reticulate pigmentary disorder: J02.53
retinal degeneration: P12.047
Retinal Dystrophies: P12.210, C15.6, EPL5.6, P02.166
Retinitis Pigmentosa: C11.5, C15.6, P02.075, P11.112, P12.088, P12.211
retinoblastoma: P06.190
Retinopathy of prematurity: P05.64
retrospective: J15.03
Rett Networked Database: P10.49
Rett syndrome: P03.115, P10.49, P12.142, P12.212, P13.46, P12.042
Revertant mosaicism: P11.078
RFLP-PCR: P09.090
RGMA: P03.092
RHD gene: P05.56
RHD genotype: P05.09
RHD: P05.42, P05.43
rheumatoid arthritis: J09.03, J09.30, P08.15, P09.117, P09.137
Rhinitis: P08.41
Rhizomelic chondrodysplasia punctata: J02.35
RHO: P12.001
rhombencephalosynapsis: P02.112
RHOXF1: P06.192
ribosome: C12.5
Ribosomopathy: S04.2
Rieger: P02.037
Right Ventricular Arrhythmogenic Cardiomiopathy (A: J02.46
RIN2 gene: P02.157
Ring 14: P03.116
ring 9 chromosome: P03.117
ring chromosome 13: P03.118
ring chromosome 18: P02.219
ring finger: P02.135
ring X: P03.136
RIPK4: P12.027
risk assessment: P08.07
risk communication: EPL6.2
risk factor: J08.04
Risk for schizophrenia: P09.141
risk prediction: P09.072
risk reducing mastectomy: EPL7.3
risk reducing surgery: EPL4.5
risk: J09.36
RLIP76: P06.130
RMRP: P12.039
RNA modifications: P09.060
RNA sequencing: P11.109
RNA: P12.033
RNAi: C05.1
RNA-seq: P08.13, P11.010, P11.106, P11.113, P11.114
RNASeq: S14.2
Robertsonian translocation: P03.119, P04.47
Roche GS junior: P12.251
roche junior: P12.150
Roferon-A: P02.220
ROGDI: C18.5
Roma (Gypsies): P10.50
Roma population: P12.139
Roma: P10.25, P10.30, P10.43
Roma, Hungarian: P10.52
Romania: J09.04
RP1 gene: P12.074
RPL: P04.48
RPL5: P02.081
RPS26: P02.080
rs12979860: J09.16
rs4132601: P06.005
rs4680: P09.093
rs601338: P09.037
RSA, CL/P: J09.26
RT-PCR: P06.114
RTTN: P12.199
Rubinstein-Taybi Syndrome: P02.221, P02.222, P12.213, P12.214
RUNX1 gene: P03.005
RUNX1: P06.022
RUNX3: C14.5
Russian population: P10.59

S
sa98lu10: P02.223
SAA: P15.01
sacs: J12.34
Saethre-Chotzen syndrome: P02.224
sagittal suture: P12.264
SALL4: J06.19
Sample Management: P15.12
sample preparation: P04.12
sample QC: P11.085, P11.087
Sanger sequencing: P11.075, P11.115, P15.13
SaOS-2: P11.116
sarcoidosis: J13.10
SCA: J12.13
SCA19: C11.3
SCA3/MJD: P14.21
SCA36: P12.216
scalp defects: P02.225
Schinzel-Geidon syndrome: P02.226
schizophrenia: C06.4, J11.05, P02.010, P09.118, P09.119, P09.120, P09.121, P09.122, P09.123, P11.117, P11.118, S05.2, S10.3
SCID: C15.4
SCN1A gene: P12.080, P12.217
SCN1A: J02.12, P02.094, P12.078
SCN1B: J02.12
SCN5A: P12.035
SCN9A gene: P02.239
SCO2: P13.30, P13.47
scoliosis: P02.197
Scoring System: P04.20
scorpion: J03.01
screening criteria: P06.031
Screening: EPL1.2, EPL1.4, EPL2.4, EPL4.2, P06.041, P06.067
SDHB: P06.051
SDHD: P06.191
Seborrheic dermatitis: P12.262
second malignant neoplasm: P07.33
SEDC: P02.115
segmental duplication: P03.105, P10.51, P10.05
segmental NF1: J02.31
Segmental overgrowth disorders: P14.20
segmental upd 14: P03.138
Seizures: P03.116, P12.101
Selection: P10.02

self renewal gene: J04.03
self-collecting saliva sample: P10.32
Self-renewal: P06.050, P06.119
SEMA3A: P12.218
Semen parameters: P04.49
Sengers syndrome: C12.3
sensory neuropathy: P12.004
sequence quality: P15.13
Sequence Read Archive: P11.141
sequence: P06.004
Sequencing non-deleted allele: P09.141
sequencing: C08.3, C17.2, P08.19, P11.119, P11.128, P12.075
Serotonin receptor gene expression: J09.30
serous endometrial carcinoma: P06.177
SERPING1 mutations: P12.110
Sertoli cell tumor: P02.227
Serum triple marker screening: P05.65
SETBP1: P02.226
severe ID: P03.093
severity: P09.104
sex chromosomes: P03.125
sex reversal: P03.108
Sex-specific: C06.2
SGA: P11.120
SH2 domains: P12.222
SH3TC2: P12.219
SHANK2: P12.021
SHANK3: J02.03
SHB gene: P06.182
SHH: P02.123, P02.228, P02.229, P02.238
SHMT: P06.090
SHOC2: P02.187
Short rib-polydactyly syndrome Majewski: P12.220
short stature: C03.5, C03.6, P02.021, P03.120, P12.221, P12.261
SHOX duplication: P03.121
SHOX: P02.152
Shox2: P12.016, P12.221
SHP2: P12.222
Shwachman-Bodian-Diamond-syndrome: P02.230
sickle cell disease: EPL3.5
Silibinin: P06.193
Silver Russel syndrome: P02.231, P02.232, C04.2, P02.091, P02.233, P02.234
Simplex EB: P02.235
Simulation Study: P08.27
simultaneous pancreas and kidney transplant: P02.084
Single cell PCR: J04.09
single nucleotide polymorphism (SNP): P09.065, P09.052
Single seq RNA-seq: C08.2
single-cell: P03.122
single-nucleotide polymorphism: P09.124
single-nucleotide primer extension assay: P06.042
sirenomelia sequence: J02.36
SIRT6: P11.121
six year old girl: P03.131
Sjögren's syndrome: P12.223
skeletal abnormalities: P03.048
skeletal dysplasia: C09.2, P02.230, P12.183, C09.1
Skeletal muscle dystrophy: C06.6
skeletal muscle: J09.44, P09.083
Ski complex: P12.238
skin biopsy: P07.32
Skin cancer: J06.21
skin disorder: P02.033
SLC22A12: P13.17
SLC22A5: J02.37
SLC26A4: P02.150, P12.224
SLC26A5: P12.224
SLC2A1: P02.110
SLC6A14 polymorphisms: P04.50
SLCO1B1: P10.52
Sleep apnea: P08.42
SLOS: P02.236
Slovenia: P12.110
SMA: C01.6, EPL3.6, P12.225, P12.265
SMAD3: P02.023, P12.226
SMAD4: C03.5, P12.227
SMAD4, BMPR1A, PTEN, STK11: P12.228
small cell lung cancer: P06.195
small supernumerary marker chromosomes (sSMC): P03.061
SMARCA2: P02.237
SMC: P03.013, P03.079
SMC(11): P03.013
SMC1A: P12.043, P12.058
SMEI: P12.081
Smith-Lemli-Opitz Syndrome: P12.229
SMMCI: P02.238
SMN1 gene: P12.265
SMN2 gene: P12.230
SMOC2: C18.4
smoking habits: J03.10, P03.030
SMPD1 gene: J13.08
SMPD1: J13.09, P12.165
SMPX: P12.108
Snapback primer: P15.04
SNP array: C04.5, P03.080, P03.137, P05.60, P11.015
SNP data management: P15.14
SNP detection: P11.034
SNP interaction: P11.122
SNP Microarray: EPL7.5, P02.116
SNP: J03.19, P02.155, P09.005, P09.138, P10.54, P10.55, P11.128
SNP-array: P03.123, P07.25
SNPs Marker (SNPforID): J10.11
SNPs: P11.143
SNRPN: C04.3
Social media: J01.09
social network: P01.28
SOCS3: P09.040
Sodium Channelopathy: P02.239
Somatic cancer gene variants: P11.014
Somatic mosaicism: C04.4
somatic mutations: P06.154
Sonic Hedgehog: J02.23
SORL1: C11.1
SOS1: P02.185, P12.172
SOS2: P06.080
Sotos: P02.240
South Romania: P10.10
SOX10: P02.258
SOX5: P02.134
SOX6: P02.241
SOX9: P09.125, P11.123
SPAST: P12.115
Spastic Paraplegia 31: P02.242
spastic paraplegia: C11.2
spastin: P12.116
specific language impairment: P09.126
sperm FISH: P04.57
Sperm morphological defects: J04.06
sperm parameters: P04.54
Spermatogenesis: J04.13, P04.22
spg4: J12.27, P12.115, P12.116, P12.117
SPG8: P02.243
Spinal Muscular Atrophy: J14.05, P12.230, P15.15
spinocerebellar ataxia type 1 (SCA1): P02.035
Spinocerebellar ataxia type 3: J14.08, P14.21
spinocerebellar ataxia: C11.3, J12.13, P12.136, P12.216, P12.231, P12.232
splice isoforms: P06.103
splice mutation: P02.046
Splice site mutation: P12.101, P12.219
splice: P11.110
Splicing Governs: P11.137
splicing mutations: P12.147, P14.23
Splicing: P06.047, P06.196, P12.194, P12.233, S14.2
split hand: P12.244
Split Metopic suture: P02.249
split-hand/foot malformation: PL2.3
Spoc1: C16.6
spondylocostal dysostosis: P12.234
spondyloepiphyseal dysplasia, luxations type: P02.059
spontaneous abortion: J03.07, P04.05, P04.18
sports success: J02.45
Sprouty genes: P08.45
SPTLC1: C01.5
squamous cell carcinoma: P06.133
squamous: P06.209
SRD5A2 gene: P06.183
SRGAP3: C13.6
SRS: P02.049, P02.231, P02.234
SRY gene: P03.135
SRY: P11.124
SS18-SSX: P06.198
SSCP: J03.22, P02.159
sSMC: P03.124
Stargardt desease: J12.16, P12.001, P12.235
Statistics: C10.1
status: P02.042
Stem cell: J04.03, J11.01, P06.085
stent implantation: P11.108
steroid-resistant nephrotic syndrome: P11.125
STGD1: J12.16
Stickler syndrome: P12.054
stillbirth: P05.34
STK11: P12.236
STK11gene: P06.197
stomach: P11.135
stratified medicine: P01.29
Strength: P09.068
stress response: P09.128, P10.26
stress sensitivity of fragile sites: S02.3
Stress: J02.42
Stroke Subtypes: J09.31
stroke: J10.15, P09.127
structural abnormalities: J03.02, P03.125
Structural variants: P11.062
structural variation: C06.1, C06.3
subfertility: P04.51
Subjective health complaints: EP04.02
submicroscobic chromosomal abnormalities: P05.07, P05.36
subtelomere deletion: P03.126
subtelomeres: C06.1
subtelomeric aberration: P03.127, P03.096
Subtelomeric deletion: P02.244
Sudden arrhythmic death: C16.4
sudden cardiac death: P02.245
sudden death: P12.237
suicidal behavior: P09.128
Suicide: P09.063
sulfatase: P06.055
SULT1A1*2: P13.48
supernumerary chromosome: P02.089
Supernumerary marker chromosome 15: P03.128
supernumerary X derivative: P02.246
supervision: EP06.04
support vector machine (SVM): P08.43
supranational database: P06.139
Surveillance: S09.1
survival analysis: P10.23
survival: J06.12, P06.170
Survivor Effect: P10.53
susceptibility: P02.024, P06.021
synaptic plasticity: C13.5
Synaptonemal complex protein 3: P04.52
syndactylies: P02.247
Syndecan: P12.241
syndrome 18p-: J03.15
Syndrome: J02.38, P08.21, P02.031, P03.012
Syndromic Diarrhea: P12.238
syndromic mental retardation: P02.248
syndromic: P02.132
synovial sarcoma: P06.198
Synphilin-1: P12.187
Syntelencephaly: P02.249
syntrophin: P12.034
SYP: J12.31
Systematic review: P01.13
Systemic lupus erymathosus: J09.32, J09.33, J09.30
Systemic primary carnitine deficiency: J02.37
systemic sclerosis: P09.129
systems biology: P11.126

T
t: P05.66
t(1;12)(p22;p12-13): J03.04
T/C- FISH: P07.34
T2*-MRI scans: J12.33
TAAD- thoracic aortic aneurysm and dissection: P12.239
Tabacco: P11.127
Taghdir: P01.19
T-ALL: C02.2
tandem mass spectrometry: J13.05, P13.15
Target Enrichment: P11.028, P11.128, P12.046
targeted next generation sequencing: P12.111
targeted resequencing: P11.077
Targeted sequencing: P11.129
targeted treatments: PL3.2
Tatarstan of Russia: J10.10
Tay-sachs: P11.080
TAZ: P02.250
Tbx4: P12.221
T-cell prolymphocytic leukemia: P06.199
TCF7L2: P13.09
TCOF1: P02.251, P12.248
TCS: P02.251
teachable moment: EP05.12
teaching: J01.06
Teeth: C18.4
Tehran: P09.067
telomerase: P06.049
telomere length measurement: P07.34
telomere length: P09.076, P10.53
telomere: P09.130
Temple syndrome: P11.120
Temporal lobe epilepsy: J09.45
Tenascin-X deficient: P12.240
Teratogenicity: P02.220
terminal deletion: P03.071
termination of pregnancy: EPL6.1
testicular cancer: P02.209
Testicular germ cell tumour: P06.200
testis cancer: P06.201
testis tumours: P06.202
testosterone/Δ4 androstenedione ratio: J04.16

Tetralogy of Fallot: P09.131
TFAP2A: P02.047
TGF beta: P09.132
TGFB1: P09.142
TGF-beta signaling: C01.3
TGF-beta signalling: P12.241
TGF-Beta: P14.13
TGFBR1: P06.082
TGIFLX gene: P06.203
TGM1: P02.027
Thai: P09.029
thalassaemia: P12.242
Thalassemia intermedia: P02.252
Thalassemia Phenotype: P12.243
thalassemia: J10.12, P05.28, P08.44
the exposure: J10.09
therapeutic agent: P14.04
Therapeutics: J14.05
Therapy: P06.179, P14.23
thermocycler variation: P15.11
thoracic aortic aneurysm (TAA): P11.130
Thoracic aortic aneurysm and dissection: P12.226
thrombocytopenia: P03.005, P03.129
Thrombocytopenia-Absent Radius syndrome: P05.67
THROMBOPHILIA: EP04.01, J04.07, P04.19, P04.53
thyroid cancer: P06.204, P06.205, P06.206, P06.207
thyroid carcinoma: P06.208
tibia: P02.194
TIE2: C01.4
time series: P11.113
tiopurine drugs: P14.12
tissue DNA: P11.131
tissue inhibitor of metalloproteinases-2: P08.01
titin: P10.13
TKI inhibitors: P06.165
TLR4: J09.45
TMEM132D: P11.104
TMEM18: P09.136
TMPRESS:ERG fusiongene pos.and neg.: P06.185
TNF alpha: P08.29
TNF- α: P09.013
TNFa, TGFb: P09.107
TNF-alpha: J09.04
TNFR1: P12.247
TNFRSF13B: J12.30
TNFα: J10.03
TNF-α, IL2, IL-2RB: J09.29
toll-like receptors: P09.023
Tooth agenesis: J12.14, P02.253, P08.45
torsinA: J12.06
total mortality: P09.134
total RNA: P11.131
TP53 mutations: P06.134
TP63 mutation: P12.244
TPO gene: J09.14
trangenic mice: P12.142
transcript: P12.247
transcription activator-like effector nucleases: P11.020
transcription factors: P11.044
transcription: P12.245
Transcriptome profiling: P12.246
transcriptome: P09.060, P11.088
Transcriptomics: S14.2
Transforming growth factor: P08.36
transitional: P06.209
translocation carrier: P04.43, P04.54
Translocation: P03.130
transthyretin: J02.44
TRAPS: P12.247
Treacher Collins syndrome: S04.2
Treacher Collins: P12.248
treatment: S15.3
Treatment-Focused Genetic Testing: EPL4.1
TRIC: P08.05
Tricho-hepato-enteric syndrome: P12.238
Trichorhinophalangeal syndrome: P12.249
tricho-rino-phalangeal: J02.39
trigeminal anesthesia: P02.112
TRIM8: P11.103
trinucleotide repeat expansion: P12.250
trio sequencing: P11.035
trio: C07.5
triploidy mosaicism: P03.131
triploidy: P05.68
trisomy 10q: P03.132
trisomy 16: P04.32
trisomy 21: P03.133, P09.056, P11.027
Trisomy 22: P02.254
trisomy 4: P03.134
trisomy rescue: P02.206
trisomy8p: P03.052
TrkC: P14.17
trombocytopenia: P02.137
trophectoderm analysis: P04.43
Trophectoderm biopsy: P04.42
Trophectoderm: P04.37
TRPS1 gene: P12.249
TRPV4: P12.049
TSC2-PKD1: P12.056
TSPX gene: P06.210
TSPY gene: P06.210
TSPY1: P04.55
TTN: C07.4
TTR: P10.21, P12.109
TTVs: J10.12
TUBB3: P02.062
tuberculosis: P09.064, P10.54
Tuberous Sclerosis Complex (TSC): PL3.2, P12.251
tuberous sclerosis: P11.132
Tubulin: P07.14
tumor associated stroma: P06.184
Tumor suppressor genes: P06.075, P06.155
tumour necrosis factor-α: J09.20
Tumour testing: EP05.08
Tunisian medical staff: P03.015
Tunisian population: P10.31
Turkish population: P12.143
Turner syndrome: J02.40, J05.05, P02.255, P03.135, P03.136, P04.58
Tuvinians: J12.32
twins: P02.232
TWIST1: P02.224, P06.143
Two partial monosomies: J05.11
two-colour FISH: P02.205
type 1 diabetes: J09.34, P09.135
type 2 diabetes: C05.2
type two diabetes mellitus: P09.137
Type two diabetes: P09.136
Types of results from Whole-Genome Sequencing: EPL7.1
TYR: P12.177
tyrosinase: P11.133
tyrosine kinase inhibitor imatinib mesylate: P07.11

U
U2AF65: P11.052
UAKD: P12.140
UBE2A: P02.263
ubiquitin-specific protease 26 (USP26): P04.56
UCP2 gene expression: P06.211
UGT1A1: P13.02
ulcerative colitis: J09.35, J10.08, P09.138
Ultrasonographic Findings: P05.22
ultrasound anomalies: P05.58
ultrasound screening: P05.69
ultrasound: J05.08
unbalanced rearrangements: P03.050
unbalanced translocation: P03.109, P03.132
unbiased screening: P06.141
UNC13D gene mutation: P12.089
Unclassified Variant: EP05.05, EP05.03, P06.117
Uncoupling protein 2: P09.082
undeleted patients: C01.2
uniparental diploidy: P05.70
uniparental disomy 14: P03.138, P02.092, P03.137, P12.204
Uniparental heterodisomy: P11.056
uniparental isodisomy: P05.14
unknown CNVs: P02.063
UPA system: P06.046
UPD 15: P02.025
UPD 9: P02.256
upd(14)mat: P03.138
UPD2: P03.006
UPF2: C13.3
uraemic: P08.21
Urea Cycle Defects: EP08.01
URG4/URGCP: J06.17
Usher syndrome: P02.075, P12.252, P12.253
uterine leiomyoma: P08.09, P09.140
UV: J06.21
Uveal melanoma: P06.212

V
VAAST: S10.1
VACTERL association: J02.41
valproic acid: C01.6, J13.13, P06.213, P09.112
VANGL2: P12.157
variability: J02.39, P12.198
Variant Acute Intermittent Porphyria: P12.118
variant detection: P11.115
Variant interpretation: P12.235
Variant of uncertain significance: P06.214
variant: P11.067, P06.138
Variation catalogue: P11.134
variation: J02.19
vascular accident: P02.125
vascular anomalies: P02.038
vascular anomaly: J03.16
Vascular remodeling: P09.075
VATER / VACTERL association: P02.130
VATER/VACTERL association: C09.4
VDR FokI polymorphism: P06.170
VDR gene expression: P06.215
VEGF gene: P06.216
VEGF: J13.11, P06.047, P09.021
Velaglucerase alfa: P13.14, P14.10
VeloCardioFacial syndrome: P09.141
velopharyngeal insufficiency: P02.214
venous blood: P11.114
venous thromboembolism: J09.36
ventricular septal defect: P02.071
VeraCode GoldenGate: P11.066
vertebral anomalies: P02.244
Vertebral malformation: P03.035
VGFR3 mutation: P12.203
VHL disease: P06.217
VHL gene: P06.062
VHL: P06.188
Vici: P12.254
virus: P05.47
visceral leishmaniasis: P12.089
visfatin: P10.55
Visual impairment: EP07.02
Vitamin D deficiency: P10.12
Vitamin D receptor gene polymorphism: P09.089
Vitamin D receptor: J08.06, P06.189
Vitiligo: P02.120
VKORC1 1639 G>A: P14.22
VKORC1: P02.199, P10.56
VMD: J11.04
von Hippel-Lindau disease: P02.257, P06.218
vps35: J12.35
Vsig1: P11.135

W
Waardenburg syndrome: P02.258, P05.50
WAGRO: P02.259
Waist-Hip Ratio: C06.2
Walker-Warburg syndrome: P02.149
WBSCR22: P11.139
WDR62: P12.145
WDR72: P02.021
Weaver syndrome: C03.4
web application: P15.14
web-based genetics resource: J01.08
web-based: P01.37, P11.141
Werner mesomelic syndrome: P12.256
whole exome sequencing: C07.1, P11.136, P11.138, S12.1
whole genome microarray analysis: EPL7.4
whole- genome microarray: P09.031
Whole Genome Sequencing: P01.16, P06.199, P11.134, P12.188
whole genome: J11.12
Whole-exome sequencing: P02.095
WHSC1: P03.139
William-Beuren syndrome: P11.139
Williams syndrome: J02.42
williams-beuren: P02.088
Wilson disease: J13.04, J13.14, P10.57, P13.49
Wnt signaling: P12.153
WNT10A: P02.253
Wolf- Hirschhorn- Syndrome: P03.139, P03.140
Wolfram syndrome: P02.260
women: J09.14
Wormian bone: P02.249
WPW: P03.141
Wrinkly skin syndrome: P02.032
written information: EP02.02
Wt1: P06.111

X
X chromosome STR: P10.50
X chromosome: J04.14
X linked: J02.53
XbaI: J04.12
X-chromosomal: P12.108
X-chromosome inactivation: P12.070
XDH gene: P14.12
X-exome next-generation sequencing: P12.259
X-inactivation: P03.130
XLID: J12.31, P02.261, P12.260
X-linked female-restricted epilepsy: P12.190
X-linked hearing loss: P12.258
X-linked intellectual deficiency: P02.265
X-linked Intellectual Disability: P11.140, P11.142, P12.259, P12.260
x-linked mental retardation: P12.178
X-linked: P12.009, P12.084, P12.131
Xmn1-HBG2: P12.243
Xp deletion: P03.142

Xp duplication: P03.059
Xp22 duplication: P03.143
XPD gene polymorphism: P06.088
Xq duplication: P02.262, P03.144
Xq22.2 duplication: J02.34
Xq23q25 duplication: P03.145
Xq24: P02.263
Xq28 deletion: P03.115
Xq28: P02.174
XRCC3gene polymorphism: P06.089
XY sex reversal: P02.058

Y
Y chromosome haplogroups: P04.55, P10.59
Y chromosome microdeletions: P04.52
Y chromosome: J04.08, J04.09, J04.11, J04.14, P10.58
Y haplogroups: P04.27
Y;autosome translocation: P04.57
Yakuts: P12.261
Yeast: P13.44
young onset: EP05.07
Yq microdeletions: P04.58
Yunis Varon Syndrome: P02.264, J02.08

Z
ZDHHC9: P02.265
zebrafish: P06.057
ZNF41: P10.09
ZNF750: P12.262
ZNF9: J10.06
ZRS: P12.256

Aalfs, C. M.: EP05.01, P01.34
Aaronson, N. K. A.: EPL6.3
Aartsma-Rus, A. M.: P11.020
Ababei, R.: J02.21, **J02.39**
Abad, M.: P06.131
Abad-Grau, M.: P09.085
Abadie, V.: P11.123
Abarca, E.: J03.11, P03.124
Abbasi, S.: **P06.045**
Abbaszadegan, M. R.: J06.19, **P06.050,** P06.091, P06.119, P12.186
Abboud, A.: P11.060
Abbs, S.: P12.254
Abdalla, E.: P12.027
Abdel Ghaffar, T.: J02.51
Abdel Hamid, M.: P12.197
Abdelhaleem, M.: P07.01
Abdel-Hamid, M.: P12.132
Abdelhedi, F.: J04.16
Abdellaoui, A.: C06.3
Abdelmoula, N. B.: **P02.039**
Abdennadher, I. M.: **P06.011**
Abdollahi Fard, S.: J03.09
Abdulhadi, K.: P12.105
Abe, K. T.: P03.108, **P03.140**
Abecasis, G.: P11.037
Abecasis, G. R.: C08.3
Abedini, S.: C15.3, P08.37, P12.243
Abeler, K.: P12.049
Abé-Sandes, K.: P10.03
Abhyankar, A.: C03.5, C09.2
Abicht, A.: **P02.064**, P02.204
Abid, M.: J04.16, P02.260
Abidi, F.: P11.142
Abidi, Z.: P06.078
Abilash, V. G.: J03.21, **J03.22**
Abildinova, G.: J04.08, J05.04, J13.05, **P02.061**
Abla, O.: P07.01
Abolhasani, M.: J12.12
Abou Jamra, R.: **C15.2**, P09.078, P11.060
Aboura, A.: P03.062
Abraham, D.: P09.129
Abrahamsen, T. G.: C15.4
Abramchik, H.: P04.08
Abramczuk-Piekutowska, D.: P07.24
Abramowicz, A.: P02.185, **P12.172**
Abramowicz, M.: P02.062
Abramycheva, N. I.: J09.37
Absher, D.: C04.4
Abysheva, S. N.: P06.036
Abyzov, A.: J11.12
ACAR, A.: P03.132
Acar, H.: J02.38, **J03.04**, P02.122, P03.119, P06.114
Acedo, A.: P06.196
Achermann, J.: P02.173
Achmann, R.: P05.53, P05.70, P15.03
Achmüller, C.: P12.121
Ackerman, M. J.: C16.4
Acquaviva, F.: P02.001
Acun, T.: P02.038
Adamaki, M.: **P07.11**, P07.18, P07.33
Adamczyk, J.: P11.048
Adamova, D.: P07.17
Adamski, J.: P13.09
Ádány, R.: P06.195
Adebamowo, C.: EP08.04
Adham, I. M.: P11.053, P11.135
Adyan, T.: **J12.26**
Adyan, T. A.: P12.249
Afanasjev, V.: P06.067
Afenjar, A.: C03.5, P02.024
Afifi, H.: P12.198
Afroozan, F.: P02.171, P03.044, P05.30
Afsharian, P.: P04.11
Aganovic, I.: J03.10, P03.030, P03.075
Agiannitopoulos, K.: J08.02, J08.04
Aglan, M.: P12.055
Agné, E.: P11.129
Agostinho, L. A.: P09.139
Agrawal, P.: P02.055
Aguado, C.: **P11.062**
Aguglia, U.: J09.45
Aguiar, J.: P03.038
Aguilar-Pimentel, J.: C12.6
Aguirre, L. A.: P12.108
Ahadi, B.: P09.116
ahangari, g.: J09.30
Ahangari, R.: P06.121
Ahmad, I.: C13.4
Ahmad, W.: P12.153
Ahmadi, A.: P12.106
Ahmadi, S.: J05.02, J05.06, J05.07, **P05.28**, P05.66, P09.089
Ahmadlou, S.: **J12.13**
Ahmadzadeh, A.: P06.006
Ahmetov, I. I.: J09.39, J09.40, J09.44, P06.151, P09.068, **P09.083**
Ahmetov, I. I.: Ahn, J.: P03.097
Ahnert, P.: P07.25
Ahting, U.: C12.4, P14.19
Aichinger, E.: P02.160
Aiello, C.: P12.147
Ailenei, C.: J02.31
Ainsworth, P.: P02.146
Ajina, M.: P04.49
Ajit Bolar, N.: C01.3
Ajlouni, K.: P03.135
Ajzner, É.: P12.015
Akalin, I.: **P05.64**
Akar, E.: P06.132
Akarsu, N. A.: P12.027
Akbari, M.: P06.015
Akbarzadeh Najar, R.: P08.36
Akbarzadeh, A.: J06.08, J06.09, J06.13, P06.043, P06.104
Akcay, T.: P12.037
Akdur, S.: P14.22
Akel Junior, A. N.: P12.181
Aken, B. L.: C08.1
Akhmadeeva, G.: **J09.27**
Akhmadishina, L.: **P06.016**, P09.041
Akhmetova, V.: P05.16, P09.124
Akhondi, M.: J04.13
Akimoto, C.: P12.011, P12.012
Akkanet, S.: P08.25
Akkoyunlu, R.: P02.014
Akkoyunlu, R. U.: J03.12
Akopyan, H.: J09.26
Akota, I.: P09.073
Akram, M.: P02.214
Akrami, S. M.: P03.082
Aksentijevich, I.: P15.07
Aksoy, B. A.: J02.36
Aksulu, H. E.: P14.22
Al Drees, A.: C11.2
Al Momani, R.: C07.1
Al Quaiti, M. M.: J03.20
Al Sannaa, N. A.: J10.13
Al-Abdulwahed, H. Y.: J02.50
Alabdulwahed, H. Y.: **J10.13**
Aladzsity, I.: P06.195
Alagozlu, H.: J14.01
Ala-Korpela, M.: C05.3
Alakulppi, N.: **P09.037**, P09.042
Al-Allawi, N.: P12.055
Alan, C.: P06.127
Alanay, Y.: C03.4
Alanova, R.: P03.004, P06.001
Alasmi, A.: P12.205
Alavi, A.: J11.08, **J12.18**
Al-Awami, A. H.: J02.50
Albagha, O. M. E.: P09.104
Albers, M.: P12.247
Albin-Kaplanski, A.: C12.1
Alblas, M. A.: C14.4, P08.32, P09.078
Albrecht, B.: C13.2, **P02.222**, P12.145
Albrecht, E.: **P10.33**
Albu, C. C.: **P02.037**, P02.074
Albu, D. F.: P02.037, **P02.074**
Albuquerque, C.: P06.109
Alcaraz Mas, L.: P03.005
AlChawa, T.: C14.4, P08.32
Alcina, A.: P09.085
Alef, T.: C13.4
Alekseeva, E.: **P06.145**
Alemany-Schmidt, A.: **P04.38**
Alembik, Y.: P02.216
Alenda, C.: P06.082
Alenius, G.-M.: C14.5
Alexander, M.: C08.5
Alexandrou, A.: P03.112, **P11.140**, P11.142
Aleynikova, T. D.: J12.07, J14.02
Alfaiate, C.: P09.025
Alfaro, A.: P06.061
Al-Fayyadh, M.: P12.128, P12.135
Al-Gazali, L.: P02.223, P02.225, P12.055
Alhabsi, M. M.: P12.205
Al-Hait, S.: P12.055
Al-Hassnan, Z.: P12.128
Al-Hassnan, Z. N.: **P12.135**
Ali Akin, M.: P12.060
Alías, L.: P06.027
Alias, L.: P12.070
Alías, L.: **P12.265**
Alice, M.: P13.25
Aliferis, K.: P05.58, P12.055
Alimat, S.: **J10.11**
Alitalo, K.: **S12.3**
Aliu, N.: P03.092
Alivi, J.: P06.108
Alix, E.: P02.141
Alizadeh Sharafi, M.: P10.22
Al-Jasmi, F.: P12.055
Alkan, C.: P10.51
Al-Khateeb, A.: P11.060
Alkhayat AlShehi, A. H.: **P12.205**
Al-Khzouz, C.: J14.07
Alkowari, M.: P12.105
Allanson, J.: P02.017, P02.175
Allebrandt, K. V.: **P10.32**
Allegue, M.: P07.28
Allen Jr, J. C.: C10.3
Allen, C.: **P11.009**
Allford, A. M.: **P01.01**
Allon-Shalev, S.: P01.18
Almadani, N.: J04.17
Almadani, S.: P05.30
Almaras, S.: P02.064
Almasi, M.: P07.27
Almeida, E.: P02.163
Almeida, M.: P09.025
Almeida, M. G.: P12.181
Almeida, R.: P11.126
Almeida, R. C.: **P09.059**
Al-Memar, A.: C11.4
Almen, M. S.: P09.136
Almomani, R.: P12.051, P12.064
Alonso, C.: P06.008
Alonso, I.: P08.45, P09.080, P09.081, P10.20, P10.21
Alonso, M.-J.: **P08.42**
Alonso, N.: **P09.104**
Alp, M. Y.: **J02.36**
Alptekin, D.: P04.39, P05.65
Al-Qahtani, M. H.: J03.20
Alrasbi, S.: P12.205
Al-Sanna'a, N. A.: **J02.50**
Alshukaili, H.: P12.205
Alsop, K.: EP05.10, EPL6.5
Altiok Clark, O.: P06.130, P12.029, P12.143
Altmann, A.: C08.5, P11.104

Altmüller, J.: C13.4, P12.108
Altunoglu, U.: P02.157, P03.026
Al-Turki, S.: C11.4
Alturkustani, M.: P02.146
Altuzarra, C.: P12.167
Álvarez Lozano, R.: P07.14
Alves, A. C.: **P12.085**
Alves, M.: **P09.063**
Alves, R. M.: P02.118
Alves, S.: P12.007
Alves-Ferreira, M.: P10.21
Alves-Ferreira, M. F.: **P08.45**
Alves-Leon, S.: P09.139
Aly, M. S.: **P06.209**
Al-Zahery, N.: P10.58
Alzeyadi, M.: **P06.164**
Amann, K.: P02.136
Amanpour, S.: P04.36, P04.36
Amaral, V. A. A. S.: **J02.42**
Amaryan, G. G.: P02.098
Amberger, A.: C18.3, C18.5
Ambrosetti, U.: P12.169
Ambrósio, A. M.: P09.025, P09.063
Ames, A. G.: **EPL1.4**
Ameye, G.: P09.125
Amiel, J.: C01.1, P02.141, P02.165, P11.123, P12.167
Amin, N.: C06.3, P10.53
Amini, E.: P03.082
Amiri, P.: J13.02, J13.11
Amirian, A.: J05.02, J05.07, P05.28, **P05.66,** J05.06
Amirian, M.: **J01.05**
Amirijavid, S.: **J06.13**
Amit, A.: P04.20
Ammar-Keskes, L.: P04.16, P04.27
Ammerpohl, O.: C10.5, P04.25, P06.073, P06.199, P11.026, P11.069
Amoli, M. M.: J13.02, **J13.11**
Amor, D.: EPL7.5
Amosenko, F. A.: **P06.076**
Amouri, A.: P06.011
Amouyel, P.: C11.1
Amparo, G.: P11.128
Amr, K.: P12.132, P12.197
Amram, D.: P02.104
Amstalden-Mendes, L. G.: P01.33
Amstutz, N.: P12.086
Amunts, K.: P09.047
Amyere, M.: **P12.202**
An, A.: P09.064
Anastasiadou, V.: EP07.01, P11.003, P11.061, P12.107
Anastasiadou, V. C.: **EP06.07**
Anastasiadou-Christophidou, V.: J02.16
Anastasovska, V.: **P12.263**
Anderlid, B.-M.: C01.3, P12.246
Anders, S.: P03.139
Andersen, J.: **EP04.02**
Andersen, M.: P06.126
Andersen, P. M.: P12.011, P12.012
Andjelic-Jelic, M.: J10.03
Andonov, S.: P10.56
Andonova, S.: **J09.32**, P02.079, P03.018, P03.073
Andrade, C. E. F.: P02.240
André, C.: C18.2
Andreescu, N.: P03.007, **P03.010**, P03.145, P05.39, P06.034
Andreeva, A.: P12.116, P12.117
Andreeva, T. V.: P09.012
Andreeva, Y.: J06.16, P06.103, P06.184
Andreoli, V.: **J12.29**
Andreotti, A.: EPL7.3
Andrew, C.: P05.14
Andrews, B.: P12.006
Andria, G.: J02.14, J02.52
Andrieux, J.: P02.161, P02.169, P03.062
Ang, A. L.: C10.3
Ang, L. C.: P02.146
Ang, M. K.: C10.3
Angel, P.: C02.1
Angelkova, N.: J02.11
Angelov, A.: P14.06
Angelov, K.: P06.171
Angelova, O.: J09.15
Anghel, A.: P06.034, P13.35
Angioni, A.: P02.184
Angisch, M.: P08.39, P10.38
Angius, A.: C08.3, P11.037, P11.119, P12.125
Angurana, S.: P13.06
Anheim, M.: P12.167
Anissi Hemaseh, K.: P03.033, P03.077, P04.09, P04.56
Annerén, G.: EPL1.3, J02.55, P12.246
Annesi, G.: J12.33, J12.34, **J12.35**, P12.081
Annette, u.: P02.190
Anney, R.: P11.117
Anni, F.: P11.037
Annilo, T.: P06.087, P11.094
Ansar, H.: P06.211, P06.215
Ansari Dezfouli, M.: **J12.24,** J12.24
Ansari, M.: P02.228, P11.123
Anthony, D.: P12.200
Antoine-Poirel, H.: P09.125
Antonarakis, S.: P09.141, **S14.1**
Antonarakis, S. E.: C04.6, P08.11, P09.056, P11.027, P12.055, PL2.3, PL2.6
Antonelli, E.: P05.58
Antonenko, V.: **J03.06**
Antoniadi, T.: P05.41
Antoniou, P.: **P11.003**
Antony, P.: P09.092
Antov, G.: P06.093
Anttonen, A.-K. K.: **P12.028**, P12.192
Anvar, S.: **P11.020**
Anvar, Y. S.: C06.6
Aoki, Y.: J02.54
Apak, M. Y.: PL2.4
Apel, M.: C14.5, **P09.111**
Apold, J.: C12.2
Apone, L.: P11.098
Apostol, G.: P14.06
Apostol, P.: J10.12, P06.010, P10.10
Apostolopoulos, M.: P09.007
Appelbäck, M.: **P02.087**, P11.005
Aquilano, A.: **P12.130**, P13.27
Arab, H.: P12.186
Arabzadeh, M.: **J06.05**, J06.06, J06.07
Aral, B.: P12.167, P12.178
Aranha, I. P.: **J03.23**
Aranko, K.: P09.037
Aráoz, H. V.: P06.008
Arasimowicz, E.: P02.232, P02.233
arastehkani, a.: J02.27, J06.05, J06.06, J06.07, J13.09, **P13.29**, P13.46
Araujo, L. F.: **P10.46**
Araujo, T. K.: P12.244
Araz, M.: J09.11, P09.082
arbabi, F.: P06.052
Arbez-Gindre, F.: P02.011
Archer, C.: P12.248
Archibald, A. D.: EPL1.4, EPL3.6
Ardañaz, M.: P07.28
Ardeleanu, C.: J02.26
Arden, N.: PL2.5
Ardeshirdavani, N.: P02.221
Aref, A.: P12.197
Arellano, J.: P06.061
Aretz, M.: C16.5, P11.038
Aretz, S.: C10.2
Argente, J.: P12.003
Arghir, A.: P03.046
Arghirescu, S.: J03.05, J06.12
Argyo, N.: P09.093
Argyriou, L.: P05.53
Ariani, F.: P02.060
Ariani, O.: P02.050
Arias, A.: P07.12, P07.28
Arias, M.: P12.216
Arias-Rivas, S.: P12.216
Arias-Vásquez, A.: **P09.105**
Ariyani, O.: J12.06
Ariyaratne, P. N.: C10.3
Arkasu, N.: C03.3
Arkhipova, A. A.: J09.40, P06.151, P09.068, P09.083
Arloth, J.: **P08.20,** P11.104
Armangil, D.: P05.64
Armelao, F.: P06.176
Armengol-Dulcet, L.: P03.019
Armor, D.: C18.1
Armstrong, J.: P10.49
Arnal, C.: P09.085
Arnedo, M.: P02.066
Arnold, C.: P06.098
Arnold, E.: P12.086
Arnold, N.: P06.117, P07.05
Arora, R.: P12.221
Arps, S.: P04.29
Arrieta, I.: P03.025, P12.094
Arroyo, C.: P08.42
Ars, E.: P02.163
Arsali, M.: P12.008
Arsenieva, E. N.: J09.10
Arshad, N.: C12.2
Arslan Ates, E.: **J12.04**
Arslan-Kirchner, M.: P03.035, P09.027, P12.239
Arslantas, A.: P07.19
Artan, R.: P12.143
Artan, S.: P06.040, P07.19
Artigas, M.: P03.016, P03.070
Arts, H. H.: **P12.126**
Artuso, R.: P11.077
Arunachalam, J. P.: P12.142
Aryan, H.: **J13.06**
aryani, o.: J02.27, J13.07, J13.09, P10.07, P13.29, P13.46
Arzhangi, S.: P12.023, P12.024, P12.025, P12.168
Asabere, A.: C06.5
Asadchuk, T.: **P05.03**
Asadollahi, R.: **P02.162**
Asadollahi, s.: **J12.09**
Asadpoor, U.: P04.56
Asadullin, A.: P08.02
Asami, M.: P03.131
Asanov, A. U.: P12.112
Asanov, A. Y.: J09.10
Asgun, H. F.: P14.22
Asharti, M.: P02.082
Ashery-Padan, R.: P14.04
Ashki, M.: P02.252
Ashrafi Kakhki, S.: **P11.124**
Ashrafi, M.: P13.29
Asia, S.: P03.033, P03.039
Askiti, V.: P12.156
Aslam, A.: P11.086
Aslam, M.: P10.33
Aslan, Y.: P05.64, P12.027
Aslanger, A. D.: P02.157
Aslanger, E.: P02.157
Asmoniene, V.: **P06.106**
Assawamakin, A.: P09.029
Asselbergs, F. W.: C18.1
Asselta, R.: P09.088, P11.018, P12.169
Assimes, T. L.: C04.4
Assis, T. M.: P12.212
Assmann, B.: P02.241
Assouline, Z.: P13.32
Assy, O.: P12.060
Ast, G.: P11.137, P14.04
Astratenkova, I.: P08.30
Astratenkova, I. V.: J09.39
Asvadi Kermani, I.: P06.133
Ataei, M.: J02.19, J11.02, P02.094
Ataman, E.: P12.171
Atanasov, P.: P10.56

Atanasova, I.: J09.15
Atanasovska, B. L.: **P12.242**
Atasoy, H. I.: P12.123
Aten, E.: C07.1, P03.129, P12.051
Ateş, Ö.: P08.06
Ates, O.: **P08.09**, P08.38
Athanasakis, E.: P12.054
Athanasiadou, A.: P07.11, **P07.18**, P07.33
Atik, S.: P06.127, P06.152, P06.205, P06.206, **P14.05**, P14.22
Atıs, A.: J02.15
Atkinson, A.: EPL3.6
Atlan, D.: J11.07
Attanasio, C.: P11.123
Attié-Bitach, T.: P12.178
Attig, A.: P02.053
Attri, S. V.: P13.06
Atzeni, A.: P09.077
Atzeni, R.: C08.3, P11.119, P12.125
Auber, B.: P05.53, P05.70, **P15.03**
Aubert, D.: P02.011
Auer, M.: C10.6, P03.021, P06.051
Augello, B.: P11.103
Augste, E.: P02.159
Aulchenko, Y. S.: P08.34
Aumann, V.: P12.103
Aumueller, E.: P13.26
Auricchio, H.: P12.053
Ausems, M. G. E. M.: EPL6.3
Austin, J.: **EPL5.4**
Australian Ovarian Cancer Study Group, T.: EP05.10
Austrup, F.: P02.230
Avatajitei, C.: P05.11
Avcılar, T.: P02.220, **P06.020**
Avdjieva-Tzavella, D.: **P02.034**, P02.063
Avdonina, M.: **P09.127**
Avela, K.: J12.31
Averiyanova, N. S.: P12.112
Avila, M.: **P02.170**, P03.008, P03.013
Avila-Fernandez, A.: **P12.074**, P12.022, P12.210
Avsar, M.: P02.220
Axelsson, O.: EPL1.3
Axenovich, T. I.: P08.34, **P08.35**
Ayadi, H.: P03.015, P10.31
Ayadi, L.: P06.011
Ayala, J.: J02.46
Ayat, H.: J04.10
Ayaz, A.: J02.07
Aycan, Z.: P02.022
Aydın, F.: P04.52
Aydın, H.: P02.249
Aydinli, K.: P05.22
Aykut, A.: P02.071, P12.099, **P12.165**
Aymé, S.: **P01.24**
Ayuso, C.: P02.248, P12.022, P12.074, P12.177, P12.210
Azad, M.: P12.090
azadegan, f.: **J12.12**
Azadmanesh, K.: P06.104
Azarkeivan, A.: P02.252, P12.243
Azarpajooh, M.: J09.31
Azevedo, A.: P06.109
Azhar, A.: **P12.020**
Azik, F. M.: P12.089
Azimi, C.: P03.082
Aziz, S. A. A.: P02.223
Azizi, F.: P09.067
Azoulay, J.-P.: P02.007
Azuma, Y.: P02.110
Azzarello-Burri, S.: P02.003, P02.162, P02.237, **P02.256**, P03.067, P12.145
Azzollini, J.: P12.058
B. Russell, M.: P02.056
Baas, A.: P02.172
Baasanjav, S.: **P02.223**
Baasner, A.: P12.201
Babadjanova, G.: P09.069
Babadjanova, G. J.: P09.053
Babal, P.: P06.083
Babali, K.: **J08.02**
Babalis, D.: J08.02
Baban, A.: P02.184
Babanejad, M.: J09.21, **P08.05**, P12.024, P12.025, P12.168
Babayan, A.: **J06.16**, P11.006
Babenko, N. N.: J09.05
Babenko, O.: P06.145
Babenko, O. V.: P11.064
Babikyan, D.: P11.059
Babikyan, D. T.: **P02.009**
Babrzadeh, F.: P06.116
Babushkina, N.: P09.064
Baccarani, A.: EPL7.3
Bach, J. E.: **P11.021**
Bachi, A.: P12.042
Bachinski, L. L.: C02.4
Bachleitner-Hofmann, T.: P06.070
Bachmann, N.: P02.049, **P02.091**, P12.068
Bacino, C. A.: P02.134
Backsch, C.: **P06.055**
Bacon, C.: **C13.6**, P11.039, P12.016
Bacon, E.: P11.025
Bacoulas, V.: P05.67
Bacu, M.: P13.10
Badens, C.: P02.161, **P12.238**
Badens, N.: P12.178
Bader, D.: P11.104
Bader, I. M.: **P02.138**
Badeschi, M.: P02.040
Badescu, E.: J06.12
Badii, R.: P12.105
Badura-Stronka, M.: P11.059
Badura▯Stronka, M.: P11.140
Bady-Khoo, M. S.: J12.32
Baena, N.: **P03.041**
Baert, A.: P12.019
Bagalà, A.: J12.35
Bagala, A.: P12.081
Bagci, G.: J06.17
Bagci, S.: J10.08
Bagerian, H.: P12.144
Baggerly, K. A.: C02.4
Bagheri, N.: J12.12, **P08.41**
Bagherian, H.: P04.36
Bagherizadeh, E.: P03.131
Bahari, G.: P10.54
Baharvand, H.: P11.089, P12.208
Bahgdadi, P.: J02.19
Bahi-Buisson, N.: P10.49
Bahman, I.: P03.106
Bahmanimehr, A.: **P10.40**
Bai, Y.-M.: P08.40
Baig, S.: P12.020, P12.211, C13.4
Baiget, M.: P06.027, P12.070, P12.210, P12.265
Bair, J. J.: C16.4
Baizigitov, D.: J09.27, P12.113
Bajelan, B.: **P06.187**
Bajrovic, K.: J09.36, P02.124
Bakir, K.: P06.132
Bakker, E.: C07.1
Baklouti Gargouri, S.: P04.27
Baklouti, S.: P04.16
Bal, J.: J12.25, P02.185, P12.127, P12.172, P12.217
Balaii, H.: J09.35
balali, m.: J02.27, J12.05, **P02.050**
Balantic, M.: **P09.021**
Balaščaková, M.: P01.11
Balat, A.: J09.11, P09.082
Balat, O.: P04.48
Bald, R.: P05.70
Baldassarre, G.: P02.040, P02.129, **P02.187**
Baldassarri, M.: P02.060
Baldi, M.: P05.05, P05.12
Baldo, C.: P11.063
Baldovic, M.: J09.28
Balducci, T.: J02.53
Bale, S. J.: J12.23
Balendran, S.: **P09.109**
Balestrieri, M.: EP01.02
Balg, S.: P02.204
Ballesta, M.: P12.005
Ballesta-Martínez, M. J.: P03.019
Ballester, Á.: P11.050
Balmaña, J.: P06.118
Balocco, M.: P02.147
Balog, J.: **P11.042**
Balogh, I.: **P12.015**
Baloira, A.: P09.075
Balschun, T.: P04.04, P09.138
Balta, G.: **P12.089**
Bambl, F.: P12.235
Banasiewicz, T.: P06.162, P12.236
Bandelier, C.: **P03.058**
Banescu, C.: P06.088, **P06.089**, P09.003, P09.093
Banin, E.: C11.5
Banka, S.: **P12.241**
Banks, E.: C07.5
Banning, M. J. G.: C04.5
Bao, S.: C08.2
Baptista, I. P.: P06.169
Baquero, C.: P02.066, **P03.070**
Bara, C.: J03.07, J09.04, P04.21
Barakat, A. Z.: P11.053
Baraldi, O.: P12.130
Baranov, V.: P12.230
Baranov, V. S.: J13.13, P03.094, **P03.113**, P11.024, P13.28
Barashkov, N. A.: J12.32, P02.109, **P10.11**, P10.29
Barasoain, M.: P03.025, **P12.094**
Barat-Houari, M.: P12.247
Barba, M.: P09.048
Barbacioru, C.: C08.2
Barbarii, L.: P02.031, P03.121
Barberá, V. M.: P06.072, P06.082
Barbieri, A.: P11.008
Barbirato, C.: P12.181
Barbova, N.: J01.03, J10.02
Barbova, N. I.: **P12.063**
Barc, J.: PL2.2
Barceló, M.: P12.265
Barcia Ramírez, A.: **P03.005**
Barcsay, E.: P05.47
Barešić, A.: **P10.50**
Bargay, J.: P07.28
Barisani, D.: P09.059
Barisic, I.: P03.096, **P03.117**
Barkane, B.: P09.073
Barkovich, J.: P02.082
Barlow-Stewart, K.: EPL4.1
Barlow-Stewart, K. K.: **EPL1.2**
Barnerias, C.: P13.32
Barnes, A. M.: J12.22, **J12.23**
Barnett, C. P.: **P02.047**
Barøy, T.: P03.051, P03.103, P03.145
Barragan, E.: P02.128
Barrenetxea, G.: P03.025, P12.094
Barresi, S.: J12.30
Barriales_Villa, R.: J12.28, P02.126
Barrionuevo Porras, J.: P02.030
Barrois, M.: P11.110
Barros, A.: P05.21
Barros, J.: P09.080, P09.081
Barshop, B.: P14.03
Barta, T.: P11.105
Bartel, F. O.: **P03.098**
Bartels, E.: C09.4, P02.026, **P02.130**, P08.08
Barterls, E.: P09.033
Bártfai, Z.: P06.195
Barth, S.: C14.4, P08.32
Barthelmes, D.: P09.091
Barthelmie, A.: P12.041

Barthlen, W.: P13.19
Bartholdi, D.: C13.2, **P02.003**, P02.112, P02.162, P03.067, P12.053, P12.145
Bartish, M.: P12.246
Barton, A.: C14.5
Barton, D. E.: P15.10
Bartos, D. C.: C16.4
Bartošová, J.: P01.11
Bartosova, Z.: P06.101
Bartram, C. R.: C18.5, P02.236, P02.241
Bartsch, D.: C13.6
Bartsch, H.: P02.082
Bartsch, O.: **P02.188**, P02.222, P03.078, P09.129, P12.137, P12.157, P12.213, P12.226
Baruchel, A.: P06.166
Barwick, K. E. S.: **C11.4**
Basaran, S.: P03.026, P05.07, **P05.22**, P05.29, P05.36, P12.030, PL2.4
Basel-Vanagaite, L.: C03.4, **C12.1**, P02.017
Basel-Vanagaite, L.: P02.132
Basmanav, F. B.: **P09.120**
Basso, M. C. S.: P01.33
Bassyouni, R.: J03.20
Batsukh, T.: **P12.045**
Battaglia, D.: P02.058
Battaglia, V.: P10.58
Battersby, B.: C12.5
Baudis, M.: P02.234, P06.176
Baudson, T. G.: P10.47
Bauer, C.: P12.260
Bauer, J.: **P11.060**
Bauer, M.: P07.25
Bauer, P.: P06.178, P12.136, **P12.260**
Bauer, S.: P11.100, **P11.101**
Bauer, T.: C01.6
Bauernhofer, T.: C10.6
Baujat, G.: C09.2, **P12.018**
Baukloh, V.: P04.29
Bauman, C.: P06.166
Baumann, C.: P02.141
Baumann, M.: C18.3
Baumann, U.: P13.49
Baumeister, F.: P02.204
Baumer, A.: P02.162, P02.256, P03.138, **P12.053**
Baumgartner, M. R.: C12.3
Bavdekar, A.: P14.10
Baxová, A.: P03.123, P10.42, P12.009
Bayanova, M.: **J05.04**, J13.05
Bayat, F.: **J05.02**, J05.07, P05.28, P05.66, J05.06
Bayat, N.: **P02.252**
Bayerl, C.: P13.33
Bayet, B.: P09.125
Baykan, B.: J12.10
Bayly, G.: P11.054
Bayrak, O.: P06.180
Bazazzadegan, N.: P08.05, P12.024, P12.025, **P12.168**, P12.023
Beach, A.: EPL2.6
Beales, P. L.: P12.126
Beaty, T. H.: C14.4
Beauloye, V.: P03.058
Beaurain, G.: PL2.2
Bebek, N.: J12.10
Bebenek, M.: P06.071
Beck, B. B.: P12.140, P12.201
Beck, M.: C16.1
Becker, A.: C08.5
Becker, A. A.: P06.117, **P07.05**
Becker, B.: P02.138, P04.43, P05.14
Becker, C.: P02.223
Becker, J.: C03.3, C14.4, **P09.004**, P11.044
Becker, K.: **P06.107**
Becker, T.: C17.4, P08.12, P08.39, P09.010, P09.047, P09.058, P09.078, P09.078, P10.38
Becker-Grünig, T.: P02.215
Beckett, W. H.: **EP05.03**
Beckmann, B. M.: **P02.245**
Beckmann, C.: P11.013
Beckmann, J. S.: P01.28
Becvarova, V.: P02.259, P03.123
Bedeschi, M. F.: J09.46, P02.060
Bednarczyk, D.: P09.099
Bednarek, N.: P09.026
Beechem, J. M.: P11.035
Begemann, M.: C04.2, **P02.092**, P02.234, P11.012
Beggs, A.: P02.055
Begiev, B. G.: P06.151
Begovic- Hadzimuratovic, S.: P03.075
Begovic, D.: P03.040, **P03.110**, P05.02, P05.19
Behalova, L.: P12.160, **P12.251**
Behboudi, A.: P02.102
Beheshti, Z.: P03.033, P03.077
Behjati, F.: P03.106, **P03.131**, P08.37
Behmanesh, M.: P06.053
Behrens, F.: C14.5, P09.111
Behzadi, R.: **P11.133**
Beke, A.: P05.47
Bekpen, C.: P10.16
Belei, O.: J02.41, J09.33, **J02.05**
Belengeanu, A.: J02.20, P02.127, P03.010, **P06.034**
Belengeanu, D.: J02.20, **P02.207**, P03.145
Belengeanu, V.: P03.007, P03.010, **P03.145**, P05.39
Beleza-Meireles, A.: **J02.17**, P03.050
Belge, G.: P14.13
Belguith, N.: J04.16, P12.194
Belitz, B.: P03.063
Belkaid, M.: P07.28
Belligni, E.: P12.227
Belligni, E. F.: J02.55, P02.010, **P02.100**
Bello, Y.: J02.03, J02.04, P13.42
Belloir, B.: P01.24
Belluoccio, D.: P06.202
Belluomo, I.: P13.27
Belmont, J. W.: C04.1
Belonogova, N. M.: P08.34
Beltcheva, O.: **J09.15**
Belusica, L.: P06.010
Belyaeva, I. A.: J09.10
Bembea, D.: P02.105
Bembea, M.: **P02.105**, P02.137
Bembea, S.: P02.105
Ben Abdallah Bouhjar, I.: **P03.066**
Ben Abdallah, F.: P04.27
Ben Abdallah, I.: P03.085, P07.31
Ben Ahmed, S.: P02.221
Ben Dridi, M.-F.: P13.14, P14.10
Ben Itto, A.: **J14.05**
Ben Jemaa, L.: P02.054
Ben Khelifa, H.: P03.066, **P03.085**
Ben Larbi, N.: P04.13
Ben Mefteh, R.: P02.053
Ben Rhouma, B.: **J04.16**
Ben Salah, G.: **P03.015**, P10.31
Ben Turkia, H.: P13.14, P14.10
Ben Yosef, D.: P04.20
Ben Zeev, B.: P10.49
Béna, F.: C04.6
Benavides, A.: P12.005
Benedek, I.: P06.089
Benedicenti, F.: P12.060
Beneken, C.: C10.6
Benet-Pagès, A.: C12.6
Bengesser, K.: P06.157, **P06.158**, P06.159, P06.160
Bengoa, A.: P03.016
Bengoechea, O.: P06.131
Beni, V.: P09.042
Benkhelifa, H.: P07.31
Benko, S.: P11.123
Benlier, N.: J09.11
Benlloch, L.: P07.28
Bennett, P.: EPL4.3
Bennett, R.: P11.009, P11.014
Benomar, A.: C11.2
Bens, S.: P04.25, P11.069, **P11.120**
Bensenor, I. M.: P02.199
Ben-Shachar, S.: P02.145, P03.142, P05.54
Ben-Shlomo, Y.: P10.37
Bensignor, E.: C18.2
Benthaus, T.: C02.3
Berberoğlu, M.: P02.022
Berdel, W. E.: C02.3
Bereczki, C.: P12.015
Bereket, A.: P12.037
Bereketoğlu, C.: P04.39
Berencsi, G.: P05.47
Beresheva, A. K.: P03.014
Berezina, G.: J12.19
Berger, I. M.: P12.016
Berger, W.: P09.091
Bergh, J.: P12.012
Berghea, C. E.: J09.04
Berghmans, R.: P01.15
Bergmann, A. K.: **P06.199**
Bergmann, C.: **C15.5**, P02.049, P02.091, P02.136, P02.200, P02.201, P12.068, P12.211, P12.252, P12.253
Bergmann, M. M.: P01.03
Bergstrom Lucas, A.: **P11.093**
Bergström, T.: P12.246
Berguiga, R.: P07.31
Béri, M.: P02.161, P09.026
Béri-Deixheimer, M.: P03.020, P12.167
Beristain, E.: **P06.191**
Berkel, S.: **P12.021**
Berland, S.: P02.087, P02.201, **P11.005**
Bermejo, E.: J02.55
Bermejo, M.: P12.005
Bernal, S.: P12.210, P12.265
Bernard, R.: J11.10
Bernardini, C.: P09.048
Bernardini, L.: P03.062
Bernasovska, J.: J12.14, P09.097
Bernateck, M.: P08.29
Berndt, S. I.: C06.2
Berneaga, D.: P07.28
Bernhart, S.: C10.5
Bernstein, I.: P06.135
Berosik, S.: P15.03
Berr, C.: C11.1
Berrade, S.: P12.003
Berry, D. J.: P10.12
Bertarnd, Y.: P06.166
Bertelli, M.: **P12.203**
Berth, H.: EPL6.1
Berthele, A.: P10.33
Bertini, E.: J12.30
Bertini, E.: P12.147
Bertola, D.: P03.023
Bertola, D. R.: J02.42, **P02.020**, P13.38
Bertula, K.: P11.131
Berulava, T.: **P09.060**
Berutti, R.: P11.037, **P11.119**, P12.125
Berwouts, S.: **P15.10**
Besana, A.: C16.4
Beskorovajna, H.: P04.31
Bessão, C.: P02.123
Bester, A. C.: S02.3
Bettecken, T.: **P03.143**, P11.136
Bettens, K.: P08.03, P12.073
Betts, H.: C14.3
Betz, R. C.: P09.010, **P09.058**, P12.123, P12.153
Beudt, U.: EP02.02
Bevot, A.: P02.076, P02.169
Beygo, J.: C13.2, **P11.026**
Bhaskar, S.: C15.6
Bhatt, S.: P03.091
Bhengu, L.: P02.208
Biagi, F.: P09.115
Białecka, M.: P03.114
Biamino, E.: **P02.010**, P02.100, P12.227
Biancalana, V.: J11.10, P02.104

Bianchi-Scarrà, G.: P06.110
Bianconi, M.: EP05.02
Bichev, S.: **P02.079**, P03.018, P03.073, P10.56
Biela, S.: P14.16
Bienert, P.: P06.004, P09.005, P10.55
Bienertova Vasku, J. A.: P06.004, P09.005, **P10.55**
Bienvenu, T.: P02.161, P10.49
Bier, A.: P02.046, P02.243
Biervliet, M.: P12.035
Biesecker, B. B.: **EPL7.1**
Biesecker, L. G.: EPL7.1
Bigler, M.: P11.073
Biktasheva, A. R.: P09.023
Bilbao, J.: P09.059
Bilbao, J. R.: P09.036, **P09.038**, P09.039, P09.040
Bilevych, O.: **P13.16**
Bilgen, A. S.: P06.097
Bilgen, T.: **P12.029**, P12.143
Bilguvar, K.: P02.051
Billieux, M.-H.: P05.58
Binder, A.: C14.3
Binder, E. B.: P08.20, P11.104
Binder, H.: C10.5
Binder, V.: C10.5
Bingemann, S.: P05.14, P13.33
Bingham, C.: P08.21
Binner, P.: J02.24, P12.239
Binquet, C.: P02.141
Biramijamal, F.: P06.095, **P06.174**
Biray Avci, C.: J06.17
Birch, J.: EP06.04
Birch, M.: P09.058
Birk, O.: P02.202
Birk, O. S.: P12.122, P12.262
BIRLING, M.-C.: **P11.016**
Birnbaum, R. Y.: P12.262
Birnie, E.: P01.06
Birot, A.-M.: EPL6.4, P01.04
Birt, L.: **EP02.05**, **EPL1.5**
Birve, A.: P12.011, **P12.012**
Biselli, J. M.: P11.030
Biselli-Chicote, P. M.: P06.047, **P06.216**
Bisgaard, A. M.: P10.49
Bisgaard, M.: **P02.257**, P06.218
Bishop, M.: EP06.03, **EPL3.1**
Biskup, S.: P02.072, P02.247, P12.054, P12.082
Biskupska-Bodova, K.: J09.12
Biswas, K. H.: C12.2
Bitsori, M.: P12.156
Bittner, R.: C18.3
Bittner, R. E.: P11.053
Bittóová, M.: P06.001
Bivoleanu, A.: P05.11
Bizot, J.-C.: P09.056
Bizzarri, V.: P02.060
Bjeda, D.: C01.3
Bjørgo, K.: **P03.103**
Black, G.: EPL5.6
Black, G. C.: **C15.6**
Black, G. C. M.: EP07.02
Blackwood, D.: C06.4
Blagova, O.: P12.017
Blair, E.: C18.6, P09.016
Blakely, R.: C11.4
Blancet, P.: P02.060
Blanchet-Bardon, C.: P02.027
Blanco, I.: EP05.12, **EPL2.3**
Blanco, O.: P06.131
Blanco-Arias, P.: P12.216
Blanco-Kelly, F.: **P02.248**, P12.022, P12.074, P12.177, P12.210
Blank, C.: C16.1
Blassnig-Ezeh, A.: P02.116
Blaszczyński, M.: P09.099
Blatny, R.: **P12.134**
Blattmann, P.: C05.1
Blau, H.: P12.200
Blaumeiser, B.: P09.010
Bleiker, E. M. A.: EPL6.3, P01.34, **S08.3**
Blesson, S.: P05.18
Blin, N.: P06.071
Bliznetz, E. A.: J04.14, **J12.15**
Bloch-Zupan, A.: **C18.4**
Bloechle, M.: P04.37
Blokland, E. A. W.: C11.5
Blom, A.: EW1
Blum, H.: C02.3
Blümel, P.: P02.234
Blume-Peytavi, U.: P09.010
Blumkin, L.: P12.179
Boari, N.: P06.057
Boavida, J.: P09.025
Bocchinfuso, G.: P12.222, P12.227
Boccone, L.: P12.227
Bochner, R.: **P14.04**
Bocian, E.: J02.55, P02.028, P02.185, P03.074, P12.172
Bockius, A.: P02.111
Bockkamp, E.: P06.111
Boday, A.: P02.159
Bodda, C.: P12.142
Boddaert, N.: P02.165, P12.178
Boddeke, H.: C11.3
Bódi, I.: P13.22
Bodmer, D.: P02.172
Boduroğlu, K.: P02.097
Boeckxstaens, G. E.: P09.004
Boemers, T. M.: P09.033
Boemio, P.: J02.14, J02.52
Boeriu, E.: **J06.12**
Boesch, S.: P12.121
Bogdanova, N.: P04.04, **P06.038**, P06.156
Bogdanova-Markov, N.: P11.001
Bögershausen, N.: C03.4
Boggan, J.: P12.264
Bognár, C.: **J09.28**
Bohacek, J.: **S03.3**
Bohiltea, L.: P04.21
Bohiltea, L. C.: **P05.20**
Bohlander, S. K.: C02.3
Bohlken, M.: P09.120
Böhm, B.: C14.5
Böhm, D.: P12.082
Böhm, J.: J11.10
Böhm, M.: P09.010, P09.058
Böhmer, A. C.: C14.4, **P08.32**
Böhmova, J.: **P05.09**, P06.210
Bohne, F.: P02.049
Bohnhorst, B.: P03.035
Bohring, A.: **P02.043**, P02.091
Boia, E. S.: P02.018
Boia, M.: **J02.22**, J13.03
Bojesen, A.: P02.144, P02.155, P03.080, P03.104, P03.130
Bojesen, A. B.: **P10.35**
Bojinova, V.: P02.079, P03.018, P03.073, P12.190
Bolar, N. A.: P02.023
Bole-Feysot, C.: P13.32
Bolz, H. J.: C15.5, P02.091, P02.136, P02.200, P02.201, P12.068, **P12.211**, P12.252, P12.253
Bolz, J. E.: P15.12
Boman, H.: C12.2
Bombois, S.: C11.1
Bonache, S.: P06.118
Bondar, C.: **P09.036**
Bondarenko, E.: P09.127
Bondarenko, M. T.: **J12.16**
Bondavalli, D.: **P03.069**
Bonduelle, M.: P12.035
Boneh, A.: EP08.01
Bonetti, M.: P09.131
Bonfig, W.: P02.255
Bonfiglio, F.: J11.11
Bongardt, S.: P13.03
Bongers, E. M. H. F.: C01.1, C01.3, P11.007, P12.126
Bonilla, C.: **P10.37**
Bonilla, X.: P09.141
Bonin, M.: P02.076, P02.149, P02.169, P12.239
Bonnefond, A.: PL2.2
Bonnefont, J.-P.: P02.161, P13.32
Bonnemains, C.: P13.32
Bönnemann, C.: C18.3
Bonnet, C.: P03.062, P09.026, P12.167
Bonnet, F.: P11.110
Bonnet, M.: P02.265, P12.167
Bono, S.: **P05.05**, P05.12
Bonora, E.: **P02.135**, P11.059, P11.070
Bonvicini, M.: P02.023
Boomsma, D.: C04.4, P08.13
Boomsma, D. I.: P11.143
Boon, E. M.: P12.173
Boon, L.: P12.202
Boon, L. M.: C01.4
Boonyaratanakornkit, J.: P11.115
Boppudi, S.: **P11.071**
Boraska, V.: P10.06
Borcan, I.: P03.046
Bordet, T.: P14.11
Bördlein, A.: C16.6
Borel, C.: P09.056, PL2.6
Borgulova, I.: P05.44, P05.50
Borgwardt, K.: C08.5
Borgwardt, K. M.: P11.122
Borisova, A. V.: J09.40, P09.068
Borjian, P.: J04.17
Borkhardt, A.: C10.5
Bornfeld, N.: P06.212
Bornkamm, M.: P02.255
Börno, S. T.: P06.185
Boronova, I.: J12.14, P09.097
Boroumand, S.: P06.119
Borras, S.: **P02.217**
Borrell, A.: P05.43
Borrelli, L.: P11.040
Borry, P.: EPL7.6, P01.03, P01.09, P01.14
Borsani, G.: P11.103
Borucka-Mankiewicz, M.: P02.041, P02.186, P02.232, P07.24, P13.47
Borun, P.: **P06.162**, P12.236
Borzov, E. A.: P01.10
Bosch, N.: P06.118
Bösl, M. R.: C13.5
Bösmüller, H.-C.: P06.013
Bost, D. A.: P04.12, **P12.093**
Bostan, O.: P02.036
Botana-Rial, M.: P06.122
Botcherby, M.: P05.14
Botelho, P.: J03.03, **P03.072**, P03.134
Both, M. I.: P11.029
Bottani, A.: P12.055
Bottomley, M.: P12.241
Bouali, N.: P07.31
Bouatia-Naji, N.: PL2.2
Bouchard, L.: P11.023, P11.049
Bouhnik, A.-D.: EPL2.1
Bouhouche, A.: C11.2
Bouma, G.: P09.115
Bouraoui, S.: P03.085, **P07.31**
Bourbon, M.: P12.085
Bourdon, V.: P11.110, P15.13
Bourrat, E.: C18.2, P02.027
Bours, V.: P02.238, P12.076
Bouslam, N.: C11.2
Boute, O.: P09.125
Boutoleau-Bretonnière, C.: C11.1
Boven, L. G.: P12.111
Bowes, J.: C14.5
Bowles, K.: P06.214
Bowling, B.: P01.25
Bowman, M.: P12.248
Bowtell, D.: EP05.10, EPL6.5
Boyadjiev, S. A.: P08.08, **P12.264**

Boyadzhieva, M.: J09.15
Boyanova, O. S.: **P06.018**
Boyce, J.: P11.086
Boyd, E.: P14.03
Boyer, T. G.: P02.192
Boys, A.: P05.34
Bozdemir, K.: P06.097
Bozhanov, A. Y.: **EP04.03**
Bozhinovski, G.: P06.042
Bozhinovski, G. L.: P12.242
Bozikova, A.: J12.14, P09.097
Bozkurt, N.: P08.25
Bozon, D.: P12.096
Bozorgmehr, B.: C09.6, **P02.171**
Braathen, G. J.: P03.128
Brackman, D.: C12.2
Braddock, S. R.: P02.043
Bradinova, I.: P02.079, P03.018, P05.62
Bradinova, I. M.: **P03.073**
Bradshaw, N.: P02.178
Brady, P. D.: **P05.15**
Braess, J.: C02.3
Bragagnolo, S.: P03.023, P03.118
Bragin, E.: EP08.03
Bragina, E.: P09.064
Bragina, E. Y.: P09.019, P09.062
Braha, E.: J02.21, J02.32, J02.39, P02.140, P03.125, **P03.136**, P03.144, P05.11
Braham, S.: P04.49
Brahem, A.: P07.31
Brahem, S.: P07.31
Brain, K.: EP05.09, EPL2.4, EPL4.3
Brain, K. E.: **EPL4.2**
Brais, B.: P12.087
Bralten, J.: P09.105
Brambilla, D.: P12.042
Bramlett, K.: P11.106
Bramon, E.: P09.120
Brancaleoni, V.: **P12.084**, P12.118
Brancati, F.: C03.1, P02.060
Brand, T.: P13.09
Brandejska, M.: P05.50
Brandmeier, B.: P12.254
Brandstätter, J. H.: P12.220
Brännström, T.: P12.012
Brase, J. C.: P06.185
Brath, H.: P11.076
Bratu, C.: P02.207
Bratu, C. D.: **J02.20**
Bratus, D.: P06.094
Braulke, F.: P07.23
Brault, V.: **P09.056**
Braun, T.: P02.209, P12.158
Braunholz, D.: P12.245
Braverman, A. C.: C01.3
Bravo-Marques, J. M.: P06.109
Bray, P. F.: P11.109
Bredt, M.: P13.49
Bregant, E.: P12.013
Brehm, A.: P12.199
Brejcha, M.: P07.17
Brena, M.: J02.53
Bressac de Paillerets, B.: P11.110
Bresson, J.-L.: C09.5, P02.120
Breuer, R.: P09.069
Breukel, C.: P11.020
Breuning, M. H.: C07.1, P03.129, P12.051, P12.173
Breunis, W. B.: C14.3
Breur, J. M.: C18.1
Brezinova, J.: P07.02
Brice, A.: C11.2, P12.167
Brilstra, E.: P02.095
Brinca, A.: P02.163
Brinkman, M.: P14.06
Brinyiczki, K.: P13.22
Brisson, D.: P11.023, P11.049
Brito, D.: P11.055
Broccoli, V.: P12.042
Brockschmidt, A.: C02.1, P06.136
Brockschmidt, F. F.: C02.1, C15.2, P09.010, P09.058, P09.078
Broda, G.: P02.117
Brody, J.: C08.4, C17.6
Broer, L.: P09.076, **P10.53**
Broggio, E.: P10.24
Bromilow, G.: P08.21
Bromley, D.: P02.217
Bronkhorst, Y.: P02.253
Bronner, M.: P11.110
Brookes, A. J.: J11.07
Brooks, A.: C03.5
Brooks, S.: EPL7.1
Brors, B.: C10.5
Brost, B.: C16.4
Brostjan, C.: P06.173
Brouillard, P.: P12.202
Brouwer, T.: **EP03.02**
Brouwers, A.: C09.3
Brovko, A. O.: P03.084
Brown, K. E.: C03.4
Brown, L.: **P03.071**
Brown, R.: C07.3
Bruce, I. N.: C14.5
Bruestle, O.: C01.6
Brueton, L.: C18.6
Brugada, P.: P12.035
Bruland, O.: P02.087, P11.005
Brunetti-Pieri, N.: C03.5
Bruneval, P.: PL2.2
Brunner, H.: C07.2, P02.132
Brunner, H. B.: C15.1
Brunner, H. G.: C01.1, C01.3, EP05.06, P02.192, P11.019, P11.035
Bruno, D. L.: P05.34
Bruno, I.: P12.054
Bruno, M. J.: P01.34
Brusco, A.: P02.010, P02.100, P12.147
Brussino, A.: P12.147
Bruttini, M.: P02.060
Brychtova, Y.: P07.17
Bryois, J.: PL2.6
Buades, C.: P11.050
Bucerzan, S.: **J14.07**
Buchert, R.: C15.2, P11.060
Buchinskaya, N. V.: **J13.12**
Büchner, T.: C02.3
Buck, D.: P10.33
Buckley, M.: P12.264
Bucur, A.: P02.108
Budisteanu, M.: P03.046
Budtz-Jørgensen, E.: P06.218
Budzeika, E.: P13.21
Bueno, G.: P03.070
Bueno, I.: P03.070
Buermans, H. P. J.: P06.033
Buescher, A.: P12.201
Buffone, A.: P06.033
Bug, S.: P04.43, **P05.14**
Buheitel, G.: P02.078
Buijsman, W.: C07.2
Buil, A.: PL2.6
Buisson, M.: P11.110
Buitelaar, J.: P09.028, P09.105
Buiting, K.: P11.026, P11.111, P11.120, P12.036
Bujoran, C.: P03.053, P03.125, P03.136, P05.11
Bukaeva, A.: P02.151
Buksch, M.: P03.063
Bulik, C.: P10.06
Bullerdiek, J.: P14.13
Bulut, Z. B.: J03.04
Bulycheva, I.: P13.39
Buniello, A.: P08.31
Buob, M.: P12.145
Buoni, S.: P02.060
Burbidge, L.: P06.214
Burcos, T.: P06.010
Burdach, S.: P02.255
Burfeind, P.: C02.5, P06.074, P06.179, P06.213
Burgemeister, A. L.: **P02.111**
Burger, M. C.: **P09.114**
Burgess, T.: EPL7.5
Burghoff, S.: P06.188, **P13.03**
Burglen, L.: P12.167, P12.178
Burgués Gasión, J.: P04.38
Burjanivova, T.: P06.102, **P12.209**
Burkart, V.: P13.03
Burkhardt, B.: C10.5
Burkhardt, H.: C14.5, P09.111
Burkhardt, T.: C16.1
Burlova-Vasylieva, M.: **J10.15**
Burnett, C.: P11.106
Burns, I.: P05.34
Burnyte, B.: **J02.25**
Burri, H.: P12.086
Burton, H.: P01.29
Burwinkel, B.: C18.5
Busch, A.: **P07.09**
Busch, J.: **P12.068**
Busch, K.: P12.253
Busche, A.: P02.112
Büscher, A.: P12.155
Busonero, F.: C08.3, P11.037
Bussini, A.: P06.059
Bustani Farkoush, H.: P12.186
Butnariu, L.: J02.21, J02.32, J02.39, P03.125
Butnariu, L. I.: **P03.144**
Büttel, H.-M.: P02.205
Buurman, R.: P06.113
Buxton, C.: **P05.41**
Buyukalpelli, R.: P06.143
Buzalkova, V.: P06.102
Bychkova, G. M.: P03.084
Byers, P. H.: C01.3
Bykov, I.: P06.099, P06.100
Byrne, M.: **P11.067**
Bystricka, D.: P07.02

C
C, A.: J06.01
C, W. T. C. C.: C06.4
C., C. E. G. E.: P09.036, P09.040
C2, W. T. C. C.: C06.4
Caba, L.: J02.32, **P03.125**, P03.144, P05.11
Caballero Salvador, E.: P07.14
Caberg, J. H.: P02.238
Cabo-Diez, M.: P11.091
Cabral, W. A.: **J12.22**, J12.23
Cabras, V.: P03.042
Cacace, R.: **P12.073**
Caccia, S.: P12.169
Cáceres, M.: P11.062
Cacheiro, P.: P12.216
Cacheux, V.: P12.088
Caggana, M.: P12.264
Caiazzo, F.: P05.05, P05.12
Cailotto, F.: PL2.5
Cain, S.: P12.241
Calado, J.: P02.163
Calaminus, C.: P14.11
Calasanz, M.: P07.28
Calcia, A.: P02.010, P02.100
Caldas, H. C.: P06.129
Caldés, T.: P06.118
Calì, G.: J11.11
Caliebe, A.: P04.25, **P11.069**, P11.120
Calini, D.: P10.04
Callewaert, B. L.: **C09.5**
Callier, P.: P02.170, P03.020, P05.48, P12.167
Calpena, E. L.: P12.114
Calpena, R.: P06.082
Calvo Medina, R.: P14.08

Calzada-Wack, J.: C12.6
Calzari, L.: P02.040
Cambon-Thomsen, A.: EP08.04, EPL7.2, EPL7.6, P01.27, **S16.2**
Camerota, F.: P02.065
Camm, N.: C07.3
Campbell, C.: P02.146
Campeau, P.: C03.5
Campion, D.: C11.1
Campo, E.: P07.12, P07.28
Campos Valenzuela, J. A.: P06.167
Campos-Xavier, B.: P02.090
Can, K.: P12.142
Candan, S.: P12.171
Cangul, H.: P02.036
Canham, N.: C01.3
Cannizzo, P.: P02.010
Cansu, A.: P12.027
Cantagrel, V.: P02.223
Cantalapiedra, D.: P11.050, P12.210, P12.237
Cantoni, N.: P06.064
Canturk, N.: P06.037, P06.175
Capella, G.: EPL2.3
Capitanio, N.: J11.11
Čapková, P.: P05.55
Capolino, R.: P02.184
Caporossi, D.: P08.30
Cappellini, M. D.: P12.084, P12.118
Cappuccio, G.: J02.14, **J02.52**
Caputo, V.: **P12.227**
Car, D.: P06.030
Caracciolo, M.: J12.33, J12.35
Carballo, M.: P12.022
CARDIoGRAM, C.: C05.5
Cardone, M.: P12.203
Cardos, G.: P06.123, **P06.124**, P10.39
Caredda, E.: P02.001
Carels, C.: P06.063
Carim, S.: P12.241
Carlier, P. G.: P12.120
Carlier, R.: C18.3
Carmack, C. E.: C02.6
Carmignac, V.: P12.167
Carneiro-Sampaio, M.: P13.38
Carney, T.: C03.3
Carnogurska, J.: P09.097
Carossa, V.: P10.58
Carracedo, A.: P12.216
Carrani, E.: P12.227
Carraro, D. M.: P11.030
Carreira, I.: P03.050, P03.017, **P06.169**, P11.055
Carrel, T.: P12.086
Carreño, O.: P12.083
Carreras, R.: **P10.28**
Carretero, G.: P06.138
Carrillo, A.: P12.094
Carrió, A.: P07.12, P07.28
Carrol, E. D.: C14.3
Carroll, C. J.: C12.5
Carsetti, R.: J12.30
Carstensen, M.: P11.047
Carta, C.: P12.227
Carter, N.: EP08.03
Carvalheira, G.: **P06.208**
Carvalho, C. M. B.: **C04.1**
Carvalho, D. R.: P03.108
Carvalho, F.: P05.21, P12.007
Carvalho, F. A. G.: C16.3
Carvalho, M.: P02.163
Casacchia, M.: P09.113
Casadei, S.: P06.028
Casanova, J.-L.: C03.5, C09.2
Casasnovas, J. A.: J09.47
cascella, r.: J09.43, **P09.110**
Cascioli, S.: J12.30
Casertano, A.: J02.14, J02.52
Casey, J. P.: **P12.174**
Caspers, S.: P09.047
Cassiman, J.-J.: EPL7.6, P01.24
Cassinat, B.: P06.166
Cassini, C.: EPL6.4, P01.04
Castagnetta, M.: P11.063
Castagnoli, L.: P12.222
Castaldo, G.: P12.002
Castaneda, J.: P02.028, P03.074
Castanho, P.: P06.109
Castano-Betancourt, M. C.: **PL2.5**
Castejon, V.: **EP05.12**, EPL2.3
Castellano, L.: P12.002
Castellanos Maroto, C.: P06.161
Castellanos-Rubio, A.: P09.039
Castilho, A. M.: P02.118, P12.066
Castillejo, A.: P06.072, P06.082
Castillejo, M. I.: P06.072, P06.082
Casto, G. T.: **J01.02**, P01.12
Castori, M.: **P02.065**
Castorina, P.: P12.169
CASTRO, R.: **P06.047**, P06.216
Castro, T. B.: **P06.090**
Castronovo, P.: J09.46
Casuga, I.: P11.014
Catacchio, C.: P10.51
Catacchio, C. R.: **C06.1**
Catalá-Rabasa, A.: P09.085
Catón, B.: P06.191
Caumes, R.: P12.018
Cavalcanti, D. P.: P12.244
Cavalcanti, F.: J12.33, **J12.34**, J12.35, P12.081
Cavalieri, S.: P12.147
Cavdarli, B.: P12.193
Cavé, H.: P02.175, **P06.166**
Caye, A.: P06.166
Cazarin-Barrientos, J.: P12.164
Cazier, J.-B.: P09., P02.126
Cebura, E.: P06.005
Cecchetti, S.: P09.131
Cecchin, S.: P12.203
Cecen, O.: P08.25
Cedeno, R. A.: **J02.33**
Cejnova, V.: **P05.68**
Celbova, L.: J03.08
Celec, P.: P09.052
Celik, D.: J03.10
Celletti, C.: P02.065
Celli, M.: P02.065
Cellier, C.: P09.115
Cen, Y.: P13.26
Censi, F.: **J15.02**, J15.03
Cepok, S.: P10.33
Cereda, A.: P12.058
Cerf-Bensussan, N.: P09.115
Cermak, J.: P07.02
Cernach, M. C.: P03.023
Ceroni, F.: P09.126
Certa, U.: P03.011
Cerutti, J. M.: P06.208
Cervera, J.: P07.28
Cervero, C.: P07.28
Cesareni, G.: P12.222
Cesca, F.: EP01.02
Cestari, M.: P12.203
Cetin, I.: C16.4
Cetin, O.: J02.07
Cetin, T.: P09.132
Çetincelik, U.: **J02.15**
Cevei, M.: J14.03, **P02.125**, P02.127
Chaabouni, H.: P02.054
Chaabouni, H. B.: P12.046
Chaabouni, M.: P02.054
Chaabouni-Bouhamed, H.: P12.055
Chabchoub, E.: **P02.053**, P02.221
Chabchoub, I.: **P02.221**,P12.194
Chaix, C.: P12.098
Chakalova, L.: P04.55, P12.242
Chakroun, N.: P04.16, P04.27
Chalmers, D.: EP08.04
Chambert, K.: P09.032
Champion, K. J.: P11.115
Chan, A. C. S.: P05.57
Chancenotte, S.: P02.265
Chang, P.: P14.03
Chang, W.: J12.22
Chang, Y.-T.: P08.40
Chantot, S.: P02.161
Chaouch, A.: P02.064
Chaoui, R.: P05.31
Chaparzadeh, N.: J04.05
Chapman, C.: EPL4.6
Charfi, N.: J04.16, P02.260
Charles, P.: **P02.007**, P02.024, P12.167
Charloteaux, B.: S01.3
Charlton, R.: C07.3
Chasnyk, V. G.: J13.12
Chatzisevastou-louKidou, C.: P08.11
Chau, K. T.: P02.250
Chau, N. M.: C10.3
Chaudhary, A. G.: J03.20
Chaudhuri, J. P.: **J07.01**
Chausheva, A.: **P06.085**
Chavarria Piudo, N.: P06.161
Chavarria-Soley, G.: **P11.017**
Cheek, J.: EPL5.4
Chekunova, N.: **P06.099**
Chellappa, V.: P12.027
Chelly, J.: J11.10, P02.265, P12.124
Chen, C.: P12.121
Chen, H.-W.: P11.109
Chen, J.: P15.04
Chen, L.-R.: P09.061
Chen, Q.: P02.107
Chen, S.-M.: **P11.014**
Chen, W.: C14.2
Chen, Y.: C01.3
Chen, Y.-T.: P09.100, P12.223, P12.234
Chen, Z.: P11.009
Cheng, Y. K. Y.: P05.57
Chenichene, S.: P02.088
Cherdyntseva, N. V.: J06.15, J06.18
Cherif, M.: **P04.16**, P04.27
Chernjak, I. J.: J09.34
Chernykh, V. B.: **J04.14**
Chertkoff, L. P.: **P06.008**
Cheuk, I.: **P09.142**
Cheung, S.: C04.1
Cheung, S. W.: C01.1
Chevalier, P.: P12.237
Chew, E.: P12.088
Chiappe, F.: P12.060, P12.125
Chiatante, G.: **P10.05**
Chien, W.-K.: **P08.40**
Chiesa, N.: P02.040, P02.129
Chin, B.: P11.086
Chin, T. M.: C10.3
Chinnery, P.: C12.3
Chirdo, F.: P09.036
Chirita Emandi, A.: **J02.40**, J06.12, P02.139
Chiru, D.: J02.41, J09.33
Chiryaeva, O. G.: P03.094, P03.113
Chitayat, D.: P12.202
Chitty, L.: EPL3.2, EPL3.3
Chiu, N. C.: P13.31
Chiu, Y.-F.: **P08.43**
Chiurazzi, P.: C01.2, **P12.004**
Chng, W. J.: C10.3
Cho, Y.: P09.065
Chomienne, C.: P06.166
Chong, K.: P12.202
Chong, S. S.: **P10.02**
Choobini, M.: J05.02, J05.07, P05.28, J05.06
Chorna, L.: J09.26
Chou, C.-H.: P09.100

Choukair, D.: P02.111
Chow, P.-H.: P06.116
Chowbay, B.: C10.3
Choy, K. W.: P05.57
Choy, R. K. W.: **S06.1**
Choynzonov, E.: P03.101
Chrast, J.: C08.1
Chris, O.: P13.25
Christensen, M. W.: C16.3
Christiansen, M. A.: C08.4, C17.6
Christodoulou, K.: P10.14
Christophi, C.: P11.003
Christova, S.: P06.044
Chrzanowska, K.: P02.041, P02.114, P02.186, P03.114, P07.24, P07.32
Chrzanowska, K. H.: P02.232, **P02.233,** P03.045
Chuah, C. T. H.: C10.3
Chuchrova, A. L.: J12.21
Chudoba, D.: P05.37
Chukhrova, A.: J12.16
Chukhrova, A. L.: J04.14
Chung, A.: P06.116
Chung, B.-Y.: **P02.250**
Chung, E.: P12.200
Chung, T.: P11.025
Chun-Hui Tsai, A.: C01.1
Cianetti, L.: P12.227
Ciara, E.: P02.041, **P02.186**, P02.232, P02.233, P07.24, P13.47
Cibulkova, P.: P02.159
Ciccone, R.: P09.030
Cichon, S.: C08.5, C14.4, P09.009, P09.047, P09.069, P09.118, P09.120, P11.015, P15.12, **S05.3**
Cichy, W.: P12.228, P12.236
Ciemniejewska, E.: P13.48
Cieślikowska, A.: **P02.114**, P02.186, P02.262
Cieza Borrella, C.: P13.18, P06.177
Ciftci, E.: P06.040, P07.19
Ciglidag Dungul, D.: **P15.06**
Cil, E.: P02.036
Cilingir, O.: **P06.040**, P07.19
Cimbalistienė, L.: P02.083
Cimpoeru, A.: P13.10
Cimponeriu, D.: J10.12, P06.010, **P10.10**
Cina, V.: **EP05.07**
Çine, N.: J03.12
Cine, N.: P02.014, P06.007, P06.014, P06.037, P06.056, **P06.175**
Cioboata, E.: P13.10
Ciolfi, A.: P12.227
Cionca, F. L.: P06.123
Cionca, F.-L. F.: **J02.26**
Cionca, L.: J02.26
Cirak, S.: C18.3, P02.131
Cirigliano, V.: P05.43
Citrigno, L.: J12.27
Cittadella, R.: J12.29
Ciuca, I.: P13.35
CIUCA, I. M.: **P13.08**, P02.083, **P03.043**
Civeira, F.: J09.47
Claassen, L.: EPL6.2
Cladovan, C.: P02.137, **P13.11**
Claes, K.: EP01.01, J02.30, P06.158, P06.159, P06.160, P12.184
Claes, K. B. M.: **P12.019**
Clancy, T.: EP06.01, **EPL3.4**, EPL4.5, PL0
Clark, O. A.: **P13.34**
Clarke, A.: P10.49
Clarke, A. J.: EP05.08
Clarke, E.: **EP07.02**
Claus, R.: P06.185
Clausen, K. O.: P03.128
Claussnitzer, M.: P13.09
Claviez, A.: C10.5
Clemens, L.: P14.11
Clementi, M.: P02.090
Clements, A.: EPL2.4, EPL4.2
Clenciu, D.: P13.10
Closset, E.: P02.070
Coassin, S.: P15.14
Coban, E.: P12.143
Cocchella, A.: P03.069
Cockburn, D. J.: **C07.3**
Cocozza, S.: P02.001
Codd, V.: P09.076, P10.53
Coe, B. P.: C06.4
Coelho, A. L. V.: P03.108
Coelho, T.: P10.20, P10.21, P10.23
Coerdt, W.: P02.188
Coeslier-Dieux, A.: P12.018
Cofán, M.: J09.47
Cogliati, F.: P10.49
Cogulu, O.: P12.099, P12.165
Cohen, D.: EP07.03
Cohen, I.: **P12.262**
Cohen, M.: P02.138, P03.090
Cohen-Woods, S.: P09.128
Coker, M.: P12.099, P12.165
Colak, A.: P06.075, **P06.127**
Col-Araz, N.: **J09.11**, P09.082
Coll, I.: P15.09
Collado, C.: P06.161, P11.050, P11.091, P12.237
Collado, R.: P07.28
Colleaux, L.: P02.165
Collet, C.: P02.070
Collier, D.: P10.06
Collier, D. A.: P03.097
Collin, R. W. J.: C11.5
Collins, F.: P12.088
Collins, M.: P09.114
Collober, C.: P14.06
Collonge-Rame, M.-A.: EPL6.4, P01.04
Collura, A.: **S09.3**
Colomb, V.: P12.238
Colombi, M.: C18.3, P02.023
Colombo, C.: P11.018, P12.002
Colombrita, C.: P10.04, P11.008
Colomer, D.: P07.12
Columbaro, M.: C07.4
Combs, R.: EP07.02, **EPL5.6**
Cömertpay, G.: P02.131, P04.39
Condorelli, G.: C07.4
Conforti, F. L.: J12.27
Conlin, L. K.: P03.118
Conrad, R.: P11.105
Conroy, J.: P02.214
Consortium, S. L. I.: P09.126
Conti, A.: J11.11
Contini, E.: P11.077, P12.203
Contreras, A.: P06.061
Cooke, M. S.: J06.21
Cooper, D.: P06.158
Cooper, D. N.: P06.157, P06.160
Copeland, K.: **P06.214**
Copetti, M.: P09.131
Copin, H.: P02.088
Coppieters, F.: P12.191
Çora, T.: J02.38, P02.122
Cora, T.: J03.04, **P03.119**, P06.114
Corazza, G. R.: P09.115
Corbo, R. M.: **P10.24**
Corda, D.: P12.203
Cordeiro, C.: P09.063
Cordeiro, I.: P03.008, P03.013
Cordier, C.: EPL5.2, **P01.20**
Cordier, M. P.: P02.141
Cordisco, M.: C01.4
Córdoba-Lanús, E.: **P06.138**
Corioni, M.: P11.028
Cormand, B.: P09.028, P12.083
Cormican, P.: C06.4, P11.117
Cormier-Daire, V.: C03.4, C03.5, C09.2, P02.141, P02.165, P12.018, P12.138, **S15.2**
Cornel, M. C.: EP08.06, P01.22, P01.23
Cornelis, F. M.: PL2.5
Cornell, M.: **C07.6**
Cornes, B. K.: C05.4
Cornet, M.: P06.027
Corominas, R.: P12.083
Coron, F.: **EPL6.4**, **P01.04**
Correia, H.: P03.038
Correia, P.: P03.134
Corsetto, P. A.: P12.002
Cortesi, L.: EPL7.3
Corton, M.: P12.074, P12.022, P12.210
Corvin, A. P.: C06.4, P11.117
Cosmi, E.: P02.090
Costa, A.: P03.072
Costa, D.: P07.12, **P07.28**
Costa, E. M. F.: P04.58
Costa, I.: P06.109
Costa, P.: P03.060, P03.122, P11.093
Costantino, L.: P11.018, P12.002
Costi, C.: **P12.107**
Cotelli, F.: P06.057
Cotugno, G.: P11.103
Coucke, P.: P12.048, P12.183
Coucke, P. J.: C09.5, P02.045, P12.207
Coulet, F.: P11.110
Courage, C.: P03.092
Courcet, J.-B.: **P02.141**
Courtens, W.: P09.125
Coutant, S.: C11.1
Couto, F. M.: P11.055
Coutton, C.: P02.161
Covic, M.: P03.125
Coviello, D.: P03.069, P03.093
Coviello, D. A.: **P11.063**, P12.002
Coxhead, J. M.: P02.217
Craciun, A.: J09.33
Craciun, A.: P10.10
Craig, I.: P09.128
Craiu, D.: P10.49
Cras, P.: P12.188
Crawford, G.: EPL5.3
Crawford, H.: P02.194
Cremers, F. P. M.: C11.5
Creton, M.: P02.253
Cretu, R.: P05.20
Creveaux, I.: **P12.096**
Criado, B.: P03.025, P12.094
Crins, F.: S01.3
Crisan, D. C.: P05.39
Crisan, M.: J14.07
Crisponi, G.: P12.060
Crisponi, L.: P12.060, P12.125
Cristea, M.: P03.046
Cristina, X.: **P11.106**
Crkvenac Gornik, K.: P03.040, P03.110, P05.02, P05.19
Croisile, B.: C11.1
Crolla, J.: P03.037
Crombez, E.: P13.14, P14.10
Croner, R.: P09.050
Crook, A.: EP05.10
Crosby, A. H.: C11.4, P12.205
Crosiers, D.: P12.188
Crotti, L.: C16.4
Crout, R. J.: J01.02, **P01.12**
Crüger, D. G.: P06.135
Cruts, M.: P12.163
Cruz Guerrero, G.: P03.005
Cruz Hernández, J.: P06.128
Cruz, M.: P08.24
Cruz-Hernandez, J. J.: P06.177
Csábi, G.: J02.02
Csep, K.: P06.089, **P09.093**
Csire, M.: P05.47
Cucca, F.: C08.3, P09.077, P11.037, P11.119, P12.060, P12.125
Cucuruz, M.: J06.12
Cueff, A.: EPL6.4, P01.04

Author Index

Cuenca, E.: P12.083
Cuesta-Lopez, E.: P12.056
Cuevas, B.: P15.09
Cuevas-Covarrubais, S. A.: P02.106, P12.100, P12.164, P10.18
Cukrowska, B.: P09.059
Cukuranovic, R.: P09.031
Cullinan, T.: EPL3.5
Cullup, T. P.: **P12.254**
Cune, M.: P02.253
Cunha, B. R.: P06.216
Cunha, M.: P05.21
Cunningham, M. L.: P12.264
Cuoco, C.: J07.04
Cuppen, E.: C18.1, P12.259
Cuppens, H.: **P11.083**, P11.114
Curcio, F.: EP01.02, P12.225
Cure, S.: P02.027
Curie, A.: PL3.3
Curiel, Á.: P06.196
Curran, S.: P03.097
Curreli, N.: P09.077
Curtis, A.: P02.217
Curtisova, V.: **P05.55**
Cury, N. M.: **P06.029**
Cusano, R.: P11.119
Cusmai, R.: J12.30
Custódio, S.: **P03.008**, P03.013
cusumano, a.: J09.43
Čutka, D.: P03.123
Czamara, D.: C08.5
Czauderna, P.: P09.099

D
D´Aloi, K.: P06.008
da Rocha, A. L. B.: **P02.123**
da Silva-Costa, S. M.: **P02.118**
Daber, R.: P03.118
Dadaei, T.: **J09.35**
Dadali, E.: J12.26, P12.166
Dadali, E. L.: J12.21
D'Adamo, A. P.: P12.054
Dadgar, J.: **P13.46**
Dadgar, S.: **J13.07**, J13.09, P13.46
Dadkhah, E.: **P12.186**
Daescu, C.: J02.05, J02.41, **J09.33**
Dafinger, C.: P12.252
Dagan, E.: P03.111, **P05.59**
Daggag, H.: **P03.135**
Dahan, K.: P12.184
Dahl, A.: P06.185
Dahl, F.: P11.129
Dahl, N.: P02.033, P12.020, P12.246
Dahlgaard Hove, H.: P02.170
Dahlum, S.: P02.230
Dahoun, S.: C04.6, P05.58, P09.141
Dal Sasso, L.: EP01.02
Dales, A.: P02.082
Dalgleish, R.: J11.07
Dallaire, S.: P05.57
Dallapiccola, B.: P02.184, P02.197, P09.131, P12.227
Daly, M. J.: C07.5
Daly, S.: P12.241
Damante, G.: EP01.02, P06.163, P12.013
Damette, A.: EPL6.4, P01.04
Damm, G. R.: **P11.127**
Dammak, R. A.: P06.011
Dan, A.: J04.07
Dan, D.: P01.37
Danda, S.: P12.068
Dander, A.: C18.5
Daneshpour, M.: P09.067
Danilenko, N.: J09.01, J10.14
Danilova, M. M.: **J13.13**
Danjou, F.: **P11.037**
Danko, J.: J09.12
Daoud, S.: P04.16
Daoutidou, E.: P10.45
D'Apice, M.: P02.147
Dara Hama-Amin, A.: P02.055
Dard, R.: P09.026
Darios, F.: C11.2
Dariy, A. S.: P04.28
Dartigues, J.-F.: C11.1
Darvish, H.: P12.243
Dasani, P.: **EP02.01**
Daser, A.: **P04.03**
Dasouki, M. J.: **P12.006**
Dathe, K.: C03.1
Datter, S.: P11.132
Dauchel, H.: C11.1
Daumer-Haas, C.: **P02.032**, P02.209, P05.35
Dauwerse, H. G.: P12.173
Davaran, S.: J12.20
Davceva Cakar, M.: P10.19
Davey Smith, G.: P10.37
David, A.: P12.018
David, V. L.: **P02.018**
Davidson, C. J.: **P11.115**
Davidson, R.: P02.178
Davies, L.: EPL1.6
Davies, S.: C16.3, P05.51
Davila, S.: C14.3, P12.088
Davis, E. C.: C09.5
Davis, T.: P11.098
Davydenko, O.: J09.01, J09.03
Day, A.: P11.054
Day, E.: P04.03
Day, S. H.: P09.068
Dayakar, S.: **P09.090**
De Almeida, S.: C09.5
De Backer, A.: P07.22
De Baere, E.: P12.191
de Bakker, P. I. W.: C07.5, C17.1, P11.134
de Bock, T.: C10.4
de Boer, E. N.: P12.111
de Boer, S.: **P06.201**, P06.202
De Boever, P.: **P10.26**
De Broca, A.: P02.088, P02.169
de Brouwer, A.: C15.1
de Brouwer, A. P. M.: C01.1, P02.192, **P12.124**
De Castro-Orós, I.: **J09.47**
De Chiara, L.: **P06.122**
De Cock, P.: P02.053
de Coo, I.: P12.199
De Crescenzo, A.: P02.040, P02.129
De Deyn, P.: P08.03, P12.163
de Diego Otero, Y.: P14.08
de Diego, J.: P06.191
de felici, c.: J09.43
De Frutos, J.: P08.42
de Geus, E.: **EP05.01**, P08.13
de Greef, J. C.: P11.042
de Groot, R.: C14.3
De Iaco, P.: P06.110
de Jonge, A.: EP08.06
de Klerk, E.: **C06.6**
de Knijff, P.: P11.068, P11.074
de Kort, A.: P12.173
de Kovel, C. G.: P09.044
de Kovel, C. G. F.: **P02.095**, P12.259
de la Cruz, L.: P06.161
de la Hoya, M.: P06.118
De las Rivas, J.: P06.131
De Leeneer, K.: P12.019
De Leenheer, E.: P12.184
de Leeuw, N.: **C04.5**, C15.1
de Ligt, J.: C02.2, C06.3, C15.1, **P11.019**, P11.035
De Lonlay, P.: P13.32
De Luca, A.: **P09.131**
De Marco, E. V.: J12.29
De Matteis, E.: EPL7.3
De Meirleir, L.: P13.23
de Moraes, V. C. S.: P12.066
de Munnik, S. A.: C04.5
De Paepe, A.: C09.5, EP01.01, P01.09, P02.045, P12.048, P12.183, P12.207
De Palma, S.: P11.008
de Reuver, R.: C07.2
de Ruiter, K.: P06.031
de Silva, D.: P12.138
de Torres, M. L.: J02.55
De Vries, B.: P02.060
de Vries, B. B. A.: C01.1, C04.5, P02.192, P11.019, P12.124
De Vries, J.: EP08.04
de Vries, P.: P11.019, **PL3.2**
de Wert, G.: P01.15
de Wert, G. M. W. R.: P01.16
de Wilt, H.: EP05.04
de Wit, M.-C.: P12.199
De Witte, A.: P03.060, **P03.122**
de Zegher, F.: J02.49
Dean, P.: **P11.054**
Deans, Z.: S16.3
Dear, P. H.: P04.03
Debiec-Rychter, M.: P06.198
Debomy, F.: EPL6.4, P01.04
Dechelotte, P.: P12.096
Decker, C.: C15.5, P12.211, P12.252, P12.253
Decker, E.: P12.080, **P12.160**
Dedic, V.: P09.022
Dedousis, G.: P10.45
Deeg, L.: P14.16
Deelchand, D.: P12.120
Deffontaine, V.: S01.3
DeFilippi, C.: P02.100
Degenhardt, F.: C09.4, P09.069, **P09.118**, P11.015, P09.078
Deggouj, N.: P12.184
Degiorgio, D.: **P12.002**, P12.203
Değirmenci, I.: J13.10
Degn, B.: C16.3
Degorce-Rubiales, F.: C18.2
Degos, B.: P02.007
Dehgan, T.: P05.22
Dehganpour, H.: P02.085, **P02.235**
Dehghan Manshadi, M.: J13.07, P13.29
Dehghan, H.: P12.075
Dehghanmanshadi, M.: **J13.09**
Dei, M.: C08.3
Deichmann, A.: C18.5
Dejmek, P.: P01.11
Dekker, N.: **EP05.04**, P01.35
DeKoninck, P.: P05.15
del Barco Morillo, E.: P06.128
Del Campo, F.: P08.42
del Castillo, I.: P11.050, P12.108, PL2.4
del Rosario, M.: C04.5, C07.2
Delabar, J.: P08.11
Delaneau, O.: **C17.2**
Delani, D.: **EP08.05**, **J10.07**
Delaporta, P.: P05.51
Del-Favero, J.: **P06.033**
Delgado, C.: P09.085
Delgado, J.: P07.12
Delgrosso, K.: P11.109
D'Elia, A.: P12.013
Delicado, A.: J02.55
Deligiorgi, M.: P02.227
Delikurt Tuncalp, T.: EP06.07
Delikurt, T.: **EP07.01**, J02.16
Deliloglu Gurhan, I.: P07.04
Delisle, B.: C16.4
Della Marina, A.: P02.064
Della Monica, M.: P02.044
delle Chiaie, B.: C13.1
Delle Vigne, S.: P12.222
Delman, M.: **P11.080**
Delrue, M. A.: P02.141
Delrue, M.-A.: P12.138

Deltas, C.: P12.008
Demartis, F.: P11.037
Dembele, D.: P09.056
Dembour, G.: P08.11
Demeer, B.: **P02.088**, P05.18
Demichelis, F.: P06.185
Demidova, I. A.: P03.014
Demirhan, O.: P05.65
Demirkan, A.: **P09.076**
Demirturk, F.: P08.09
Demosthenous, P.: P12.008
Demuth, S.: P03.139
den Dunnen, J. T.: C06.6, C07.1, P06.033, P11.020, P11.074, P11.084, P12.051
den Dunnen, W.: C11.3
den Heijer, M.: **EPL2.2**, P01.17
den Hertog, J.: P09.131
den Hollander, A. I.: C11.5
den Hollander, N. S.: P12.051
den Ouden, E.: P07.13
Denapiene, G.: P09.138
Denecke, B.: P02.234
Denes, C.: J14.07
Dengler, L.: P12.136
Denisenko, S. V.: P04.28
Denisov, E. V.: **J06.15**
Denisova, G.: P10.40
Dentici, M.: P02.184, **P02.197**
Dentici, M. L.: P12.227
Depienne, C.: P12.167
Depping, R.: P12.245
Deprest, J. A.: P05.15
DePristo, M. A.: C07.5
Depuydt, J.: P12.019
Dequeker, E.: P01.24, P15.10
Derakhshan, S. M.: J12.08, P02.164, **P14.23**
Derakhshandeh-Peykar, P.: **P06.108**
Derbois, C.: C18.2, P02.027
Derenko, M.: P10.40
Derks, A.: EP08.01
Derks, R.: C07.2, P02.006
Dermitzakis, E. T.: PL2.6
Derrien, T.: C08.1
Dervent, A.: J12.10
Derwinska, K.: P02.028
Derwinska, K. W.: **P03.074**
Des Portes, V.: P02.161
des Portes, V.: PL3.3
Desai, A.: P14.03
Deschênes, M.: EP08.04
Desguerre, I.: P02.165, P13.32
Desmyter, L.: **P09.125**
Desportes, V.: P02.265
Dessì, V.: P12.125
Destouni, A.: **C16.3**, P05.51
Destrée, A.: C03.5, P02.043
Desviat, L. R.: **P13.01**, P13.07
Deutsch, E.: P14.09
Deutsch, S.: P08.11
Deutsch, U.: P11.013
Deutschbauer, S.: P03.047, P06.013
Deutschmann, A.: C18.3
Devereau, A.: C07.6, P11.095
Devery, S.: EPL5.6
Devos, H.: **P07.22**
Devriendt, K.: J02.49, P05.15, PL2.3, **S06.2**
DeWitte, A.: P11.093
Dey, S. K.: **P04.14**
Deyling, M.: C10.5
D'haene, B.: P12.191
Dhaifalah, I.: P05.55
D'Hooghe, M.: J02.49, P12.019
Di Donato, N.: C13.2, P02.101, P02.133, **P02.203**
Di Fiore, P. P.: PL2.4
Di Gregorio, E.: P02.010, P02.100
Di Lallo, V.: P13.27
Di Maria, E.: P02.147, P03.069, P03.093, P09.113
Di Medio, L.: P02.044
Di Palma, G.: J12.29
di Pasquale, E.: C07.4
Di Pasquale, F.: P11.013
Di Pierro, E.: P12.084, P12.118
Di Rocco, C.: P09.048
di Rocco, M.: C03.5
Dias, B. L. S.: P03.140
Dias, L.: P12.170
Dias, P.: P03.008
Dibbens, L. M.: **P12.079**
Dibilio, V.: J12.34
Diblík, J.: P04.51
Dibskaya, Y. B.: J07.03
Dibskiy, S. S.: J07.03
Dick, K.: C11.4
Dickerson, J.: C15.6
Dickhaus, T.: P11.100, P11.101
Diderberg, V.: P02.238
Didziapetriene, J.: P06.148
Diekstra, A.: P02.055
Diener, S.: C12.6, **P13.12**
Dierking, A.: **P01.07**
Dierks, S.: **C02.5**
Dierssen, M.: P09.056
Dietz, H.: **PL3.1**
Dietz, H. C.: C01.3
Dietze-Armana, I.: P02.205
Diez, O.: **P06.118**
Díez-Gómez, B.: P06.196
Digilio, M.: P02.184
Digilio, M. C.: P09.131
Dikov, T.: P06.044
Dimalanta, E. T.: P11.098
Dimitrov, B.: P02.221
Dimitrov, P.: P09.031
Dimitrov, R.: P06.171
Dimitrova, V.: P05.62
Dimkovic, N.: P09.022
Dimopoulou, A.: P12.061
Dimos, J.: C01.6
Dimos, L.: P05.38
Dimova, I.: P06.018, P06.054, **P06.093**, P06.164, P06.171, P09.031, P11.118
Dimova, P.: P12.190
Dimovski, A. J.: P06.068, P06.077
Dinant, G.: P01.22
Dinardo, C. L.: P02.199
Dinarvand, A.: **J02.01**
Dinarvand, R.: J02.01
Dincic, E.: P09.084
Diness, B. R.: EP02.03
Dioikitopoulou, K.: P07.18
Dion, P. A.: P09.119
Dionisi-Vici, C.: P12.254
Diplas, A. I.: P03.058
Disciglio, V.: **P09.030**
Distler, C.: P13.13
Dittrich, M.: C16.5
Divane, A.: P07.11, P07.18, P07.33
Dixon, J.: S04.2
Dixon, M. J.: **S04.2**
Djonov, V.: P09.031
Djukic, A.: P10.49
Djuric, M.: P10.49
Do Ngoc Thanh, C.: P12.018
Doan, T. B.: P09.024
Dobnig, H.: P09.103
Dobreanu, M.: P06.088, P06.089
Dobrowolska-Zachwieja, A.: P14.12
Dobsakova, Z.: P05.10
Dobson, K.: P09.058
Dobyns, W.: P12.199
Dobyns, W. B.: P02.082
Dockhorn-Dworniczak, B.: P06.079
Doco.Fenzy, M.: **P09.026**
Dodova, R.: **P06.044**
Dodurga, Y.: **J06.17**
Doelken, S. C.: **P02.004**, PL2.3
Doerr, H.-G.: C03.6, P03.120
Dogan, O. T.: P14.22
Doganov, N.: P06.171
Doğruoğlu, B.: J03.12
Doherty, M.: PL2.5
Doherty, S. A.: PL2.5
Döhla, J.: P07.34
Doimo, M.: P13.44
Dokal, I.: P11.078
Dolashka, P.: P06.018
Dolatkhah, R.: P06.133
Dölken, S.: P12.031
Dollfus, H.: C18.4, P12.046, P12.178
Dom, L.: P13.23
Dóme, B.: P06.195
Domenice, S.: P04.58
Domínguez de Bidaurreta, A.: P03.025
Dominguez, N.: P05.43
Dompmartin, A.: C01.4
Donahue-Hjelle, L.: P11.010
Dondorp, W. J.: P01.16
Donnai, D.: EPL1.6
Donnelly, P.: C06.4
Donohoe, G.: C06.4, P11.117
Dopazo, J.: P12.114
Dopudja-Pantic, V.: J10.03
Dörge, P.: C02.3
Dória, S.: **P05.21**
Doriani, A.: P06.108
Dörk, T.: P06.038, P06.156
Dorn, S.: P12.121
Dorn, T.: P12.082
Doros, G.: P01.37, P02.073
Dosa, L.: P02.060
Dosen, M.: P04.07
Dossow-Scheele, D.: P04.57
Dostál, O.: P01.21
Dostalova, L.: P07.17
Dostalova, V.: P13.36
Dott, B.: P02.216
Dougherty, M.: **P01.25**
Douglas, G.: C02.6
Doummar, D.: P12.167
Douw, K.: EPL7.6
Douzgou, S.: P02.134
Downes, S.: EPL5.6
Downing, S.: EPL1.5
Doyle, J.: C01.3
Draaken, M.: C09.4, C10.2, P02.026, **P08.08**, P09.033
Drabova, J.: P02.168, P02.174,P03.004, P03.079, P03.089, P03.102, P05.37
Dragomir, C.: **J10.05**, P05.42
Dragotoiu, R.: **J01.06**
Draisma, H.: **P11.143**
Dreesen, J.: C16.3
Drexler, H.: P02.153, P02.154
Drichel, D.: C17.4, P08.12, **P08.39**, P09.047, P09.058, P10.38
Drielsma, A.: **P02.062**
Driscoll, D. A.: P02.006
Dritsoula, A.: P12.200
Drozhzhina, G.: J09.19
Drozniewska, M.: **P03.065**
Drunat, S.: P02.161
Druschel, C. M.: P12.264
Druzhevskaya, A.: **P08.30**
Duarri, A.: C11.3
Duba, H.-C.: P03.047
Dubau, D.: P13.11
Dubé, M.-P.: P09.119
Dubina, M. V.: J09.07
Dubois, A.: P03.093
Dubois, B.: P10.33
Dubois, M.: P03.093
Dubourg, C.: P02.161

Dubovik, S.: J13.04, P13.21
Dubsky de Wittenau, G.: EP01.02, **P12.225**
Dudkiewicz, Z.: P02.028
Dudkina, V. S.: P03.094, P03.113
Dudová, S.: P06.190, P12.159
Dudutz, G.: P09.093
Dufke, A.: C04.2, C13.2, P02.076, P02.169, P12.260
Dufke, C.: P12.260
Dufour, A.: C02.3
Duga, B.: J09.16, P10.25, **P10.30**, P10.43, P10.52
Duga, S.: P09.088, P11.018, P12.169
Dührsen, U.: P06.199
Duicu, C.: **P06.088**, P06.089, P09.003
Duijkers, L.: P12.126
Dultseva, T. S.: J06.15
Duma, M.: P11.043
Dumanski, J. P.: C04.4
Dumic, K.: P03.117
Dumitrascu, V.: **P02.108**, P05.17, P11.043
Dumitriu, S.: J02.13, P02.139
Duncan, G.: J12.23
Duncan, R.: EP08.01, EPL1.4
Duncombe, A.: C09.2
Dunkhase-Heinl, U.: P03.080
Dunlop, K.: EPL1.2
Dunn, G.: EPL1.6
Dunna, N.: J06.01
Duplomb, L.: P12.167
DuPont, B. R.: P03.098
Dupuis, J.: C05.4
Durak Aras, B.: P06.040, P07.19
Durakbasi-Dursun, G.: P06.137
Durakbasi-Dursun, H. G.: **P07.20**
Durán Domínguez, M.: P06.021, P06.023
Duran, K.: C18.1, P12.259
Durán, M.: **P06.065**, P06.196, P08.42
Duret, H.: S09.1
Duriaux-Sail, G.: P12.055
Dürig, J.: P06.199
Durlik, M.: P02.084, P09.107
Durr, A.: C11.2, P12.120
Dürst, M.: P06.055
Duske, I.: P12.232
Dusl, M.: P02.064
Dutescu, M. I.: J09.04
Dutly, F.: P12.086
Dutra, A. R.: P03.023
Dutra, R. L.: J02.42
Duvak, E. V.: J12.32
Duval, A.: P06.078, S09.3
Duzcan, F.: J02.07
Dvořáčková, J.: P06.165
Dvorak, P.: P07.08
Dvořáková, L.: P05.37, **P13.36**
Dvorakova, M.: **P02.159**
Dwivedi, A.: P03.098
Dworniczak, B.: P03.006, **P11.001**
Dworschak, G.: C09.4
Dworschak, G. C.: **P02.026**
Dyatlov, D. A.: J02.45, P10.01
Dzemeshkevich, S.: P12.017, P12.034, P12.104
Dzemidovitch, T.: P13.21
Dzhemileva, L.: P12.224
Dzhemileva, L. U.: P02.109, P10.11, **P10.29**
Dzimiri, N.: P12.128

E
E. N. 1 Study Group, M.: P06.139
eandi, c.: J09.43
Earley, M.: P02.214
Eberhard, D.: P13.03
Eberlein, K.: P11.060
Ebermann, I.: P12.252
Ebert, A. K.: P09.033, P08.08
Ebrahimi, A.: J02.12, P02.085, **P02.094**, P02.235, P06.121, P06.133, P06.134, P10.22, P12.075
Ebrahimian Motlagh, S.: P03.131
Ebrahimzadeh Vesal, R.: P06.108
Ecay, M.: P12.032
Echaniz-Laguna, A.: J11.10
Eck, S.: C12.6
Eck, S. H.: C15.2
Eckel, J.: P13.03
Eckhold, J.: P12.245
Eckstein, G.: P11.047
Economides, J.: P12.067
Edalatkhah, H.: J04.13
Edelstein, L.: P11.109
Edkins, S.: P09.120
Edmont, M.: P01.20
Edmunds, B.: C14.1
Eek, A. K.: **P03.128**
Effat, L. K.: **J13.15**
Efimova, O. A.: P03.094, P11.024
Eftimov, A.: P06.077
Egger, G.: P06.070
Eggermann, K.: P03.081
Eggermann, T.: **C04.2**, P02.049, P02.092, P02.234, P11.012
Eggert, M.: P02.067, P02.160
Eggington, J. M.: P06.214
Eghbali, M.: **J04.13**
Egoavil, C. M.: P06.082
Egorov, V. V.: **J10.02**, J11.06, P12.063
Egorova, A.: P12.230
Egritas, O.: P12.238
Egusa, H.: P04.41
Ehlers, C.: P02.181
Ehlers, K.: P13.09
Ehlken, N.: P02.215
Ehrenreich, H.: P12.142
Ehret, J. K.: P02.169
Ehrler, M.: C02.1
Eichler, E.: **PL4.1**, **S10.2**
Eichler, E. E.: C06.1, C06.4, P10.16, P10.51
Eichorn, B.: P13.05
Eidem, H.: EPL7.1
Eider, C.: P02.049
Eigelshoven, S.: P09.010, P09.078
Eijzenga, W.: **EPL6.3**
Eiklid, K. L.: P12.148
Eilers, M.: P06.113
Eilers, P. H. C.: P11.029
Eils, R.: C10.5
Einarsdottir, E.: P09.037
Eisenberger, T.: C15.5, P02.201, P12.211, **P12.252**, P12.253
Eisner, F.: C10.6
Eitel, C.: C15.5
Ekici, A.: C16.6, P02.225, P09.050, P11.060
Ekici, A. B.: C03.6, C14.5, C15.2, P03.068, P03.120, P09.111, **P11.090**, P12.218
Ekström, T.: J11.05
El Chehadeh, S.: **P02.161**, P02.265
El Gammal, M.: P12.197
El Hajj, N.: **C16.5**, P11.033, P11.038
el Khassawna, T.: P12.162
El Maarri, O.: P09.009
El Malik, S.: C11.2
Elahi, E.: J11.08, J11.09, J12.17, J12.18, J12.20, J12.24
Elalaoui, S.: P12.055
Elbeyli, L.: P06.132
Elchinova, G. I.: **J10.10**
Elefteriou, F.: P12.162
Elert-Dobkowska, E.: **P12.115**
El-Gammal, M. A.: **P12.132**
Elghezal, H.: P03.066, P07.31
Eliez, S.: P09.141
Eljuga, D.: P06.030
Elkhartoufi, N.: P12.178
El-Khateeb, M.: P03.135
Elleder, M.: P13.36
Ellis, J. A.: P09.078
Ellouze, S.: P06.011
Elmslie, F. V.: C01.1
El-Murr, N.: **P06.078**
El-Osta, A.: **S03.2**
El-Ruby, M. O.: P03.126
Elsayed, S.: **J02.51**
Elstein, D.: P13.14
Elwyn, G.: EP05.04, EP05.09
Emamgholipour, S.: P06.211, P06.215
Emanuel, B. S.: P02.006
Emelyanov, A.: P12.189
Emery, J.: EP02.05, EPL1.4
Emery, J. D.: EPL1.5
Emhoff, J.: P11.086
Emiroglu, M.: PL2.4
Emmerich, D.: **P02.210**
Emmert, G.: P12.098
Emons, G.: P06.032
Emonts, M.: C14.3
Empting, S.: P13.19
Emrahi Govar, L.: J09.02, **J09.18**, P06.121
Encha-Razavi, F.: P12.178
Endele, S.: C13.2, P02.237
Endris, V.: C13.6
Endt, D.: **P06.186**
Engel, J.: P13.20
Engel, U.: P06.032
Engel, W.: P06.179, P11.053, P11.135
Engelborghs, S.: P08.03, P12.163
Engelen, E.: P12.199
Engels, H.: C09.4, C13.2, **P02.012**, P02.026, P02.169
Engert, S.: P06.117
Enjolras, O.: C01.4
Enklaar, T.: P02.049
Ennis, S.: P02.214, P12.174
Enomoto, K.: P02.029
Enquobahrie, D.: C08.4, C17.6
Entezami, M.: C16.1
Entezari, M.: **J06.11**, P05.23, P06.009
Eperon, I.: P05.58
Eraslan, S.: P15.15
Erasmus, S.: **J01.08**
Erb, S.: P06.188
Erbel, R.: P09.078
Ercin, C.: P06.037
Erciyas, K.: J12.02
Erdel, M.: P06.013
Erdem, G.: P12.241
Erdman, V. V.: **P08.22**
Erdmann, J.: **C05.5**
Erdöl, H.: P05.64
Eren, S.: **J03.12**, P02.014
Ergec, D.: P06.020, P12.037
Ergin, S.: **P05.07**, P05.29
Ergunsu, S.: P02.220
Erhardt, A.: P11.104
Erichsen, H. C.: C15.4
Eriksson, F.: C04.4
Eriksson, J. G.: C05.3, P09.086
Erjavec Skerget, A.: **J05.03**, P04.07
Erm, T.: P06.067
Ermis, H.: P05.22, P05.29
Ernst, F.: P11.034
Eroglu, T. F.: P06.137
Erogullari, A.: P12.245
Ershov, N.: P04.40
Ertel, A.: P11.109
Ertl-Wagner, B.: P02.204
Erturhan, S.: **P06.180**
Erzik, C.: P02.220
Escobedo-de La Peña, J.: P08.24
Escudier, E.: P12.178
Esfahani moghaddam, S.: J11.01
Eskandari Nasab, E.: P06.052, P10.54
Eskandari, G.: **J03.01**
Esko, T.: **C08.4**, C17.6
Eslamizadeh, S.: J06.05, J06.06, **J06.07**
Esmaeili, R.: P06.046

Esmail, A.: P11.086
Esmailnia, G.: P06.125
ESPEJO, R.: **P04.15**
Espinós, C.: **P12.114**
Espinoza-Jiménez, A.: P06.138
Esposito, L.: P12.054
Essand, M.: C04.4
Esserlind, A.-L.: J09.25
Esslinger, U.: C05.5
Esteba, S.: P03.041
Esteban Cardeñosa, E.: **P06.023**, P06.021, P06.065
Esteban, E.: P10.28
Esterling, L.: P06.214
Esteves, S.: P06.109
Estivill, X.: P08.11, P11.062, PL2.2
Estournet, B.: P12.018
Estrada-Cuzcano, A.: C11.5
Estrella, M. P.: P06.061
Etard, C.: C18.4
Etcharry-Bouyx, F.: C11.1
Etkina, E.: P09.018
Etkina, E. I.: P09.023
Euro, L.: C12.5
Eusebio, A.: P02.007
Evangelidou, P.: P11.061
Evangelidou, P. C.: **P03.112**
Evans, D. G. R.: EP05.03
Evans, G.: EPL2.1, EPL2.2
Evans, M. D.: J06.21
Evdokimova, A. N.: J09.10
Evers, C.: P02.246, **P03.059**
Evers-Kiebooms, G.: **S08.1**
Extermann, P.: P05.58
Eydoux, P.: P03.071, P03.137
Eyre, D.: J12.22
Eyre, D. R.: J12.23
Ezzati, J.: **J05.05**

F
F. Skjelbred, C.: P02.056
Faas, B. H. W.: C04.5
Fabre, A.: P12.238
Fabretto, A.: **P02.025**
Facchinetti, F.: C16.4
Fachal, L.: **C14.3**
Facio, F. M.: EPL7.1
Fagan, K.: P03.062
Fagerberg, C.: P02.152
Fagerheim, T.: **P12.049**
Fagerholm, R.: P04.10
Faggian, G.: C07.4
Fahey, C.: P11.117
Fahimi, P.: P03.033
Fahrleitner-Pammer, A.: P09.103
Fahsold, R.: P13.05
Failla, P.: P09.030
Faivre, L.: EPL6.4, P01.04, P02.141, P02.161, P02.170, P02.265, P03.020, P12.167, P12.178, P13.25
Fajardo, S.: P06.082
Fajkusová, L.: **P12.152**
Fakher, M.: P11.060
Fakhfakh, F.: J04.16, P02.260, P03.015, P04.16, P04.27, P10.31, P12.194
Fakhrjou, A.: P06.121, P06.133, P06.134
Falah, M.: **J12.05**, P12.144
Falbo, V.: J15.02, J15.03
Falconnet, E.: PL2.6
Faletra, F.: **P12.054**, P12.105
Falik-Zaccai, T. C.: C11.5
Fallah, M.: J02.12, P02.094, P09.067
Fallah-Zadeh, K.: **P04.11**
Falsafi Zadeh, S.: **J11.04**
Fälth, M.: P06.185
Fam, B.: P09.067
Famil- Tokhmehchi, M.: P06.203
Famoso, L.: P12.203
Fan, T.-B.: J12.20
Fan, X.: P10.08
Fanelli, F.: P13.27
Fang, E.: C02.6
Fang, N.: **P11.013**
Fang, Y.-H.: P08.43
Fannemel, M.: P03.103
Fanning, K.: P15.10
Faramarzi Garous, N.: P02.252
Faraone, S.: P09.105
Farashi, S.: J09.08, **P09.112**
Faravashi, N.: P03.060
Faravelli, F.: **P02.147**, P03.069, P03.093, P09.113
Farcas, S.: P03.007, P03.010, P03.145, **P05.39**
Farhadi, M.: J12.05
Faria, C.: P06.109
Faria, R. G.: P12.244
Farjadian, S.: P10.40
Farmaki, A.-E.: P10.45
Farndon, P.: EP06.03, EPL3.1
Farnood, A.: J09.35
Farokhi, B.: J09.24
Farooq, M.: C13.4
Farooqi, S.: P12.132
Farrashi, S.: P02.252
Farrokhi, E.: J09.22, J12.12
Farshidi, S.: **J09.24**
Farzanfar, F.: P03.082
Faskhutdinova, G.: **P08.02**
Fasshauer, M.: P13.24
Fatahi, F.: P08.41
Fatemi, N.: **J04.10**
Fattahi, Z.: P08.05, P08.37, P12.023, **P12.025**
Fauler, B.: P12.061
Fauth, C.: **C18.3**, C18.5, P02.008, P02.116, P12.041
Fauveau, L.: P09.005
Favaedi, R.: P04.11, P11.089, P11.124
Fawcett, H.: P12.050
Fazio, C.: P13.27
Fea, A. M.: P02.010
Federico, M.: EPL7.3
Fedetz, M.: **P09.085**
Fedorova, E.: P03.027
Fedorova, I.: P04.54
Fedorova, I. D.: P11.024
Fedorova, S. A.: P02.109, P10.11
Fedorova, Y.: **P09.018**
Fedorova, Y. Y.: P09.023
Fedotov, V.: P12.166, **P12.256**
Fedotov, V. P.: P02.176, P12.249
Fedotova, E. E.: P02.109, P10.11
Fedotova, T. V.: **P12.249**
Fedotovskaya, O. N.: **J09.44**
Feenstra, I.: C07.2
Fees, S.: P06.111
Fehrenbach, H.: **P02.136**, P02.201, P12.201
Fehrmann, R.: C08.4, C08.6, C10.1
Fehrmann, R. S. N.: C17.6
Fei, Y.: C10.3
Feillet, F.: P13.25
Feingold, M.: **P02.132**
Feinstein, M.: **P12.122**
Feki, A.: C04.6
Felbor, U.: C11.6, P02.107, P12.041, P12.204
Felice, A.: P10.36
Felice, M. S.: P06.008
Felipe, V.: P06.161, P12.237
Fend, F.: P06.178
Fenneteau, O.: P06.166
Fenwick, A.: EPL5.3
Ferandez, I.: P08.42
Ferbert, A.: P02.200, P12.041
Fereidooni, F.: J06.04, P06.142, P06.150
Ferese, R.: P09.131
Ferlazzo, E.: J09.45
Fernandes, A. R.: P11.055
Fernandes, S.: P02.177
Fernandez Carvajal, I.: **P14.08**
Fernández García, D.: P09.051, **P13.18**
Fernández Mateos, J.: **P06.128**
Fernandez, B.: P09.095
Fernández, L.: J02.55
Fernández, O.: P09.085
Fernández, P.: P11.050
Fernández, X.: P02.126
Fernandez_Otero, C.: J12.28
Fernández-Castillo, N.: P12.083
Fernández-de-Misa, R.: P06.138
Fernández-Jaén, A.: P09.030
Fernandez-Jimenez, N.: P09.036, P09.038, **P09.039**
Fernández-Pedrosa, V.: P06.161, P11.050, P11.091, P12.237
Fernández-Villar, A.: P06.122
Ferrão, J.: P06.169
Ferrari, L.: **P06.057**
Ferrari, P.: C03.1
Ferraz, V. E. F.: P06.029
Ferreira, C. A.: P06.029, P06.096, P10.46, P12.212
Ferreira, E. N.: P11.030
Ferreira, G. D.: P06.115
Ferreira, S. I.: P03.017
Ferreli, L.: P09.077
Ferrero, G. B.: P02.010, P02.040, P02.100, P02.129, P02.187
Fert-Bober, J.: C01.3
Feuth, T.: C01.1
Fic, M.: P09.107
Ficcadenti, A.: P12.057
Ficek, A.: P12.139
Fichera, M.: P09.030
Fiedler, E. E.: **P02.247**
Fiedler, J.: P02.243
Figueiredo, A. L. S.: P09.139
Figueiredo, J.: P06.063
Fila, L.: P01.11
Filipce, I.: P10.19
Filipova, H.: P06.210
Filkova, H.: P07.06
Fındık, D.: P07.20
Finelli, P.: P12.057, P12.058
Finocchi, A.: J12.30
Fiorentino, F.: P05.05, **P05.12**
Fiorillo, E.: C08.3, P09.077
Fiorini, P.: P02.044
Fiott, A.: P10.36
Firth, H.: EP08.03
Firth, H. V.: P02.169
Fischer, A.: P06.185
Fischer, B.: C09.6, P02.032, P02.223, P12.061, P13.45
Fischer, C.: C18.5, P02.215
Fischer, J.: **C18.2**, P02.027, P02.112
Fischer, K.: P01.02, P06.087, P11.121
Fischer, K.-D.: C13.5
Fischer, M.: C18.5
Fischer, U.: P12.042
Fischer, W.: P03.063
Fisher, E.: C12.1
Fisher, J.: EPL3.3
Fisher, S.: P09.126
Fisher, T.: EPL7.1
Fishman, E. K.: C01.3
Fishman, S. J.: C01.4
Fiskerstrand, T.: **C12.2**, P02.201
Fitze, G.: P06.080
Fitzgerald, O.: C14.5
Fitzner, A.: C16.6, **P11.038**
FitzPatrick, D.: P11.123
FitzPatrick, D. R.: P02.228
Flahaut, P.: C09.2
Flaherty, M.: P12.088
Flatø, B.: C15.4
Fleige-Menzen, S.: P11.001
Fleischer, S.: P02.153, P02.154
Fleischhauer, J.: P09.091
Fléjou, J.-F.: P06.078

Flex, E.: P12.222
Floettmann, R.: **P02.081**
Flögel, U.: P13.03
Flom, J.: P08.07
Flori, E.: P02.104
Floridia, G.: J15.02, **J15.03**
Floris, M.: P09.077
Floyd, J.: P10.06
Flusser, H.: P12.122
Fockens, P.: P01.34
Foertsch, T.: P09.050
Foja, S.: **P14.18**
Fojkar, M.: J03.13
Fokkema, I. F. A. C.: P11.084
Fokkens, M.: C11.3
Fokstuen, S.: P05.58, P12.055
Fomina, N.: P04.33
Foncuberta, M. E.: P06.008
Fonseca, C.: P09.129
Fonseca, E.: P06.131
Fontaine, J.: C18.2
Fontalba, A.: P06.161
Fontanillo, C.: P06.131
Fontcuberta, J.: P15.09
Fonte Santa, J.: P09.063
for the MORGAM Project, ..: P09.134
Forbes, R.: **EPL7.5**
Forejt, M.: P10.55
Forer, L.: P15.14
Forghanifard, M. M.: J06.19, P06.050, P06.091, P06.119
Formigli, L. M.: P03.140
Forni, A.: C07.4
Forni, G.: P02.147
Foroutan, M.: **P11.057**
Forsberg Nilsson, K.: P12.246
Forsberg, K.: P12.012
Forsberg, L. A.: C04.4
Forte, R.: P14.03
Forteza, A.: P12.237
Fortina, P.: P11.109
Fortuna, A.: EP03.01, P03.038
Forya, F.: EPL3.3
Forzano, F.: P02.147, P03.069, **P03.093**, P09.113
Fostira, F.: **P06.028**
Fouladi, P.: P04.36
Foulds, N.: C03.2
Fountzilas, G.: P06.028
Fourati, A.: P10.31
Foy, C. A.: P11.116
Francannet, C.: P12.018, P12.096
Francelin, P. R.: P06.115
Francioli, L.: C06.3
Francioli, L. C.: **C07.5**, P11.134
Francisco, I.: P06.109
Francisco, J. L. E.: P06.047
Francks, C.: P09.126
Franco, B.: P12.178
Franco, J. F. S.: P13.38
Franco, L. M.: C04.1
François, G.: P09.125
Francois, I.: J02.49
Frank, G.: P06.103, P06.184
Frank, J.: P09.009
Frank, V.: C15.5, P02.136, P02.200, **P02.201**, P12.068
Franke, A.: P09.138
Franke, B.: P09.028, P09.105, P11.007
Franke, L.: C08.4, C08.6, **C10.1**, C17.6, P08.13, P08.15, P08.23, P09.059, P11.126
Frankenbach, N.: P12.098
Frankish, A.: C08.1
Franklin, C.: P10.06
Fransen, E.: P02.023
Fransen, K.: P08.15
Franzoni, A.: **P12.013**
Fraser, L.: EPL2.4, EPL4.2
Fratzl, P.: P12.162
Frayling, T.: C08.4, C17.6
Frazao, R.: P02.185, P12.172
Frebourg, T.: C11.1
Frederiksen, A. L.: **P02.152**, P03.130
Freiberger, T.: P12.233
Freidin, M.: P09.064
Freidin, M. B.: P09.019, **P09.062**
Freisinger, P.: C12.4, P14.19
Freitag, C.: P09.028
Freitag, H.-J.: P02.072
Freitas, A. T.: P11.055
Frengen, E.: P03.007, P03.010, P03.051, P03.103, P03.145
Freshour, J.: P12.200
Freson, K.: J02.49
Freudenberger, P.: **P09.035**, P09.130
Frezzotti, P.: C14.1
Fridmanis, D.: P09.098
Frieauff, E.: P02.254
Friedburg, C.: P12.211
Friedl, R.: P11.136
Friemel, C.: **P04.25**
Friesema, E. C.: **P12.206**
Friez, M.: P11.142
Frisch, E.-M.: C12.4
Fritsche, L. G.: P12.235, **C14.2**, P09.008
Fritz, B.: **P07.34**
Frnandez-Jimenez, N.: P09.040
Froguel, P.: PL2.2
Fröhlich, H.: C08.5, P09.078
Frohlich, J.: P07.27
Frolova, U.: P12.104
Frolova, Y.: P12.017
Frommolt, P.: C03.3, C13.4, P12.108
Frost, M.: P02.152
Frühmesser, A.: **P02.116**
Frumkin, A.: P02.263
Fruscione, F.: P11.063
Fryns, J. P.: P02.053, P02.221, P05.15
Frysira, H.: P02.261, P03.012, **J02.30**, P08.11, P09.015, P12.040, P12.055
Fu, C.: P06.160
Fuchs, S.: P02.179
Fuciarelli, M.: P10.17
Fuentes, M.: P06.131
Fuh, J.-L.: P09.061
Fujio, K.: P09.117
Fujita, A.: P12.240
Fukushima, Y.: C04.3
Fulmer-Smentek, S.: P03.060, P03.122, P11.093
Fumoleau, P.: EPL6.4, P01.04
Funayama, C.: P12.231
Fundarò, C.: P02.058
Furlong, S.: **P11.117**
Furquim, I. M.: **P02.240**
Furuya, N.: P02.029
Fusco, C.: P11.103
Füst, G.: **P06.195**
Fylaktou, E.: **P12.156**
Fylaktou, I.: P02.227

G
Gabaldo, M.: **J02.53**
Gabau, E.: P03.041
Gabbasov, R. T.: **P09.068**
Gabbett, M.: P02.172
Gabert, M.: P02.078
Gabidulina, T.: P04.40
Gabriel, H.: J02.25, P02.089, **P02.143**, P05.06, P12.080
Gabriel, H.-D.: P02.167, P12.160, P12.176
Gabriel, M.: P08.01, P09.002
Gabrikova, D.: **J12.14**, P09.097
Gabrovsky, N.: P06.048, P06.144
Gacina, L.: J02.06, J03.13
Gadea, N.: P06.118
Gafari, S.: J09.24
Gafencu, M.: **P01.37**
Gaff, C.: **EPL6.6**
Gagliardi, M.: J12.33, J12.34, J12.35, **P12.081**
Gaildrat, P.: P11.110
Gaillard, D.: P09.026
Gaillyová, R.: **P01.21**, P03.051, P06.190, P12.159
Gaily, E.: P12.028
Gaivão, I.: J03.03
Gakh, O.: P14.09
Gál, A.: J02.02
Gal, A.: P12.108, P12.211
Galabov, A. S.: P06.054, P10.58
Galaktionova, D.: **P09.123**
Galanello, R.: P09.077, P11.037
Galbiatti, A. L. S.: P06.090, P06.115, **P06.129**
Galeeva, N.: **J12.11**, P12.256
Galeeva, N. M.: P02.176
Galehdari, H.: J11.04, **J13.08**
Galembo, I. A.: P03.094, **P11.024**
Galesi, O.: P09.030
Galesloot, T. E.: **P09.096**
Galetzka, D.: P12.137, **P12.213**
Gálffy, G.: P06.195
Galibert, F.: C18.2
Galieva, D.: **J02.18**
Galimova, E.: P09.018, **P09.124**
Galjaard, R.-J.: EPL7.4
Galjaard, R.-J. H.: P05.60
Gallagher, L.: P11.117, P12.167
Gallano, P.: **P12.070**
Gallati, S.: J02.29, P03.092, P11.073
Gallenmüller, C.: P02.064
Gallo, E.: C01.3
galluccio, a.: P09.110
Gamage, T. H.: P03.007, P03.010, P03.145
Gamba, B. F.: **J02.23**, P03.095
Gambardella, A.: J09.45, J12.33, J12.34, J12.35, P12.081
Gambina, G.: P10.24
Gambineri, E.: P02.044
Gamerdinger, U.: P03.081
Gamulin, M.: P06.200
Ganaha, A.: **P02.150**
Ganchev, G.: P06.054
Gandhi, G.: P03.100
Gandia, M.: P12.108
Ganguly, B. B.: J05.11, **J07.02**
Ganos, C.: P02.072
Ganster, C.: P07.23
Ganter, B.: P11.141
Gao, F.: P02.192
Gao, H.: P11.086
Gaponova, A. V.: **P06.103**
Garaeva, A.: P09.064
Garagnani, P.: P13.27
Garavelli, L.: P02.023, P02.090, P12.058, P12.227
Garbes, L.: **C01.6**
Garcia Bartels, N.: P09.010
García Miñaur, S.: J02.55
Garcia, A.: P03.023
Garcia, D.: J12.28
García, J.: P06.105
García, M.: J03.11, **P03.124**
Garcia-Alfonso, D.: **P12.237**
Garcia-Barcina, M.: P12.005
Garcia-Giustiniani, D. A.: P02.126
Garcia-Hoyos, M.: P12.005
Garcia-Iglesias, A.: P06.177
Garcia-Minaur, S.: C18.1
Garcia-Molina, E.: J02.46, J02.47, J02.48
Garcia-Murias, M.: **P12.216**
Garcia-Planells, J.: **P12.005**
Garcia-Sandoval, B.: P12.074, P12.210
García-Valiente, R.: P06.105
Gardeitchik, T.: P12.061, **P13.45**
Gareeva, A.: **P09.121**, P09.122, P09.123
Gargiulo, G.: P02.023
Gargouri, R.: P06.011
Garibay, N.: P02.128

Garimella, K. V.: C07.5
Garin, I.: **P11.056**
Garshasbi, M.: C15.3, P08.37
Gaspar, H.: C09.5, **P02.189**, P02.236
Gaspar, I. M.: P11.055
Gasparini, F.: P14.07, PL3.3
Gasparini, P.: P02.025, P08.31, P12.054, **P12.105**
Gasparyan, A.: P02.009, P03.052, P03.055, P03.057
Gass, P.: P12.021
Gassmann, M.: P11.087
Gassner, C.: P05.45
Gatti, R. A.: P12.147
Gaub, M.-P.: P06.078
Gaudet, D.: P11.023, P11.049
Gauguier, D.: P09.046
Gaussen, M.: C11.2
Gautel, M.: P12.254
Gauthier, J.: P09.119
Gauthier, M.-P.: P11.115
Gauthier, S.: EPL6.4, P01.04
Gavrilov, D. N.: P09.068, P14.09
Gayner, T. A.: J03.15
Gaysina, D.: P09.128
Gazal, S.: P06.166
Gebesoglu, I.: P02.191
Gecz, J.: C13.3
Geer, J. S.: P03.098
Gehrig, A.: **P06.026**, P11.021
Gehrig, C.: P09.141, PL2.6
Geigl, J. B.: C10.6, P03.021, **P06.051**
Gelb, B. D.: P12.222, P12.227
Gelfi, C.: P11.008
Gellermann, J.: P02.201
Gelmetti, V.: P09.131
Geloso, M.: P09.048
Gencik, A.: P02.089, P12.160, P12.176, P12.251
Gencik, M.: P02.143, P05.06, P12.080, P12.150, P12.160, P12.176, P12.251
Gencik-Guillier, Z.: **P02.089**
Generoso, A.: P12.227
Genesio, R.: J02.14, J02.52
Geneviève, D.: P02.141
Gennarelli, M.: P09.113
Genser, B.: P13.41
Gentile, F.: J11.11
Gentile, P.: J02.53
Geoffroy, S.: P09.119
Geoffroy, V.: C18.4, P12.046
George Jr., A. L.: C16.4
Georges, M.: S01.3
Georghiou, A.: P10.14
Georgiev, S.: P06.164
Georgieva, L.: P09.032
Georgoutsou, M.: J08.02
Gérard, M.: P05.48
Gerasimenko, N.: J03.06
Gerasimova, P.: P04.05
Gerdes, A.-M.: P02.113
Gerike, J.: P04.37
Gerlach, E. M.: P06.167
Gerlach, E.-M.: P02.133, P06.107
Gershoni-Baru, R.: P03.111
Gershoni-Baruch, R.: P05.59
Gerstein, M.: C06.5, J11.12
Gerull, B.: P12.237
Gervasini, C.: **P12.058**
Geschwind, D. H.: **S13.2**
Geurts van Kessel, A.: C02.2, P06.080, P11.078
Gezdirici, A.: J02.35, **P02.069**
Ghaderian, S.: P08.36
Ghaderi-Sohi, S.: C18.6, P03.034, P03.048
Ghaedi, K.: P12.208
Ghaffari Novin, A.: **P06.203**
Ghaffari, H.: P06.187
Ghaffari, S.: P12.211
Ghaffarpour, M.: J06.04, J06.05, J06.06, J06.07, **P06.150**
Ghafouri-Fard, S.: **P06.192**
Ghalandari, M.: P05.30, **P06.053**, P06.125
Ghandour, H.: P11.086
Gharehmohammadlou, R.: P06.121, P06.133, P06.134
Gharesouran, J.: J09.02, J09.18, **P09.011**
Gharib, S. A.: C08.4, C17.6
Gharizadeh, B.: P06.116
Ghasemi, M.: J09.31
Ghassemi, S.: P06.104
Ghassibe, M.: P09.125
Ghassibe-Sabbagh, M.: **P09.046**
Ghavami, Y.: J12.05
Ghidoni, A.: **C16.4**
Ghodsinejadkalahroudi, V.: **J02.27**, J06.05, J06.06, J06.07, J12.06, P02.050, P13.29
Gholamin, M.: **J06.19**
Ghorbel, M.: P04.16, **P04.27**
Ghosh, S.: P04.14
Gialluisi, A.: **P10.57**
Giannikou, K.: J02.34, **P02.261**, P03.012, P09.015
Giannini, G.: P06.033
Giannuzzi, G.: **P10.16**
Giardina, E.: C14.5, J09.43, P09.110
Gibson, S.: P02.178
Giedraitis, V.: C04.4
Gieger, C.: C14.5, C17.3, P10.33, P11.047
Giehl, K. A.: P09.010, P09.058
Gieruszczak-Białek, D.: P02.186
Giesemann, A.: P12.258
Gießelmann, S.: P12.108
Giessl, A.: P12.220
Giger, T.: PL2.6
Giglio, S.: **P11.077**, P11.125
Gigot, N.: P12.178
Gijsbers, A.: P03.054
Gijselinck, I.: P12.163
Gijsen, S.: C11.5, P02.132
Gijzen, M.: C18.6
Gil, M.: P06.161, P12.237
Gilabadi, S.: P03.077
Gilbert-Dussardier, B.: P02.141
Gil-Borja, M.: P11.050
Gil-da-Silva-Lopes, V. L.: P01.33, P12.244
Gilhuis, J.: P02.055
Gilissen, C.: C01.1, C02.2, C07.2, P02.132, P02.192, P11.035, P12.051, P12.126
Gill, M.: C06.4, P11.117
Gillan, T.: P03.071
Gillerot, Y.: P03.058
Gillessen-Kaesbach, G.: P02.072, P02.093, P11.069, P11.120, P12.245
Gil-Rodríguez, M. C.: P02.066
Gilyazova, I.: J09.27, P05.16, **P06.062**, P06.183, P06.189
Gimalova, G. F.: **P09.023**
Gimelli, S.: C04.6, P05.58, P12.055
Giménez, A.: P12.022, P12.177
Gimeno, J.: J02.46, J02.47, J02.48
Gimsing, S.: P02.257
Ginalska-Malinowska, M.: P02.233
Ginevičienė, V.: **P08.17**
Ginocchio, V.: **J02.14**, J02.52
Ginter, E. K.: P01.10, P10.27
Ginzburg, B.: **J04.01**
Giovannucci Uzielli, M.: **P02.044**
Giraldo, P.: P13.14, **P14.10**
Girard, S. L.: **P09.119**
Giraud, E.: C11.2
Girgin, G.: P02.220
Girisha, K. M.: **P02.059**
Girlando, S.: P06.176
Girotto, G.: **P08.31**, P12.105
Giuffre, A.: P11.128
Giunta, C.: C18.3
Giunti, L.: P11.077
Giza, A.: J12.25
Gkalympoudis, S.: C13.2
Glad, R.: P12.049
Glaeser, D.: P13.13
Glaser, A.: P07.09, P12.016, **P12.221**
Gläser, B. C.: P02.247
Gläser, C.: P09.106, P13.24
Glavač, D.: P11.052
Glebova, M. A.: P13.28
Gleeson, J.: C11.2
Gleeson, J. G.: P02.082
Gleeson, M.: EP05.10, EPL4.1, EPL6.5
Glentis, S.: P06.028
Gloning, K.-P.: P05.35
Glotov, A.: J09.17
Glotov, A. S.: J13.13, P09.083
Glotov, O. S.: **P13.28**
Gluzman, D.: P06.014
Gobbi, G.: P03.116
Gockel, I.: P09.004
Gödde, E.: P02.230
Godefroy, O.: P02.088
Godet, S.: P09.026
Godino, L.: **EP05.02**, **EP06.06**
Goeman, J. J.: C06.6
Goes, L. D.: P02.123
Goggin, N.: P02.214
Gogiel, M.: C04.2, P02.092, **P03.081**
Goh, Y. T.: C10.3
Goizet, C.: C11.2
Gokce, E.: P02.191
Goker-Alpan, O.: **P14.03**
Golberg, N. D.: P09.083
Gold, V.: **P04.20**
Goldberg, Y.: P05.59
Goldenberg, A.: C09.2, P02.141, P02.161, P11.123
Goldschmeding, R.: P12.154
Goldsmith, L.: **P01.13**, P01.31
Goldstein, M.: P05.54
Goloni-Bertollo, E. M.: P06.047, P06.090, P06.115, P06.129, P06.216, P11.030
Golovkine, N.: P09.026
Goltsov, A. Y.: P09.012
Gombos, É.: P12.015
Gomes, C.: P12.178
Gomes, Z.: J03.03
Gómez González, J.: P06.128
Gomez Milanes, I.: J02.47, J02.48
Gómez -Rosas, E.: P11.050
Gómez, C.: P07.12, P07.28
Gomez, D.: P02.223
Gómez, V.: P07.28
Gomez-Mancilla, B.: P14.07, PL3.3
Gomez-Milanes, I.: **J02.46**
Gómez-Moreta, J.: P06.105
Gómez-Puertas, P.: P13.07
Gomy, I.: J02.42, P13.38
Gonçalves, R. L.: P12.097
Gonçalves-Rocha, M.: P12.097
Gondry, J.: P05.18
Gonsales, M. C.: **P12.078**
Gonzaga-Jauregui, C. G.: C15.4
González Ramírez, A.: P02.030
González Ruano, E.: P13.18
González Sarmiento, R.: P06.128, P06.146, P07.14, P09.051, P13.18
Gonzalez, D. E.: P13.14, P14.10
González, J.: P03.025, P12.094
Gonzalez, J. M.: C08.1
Gonzalez, L.: P02.128, P06.027
Gonzalez, P.: P12.056
González-Casado, I.: J02.54
Gonzalez-Gonzalez, M.: P06.131
González-Hernández, A.: P06.138
Gonzalez-Huerta, L. M.: P12.100, P12.164
Gonzalez-Quereda, L.: P12.070, P12.265
González-Sarmiento, R.: P06.105, P06.177
Gonzalez-Valero, J.: P06.105
Gonzalo, F.: **J02.03**
Goobie, S.: **P02.146**

Goodship, T. H. J.: P08.21
Goodwin, W.: J10.11, J11.03
Goossens, D.: P06.033
Gopie, J. P.: **EPL4.4**
Goracci, M.: **P11.040**
Goranova, T.: P06.048, P06.144
Gorbunova, V.: J08.05, P08.16, P09.083
Gordon, A.: P05.05, P05.12
Gordon, C. T.: **P11.123**
Gorduza, E.: J02.21, J02.39, P03.125, P03.136, P03.144, **P05.11**
Gorduza, E. V.: J02.32, P03.053
Gorenflo, M.: P02.215
Göretzlehner, U.: P02.092
Gorfien, S.: P11.010
Gori, A.-S.: S01.3
Göring, W.: P11.135
Goris, A.: P10.33
Gorovenko, N.: **J05.13**, J05.14, J06.20
Goryluk-Kozakiewicz, B.: **P02.262**, P07.32
Gos, M.: **P02.185**, P12.172
Gosendi, M. E.: C09.5
Gospodinova, M.: P12.109
Goswami, N.: P10.26
Goto, Y.: **J05.01**
Gottwald, B.: P11.136
Götz, A.: C12.5
Götz, M.: P11.058
Gourabi, H.: J04.05, J04.10, J04.17, P03.033, P03.039, P03.077, P04.09, P04.56
Gourraud, P.-A.: P09.086
Gozalpour, E.: J09.41
Gra, O.: P09.123
Graakjaer, J.: **P02.155**, P03.080, P03.104
Grabert, K.: P06.055
Grabli, D.: P02.007
Gracia, R.: J02.54
Grad, I.: C04.6
Graefen, M.: P06.185
Graeser, M. K.: P07.05
Graf, A.: C02.3, P14.06
Graf, E.: C12.3, C12.4, C12.6, C13.2, C15.2
Gráf, L.: P06.195
Grall, A.: C18.2
Grallert, H.: C13.2, P13.09
Gramer, E.: C14.1
Gramescu, M.: P03.053, P03.125, P03.136, P03.144, P05.11
Grammatico, P.: P02.065
Granada, I.: P07.28
Granata, F.: P12.084, **P12.118**
Grande, M.: P05.43
Grandemange, S.: **P12.247**, P15.01
Granese, B.: J02.52
Grange, D. K.: P02.043
Grankina, E.: P07.29
Grasshoff, U.: P02.076, **P02.149**, P02.169, P12.145, P12.260
Grasshoff-Derr, S.: C09.4, P02.026, P02.130
Grassmann, F.: **P09.008**
Grassmann, H.: C02.1
Grasso, R.: P02.060
Grattau, Y.: P08.11
Graul-Neumann, L.: P02.004, P02.043
Graul-Neumann, L. M.: P02.081, **P02.181**
Graunteanu, C.: P13.10
Graur, E.: P03.053
Graves, T. A.: C06.1, P10.51
Gravholt, C. H.: P02.144, P10.35
Graw, M.: P02.245
Gray, M. J.: **P02.194**
Grayton, H.: P03.097
Graziano, C.: P02.135, P03.083, P03.084, **P11.059**, P11.070, P11.140, P12.130
Greco, L.: P09.059
Greely, H. T.: **S06.3**
Greenberger, S.: P12.202
Greenslade, M.: P11.054, **P12.050**
Gregersen, P.: P08.15
Gregor, A.: P02.158, **P12.102**
Gregor, M.: P12.219
Grehan, N.: EP02.05, EPL1.5
Greif, P. A.: **C02.3**
Greil, S.: P02.245
Greither, R.: P15.03
Grejtakova, D.: J12.14
Greslikova, H.: P07.26, P07.27
Gretz, N.: P12.016, P12.221
Grgic, M.: P06.200
Gribaa, M.: P07.31
Gribova, O.: P03.101
Griesche, N.: P12.121
Grigg, J.: P12.088
Grigore, A. G. M.: P06.123
Grigore, S.: P03.024
Grigorescu-Sido, P.: J14.07
Grigoriadou, M.: P12.067
Grigoroiu-Serbanescu, M.: P09.069
Grijalvo-Perez, A.: P02.082
Grillo, E.: **P10.49**
Grimm, C.: P06.185
Grimm, T.: P02.254
Grimme, A.: P12.042
Griskevicius, L.: P06.024
Gritzan, D.: P11.026
Grochova, D.: **P05.10**
Grochova, I.: P05.10
Grodecká, L.: **P12.233**
Grohe, B.: P12.201
Grohmann, J.: P12.162
Grömminger, S.: C16.1
Gromyko, O. E.: **J09.10**
Grond, S.: C18.2
Grond-Ginsbach, C.: C18.5
Groselj-Strele, A.: P13.41
Groß, B.: P04.43
Groß, U.: **P02.153**, P02.154
Großhans, M.: P09.009
Grosveld, F.: P12.199
Groza, T.: C09.1
Grozavu, A.: J02.39
Grozavu, M.: **J02.21**
Grozdanov, P.: **P06.054**
Grubert, F.: C06.5
Grubisa, I. I.: **P09.022**
Grudinina, N. A.: J02.44, J12.07, J14.02
Grugni, V.: P10.58
Grummel, V.: P10.33
Grünewald, A.: P12.245
Grünhagen, J.: C03.2
Grünig, E.: P02.215
Grutupa, M.: P06.005
Grzebieniak, Z.: P06.071
Grzmil, P.: P04.22
Gschwind, A.: **P03.011**
Guadalupe, M.: P11.128
Guaguère, E.: C18.2
Guarducci, S.: P02.044
Guarinos, C.: P06.082
Guay, S.-P. P.: **P11.049**
Guchelaar, H. J.: **S07.3**
Gudkova, A. Y.: J02.44
Gudmundsson, B.: P11.036
Gueneau, L.: P03.020
Guergelcheva, V.: P12.109
Guergueltcheva, V.: P02.064
Guerra, I.: P06.191
Guerreiro, M. M.: P12.078
Guerrot, A.-M.: C09.2
Gug, C.: EP04.01, **EP06.08**, J04.07, P02.108, **P05.17**, P05.24, P05.69, P11.043
Guida, V.: P09.131
Guigo, R.: C08.1, **S14.2**
Guilherme, R. S.: P03.023, **P03.118**
Guillén-Navarro, E.: P03.019
Guillermet, C.: P13.32
Guimarães, L.: EPL2.5
Guinchard, E.: **P05.56**
Guipponi, M.: **P09.141**, P12.055, PL2.6
Guitart, M.: P03.041
Guity, K.: P09.067
Gul, A.: P12.153
Gül, E.: P02.051
Gül, Ü.: P08.06
Gülçebi, M.: P02.220
Gulel, A.: **J09.29**, J12.02
Gulkovskyi, R.: J09.19
Gultekin, Y.: P06.127
Gültepe, P.: P02.220
Gumennaya, E. R.: P09.023
Gumerova, O.: P08.33
Gümüşlü, E.: J03.12
Gumuslu, E.: **P02.014**
Gün, D.: J02.38, **P02.122**
Günder, M.: P06.178
Gunduz, C.: J06.17
Günel, M.: P02.051
Güneş, H. V.: J13.10
Gunes, S.: P02.191, **P06.143**
Guney, A. I.: P06.020, P09.132, P12.037
Guney, I.: P02.220
Gungor, B.: P14.22
Gunnell, D.: P10.37
Guo, L.: C12.1
Gupta, N.: P14.10
Gur, S.: P14.05
Guran, T.: P12.037
Gurban, C.: P11.043
Gurbuz, Y.: P06.175
Gurgey, A.: P12.089
Gurieva, P. I.: **J12.03**
Gurinova, E. E.: P02.035
Gurkan, H.: **P04.52**
Gurler, A. I.: P07.19
Gürlevik, E.: P06.113
Gurling, H.: C06.4
Gurses, C.: J12.10
Gurtubay, P.: P03.025
Gurwitz, D.: P10.42
Guryev, V.: **C06.3**
Gusareva, E. S.: P08.28
Gusina, A.: **J13.04**
Gusina, N.: J13.04, P13.15, **P13.21**
Gussi, I.: P05.38
Gutierrez, B.: P06.191
Gutierrez, G.: P06.061, P09.040
Gutierrez, J.: P08.23, P11.126
Gutierrez, M.: P06.131
Gutierrez-Achury, J.: **C17.1**, P09.059
Gutierrez-Arcelus, M.: **PL2.6**
Gutiérrez-Enríquez, S.: P06.118
Gutkowska, A.: P02.005, P02.262, P03.045, **P03.049**, P03.114
Gutmajster, E.: P02.103, P02.244
Gutwein, J.: P04.25, P06.073, P11.069
Güven, G.: J02.35
Guzel, A. I.: P05.65
Guzmán, M.: P03.124
Güzünler, M.: P02.131
Gwanmesia, L.: **P05.58**, P12.055
Gyapay, G.: C11.2
Gyftodimou, Y.: P02.258
Gyulai, N.: P06.195
Gzgzyan, A.: P04.54
Gzgzyan, A. M.: P11.024

H
Haack, T.: **C12.3**, C12.4, P14.19
Haaf, T.: C16.5, P04.03, P11.033, P11.038
Haake, A.: **P06.098**, P11.120
Haas, D.: P02.236

Haas, S.: J12.31
Haase, D.: P07.23
Haas-Lude, K.: P02.076
Haataja, R.: P09.034
Haavik, B.: C12.2
Habbig, S.: P12.201
Habegger, L.: C06.5
Habenicht, R.: PL2.3
Haberberger, B.: C12.3, C12.4, **P14.19**
Haberlandt, E.: C18.5
Habib, R.: **P07.32**
Habibi Roudkenar, M.: **J15.01**
Habibi, M.: J09.35
Habibi, R.: J04.05
Habiboglu, A.: P08.38
Hachicha, M.: J04.16
Hacihamdioğlu, B.: P02.022
Hackmann, K.: P02.121, **P02.133**, P02.203, P06.107, P06.167
Hadchouel, J.: PL2.2
Haddad, B.: P02.104
Haddad, V.: **P03.062**
Hadipour, F.: J02.08, **J14.04**, P03.131, P12.208, P13.37
Hadipour, Z.: **J02.08**, J14.04, P03.131
Hadjidekova, S.: P02.034, **P02.063**, P06.018, P06.054, P11.118
Hadjigavriel, M.: P12.008
Hadjisavvas, A.: P11.072
Hadzsiev, K.: **J02.02**, P10.52, P13.22
Haenecour, A.: **P12.184**
Haenisch, B.: P09.069
Hafezi-Nejad, N.: C15.3
Haffen, E.: P12.167
Häffner, K.: P02.136, P02.201
Hageman, G.: C11.3
Hagemann, M.: P02.057
Hager, A.: P02.215
Hagh, J.: P03.081
Haghighatfard, A.: **P01.19**
Hagnefelt, B.: P12.047
Hahn, D. E. E.: EPL6.3
Hahn, G.: P02.203
Hahn, T.: P04.03, P11.033
Hahnen, E.: P06.117
Haivas, C.: P02.139
Haj Salem, I.: P02.260
Hajdinjak, T.: P06.094
Hajebrahimi, Z.: **P14.17**
Hajek, R.: P07.26, P07.27
Hakimullina, A. M.: P09.068
Halabudenco, E.: J01.03
Halabudenco, E. A.: J10.02
Halayenka, I.: **J09.01**
Halbhuber, Z.: P12.134
Hall, A. E.: **P01.29**
Hall, G.: C15.6, EP07.02, EPL5.6
Haller, T.: P01.02
Hallman, M.: P09.034
Hallowell, N.: EP05.10, **EPL6.5**
Hamamy, H.: P12.055
Hamanoue, S.: P02.029
Hambrock, M.: **P12.042**
Hamdan, S.: P11.060
Hamel, B. C.: P02.192
Hamel, B. C. J.: C15.1, P12.124
Hamelin, R.: P06.078
Hami, H.: **P10.15**
Hamid, A.: P03.063
Hamid, M.: P02.119, **P06.015**
Hamidieh, A.: P13.37
Hamilton, A.: P12.093
Hammami, M.: P08.04, P08.18
Hammer, C.: **P09.069**
Hammerschmidt, M.: C03.3
Hammoudeh, Z.: P11.118
Hampe, J.: P06.073
Hampel, T.: P02.136
Hamude, Z.: P06.164
Han, B.: P09.066
Hana Utami, K.: P12.088
Hancarova, M.: P02.168, P03.004, P03.089, **P03.102**
Haney, M.: J11.12
Hann von Weyhern, C.: P02.209
Hannachi, H.: P03.066, P03.085
Hanneken, S.: P09.010, P09.078
Hannequin, D.: C11.1
Hannibal, M.: P02.043
Hans, V. H.: C02.1, P06.136
Hansakunachai, T.: P09.029
Hansen, P.: P12.031
Hansen, S. J.: P02.152
Hansikova, H.: P13.30
Hansmann, M.-L.: C10.5
Hansmann, T.: P11.033
Hanson, C. I.: C15.4
Hansson, K.: **P03.054**
Happe, S.: P11.128
Haque, F.: C11.6
Har Zahav, A.: C12.1
Har Zahav, A.: P02.132
Harada, N.: P03.002
Harakalova, M.: C18.1, **P12.259**
Haraksingh, R.: J11.12
Haralambos, K.: P11.054
Haratian, K.: **P06.041**
Harborth, S.: P11.086
Hardekopf, D.: P03.091
Hardouin, A.: P11.110
Harel, T.: P12.146
Hariharan, M.: C06.5
Harinck, F.: **P01.34**
Harmas, V.: P05.68
Harms, K. C.: **P08.29**
Harrap, S.: P09.078
Harris, H.: EPL2.1, EPL2.2
Harris, J. R.: C04.4
Harrow, J.: C08.1
Hart, D. J.: PL2.5
Hartman, C.: C12.1
Hartmann, B.: **P02.027**, P12.145
Hartmann, H.: P12.258
Harton, G.: C16.3
Harutyunyan, A.: J10.01
Harvey, R. P.: P09.024
Has, R.: P05.22, P05.29, P05.36
Hasanzade, K.: J03.09
Hasegawa, T.: P12.138
Hasenclever, D.: P06.098
Hashemi, M.: J02.01, J04.03, J05.05, J06.02, J06.03, J06.08, **J06.09**, J06.10, J06.11, J06.13, J11.08, P06.003, P06.009, P06.043, **P06.052**, P06.187, P06.193, P06.197, P10.54, P14.15
Hashemi, S.: P06.052
Hashemzade Chaleshtori, M.: J08.01, J09.22
Hashemzadeh, M.: P08.41
Hashiguchi, T.: P02.223
Hashmi, M.: **P05.23**
Hashmzadeh Chaleshtori, M.: J12.12
Haslberger, A. G.: P11.076, **P13.26**
Hassan Tonekaboni, S.: P10.07
Hastings, P. J.: C04.1
Hatakeyama, M.: P12.240
Hatamnejadian, N.: J02.12, P02.085, P02.094, P02.235, P12.075
Hauenstein, N.: P10.33
Haug, K.: C16.1
Haug, U.: P03.059
Haukanes, B. I.: C12.2
Hauke, J.: **P06.117**
Haun, M.: P15.14
Hauner, H.: P13.09
Haus, O.: P03.065
Hauser, A.: C02.3
Hauser, J.: P09.069
Hausser, I.: C18.2
Havlovicová, M.: P02.068, P02.168, P02.174, **P02.213**, P11.059
Hawash-Moustafa, N.: P03.111
Haydon, J.: EPL3.1
Hayeeduereh, F.: P09.029
Hayrapetyan, H. S.: P02.098
Hazan, F.: J12.04, **P02.052**, P02.071
Hazelzet, J. A.: C14.3
He, L.: P15.03
Healy, J.: P11.086
Heard, E.: C08.2
Heberle, C.: P12.038, P12.155
Hecht, J.: C03.1, C13.1, P11.100, **P12.031**
Hecht, S.: P04.04
Heck, A.: P11.104
Heckel, T.: P03.011
Hedayati, M.: **J09.41**, **P06.140**
Hedman, A.: P09.120
Heffelfinger, C.: C06.5
Hegele, R.: C12.1
Hehir-Kwa, J. Y.: C04.5, C06.3, P11.078
Hehr, A. J.: **P05.45**
Hehr, U.: J02.17, **P02.101**, P02.149, P02.203, P02.242, P05.45
Heid, I. M.: C06.2, P09.008
Heidari, M.: P06.203, **P11.041**
Heidenreich, B.: P06.154
Heidtke, K. R.: P05.31
Heijboer, K.: P03.054
Heijdra, Y.: P11.078
Heilbronner, H.: P02.247
Heiliger, K.: P02.255, P03.090
Heilmann, S.: **P09.078**
Heim, K.: **P11.047**, P14.19
Heimbach, A.: P06.136
Heimdal, K.: C09.5, C15.4
Hein, H.: C03.2
Heine, C.: P02.121
Heine-Suñer, D.: P04.38
Heinimann, K.: P06.176
Heinrich, S.: P12.232
Heinrich, T.: **P02.254**
Heinrich, U.: P02.078, **P02.180**
Heinrich, V.: **P11.100**, P11.101
Heinritz, W.: **P02.224**
Heinzova, V.: P07.17
Heise, M.: P02.131
Heitmann, M.: P11.026
Heitzer, E.: **C10.6**, P06.051
Hejtmankova, M.: **P02.259**, P03.123
Held, K.: , **P04.29**, P05.53, P05.70
Hellenbroich, Y.: P02.072, P02.131, P12.041
Heller, R.: C01.6
Hellin, A. C.: P02.238
Helsper, K.: **P12.091**
Hemmer, B.: P10.33
Hempel, M.: C13.2, P02.255, P03.090, PL2.3
Henderson, A.: P02.184
Hendrikx, M. T. M.: P15.11
Henggeler, C.: P12.086
Hengstenberg, C.: C05.5
Hennekam, F. A. M.: P02.253
Hennekam, R.: C03.5
Hennekam, R. C. M.: J02.55, P12.138, P12.259
Henneman, L.: EP08.06, **EPL6.2**, P01.22, P01.23
Henneman, P.: P10.53
Hennermann, J. B.: C12.4
Hennies, H. C.: C13.4
Henrique, T.: P11.030
Henriques, S.: EPL2.6
Henry, C.: P03.020
Henry, P.-G.: P12.120
Hens, K.: **P01.16**
Hentschel, J.: P02.021
Herault, Y.: P09.056
Herberg, U.: C12.4

Herberz, R.: C14.4
Herbst, S.: **P13.05**
Herder, C.: P11.047
Heredia Farfan, R.: P14.08
Hermanns, P.: P12.039
Hermans, P. W. M.: C14.3
Hermens, R. P. M. G.: EP05.04
Herms, S.: C14.4, P08.08, P08.32, P09.047, P09.118, P09.120, P11.015, **P15.12**
Hernan, I.: P12.022
Hernández Sanz, L.: P06.021
Hernández, J.: P07.28
Hernández, M.: P02.066
Hernandez-Alcantara, P.: **P12.164**
Hernández-Charro, B.: **P03.016**
Hernández-Illán, E.: **P06.072**, P06.082
Hernández-Jiménez, J.: P06.138
Hernández-Moro, C.: P06.196
Hernandez-Viquez, R.: P12.100
Herold, C.: C17.4, P08.12, P08.39, P09.010, P09.047, P09.078, P10.38
Héron, D.: C03.5, P02.007, P02.024, P12.167
Herrmann, D.: P06.098
Hersmus, R.: P06.201, P06.202
Hertz, J.: P02.144
Hervás, A.: P09.028
Hessen, L.: C01.6
Hettinger, J. A.: P03.084, P11.059, **P11.061**, P11.070, P11.140
Heuzé, Y.: P12.264
Heydari, R.: P05.30
Hibaoui, Y.: **C04.6**
Hibberd, M. L.: C14.3
Hiber, M.: P11.111
Hickerton, C.: EPL3.6
Hiddemann, W.: C02.3
Hikkel, I.: P12.251
Hikkelová, M.: P12.080, P12.160, P12.251
Hildebrandt, F.: P13.44
Hilger, A.: P02.026, **C09.4**
Hilger, T.: P07.05
Hilhorst, M. T.: EPL4.4
Hilhorst-Hofstee, Y.: P12.051
Hiljadnikova Bajro, M.: **P06.068**
Hill, M.: EPL3.2
Hill, M.: **EPL3.3**
Hillman, R.: P14.03
Hillmer, A.: C08.5, **C10.3,** P09.078, P12.088
Hills, L.: P05.34
Hinderhofer, K.: P02.077, P02.189, P02.215, **P02.236**, P02.241, P03.059, P12.232
Hinghofer-Szalkay, H.: P10.26
Hingorani, A. D.: P10.12
Hinrichsen, T.: **P06.079**
Hiort, O.: P04.02
Hipp, S.: C11.5
Hippe, B.: P13.26
Hippman, C.: EPL5.4
Hirsch, K.: P08.08
Hirst, R. A.: P12.200
Hirst-Stadlmann, A.: P02.008
Hitchcock, J.: P01.29
Hitte, C.: C18.2
Hitzler, J.: P07.01
Hivert, M.-F. F.: P11.023
Hlaváčová, M.: P06.001
Hlavata, A.: P06.168
Hlavová, E.: P03.123
Hlebic, G.: P06.094
Hmida, D.: P07.31
Hmida, N.: P12.194
Hnateyko, O.: J09.26
Hnizdova-Bouckova, M.: P13.36
Hnykova, L.: P02.259, P03.123
Ho, C. S.: P13.31
Ho, L.-T.: P09.061
Ho, P.: C14.5
Hodgson, J.: EP02.04, EPL6.6
Hodicka, Z.: P09.005
Hoefele, J.: **P12.155**
Hoefers, C.: P02.057
Hoefsloot, L. H.: P02.006
Hoejland, A. T.: **P03.104**
Hoelker, I.: C01.6
Hoell, J.: C10.5
Hoertnagel, K.: P05.35
Hoesch, J.: P14.16
Höfele, J.: P02.180
Hoffjan, S.: P12.239
Hoffman, P.: P09.120
Hoffmann, D.: P12.036
Hoffmann, E.: C10.6
Hoffmann, G. F.: C12.4
Hoffmann, K.: P02.057, P02.223, P06.188, P13.24
Hoffmann, P.: C08.5, C10.2, C14.4, P02.026, P09.033, P09.047, P09.118, **P11.015**, P15.12
Hoffmann, S.: C10.5
Hoffmann, U.: P02.160
Hoffman-Zacharska, D.: **P12.217**
Hofland, N.: EP05.05
Höfler, G.: C10.6
Hofman, A.: C05.2, C08.4, C17.6, PL2.5
Hofmann, B.: P09.106
Hofmann, K.: **P12.218**
Hofmann, W.: **C16.1**
Hofner, M.: **P06.070**
Hofner, M.: P11.022
Hoischen, A.: C02.1, P02.192, P02.226, **P11.035**, P12.126
Holden, S.: C01.4
Holder, S.: C13.1
Holder-Espinasse, M.: P02.169
Holicov, M.: P05.11
Holida, M.: P14.03
Holinski-Feder, E.: C01.5, C10.2, P02.086, P02.204, P04.42, P06.154
Holland, H.: P07.25
Holland-Cunz, S.: C09.4, P02.026, P02.130
Hollódy, K.: J02.02
Holloway, L.: P11.142
Holm, I.: **P12.148**
Holme, I.: **EPL5.3**
Holubová, A.: P01.11, P02.213
Homuth, G.: P11.047
Honer, W.: EPL5.4
Hong, M.-Y.: C06.5
Honjo, R. S.: J02.42
Honzik, T.: P13.30
Hoogeboom, A. M.: C03.2
Hoogenboom, J.: P11.084
Hoogerbrugge, N.: EP05.04, EP05.06, P01.35, P06.063, P06.080, P11.078
Hoogerbrugge, P. M.: C02.2, P06.080
Hoogerheide, L. F.: P11.068
Hook, D.: P14.09
Hopfner, K.-P.: C02.3
Hoppe, B.: P12.201
Hoppe, N.: EP08.04
Hoppe-Gołębiewska, J.: **P06.084**, P06.204
Horáček, J.: P03.123
Hordijk, R.: P12.095, P12.124
Hordijk-Hos, J. M.: P12.124
Horelli-Kuitunen, N. J.: **P05.52**
Horinek, A.: P09.054
Horn, D.: **C09.3**, C13.1, C13.2, P02.099, P02.223, P12.031, PL2.3
Hornemann, T.: C01.5
Hornig, N.: C10.5
Hornung, V.: P11.044
Horpaopan, S.: C10.2
Horsthemke, B.: P09.060, P11.026, P12.036
Horstkötter, D.: **P01.15**
Horvath, G.: P03.071
Horvath, J.: P12.033
Horvath, P.: P06.178
Hoseineian, Z.: P06.043
Hosen, M. J.: P02.045, **P12.207**
Hosie, S.: C09.4, P02.026, P02.130
Hosoya, T.: P09.071
Hosoyamada, M.: P09.071
Hossain, F. N.: P14.06
Hosseini, M.: P02.094, P08.37
Hosseini, S.: P06.035, **P06.197**
Hosseinian, Z.: **J06.08**, P06.104
Hosseinifar, H.: P04.56
Hosseinnejad, A.: P06.108
Hossein-Nezhad, A.: **P06.095**, P06.211, **P06.215**, **P09.089**
Hosseinpanah, F.: P09.067
Hosseinzadeh, N.: P09.067
Hoster, E.: C02.3
Hosterey-Ugander, U.: EPL5.2
Hottenga, J.: P11.143
Hotz, A.: P02.167, P12.145
Houdayer, C.: **P11.110**
Houde, A.-A. A.: **P11.023**
Houge, G.: P02.087, P02.162, P11.005
Hourlier, T.: C08.1
Houshmand, M.: J02.19, J02.27, J06.04, J06.05, J06.06, J06.07, J09.38, J12.05, J12.06, J13.07, J13.09, P02.050, P02.094, P04.53, P06.150, P10.07, P13.29, P13.46
Houwink, E. J. F.: **P01.22**
Hovdenak, N.: C12.2
Hovhanissyan, A.: **P03.057**, P02.009, P03.052, P03.055
How, G. F.: C10.3
Howald, C.: **C08.1**
Howard, H. C.: **EPL7.6**, P01.14
Høyer, H.: **P02.056**
Hoyer, J.: C13.2, P02.237, P03.068, P12.218
Hozak, P.: P12.134
Hrabé de Angelis, M.: C12.6
Hrabíková, M.: P06.001
Hrdlickova, B.: **P09.115**, P11.126
Hrebicek, M.: P13.36
Hristova, T.: **J04.12**
Hruba, M.: **P07.08**
Hryhorowicz, S.: P06.084, P06.204, P09.002
Hryniewiecka-Jaworska, A.: P10.49
Hryshchenko, N.: **P03.084**, P11.070
Hsiao, L.-C.: P09.061
Hu, H.: C15.3, J12.31, P12.124
Hu, X.: C02.4
Hu, Y.: C18.3
Huang, S.: P03.037
Hubbard, T.: C08.1
Huber, A.: P06.064
Huber, C.: P12.018
Huber, F.: P06.181
Huber, W.: C06.5
Hübner, A.: P02.064
Hübner, C. A.: P12.108
Huckins, L. M.: **P10.06**
Huebner, A. K.: P12.108
Huebner, C.: **P09.027**
Huerta, I.: **P03.025**, P12.094
Huet, F.: P02.141, P02.170
Huff, C. D.: S10.1
Hüffmeier, U.: **C14.5,** P09.111
Huggett, J. F.: P11.116
Hughes, A.: C14.6
Hühne, K.: P09.050
Huizinga, T.: P08.15
Huleyuk, N.: P04.31
Huljev Frkovic, S.: P03.040, P03.110, P05.02, P05.19
Hulshoff Pol, H.: P09.120
Hummel, M.: C10.5
Humphreys, M.: P02.173
Hundsdörfer, P.: P02.004
Hunfeld, J.: EPL7.4
Hung, C. C.: **P11.102**

Hung, T.: P11.086
Hünlich, M.: P11.053
Hunt, K.: C17.1, P09.059
Hunter, J.: C09.1
Huntley, D.: P02.217
Hureaux, M.: **P02.120**
Hurles, M.: C11.4, EP08.03
Hussain, M. S.: **C13.4**
Hussein, H. A.: P03.126
Hussein, I. M. R.: **J03.20**
Huth, C.: P13.09
Hutter, B.: C10.5
Hutter, P.: EP05.07
Huusko, J. M.: **P09.034**
Hwu, C.-M.: P09.061
Hyppönen, for the D-CarDia collaboration, E.: P10.12

I
Iacob, C.: J02.21, J02.39
Iacob, D.: J02.22, J13.03, P05.39
Iacob, R.: J13.03
Iafusco, D.: P02.001
Iancu, B.: P03.121
Iancu, D.: P03.121
Ibala, S.: P04.49
Ibrahim, D.: C03.1, P12.031
Ibrahim, J.: P14.03
Ibrulj, S.: **J03.10**, **P03.030**, P03.075
Ichida, K.: **P09.071**
Iglauer, P.: P12.251
Iglesias, S.: EP05.12
Ignatovica, V.: **P09.098**, P09.136
Igumvov, P. S.: J10.10
Ijntema, H.: C07.2
Ikbal, M.: J02.36
Ikels, K.: P13.03
Ikonnikova, A.: P09.127
Ilencikova, D.: P02.211, **P06.083**, P06.168, P12.161
Ilgın Ruhi, H.: P02.022
İlhan, N. E.: P02.226
İlkay, Z.: J03.12, **P06.056**
Illarioshkin, S. N.: J09.37
Illig, T.: P11.047, P13.09
Ilyina, E. S.: P02.019
Imanian, H.: P02.252, P05.30, P06.125, P12.090
Imkeller, P.: P11.100
Immesberger, A.: P04.03
Imyanitov, E. N.: P06.036
Inagaki, A.: P04.41
Inaloz, H. S.: J09.29
Inaloz, S.: J12.02
İnanır, A.: J09.13, P08.25, **P08.38**
İnanır, S.: J09.13
Inanloo Rahatloo, K.: **J12.20**
Inashkina, I.: J09.09, **P11.066**
Incollu, S.: P10.57
Indráková, V.: P06.001
Infante Sanz, M.: **P06.021**, P06.023
Infante, M.: P06.065, P06.196
Ingelsson, M.: C04.4
Inglis, A.: EPL5.4
Ingvoldstad, C.: **EPL1.3**
Innaskina, I.: P11.065
Insolia, R.: C16.4
Instone, J.: **EP06.03**
Insunza, A.: P07.28
Intermesoli, T.: P06.059
Inwald, D.: C14.3
Ioan, A.: P08.15
Ioana, M.: P13.10
Ioannides, M.: P03.112
Ion, A. D.: J10.12, P10.10
Iordache, C.: P02.140
Iotchkova, V.: **C17.3**
Iourov, I. Y.: P03.009, **P03.014**, P03.022, P03.115
Irabe, M.: **J02.43**, P02.120
Irani, S.: P06.142
Irastorza, I.: P09.036, P09.038, P09.039, P09.040
Iredale, R.: EPL4.2
Irfanoglu Timuragaoglu, A.: P06.130
Iribarren, C.: C04.4
Irles, E.: P06.072, P06.082
Irvanloo, G.: P06.095, P06.174
Irvine, A. D.: C01.4
Isaacs, A.: **C05.2**, P09.076, P10.53
Isakovich, L.: J01.01
Isaksson, M.: P11.129
Isau, M.: P06.185
Isaya, G.: P14.09
Isbir, T.: P12.037
Iseri, S. U.: J12.10
Ishihara, N.: **P02.110**
Ishimoto, H.: P02.196
Isidor, B.: P06.166
Isik, A. F.: P06.132
Islamgulov, D.: P06.058
Ismael, A.: P11.060
Isobe, K.: C10.3
Isohanni, P.: C12.5
Itagaki, Y.: P12.131
Iturbe, A.: P06.027
Ivanov, I.: P03.125
Ivanova, E.: **P12.166**, P02.176
Ivanova, L. Y.: P01.10
Ivanova, N.: P12.117
Ivashchenko, T. E.: P03.113, P13.28
Iwanicka- Pronicka, K.: P02.117
Iyevleva, A. G.: P06.036
Izadi, P.: P08.10
Izhevskaya, V. L.: **P01.10**
Izhevskij, P.: **J10.09**
Izmailov, A.: P06.016
Izmailova, S.: P06.016
Izquierdo, D.: P11.062
Izquierdo, G.: P09.085
Izumi, S.: J05.01, P02.196
Izzi, B.: **J02.49**
Izzo, A.: **J11.11**

J
J. Braathen, G.: P02.056
Jaal, J.: P06.066, P06.067
Jaberi, E.: **J12.17**
Jackson, A.: P02.208
Jackson, L.: P01.13, **P01.31**
Jacob, D. E.: P12.201
Jacobs, I.: EPL4.2
Jacobs, P.: P03.037
Jacobsen, U.: P11.069
Jacobsson, J. A.: P09.136
Jacquemont, M. L.: P02.141
Jacquemont, S.: C03.5, **PL3.3**
Jacquette, A.: P02.007, **P02.024**
Jaecklin, T.: **P14.06**
Jafari, H.: **P06.133**, P06.134
Jafarieh, H.: **P02.002**
Jafarshad, R.: P02.002
Jäger, N.: C10.5
Jaggi, R.: P06.181
Jaggs, N.: C07.3
Jagielska, D.: P09.010
Jahantigh, M.: P06.052
Jahnova, H.: P13.36
Jaiswal, P. K.: **P06.019**
Jakaitienė, A.: P08.17
Jakkula, E.: P09.086, P12.192
Jaklič, H.: **J04.15**
Jaklin, C.: **P03.090**
Jakob, F.: P13.20
Jakubiak, A.: P09.099
Jakubiczka, S.: **P12.175**
Jakubowska-Burek, L.: P06.084, P14.12
Jalalvand, K.: P12.024, P12.025, P12.168
Jalili, A.: **J03.09**
Jalilian, N.: P08.10, **P12.106**
Jalkh, N.: P12.055
Jallad, S.: **P04.49**
Jalůvková, M.: P06.165
Jamali Zavarei, M.: J04.10
Jamar, M.: P02.238
Jambou, M.: P13.32
Jameel, M.: P02.033
Jamet, X.: C18.4
Jamieson, R. V.: **P12.088**
Jamsheer, A.: **P02.193**, P12.237, PL2.3
Jamshidi, J.: J04.06, **J08.06**
Janashia, M.: J03.08
Janavicius, R.: **P06.024**
Janecke, A. R.: **P02.166**
Janicsek, I.: P10.25, P10.30, **P10.43**, P10.52
Jankevics, E.: J09.09, P11.065, P11.066
Jankulovski, N.: P06.077
Jans, S. M.: **EP08.06**
Jansen, L.: C10.4, P06.055
Jansen, R.: C08.4, P08.13, C17.6
Janssen, A.: P02.045
Janssen, J.: P02.077, P02.189, P02.241, P02.246, P03.059
Janssen, N.: **P02.006**
Janssens, S.: **P01.09**
Janusicova, V.: J09.12, P12.209
Janvareva, O. K.: **P02.042**
Jaquette, A.: P02.141
Jardim, A.: P03.017
Jarolim, L.: P06.182
Jarolimova, L.: P12.134
Járomi, L.: P10.25, P10.30, P10.43, P10.52
Jaroudi, S.: P04.30
Järvelä, I.: J12.31
Järvelin, M. R.: P10.12, C05.3
Jaspers, M.: P11.083
Jastrzebska, E.: P12.121
Jauch, A.: P02.077, P02.189, P02.234, P02.241, P02.246, P03.059, P03.090
Jauhiainen, M.: C05.3
Javadi, G.: J06.04, P06.043, P06.104
Jazayeri, R.: **J09.21**
Jazayeri, S.: J09.21
Jeannin, P.: P02.045
Jędrzejowska, M.: P02.186, P02.262, P03.049
Jekel, M.: EP08.01
Jelsig, A.: P02.113
Jenetzky, E.: P02.130
Jensen, A. S.: **P02.144**, P10.35
Jensen, L. R.: **P10.09**
Jensen, T. D.: **P03.130**
Jeong, K.: **P11.028**
Jessen, W.: P02.049
Jeunemaitre, X.: PL2.2
Jezela-Stanek, A.: P02.005, P02.041, P02.186, P02.262, **P03.045**, P03.114
Jezierska, J.: C11.3
Jia, X.-Y.: P11.025
Jiang, C.: P02.102
Jimenez Criado, C.: **P06.146**, P07.14
Jimenez Orozco, E.: P06.161
Jin, Y.: P06.116
Jissendi, P.: P02.062
Jmel-Hammami, K.: P03.015
Jochim, A.: P10.33
Jöckel, K. H.: P09.078
Joda, H.: **P09.042**
Joensuu, A.: **P09.134**
Joensuu, T.: P12.192
Johannsson, O. T.: EPL4.6
Johansson, H.: **P11.129**
Johansson, L. F.: P12.111
Johansson, S.: C12.2
Johnsen, H.: P11.096
Johnson, M.: EP05.09
Jonaitis, L. V.: P09.138

Jonasova, A.: P07.02
Joncourt, F.: **J02.29**
Jones, C.: P11.037, P11.119, P12.125, C08.3
Jones, J. R.: P11.115
Jones, W.: EP05.09
Jongbloed, J. D.: P12.064, P12.111
Jongmans, M. C. J.: **P06.080**, P11.078
Jonsson, J. J.: EPL4.6, **P11.036**
Jonveaux, P.: P02.011, P03.020, P09.026, P12.167
Joost, K.: P02.231
Joosten, H.-J.: P11.097
Joosten, S. J. R.: **P01.05**
Jordanova, A.: P12.116, P12.117
Jorde, L.: **S10.1**
Jorge, P.: P12.097, P12.250
Jorge, S.: P02.163
Jørgensen, A.: P03.141
Joset, P.: C13.2
Josifovski, T.: P06.068, P06.077
Jost, B.: J11.10, P12.046
Joun, D.: P11.014
Jourkova, N. V.: P12.112
Jovanovic Privrodski, J.: J03.13
Jovanovic-Lassalle, M.: P01.24
Jovanovic-Privrodski, J.: **J02.06**
Juan, W. C.: C10.3
Juan-Mateu, J.: P12.070
Juengst, E. T.: EP08.04
Jugold, M.: C02.1
Jula, A.: C05.3
Julia, S.: EPL7.2, P01.27, P02.161
Julian-Reynier, C.: **EPL2.1**, EPL2.2
Juneblad, K.: C14.5
Jung, M.: P14.18
Jungbauer, S.: P14.16
Jungbluth, H.: P12.254
Junge, A.: P03.139
Junior, N. A. B.: P06.047
Junker, S.: P06.178
Jurajda, M.: P06.004
Jurca, A.: P02.105, P02.137
Jurca, C.: P02.105, P13.11
Jurca, C. M.: **P02.137**
Jurkiewicz, D.: **P02.041**, P02.186, P02.232, P02.233, P07.24, P13.47
Juronen, E.: P10.13
Just, S.: P12.016
Just, W.: P12.011, P12.012
Justice, C. M.: P12.264
Juul, S.: P10.35

K
Kääb, S.: P02.245
Kaabak, M. M.: J09.05
Kabashima, K.: P12.068
Kabiraj, M. M.: C11.2
Kabra, M.: P13.14, P14.10
Kachakova, D.: P06.044
Kacmaz, E.: J02.36
Kaczmarek-Ryś, M.: P06.084, **P06.204**
Kadam, N. N.: J05.11
Kadasi, L.: J09.28, J10.06, P06.168, P09.052, P12.139, P12.161
Kaderali, L.: P06.107, P06.167
Kadıoglu, A.: P04.52
Kadir, R.: **P12.146**
Kadlecová, J.: P06.190, **P12.159**
Kaeinimoghaddam, Z.: P05.66
Kaerrstedt, E.-L.: P02.102
Kähler, C.: P05.53
Kähönen, M.: C05.3
Kahraman, E.: J06.14, P06.012, **P07.04**
Kahre, T.: **P02.231**, P04.19
Kahrizi, K.: **C15.3**, P08.05, P08.37, P12.023, P12.024, P12.025, P12.168
Kahveci, H.: J02.36
Kai, J.: EPL3.5, P01.01
Kainimoghadam, Z.: J05.02, J05.07, P05.28, J05.06
Kaiser, A.: P09.092, P14.21
Kaiser, F.: C05.5, **P12.245**
Kaizer, L.: P13.22
Kakadia, P. M.: C02.3
KaKavand Hamidi, A.: **P02.085**, P12.075
Kakavand, A.: P02.235
Kakourou, G.: **P05.51**
Kalabay, L.: P06.195
Kalantar, S.: P04.53, J12.09
Kalashnikova, O. V.: J13.12
Kalay, E.: **P12.027**
Kalaycı, A.: P03.119, **P06.114**
Kalb, S.: P03.056
Kalelioglu, I. H.: P05.07, P05.22, P05.29
Kalina, D.: **P06.004**
Kalina, M.: P02.232, P02.233
Kalkan, S.: P12.099, P12.165
Kalla, C.: C02.1
Kallabi, F.: P03.015, P10.31
Kallel, N.: P02.260
Kalman, M.: P12.209
Kalnina, I.: P09.098, **P09.136**
Kalscheuer, V.: J12.31, P12.124, P12.042
Kalthoff, H.: P06.073
Kamali, K.: P09.013
Kamaliyeva, B.: **J04.08**, J13.05
Kamarainen, O.: P15.15
Kamatani, Y.: P09.115
Kamel, A. K.: P03.126
Kamenova, M.: P06.048, P06.144
Kamien, B.: P12.060
Kammoun, B.: P12.194
Kammoun, H.: **P12.194**
Kamnasaran, D.: P12.006
Kamoun, H.: J04.16, P02.053, P03.015, **P10.31**
Kamoun, M.: J04.16
Kamoun, T.: J04.16
Kamphans, T.: P11.099
Kamping, E. J.: P06.080, P11.078
Kamsteeg, E.-J.: P02.055, P12.126
Kam-Thong, T.: P11.122
Kanaeva, M.: **J09.17**
Kanafani, S.: P02.169
Kanaka-Gantenbein, C.: P02.227
Kaname, T.: P02.150, **P12.131**
Kanavakis, E.: C16.3, J02.30, P01.30, P02.227, P02.261, P03.012, P05.51, P08.11, P09.015, P11.081, P12.001, P12.040, P12.055, P12.156
Kanber, D.: P11.111
Kancheva, D.: P12.116, **P12.117**
Kandemir, N.: P02.153
Kandioler, D.: P06.172, P06.173
Kandratsenka, H.: J09.01
Kaneva, R.: J09.15, P06.044, P06.048, P06.144, P10.56
Kangas, A. J.: C05.3
Kanno, E.: P09.117
Känsäkoski, J.: P04.10
Kansikas, M.: P02.172
Kant, S. G.: P12.051
Kanterakis, A.: **P08.23**
Kanzapharova, R. F.: P09.049
Kaphingst, K. A.: EPL7.1
Kapitza, K. P.: P08.29
Kaplan, Y.: P02.145
Kapoor, R.: P06.019
Kapovic, M.: P09.084
Kappel, S.: P06.172, **P06.173**
Kappelmayer, J.: P12.015
Kaprio, J.: P09.086
Kapur - Pojskic, L.: J09.36, P02.124
Kapusta, L.: P13.04
Kapytau, A.: J09.01
Kara, B.: P02.014, 101
Karabulut, H. G.: P02.022
Karaca, E.: P02.052, **P12.099**, P12.165
Karacan, I.: J12.10, **P12.101**
Karachanak, S.: **P10.58**
Karagrigoriou, A.: C16.2
Karakok, M.: P06.180
Karakurt, C.: P07.19
Karakus, N.: **J09.13**
Karall, D.: C18.3, P02.008
Karaman, B.: P03.026, P05.07, P05.22, **P05.29**, P05.36, PL2.4
Karamysheva, T.: P10.08
Karatas, O. F.: P02.069
Karbach, J.: P02.188
Karbalai, N.: C08.5, P11.122
Karbalaie, K.: P12.208
Karbaschi, M.: **J06.21**
Karcagi, V.: P15.15
Karch, S.: P02.077, P02.111
Karczewski, K.: C05.6, C06.5
Karimi, H.: P03.033, **P03.077**
Karimi, R.: **J06.02**
Karimi, Z.: J11.04
Karimian, N.: J09.02
Karimian, S.: **P14.15**
Kariminejad, A.: **C09.6**, C18.6, P02.002, P02.171, P03.034, P03.044, P03.048, P05.30
Kariminejad, M.: C09.6, P02.002, P03.034
Kariminejad, M. H.: P03.044, P03.048
Kariminejad, R.: P02.002, P02.171, P03.034, P03.044, P03.048, P03.106, P05.30
Karimipoor, M.: J05.02, P06.006, **P06.046**, P08.44, J05.07, P05.28, P05.66, J05.06
Karimiyan, N.: J03.09
Karimloo, M.: P09.013
Karjalainen, J.: C08.4, **C08.6**, C10.1, P11.126
Karjalainen, J. M.: C17.6
Karjalainen, M. K.: P09.034
Karkucak, M.: J10.08
Karlinsky, L.: P02.017
Karout, M.: P09.056
Karpinski, P.: P06.071
Karpov, I. V.: P06.076
Karssen, L. C.: C06.3, P11.122
Karst, M.: P08.29
Karsunke, T.: P12.136
Karunas, A.: P06.058, P09.018, P09.023
Karwoski, J.: **J01.09**
Karyakin, O.: P11.006
Karytianou, A.: P07.33
Kasap, E.: **J06.14**, P06.012
Kasap, H.: P05.65
Kasap, M.: P04.39, P05.65
Kasapçopur, Ö.: P02.051
Kaschnitz, W.: P03.021
Kaserer, K.: P06.207
Kashef, A.: **P12.023**
Kashevarova, A.: **P03.083**, P04.32
Kask, M.: **P06.067**
Kaski, K.: C05.3
Kasnauskiene, J.: P02.013, P02.083, P03.043, P03.083, P11.059, P11.140
Kasowski, M.: **C06.5**
Kaspar, E. C.: P06.020, P09.132, P12.037
Kasper, B. S.: P02.101
Kastelan, Z.: P06.200
Kastenmüller, G.: P13.09
Kasuga, H.: P09.071
Katakis, I.: P09.042
Katharina, E.: P13.41
Kathiravel, U.: **P12.239**
Katmer, B.: **P02.205**
Kato, K.: EP08.04
Katosova, L. D.: P06.086, P07.30
Kattamis, A.: P05.51
Kattentidt, A. A.: **P01.17**
Kaufmann, D.: **P14.16**
Kaukinen, K.: P09.037
Kaukola, T.: P09.034
Kaulfuß, S.: C02.5, **P06.074**, P06.213

Kaur, G.: **P03.100**
Kautza, M.: P12.253
Kavecan, I.: J02.06
Kavecan, I. I.: **J03.13**
Kaviani, S.: P14.15
Kawabata-Yoshihara, L.: P02.199
Kawamura, R.: **C04.3**
Kawamura, Y.: P09.071
Kay, D. M.: P12.264
Kaya Atabey, Z.: P06.020
Kaye, J.: **EP08.04**
Kayirangwa, H.: P02.141, P12.018
Kayserili, H.: C03.4, **P02.157**, P05.07, P05.22, P05.29, P05.36, P12.027, P12.030, P12.061, PL2.4
Kayserova, H.: P09.052
Kayumova, L.: **P08.16**
Kayumova, R.: P08.16
Kazan, H.: P02.220
Kazantseva, A.: P09.014
Kazemi Noughabi, M.: P12.186
Kchorobrich, T.: P06.099, P06.100
Keating, S.: EPL4.5
Kefer, N.: **P11.105**
Kehrer, M.: P12.260
Kehrer-Sawatzki, H.: P06.157, P06.158, P06.159, **P06.160**
Keikhai, B.: P02.119
Keilhauer, C.: P09.008
Keiss, J.: P13.02
Kekeeva, T.: P06.103, P06.141, **P06.184**, P11.064
Kekkou, K.: P11.081
Kekou, K.: P01.30, P12.040
Keles, B.: P06.114
Keller, G.: P06.154
Keller, K. E.: C14.1
Kemkemer, R.: P14.16
Kempa, I.: **P09.073**
Kemper, M. J.: P12.201
Kempers, M. J. E.: C01.3, C04.5
Kendler, K. S.: C06.4
Kennerknecht, I.: P10.47, P12.253
Kenny, E. M.: C06.4, P11.117
Kent, A.: EP08.04
Kepák, T.: P06.190
Keppler, R.: P06.111, **P06.217**, P12.039, P14.20
Keramatipour, M.: J12.13, **P09.116**
Kerber, S.: P05.53, P05.70
Kerem, B.: **S02.3**
Keren, B.: P02.161, P12.167
Kerick, M.: P06.185
Kerin, S.: P02.229
Kerkhof, H.: PL2.5
Kerkhoffs, J.-L.: P07.13
Kerr, B.: P02.134, P02.175, P12.241
Kersten, F. F. J.: PL2.4
Kerstjens-Frederikse, W. S.: P02.006
Kerzin-Storrar, L.: EP06.01, EPL2.6
Keser, I.: P12.029, **P12.143**
Keshavarz, S.: P06.211, P06.215
Keshavarzi, F.: J13.09, **P06.025**, P13.29
Keskes, R.: P04.27
Keskin, O.: J12.02
Kessentini, M.: P02.053
Kessler, K.: **P12.220**
Kessler, R. W.: P03.064
Kestelyn, P. G.: P12.191
Kets, M.: P01.35
Kettunen, J.: C05.3, C08.4, C17.6
Keupp, K.: **C03.3**
Kevelam, S.: P12.154
Keyser, B.: **P12.226**, P12.239
Keyvani, S.: P05.30
Khachatryan, Z.: J10.01
Khademi, M.: J06.02
Khairi, S.: J09.22
Khalchitsky, S.: **P13.39**
Khalifa, M.: **P02.142**
Khalili, S.: P06.125
Khalilova, Z.: P09.128
Khaliullin, A.: P06.062, P06.189
Khamit, A.: P11.135
Khan, S.: **P08.27**, P12.153
Khaniani, M. S.: **J12.08**, **P02.164**, P14.23
Kharkov, V.: P10.34
Khatami, M.: P11.041
Khatami, S.: P06.069
Khazaeii, N.: P12.144
Khazraei, H.: P08.41
Kheradmand Kia, S.: P12.199
Khidiyatova, I.: J02.18, J09.27, P12.113
Khismatullina, E. A.: P09.083
Khojasteh-Fard, M.: J13.02
Khor, C. C.: C14.3
Khorov, O. G.: J12.15
Khorram Khorshid, H.: P09.013
Khosravifar, M.: **J04.05**, P04.11
Khudoyan, A.: J10.01
Khurs, O.: J01.01
Khusainova, R.: P06.062, P06.189, P09.101, P09.102, P12.180
Khusnutdinova, E.: J02.18, J09.27, P05.16, P06.058, P06.062, P06.183, P06.189, P08.02, P09.014, P09.018, P09.101, P09.102, P09.121, P09.122, P09.123, P09.124, P09.128, P12.113, P12.180, P12.224, P02.109, P09.023, P09.049, P10.11, P10.29
Kianfar, S.: P04.36
Kiani, S.: P11.086
Kibaek, M.: P02.003
Kido, Y.: P02.206
Kiefer, F.: P09.009
Kielbasa, S.: C13.1
Kielty, C. M.: P12.241
Kiess, W.: P12.204
Kießling, F.: **P02.121**
Kigasawa, H.: P02.029
Kihan Ghatreh, K.: P08.41
Kiiski, K. J.: P05.52
Kilciler, G.: J10.08
Kılınc, D.: P06.127
Kilstrup-Nielsen, C.: P12.042
Kim, C. A.: J02.42, P02.020, P03.023, P03.118, **P13.38**
Kim, G.-J.: C18.2
Kim, H.: **P06.120**
Kim, H.-G.: C13.3
Kim, J.: P12.158, P12.264
Kim, N.: P09.066
Kim, S.: **P11.044**, P12.264
Kim, Y.: P09.065, P09.065, P09.066, P12.264
Kim, Y.-D.: P09.066
Kimonis, V.: P12.264
Kimura, R.: P02.048
Kinasheva, K.: P09.128
King, M.-C.: P06.028
Kingo, K.: P09.124
Kingston, H.: P12.241
Kinnett, K.: EPL5.5
Kinning, E.: C09.2
Kinoshita, T.: C13.1
Kinsley, N.: J01.08
Kirac, D.: P06.020, **P12.037**
Kirchhoff, M.: P02.003, P02.170
Kirgiz, M.: P05.22
Kirgizov, Y. V.: P12.112
Kirk, E.: C18.1, P09.024
Kirk, J.: EPL4.1
Kirk, M.: **EP06.02**, **EP07.03**, P01.08
Kirkgoez, M.: P14.13
Kirmes, I.: **P12.157**
Kirov, A.: P12.109, **P12.190**
Kirov, G.: P09.032, P11.118
Kirschner, J.: C12.3
Kirsten, H.: P07.25
Kiselev, A.: **P12.230**
Kisfali, P.: **J09.16**, P10.25, P10.30, P13.22
Kishnani, P. S.: P14.10
Kisinovsky, I.: P13.14, P14.10
Kitajima, H.: **P09.057**
Kitsiou, S.: P08.11, P12.001, P12.055
Kitsiou-Tzeli, S.: J02.30, J02.34, **P02.227**, P03.012, P09.015, P11.081, P12.156
Kitsos, G.: P09.007
Kiudelis, G.: P09.138
Kivirikko, S.: P09.125
Kivity, S.: P12.179
Kjaergaard, S.: P02.003, P02.170
Klabanova, M.: P06.182
Klapecki, J.: P02.028, P02.185, P12.172
Klapper, W.: C10.5, P06.098, P07.03
Klar, J.: P02.033, P12.020
Klareskog, L.: C14.5
Kleefstra, T.: C15.1, P02.192, P11.019, P12.124
Kleffmann, J.: **P02.200**
Kleffmann, W.: P02.012
Klein, C.: C09.2, P12.245
Klein, E.: P03.052, P03.091
Klein, H. G.: P06.079, P02.180, P12.038, P12.155
Klein, M.: P04.37
Klein, O.: P12.264
Klein-Hitpaß, L.: P09.060
Kleinjan, D.-J.: P11.123
Kleinmann, A.: P02.245
Klemenc Ketis, Z.: P09.084
Klengel, T.: P08.20
Kleopa, K.: P10.14
Klevering, B. J.: C11.5
Kleymenova, I.: J12.11
Kliesch, S.: P04.51, P04.57
Klimentova, E.: P06.062, P06.189
Klímová, A.: **P04.47**, P05.68
Klincova, M.: P07.26
Klink, B.: P02.133, P06.107, P06.167
Kloeckener-Gruissem, B.: **P09.091**
Kloosterman, W.: C06.3, C18.1, P12.259
Klopocki, E.: C03.1, C03.2, P02.004, P02.081, P02.099, **PL2.3**
Klopstock, T.: P14.19
Kloss-Brandstätter, A.: P15.14
Klotchenko, S. A.: J11.06
Klovins, J.: P09.073, P09.098, P09.136
Kluck, N.: C08.5, C14.4, P09.058, P09.078
Kluijt, I.: EPL6.3, P01.34
Kluwe, L.: P06.158, P06.159
Knapp, M.: C14.4, P08.32, P09.004
Knappskog, P. M.: C12.2
Knebel, S.: P04.29
Knies, K.: **P11.136**, P11.138, P12.091
Knight, S. J. L.: P09.126
Knijnenburg, J.: **P05.60**
Knoers, N.: P12.154, C18.1, P11.007, P12.126, P02.055, P12.259
Knolle, P. A.: C02.1
Knopp, J.: EPL2.6
Knoppers, B. M.: EP08.04
Ko, T. K.: C10.3
Koc, G.: P02.220, P06.020, P12.037
Kocabıyık, B.: P02.122
Koçak, N.: P02.122, J03.04, P03.119, P06.075
Kocaoglu, M.: P12.030
Kočárek, E.: P03.079
Koch, K.: P06.098
Koch, M. J.: C18.5
Kochetova, O.: **J09.14**, P06.016
Kochi, Y.: P09.117
Kocova, M.: J02.11, P12.263
Kocsis, J.: P06.195
Koehler, U.: **P04.42**
Koeleman, B. P. C.: P02.095
Koelvraa, S.: P03.104
Koenig, R.: P12.138
Koeppel, T.: P02.160

Koh, L. P.: C10.3
Kohl, S.: C11.5, P11.112
Köhler, A.: P12.232
Köhler, J.: P06.117
Kohlhase, J.: P02.204
Kohlschütter, A.: C18.5
Kohoutova, M.: J03.08
Koivisto, K.: P09.086
Kok, E.: P07.13
Kokabee, L.: **P06.006**, P06.046
Kokabee, M.: P06.006
Kokalj Vokač, N.: J05.03, P04.07, P05.33, P06.094
Koker, S. A.: P02.052
Kokkali, G.: P05.51
Kokocinski, F.: C08.1
Kokotas, H.: P09.007, **P12.067**
Kõks, S.: P09.023, P09.124
Kolanczyk, M.: P02.210, P12.158, P12.162
Kolarov, Z.: J09.32
Kolarova, J.: **P06.073**, P11.120
Kolarski, M.: J03.13
Kolb, S.: P11.048
Kolde, R.: P06.087, P11.094
Kołodziejczyk, H.: P02.233
Kolotii, A. D.: P03.014, P03.022
Kolousková, S.: P02.174, P09.135
Koltsina, M.: P06.087
Kolvraa, S.: P02.155
Kölzsch, K.: P02.224
Komarova, N.: P12.071
Komlósi, K.: J02.02, P10.52, P12.139, **P13.22**
Komrska, V.: P02.174
Kondakova, O. B.: J09.10, P12.112
Kondo, A.: J05.01, **P02.196**
Kondov, G.: P06.042
Kondrat'eva, E. I.: J09.34
Konecny, M.: P02.211, **P06.101**
Konfortov, B.: P04.03
Kong, B.: P11.014
Konialis, C.: P12.047
König, C.: P04.37
König, J.: P11.052
Königsrainer, A.: P06.178
Kononenko, M. I.: P04.28
Konrad, M.: P12.155
Konstandin, N.: C02.3
Konstantinidou, A.: J02.34
Konstantopoulou, I.: P06.028
Konvalinka, D.: **P06.165**
Kooi, K. A.: P12.095
Koole, R. H.: P12.206
Koolen, D. A.: **C01.1**
Koopmans, M.: P03.054
Koparır, A.: J02.35, **P02.249**
Koparir, E.: **J02.35**, P02.249
Koperek, O.: P06.207
Kopra, O.: P12.192
Korabecna, M.: **P09.054**
Korbel, J.: C06.5, C10.5, **PL2.1**
Korcz, A.: P08.01, **P09.002**
Kordafshari, A.: J05.02, J05.07, P05.28, P05.66, J05.06
Kordaß, U.: PL2.3
Korecka, K.: P09.099
Korendowych, E.: C14.5
Korenke, C.: P12.041, P12.145
Korenkov, M.: C16.5
Korkmaz, H. A.: P02.052
Korkmaz, M.: J06.14, **P06.012**, P07.04
Kornak, U.: C09.5, P02.032, **P12.061**, P12.162, P13.45
Kornetov, A. N.: J09.06
Kornienco, V. Y.: P12.112
Korniszewski, L.: P02.117, P02.156
Kornmann, E.: P07.34
Korošec, P.: P09.021, P12.110
Koroteev, A. L.: P03.113
Korrovitš, P.: P03.078
Korte, K.: **P07.03**
Kortschak, A.: P03.021
Kortyczko, E.: P02.264
Korytina, G.: P06.016, **P09.041**
Koschny, R.: P07.25
Koskinen, S.: P09.086
Kosma, K.: **J02.34**, P01.30, P02.261, P03.012, P09.015
Košnik, M.: P09.021, P12.110
Kossler, N.: P12.158
Kostik, M. M.: J13.12
Kostina, A.: J05.04, J13.05
Kostukova, N.: J06.20
Kosyakova, N.: P03.063, P03.091, P10.08
Kosztolányi, G.: J02.02
Kotelevskaya, E.: **J10.04**
Kotschote, S.: P11.112
Kotti, A.: EP06.07, J02.16
Kötting, J.: P12.239
Kotzot, D.: C18.5, P02.116
Koudova, M.: P02.168, P03.004, **P06.001**
Koumaras, B.: P14.06
Koumbaris, G.: C16.2
Kousar Jahan, S. K.: **P07.16**
Kousoulidou, L.: P11.003
Koutroumpe, M.: P12.067
Koutsothanasi, C.: J08.04
Koutsou, P.: P10.14
Kouwenberg, D.: P12.061, P13.45
Kouznetsova, D.: EP08.02
Kovacs, L.: P06.083, P06.168
Koval, S.: P06.014
Koval, V.: C06.3
Kovalenko, K.: P04.33, **P04.44**
Kovalenko, S. P.: P06.076
Kovaleva, N. V.: **P03.133**
Kovalova, Z.: P06.005
Kowalewski, C.: J12.25, P12.127
Kowalski, P.: P02.041, P02.186, P02.232, P07.24, P13.47
Kowalzyk, Z.: P05.45
Kozlova, J.: P03.099
Kozlova, Y.: J03.06, **J05.08**
Kozlova, Y. O.: J05.09
Kozłowska, J.: P02.244, P06.071
Kozma, K.: P02.105, P02.137
Kozmus, C. E. P.: **P09.020**
Kraaij, R.: P12.206
Krabichler, B.: C18.3, **P02.008**, P02.116
Kraft, M.: P03.067
Krahe, R.: **C02.4**
Krajc, M.: P11.072
Krajewska-Walasek, M.: **P02.005**, P02.041, P02.114, P02.186, P02.232, P02.233, P02.262, P03.045, P03.049, P03.114, P07.24, P13.47
Kramer, F.-J.: C14.4
Kramer, J. M.: C01.1, P12.102
Kramer, P. L.: C14.1
Kranewitter, W.: P06.013
Kraoua, L.: P02.054
Krásničanová, H.: P02.068
Kratochvilova, R.: P05.09
Kraus, C.: **P02.158**
Krause, R.: P10.09
Krauss, S.: **P12.121**
Kravchenko, S.: J10.15
Krawczak, M.: P04.04
Krawitz, P.: C03.1, C07.4, P12.031, **C13.1**, **P11.099**, P11.100, P11.101
Krebs, S.: C02.3
Krech, T.: P13.49
Křečková, G.: P03.123
Kreienkamp, H.-J.: C13.5
Kreiger, E.: P12.027
Kreile, M.: **P06.005**, P13.02
Kreimer, A.: **C17.5**
Kreipe, H.: P06.113
Krejcik, Z.: P07.02
Kremensky, I.: P02.079, P03.018, P03.073
Kremer, H.: C11.3, PL2.4
Křen, R.: P05.44
Křenková, P.: **P04.51**, P05.37
Krenova, Z.: P06.004
Křepelová, A.: P02.068, P02.213
Kress, W.: P03.143, **P12.069**
Kreuz, F.: EPL6.1, **EP02.02**, P02.046, **P02.243**
Kreuz, M.: C10.5, P06.098
Krex, D.: P06.107, P06.167
Kriegel, A.-K.: C08.5
Krieger, J. E.: P02.199
Krieger, S.: P11.110
Kriegner, A.: P11.022
Kriegova, E.: P11.108
Kriek, M.: **C07.1**, P03.054, P12.051
Krispin, M.: **P11.025**
Kristiansen, B. S.: **P02.113**
Kristiansen, G.: P06.181
Kristiansson, K.: P09.134, P09.137
Kristoffersson, U.: P01.23
Krithika, S.: **P08.24**
Krivjanska, M.: P12.134
Krivjansky, V.: P12.134
Krizova, K.: P06.210
Kroes, T.: P11.019
Kroes, W. G.: **P07.13**
Kroisel, P. M.: **P03.021**
Kroisl, P.: P02.234
Kroll, C.: P14.09
Kronberger, G.: P03.056
Kronenberg, F.: P15.14
Kroupis, C.: P09.007
Krueger, G.: P12.138
Kruer, M.: J02.50
Krug, S.: P13.09
Krüger, S.: P02.046, P02.243
Krüger, U.: C13.1, P11.100
Krugers, H. J.: C13.5
Krulišová, V.: P01.11
Krumbiegel, M.: P02.158
Krumina, A.: P06.005, P09.073, P10.59, P13.02
Krupp, W.: P07.25
Kruse, R.: P09.010, P09.058, P09.078
Krutílková, V.: P03.123, P05.44, P05.50
Krysa, W.: P12.115
Ksienzyk, B.: C02.3
Ktena, Y. P.: P07.11, P07.18, P07.33
Kubala, M.: P12.009
Kube, D.: C10.5
Kubisch, C.: P06.200, P12.011, P12.012, PL2.4
Kubová, B.: P06.165
Kucharczyk, M.: P02.005, P02.262, P03.045, P03.049, **P03.114**
Kuchenbecker, K.: C13.5
Kucherenko, A.: **J09.19**, P11.070
Küchler, A.: C03.4
Kučinskas, V.: J02.25, P02.013, P02.083, P03.043, P03.083, P08.17, P10.41, P11.059, P11.140
Kucukalic, E.: P03.075
Kucukosmanoglu, E.: J12.02
Kuechler, A.: **P02.021**, P12.145
Kuehl, J.: **P08.14**
Kuesters, B.: C11.3
Kugaudo, M.: P02.041, P02.114, P02.186, P02.232, P02.233, P03.049, P03.114
Kuglík, P.: P01.21, P03.051, P07.06, P07.26, P07.27
Kuhn, M.: P02.201, **P13.13**
Kühnel, F.: P06.113
Kühnisch, J.: C09.3, P12.158, **P12.162**
Kührer, I.: P06.173
Kuhtz, J.: **P11.033**
Kuijpers, T. W.: C14.3
Kuiper, R. P.: **C02.2**, P06.080, P11.078, P11.097
Kujath, C.: P02.130
Kuklina, N.: P12.049
Kukulguven, F. M.: P14.22
Kulawonganunchai, S.: P09.029

Kulikov, L. M.: J02.45, P10.01
Kulikowski, L. D.: J02.42, **P03.107**, P03.118, P13.38
Kulinna-Cosentini, C.: P06.173
Kulis, T.: P06.200
Kulkarni, V.: P03.060, P11.093
Kulseth, M.: **P11.096**
Kulseth, M. A.: P02.212
Kumanov, P.: J09.32
Kumar, V.: P09.115, **P11.126**
Kumar, Y.: C16.1, P11.034
Kumate-Rodríguez, J.: P08.24
Kumnova, A.: P04.05
Kuncnik, V.: J05.03
Kundu, S.: P12.246
Kuner, R.: P06.185
Kungurova, T.: P07.29
Kunijeva, S. S.: P09.012
Kunstmann, E.: P03.120, P12.041, **P13.20**
Kuo, P.: P04.45, P11.004
Kupcinskas, L.: P09.138
Küppers, R.: C10.5, P06.098, P06.199
Kupska, R.: P07.26, P07.27
Kurahashi, H.: **P04.41**
Kurbatov, S.: P12.166, **P02.176**
Kurg, A.: P02.009, P03.004, P03.078, P03.102, P11.059, P11.139, P11.140
Kurg, R.: P11.139
Kurilo, L. F.: J04.14
Kurinnaia, O. S.: **P03.009**, P03.115
Kurkina, M. V.: **J12.01**
Kurosawa, K.: **P02.029**, P12.131
Kurppa, K.: P09.037
Kurt, E.: J13.10
Kurtanov, K.: P10.34
Kurth, I.: P02.021, **P12.108**, PL2.3
Kuru, K.: **P02.096**
Kurvinen, E.: P03.032, **P04.19**
Küry, S.: C18.2
Kurz-Levin, M.: P09.091
Kuss, A. W.: P10.09
Kutalik, Z.: C06.2
Kutkowska, A.: P12.126
Kutkowska-Kaźmierczak, A.: J12.25, P12.127, P02.028, P02.185, P12.145, P12.172
Kutlay, N. Y.: **P06.097**
Kutluhan, A.: P06.097
Kutlyeva, L.: P06.062, **P06.189**
Kutsche, K.: **C13.5**
Kutsev, S.: **P07.29**
Kuuse, K.: J03.18, P03.032
Kuzbari, S.: P02.060
Kuželová, H.: P05.37
Kuznetsova, A. V.: J09.40, P09.068
Kuznetsova, E.: P06.145, P06.141, P11.064
Kuznetzova, T.: P03.027, P03.094, P03.113, P11.024
Kvapilova, M.: P05.09, P06.210
Kwint, M.: P02.132, C11.5
Kwok, Y. K.: **P05.57**
Kwong, D.: P09.142
Kyamides, Y.: P12.107
Kyriacou, K.: P11.072
Kyriakides, T.: P10.14
Kyryachenko, S.: J05.13, **J05.14**, J06.20

L
L. Busk, Ø.: P02.056
L. Holla, Ø.: P02.056
L. Sieroń, A.: P02.244
La Calle Marto, M.: P06.161
La Manna, G.: P12.130
Laari, A.: **P12.192**
Labarque, V.: J02.49
Labate, A.: J09.45, P12.081
Labrèze, C.: C01.4
Lacassie, Y.: **J02.10**
Lāce, B.: J09.09, P06.005, P09.073, **P11.065**, P11.066
Lachenaud, J.: P06.166
Lachmann, H.: P15.01
Lackner, C.: C10.6
Lacombe, D.: P02.141
Lacour, A.: **C17.4**, P08.12, P08.39, P10.38
Lacueva, J.: P06.082
Łaczmańska, I.: P02.244
Lagarde, N.: P03.020
Lagoutte, L.: C18.2
LaGrave, D.: P02.173
Lagro-Janssen, A. L. M.: EP08.06
Lahtinen, T.: C12.5
Lai, S.: C08.3
Laible, M.: P06.185
Laidre, P.: P06.067
Laird, S.: P03.037
Laitinen, E.-M.: P04.10
Laitinen-Forsblom, P. J.: **P13.33**
Lakeman, P.: P12.173
Lakpour, N.: J04.13
Laku (Babameto), A.: **J03.16**
Lalani, S. R.: P02.060
Lalchev, S.: P09.031
Laleva, M.: P06.048, P06.144
Läll, K.: P01.02
Laloglu, F.: J02.36
Lalowski, M.: P12.121
Lam, U.: **P13.41**
Lamantea, E.: C12.3
Lamarche, B.: P11.049
Lamari, F.: C11.2
Lamb, T.: C07.3
Lamberg, A.: P11.131
Lambert, D.: EPL5.2
Lambert, J.-C.: C11.1
Lambrechts, D.: J02.49
Lambrou, G.: P07.18
Lameijer, E.-W.: C06.3
Lameiras, L.: P12.170
Lammens, M.: P02.055
Lamnisou, K.: C16.2
Lamnissou, K.: J08.02, **J08.04**
Lamorte, G.: P09.131
Lancaster, O.: **J11.07**
Lancastle, D.: EPL2.4, EPL4.2
Lanciotti, M.: J07.04
Landais, E.: P09.026
Landolsi, A.: P02.221
Landwehr, C.: P07.05
Laner, A.: **P02.086**, P06.154
Lanfranchi, A.: P06.059
Langbein, K.: P09.120
Lange, C.: C14.4, P12.162
Lange, M.: P02.101
Lange-Freitag, S.: C16.1
Langer, D.: P02.049
Langer, R.: P02.223
Langerberger, D.: C10.5
Langhorst, B.: P11.098
Langnaese, K.: C13.5
Lannfelt, L.: C04.4
Lanni, S.: P11.040
Lannoy, A.: P09.026
Lanoel, A.: C01.4
Lanta, S.: P05.18
Lanthaler, B.: P12.229
Lao, K.: P11.035, **C08.2**
Lapi, E.: P02.044, P02.060
Laporte, J.: J11.10
Lappalainen, T.: PL2.6
Lapunzina, P.: J02.54, J02.55, P03.076, P12.056, P12.060, P13.01
Larbi-Masmoudi, M.: P03.015
Larizza, L.: P02.040, P12.057, P12.058
Laros, J. F. J.: P11.020, **P11.051**, P11.074, P11.084
Larsen, M.: **P12.098**
Larti, F.: P08.37
Lasa, A.: **P06.027**
Lasabova, Z.: **J09.12**, P06.102, P12.209
Lasan Trcic, R.: P03.040, P03.110
Lasan, R.: P05.02, P05.19
Lassuthova, P.: **P12.219**
Lastra Aras, E.: P06.021
Lastra, E.: P06.065
Lastuvkova, J.: P05.68
Latham, D.: EPL3.1
Lathrop, M.: C18.2, P09.115
Latos-Bielenska, A.: P02.193, P11.059
Latta, K.: P02.201
Lattante, S.: C01.2
Lattanzi, G.: C07.4
Lattanzi, W.: **P09.048**
Latz, E.: C02.1
Laugel, V.: C18.4, J11.10
Laumen, H.: P13.09
Laurense-Bik, M.: P03.054
Laurent, N.: P05.48
Lausch, E.: P06.111, P06.217, P12.039, **P14.20**
Lavarone, E.: **P06.163**
Lavebratt, C.: J11.05
Lavická, E.: P06.001
Lavie, J.: C11.2
Lavoie, P. M.: P09.034
Lavrov, A. V.: P06.086, **P11.011**
Lavtar, P.: **P09.084**
Lawerenz, C.: C10.5
Lawlor, D. A.: P10.37
Lawton, S.: EPL3.6
Lax, S.: C10.6
Lázár, L.: **P05.47**, P05.49, P05.63
Lazaro, C.: EP05.12
Lázaro, M.: P06.161, P11.050
Lázaro, R.: P06.072, P06.082
Le Bastard, N.: P08.03
Le Boette, E.: P01.20
le Gallo, M.: C18.2
Le Goff, C.: **C03.5**, C09.2
le Goff, W.: C03.5
Le Gras, S.: P12.046, J11.10
Le Merrer, M.: C09.2, P02.141, P12.018
Le Quan Sang, K.: P12.018
le Sage, S.: EPL7.4
Le Saux, O.: P12.207
Leamon, J.: P11.014
Leandro, B.: **J01.04**
Leavitt, R.: P11.025
Lebedev, I.: P03.083, P03.101, P11.059, J06.18, P04.32, P11.032
Leber, M.: P08.39, **P10.38**
Lebioda, A.: P09.099
Lebre, A.-S.: **P13.32**
Lecaignec, C.: P02.161
Lechner, A.: P13.09
Lechno, S.: P12.137, P12.213
Lechowicz, U.: P02.117, **P02.156**
Lecut, C.: S01.3
Lederer, G.: P02.255
Ledig, S.: **P04.02**
Lee, A.: P11.087, P11.128
Lee, B.: **S15.3**
Lee, C.: C08.2
Lee, C. G.: **P06.116**
Lee, C. N.: P11.102
Lee, J.: **P09.066**, P14.06
Lee, J. A.: P02.012, **P02.169**
Lee, J. G. H.: C09.5
Lee, J.-Y.: P09.066
Lee, M.: **P09.100**
Lees, M. M.: C18.1
Lefeber, D.: P13.04
Lefever, S.: P12.191
Lefol, C.: P11.110
Legallic, S.: C11.1
Legius, E.: J02.49
Legnaro, C. C.: J02.23, P02.123, **P03.095**

Lehesjoki, A.E. E.: P12.028, P12.192
Lehmann, A.: P12.050
Lehnen, H.: C16.5, P11.038
Lehnert, S.: P11.083, **P11.114**
Lehoczka, V.: P06.092
Lehrach, H.: P06.185
Lehtimäki, T.: C05.3, P09.086
Leibson, K.: P12.262
Leich, E.: C10.5
Leijsten, N. F. A.: C04.5
Leikin, S.: J12.22, J12.23
Leiro-Fernández, V.: P06.122
Leisz, S.: P06.188
Leitsalu-Moynihan, L.: **P01.02**
Leja, M.: P09.138
Lekontsev, E. V.: J02.45, **P10.01**
Lemeur, N.: P02.161
Lemke, J.: P02.203, P03.092, **P12.082**
Lemmers, R. J. L. F.: P11.068
Lemmink, H. H.: **P15.15**
Lemos, C.: P08.45, P09.080, P09.081, P10.20, **P10.21**, P10.23
Lempp, F.: P09.026
Lenglet, T.: P02.007
Lenze, D.: C10.5
Leohold, J.: P11.120
León, M.: J09.47
Leonard, N. J.: P02.043
Leonard, S.: EPL7.2, **P01.27**
Leonardo, A.: **EP01.03**
Leone, M.: P11.110
Leonhardt, J.: P02.130
Lepage, P.: P02.185, P12.172
Lepore, S. M.: P02.147
Lepori, M. B.: P10.57
Leppä, V. M.: **P09.086**
Lepre, T.: **J09.43**, P09.110
Lepri, F.: P09.131, **P02.184**
LeProust, E.: P03.060, P11.028, P11.093, P11.128
Lequin, M.: P12.199
Lerchbaum, E.: P09.103, P13.41
Lerche, H.: P12.082
Lerman-Sagie, T.: P02.263, P12.179
Leroy, B.: P12.191
Lesende_Rodriguez, I.: J12.28
Leshinsky-Silver, E.: P12.179
Lesnik Oberstein, S. A. J.: P12.173, C07.1
Lessel, D.: **P06.200**
Lester, T.: P12.248
Lesuffleur, T.: P06.078
Letica, L.: **P03.040**, P03.110, P05.02, P05.19
Letourneau, A.: C04.6, **P11.027**, PL2.6
Letteboer, S. J. F.: C11.5
Leube, b.: EP02.02
Leubner, S.: **P03.139**
Leung, K. O.: P05.57
Leung, T. Y.: P05.57
Lev, D.: P02.263, **P12.179**
Levačić Cvok, M.: P06.030
Levanat, S.: P06.030
Levashova, S. V.: P09.023
Levin, M.: C14.3
Lev-Maor, G.: P11.137
Levy, A.: P11.137
Levy, F.: P02.265
Levy, N.: J11.10, P12.238
Levy, S. E.: C12.2
Lewinska, M.: **P13.43**
Lewis, C.: **EPL3.2**, P01.01
Lewis, M.: P08.31
Lewis, S. J.: P10.37
Leyser, M.: P03.140
Lezhnina, J. G.: P03.094
Lezirovitz, K.: PL2.3
Leznarova, D.: P05.10
Li, A.: P11.014
Li, L.: P12.016, P12.221
Li, M.: P10.41, P11.074, P11.105
Li, M. M.: **C02.6**
Li, X.: C05.6
Li, Y.: C02.6, C03.3, C03.4, P12.193, PL2.4
Liang, J.: P12.169
Liaugaudienė, O.: **P02.083**
Libarkin, J.: P01.25
Licastro, D.: P12.105
Lichter, P.: C10.5, **PL1.2**, PL2.1, **PL5.1**
Lichtner, P.: P10.32, P10.33
Lidereau, R.: P11.110
Liebe, D.: P05.31
Liebisch, G.: P09.076
Liebrecht, D.: **P02.209**
Liegl-Atzwanger, B.: P06.051
Liehr, T.: P02.009, P03.014, P03.022, P03.052, P03.055, P03.057, P03.063, P03.091, P05.32, P10.08
Liem, M. K.: P12.173
Lien, H.: P14.03
Lifford, K. J.: **EPL2.4**, EPL4.2
Ligtenberg, M.: P06.063, EP05.04, EP05.06, P06.080, P11.078
Liguori, M.: J12.27, J12.29
Lilien, M.: P12.154
Lim, C. C.: C14.3
Lim, K. S.: C14.3
Lim, L. C.: C10.3
Lim, W. T.: C10.3
Lima Cabello, E.: P14.08
Lima, M.: P06.110
Lima, V.: P05.21
Limaye, N.: C01.4
Limborska, S. A.: P10.59
Limborskaya, S.: P09.127
Limon, J.: P02.066
Limprasert, P.: P03.127, **P09.029**
Lin, D.-S.: **P13.31**
Lin, E.: P11.087, P11.128
Lin, I.-H.: P08.40
Lin, M.-W.: P08.40, **P09.061**
Lin, S. Y.: P11.102
Lind, K.: P09.042
Linde, L.: P03.111
Lindfors, K.: P09.037
Lindgren, C. M.: C06.2
Lindgren, P.: EPL1.3
Lindhout, D.: P02.253
Lindner, M.: P02.032, M.: P13.19
Lindner, T.: P02.223
Lindsay, H.: C07.3
Lindsay, M. E.: C01.3
Linek, B.: P11.033
Link, S.: P11.112
Linke, M.: **P11.121**, P12.213
Linn, A.: EPL7.1
Liou, W.: P06.002
Lipiński, D.: P12.236
Lipp, H.-P.: C13.5
Lippke, B.: P08.32, P09.078
Lisiansky, V.: P04.20
Lisicina, S.: J02.37
Lisik, M.: **P02.103**
Lissens, W.: **P04.24**, P13.23
Lissewski, C.: **P02.175**, P12.175
Littmann, E.: P06.136
Litviakov, N. V.: J06.15
Litvinova, M. M.: **P11.107**
Liu, E.: P12.088
Liu, H. L.: P13.31
Liu, L.: P06.116
Liu, M.: P11.010, P11.085
Liu, P.: P11.098
Liu, S.: P11.135, P12.145
Liu, X.: P12.169
Liukkonen, E.: P12.028
Livshits, L.: J10.15, P11.140, J09.19, P03.084, P11.059, **P11.070**
Llewellyn, M.: EP07.03
Lo Curto, F.: P06.059
Lo Rizzo, C.: P09.030
Lobato, S.: P09.051
Lobina, M.: P09.077
Lobo Antunes, J.: P06.109
Lobov, S.: P10.29, **P12.224**
Locher, M.: P04.42, **P06.154**
Lochmüller, H.: P02.064
Loeffler, M.: C10.5
Loeys, B.: P02.023, P11.130, C01.3
Loeza-Becerra, F.: **P10.18**
Löffler, M.: P06.098, P06.178
Loges, N. T.: P12.200
Loginova, A.: J12.16
Lohan, S.: C03.1, C03.2, PL2.3
Loher, P.: P11.109
Lohmann, D.: P11.111, P06.212
Lohmann, J.: C14.5, P09.111
Lohmann, K.: P12.245
Lohse, P.: P02.230, P02.255
Loizidou, M.: **P11.072**
Lojo - Kadric, N.: **J09.36**, P02.124
Lokar, L.: P04.07
Lokk, K.: P06.087
Lomax, B.: P03.071
Londin, E.: **P11.109**
Longley, M.: EP07.03
Longmuir, M.: P02.178
Longo, I.: P02.060
Lonigro, R.: EP01.02, P12.225
Looijenga, L.: P06.201, P06.202
Loos, R. J. F.: C06.2
Lopes, A.: P12.170
Lopes, F.: **J03.03**
Lopes-Cendes, I.: P12.078
Lopez Ayala, J.: J02.47
López Bartolomé, M.: **P09.051**
Lopez, C.: **P07.12**
Lopez, E.: P12.167, P12.178
Lopez-Ariztegui, M.: P12.005
Lopez-Cuenca, D.: J02.46, J02.47, J02.48
López-González, V.: **P03.019**
Lopez-Larios, A.: P09.040
Lopez-Martínez, M. Á.: P12.177
Lorda, I.: P02.248
Lorente, B.: PL2.4
Lorente-Cánovas, B.: P08.31
Lorenz, A.: P03.064
Lorenz, B.: P12.211, P12.253
Lorenz-Depiereux, B.: **C12.6**, P13.12
Lories, R. J.: PL2.5
Loscher, C.: P04.13
Lossos, A.: C11.2
Lototska-Savchak, O.: P04.31
Lott, A.: P02.089, **P05.06**
Lotufo, P. A.: P02.199
Louati, R.: P02.039
Loudianos, G.: P10.57
Louis dit Picard, H.: **PL2.2**
Louis, E.: S01.3
Loureiro, J.: **P12.250**
Lourenço, C. M.: P02.038, **P12.231**
Louro, I. D.: P12.181
Loustalot, C.: EPL6.4, P01.04
Loviglio, M.: **P11.103**
Lovrečić, L.: J05.12, P09.084
Low, S.: P06.171
Lowndes, J.: EPL5.6
Lozano-Mejorada, R.: P06.177
Lubusky, M.: P05.09
Lucas, M.: P09.085
Lucassen, A.: EPL5.3
Lucchini, C.: P12.147
Luce, A. L. P.: P03.107
Luckow, S.: **P03.064**
Łuczak, S.: P02.041, P02.186, P02.232, P07.24, **P13.47**

Ludolph, A.: P12.011, P12.012
Ludwig, K.: **P02.090**, **C14.4**, P08.32
Ludwig, M.: C09.4, C10.2, P02.026, P02.130, P08.08, P09.033, P12.123
Luedecke, H.-J.: P02.021
Lugtenburg, I. C.: P11.084
Lührig, S.: P11.048
Luizon, M. R.: P06.029, P10.03, P10.46
Lukassen, S.: C16.6
Lukina, E. A.: P13.14, P14.10
Luleyap, U.: P05.65
Lun, K. S.: P02.250
Luño, E.: P07.28
Lunt, P.: P05.41, P12.050
Lupo, V.: P12.114
Lupski, J. R.: C04.1, C15.4
Lustig, J.: P14.06
Lutz, G.: P09.010, P09.058
Lutze, A.: P09.106
Lux, S.: P09.047
Luyten, F. P.: PL2.5
Lyle, R.: C04.4, C15.4, P02.080
Lynch, D.: P14.09
Lynch, S.: P12.174
Lynch, S. A.: J02.55, **P02.214**
Lyonnet, S.: C09.2, P02.141, P11.123, P12.178
Lyons, M. J.: P03.098
Lysogorskaia, E. V.: **J09.37**
Lyubaeva, E. V.: J09.44, P09.083
Lyubchenko, L. N.: P06.076
Lyytikäinen, L.-P.: C05.3

M
M. Boemers, T.: P08.08
Ma, P.: P11.075
Maak, A.: P12.108
Maas, B.: P02.077, P02.189, P02.236, **P02.241**, P03.059
Maas, E.: P14.20
Maassen, R.: P11.068
Maatoug, S.: P03.015, P10.31
Maazoul, F.: P02.054
Mäbert, K.: **P02.033**
Mac Neal, M.: C09.5
Macarie, I.: P06.088
Macaya, A.: P12.083
Macek Jr., M.: **P01.11**, P01.21, P04.51, P05.37
Macek sr., M.: P04.51, P05.37
Macekova, S.: J12.14, **P09.097**
Mach, C.: P12.167
Mach, M.: P03.021
Machado, C. M.: P11.055
Machalová, E.: P12.219
Mache, C.: P12.201
Machielsen, T. C.: C04.5
Maciejewski, H.: P09.099
Maciel, P.: P05.21
Maciewicz, R. A.: PL2.5
Macip, S.: J06.21
Mackay, D.: P11.005, P11.056
MacKay, S.: P02.228
Mackic, M.: J03.10, P03.030
Mackic-Djurovic, M.: **P03.075**
Mackintosh, C.: P06.131
Macleod, R.: EP06.01, EPL1.6, **EPL2.6**
Macovei, M.: J02.32, P03.125, P03.144, P05.11
Madan-Khetarpal, S.: C09.5
Madjunkov, M.: P04.05
Madjunkova, S.: **P04.05**
Madrigal, I.: P03.076
Maerz, W.: J02.24
Mafra, M.: P06.109
Magagnotti, C.: P12.042
Magariello, A.: **J12.27**
Magg, J.: P14.11
Mägi, A.: P06.066
Magi, F.: P08.30
Magic, Z.: P06.170
Magini, P.: P02.044, P03.083, P11.059, P11.070
Magna, L. A.: **P01.33**
Magnin, E.: P12.167
Magyari, L.: P10.25, P10.30, P10.43, P10.52
Magzhanov, R.: J02.18, P12.113
Mahachie John, J. M.: **P08.28**
Mahaut, C.: C03.5
Mahdavi, M.: P02.085, P02.235, **P12.075**
Mahdian, R.: J05.05, J06.09, P06.043, P06.046, P06.104
Mahdizadeh, M.: P05.30
Maher, B. S.: C06.4
Mahjoubi, A.: **J04.09**
Mahjoubi, B.: J09.23, P06.069
Mahjoubi, F.: J04.09, J09.23, P06.049, P06.069, **P06.149**, P11.057
Mahlberg, N.: P12.086
Mahlman, M.: P09.034
Mahmood Baig, S.: P02.033
Mahmoud, A. A.: P06.209
Mahmoudi, H.: **P12.123**
Mahoney, K.: P09.095
Maia, S.: P03.050
Maier, H.: P12.108
Maier, L. S.: P11.053
Maier, W.: P09.118
Mair, M.: P12.041, P12.226
Maire, G.: P07.01
Majewski, J.: P02.185, P12.172
Majidzadeh-A, K.: P06.046
Makareeva, E.: J12.22, J12.23
Makarova, N. V.: P09.083
Makasheva, V. A.: J03.15
Makchrova, I. A.: P13.28
Makhmutova, Z.: **J12.19**
Mäki, M.: P09.037
Makita, Y.: P02.048
Mäkitie, O.: PL2.3
Makowski, C. C.: P03.090
Makrythanasis, P.: J02.34, P08.11, P11.027, **P12.055**, PL2.6
Maksimova, N. R.: P02.035, **P12.261**
Makukh, H.: **J09.26**, P04.18, P13.16
Malacarne, M.: P03.069, P03.093
Malakooti Nejad, M.: J12.18, **J11.08**
Malaktari-Skarantavou, S.: P02.227
Malamut, G.: P09.115
Malan, V.: C01.1, P02.161, P02.165
Malbin, j.: **P12.144**
Malcikova, J.: P07.17
Malcov, M.: P04.20
Maleva, I.: **P06.042**
Malfait, F.: C09.5, P12.048, **P12.183**
Malievsky, O.: P05.16
Malig, M.: P10.16
Malikova, M.: P05.50
Malli, T.: **P06.013**
Maloney, V.: P05.21
Maltsev, A.: **P09.101**
Malyarchuk, B.: P10.40
Malykh, S.: P09.014
Mamai, O.: P07.31
Mämecke, L.: **P02.057**
Man, E.: P02.250
Mancini, C.: **P12.147**, **P12.199**
Mancini, T. I.: P03.023
Manco, R.: J11.11
Mandal, S.: **J05.11**
Mandel, H.: P03.111, P13.04
Mandel, J.-L.: J11.10, **P01.28**, P12.046, P13.25
Mandengue, V.: J02.43
Mander, A.: P12.203
Mandrile, G.: P03.093
Manea, A.: J02.22
Manea, A.-M.: **J13.03**
Maneiro, E.: P02.126
Manfellotto, D.: P10.17
Manfred, S.: P12.239
Mangino, M.: PL2.5
Mangold, E.: C14.4, P02.012, P02.086, P08.32, **S04.1**
Maniglia, J.: P06.216, P06.090
Manna, I.: **J09.45**
Mannan, A. U.: **P12.142**
Manners, S.: P02.194
Männik, K.: P02.009, P03.004, P03.102, P11.059
Mannion, G.: **EP06.04**
Mannucci, E.: P11.077
Mano, H.: C10.3
Manolakos, E.: P02.258, **P05.67**
Manouvrier, S.: P02.070
Mans, D. A.: C11.5
Mansilla, E.: J02.55
Mansour, A.: P12.252
Mansour, S.: C03.5, C09.5
Manta, P.: P12.040
Mantovani, V.: P12.130, **P13.27**
Manzoni, M.: P11.103
Manzoor, S.: P08.27
Maraleva, A.: **P08.33**
Marangi, G.: C01.2
Maraş, H.: P12.101
Marasco, E.: P12.130, P13.27
Marazan, M.: J02.40
Marazita, M. L.: C14.4
Marcelli, M.: C08.3, P11.119
Marchini, J.: C17.2, C17.3, P08.19
Marciano, C.: P03.069
Marcos García, G.: P06.021
Marcos, G.: P06.065
Marcovici, T.: J02.05, **J02.41**, J09.33
Marcus, B.: P12.122
Marczak, A.: P03.114
Marczak-Hałupka, A.: P02.232, P02.233
Mardach, R.: P14.03
Mardin, C. Y.: C14.1
Mareckova, J.: P05.68
Marek, J.: P12.152
Marek-Yagel, D.: P12.202
Mares, J.: **P06.182**
Maretina, M.: P12.230
Margaryan, A.: **J10.01**
Marginean, O.: J02.41, P05.17, **P09.003**, J09.33
Mari, F.: P02.060, P09.030, P10.49
Marián, E.: P12.015
Marikova, T.: P02.213
Marini, J. C.: J12.22, J12.23
Marino, B.: P02.184, P09.131
Marion, V.: C18.4
Maris, I.: J09.33
Maristany, M.: P09.028
Markaki, Y.: P12.036
Markie, D.: P02.194
Marklund, S. L.: P12.012
Markoff, A.: P04.04
Markova, J.: P03.099
Markova, T. G.: J12.15
Markova, Z. G.: J05.09
Markus, J.: **P06.092**
Markus-Bustani, K.: **P05.54**
Marle, N.: P02.170, P03.020, **P05.48**, P12.167
Marlin, S.: C03.5
Marohn, B.: P04.22
Marom, D.: C12.1
Marongiu, M.: P09.077
Marques Jr, W.: P02.038, P12.231
Marques, A.: **P09.025**, P06.029, P10.46
Marques, B.: P03.038
Marques, C.: P09.063
Marques, F.: P06.169
Marques, I.: **P12.097**, P12.250
Marques, V.: P11.055
Marques-Bonet, T.: C06.1, P10.16, P10.51
Marr, S.: P04.37
Marra, G.: P06.176
Marra, M.: J15.02, J15.03

Marrocco, G.: P02.058
Marroquin, N.: P12.011
Marschall, C.: P02.195, P02.245, **P12.038**
Marsche, G.: P09.130
Marschon, R.: P06.013
Marshall, P.: EP08.04
Marszalek-Kruk, B. A.: **P02.251**
Martasek, P.: **P10.42**
Marteau, T.: **S08.2**
Marthinet, C.: P05.56
Martin, I. C. A.: P09.024
Martin, J.-J.: P08.03
Martín, M.: P07.28
Martin, R.: P14.03
Martin, T.: P02.086
Martin-DeSaro, M.: P02.106
Martiné, U.: P02.049
Martinelli, S.: **P12.222**
Martínez Fernández, M. L.: J02.55
Martínez Frías, M. L.: J02.55
Martínez García, G.: P06.023
Martínez Martín, N.: P06.021
Martinez, D. I.: P06.061
Martinez, P.: J02.47, J02.48
Martínez-Atienza, M.: P12.005, P12.237
Martínez-Cantó, A.: P06.072, P06.082
Martínez-García, M.: **P12.177**
Martínez-Glez, V.: J02.55
Martínez-Hernández, R.: P12.265
Martínez-Pardo, M.: P12.032
Martínez-Rubio, D.: P12.114
Martínez-Sánchez, E.: P15.09
Martinez-Vinson, C.: P12.238
Martiniuc, V.: P03.125, P05.11
Martinon-Torres, F.: C14.3
Martinon-Torres, N.: C14.3
Martins, C.: P06.109
Martins, F. T. A.: P02.118
Martins, M.: J03.03, P03.072, P03.134, P12.250
Marton, M.: P02.138
Marton, V.: P03.141
Martorell, L.: P11.056
Martsul, D. N.: J12.15
Marucci, G. H.: P06.216
Marullo, L.: **C05.4**
Maruniak-Chudek, I.: P02.264
Marusin, A. V.: **J09.06**
Marussin, A.: P10.34
Mary, P.: P11.086
Maryami, F.: J05.02, J05.07, P05.66, J05.06
Märzheuser, S.: C09.4, P02.026, P02.130
Masas, M.: P06.118
Mascarenhas, A.: P03.017
Maschio, A.: P11.037
Masciadri, M.: P12.057, P12.058
Mashhadi, M.: P06.052
Mashkina, E.: **P04.33**, P04.44
Maslennikov, A. B.: J04.02
Maslinskaya, L.: J09.03
Maslova, E. V.: J04.02
Maslyankov, S.: P06.093
Masneuf, S.: C13.5
Masodifar, M.: P12.144
Mason, D.: P11.116
Masoudi, N.: P04.09
Masri, A.: P12.055
Massimi, L.: P09.048
Masters, B. S.: P10.42
Mastrominas, M.: P05.51
Masuno, M.: P02.029
Masurel-Paulet, A.: P02.170, **P02.265**, P05.48
Mata, C.: P03.041
Mata-Balaguer, T.: P06.082
Mateo, J.: P15.09
Materassi, M.: P11.125
Matern, D.: P14.09
Matesanz, F.: P09.085
Matevska, N.: **P06.077**
Mathieu, M.: P05.18
Mathieu-Dramard, M.: P02.088, P02.169
Matko, S.: P02.133
Matos, A.: P03.050
Matos-García, I.: P06.105
Matoso, E.: P03.050
Matsko, D. E.: P06.036
Matsui, T.: P03.002
Matsumoto, K.-I.: P12.240
Matsumoto, N.: P03.002, **P12.052**
Matsuo, H.: P09.071
Mattheeuws, E.: P05.15
Mattheisen, M.: C08.5, C14.4, P08.08, P09.069, P10.38, P12.153
Mattila, K.: P12.028
Mättö, J.: P09.037
Máttyus, I.: P12.015
Matuska, M.: P07.17
Matusz, A.: P02.108, P11.043
Matveeva, V. A.: P09.045
Matyas, G.: **P12.086**
Mauermann, O.: C10.6
Maurer-Morelli, C. V.: P12.244
Mauri, L.: P12.013
Mautner, V.-F.: P02.179, P06.158, P06.159
Maver, A.: J05.12, P09.084, **P11.079**
Mavrogiannis, L.: C07.3
Mavrou, A.: J02.30
Maximova, N.: P10.34, J12.03
Mayer, K.: P02.180, **P11.132**
Mayer, V.: P04.42
Mayet, J.: C14.6
Mayr, J.: P14.19, C12.3, C12.4
Mazen, I.: P12.132, **P12.197**, P03.126
Mazilli, M.: P09.059
Mazoyer, S.: P11.110
Mazzanti, A.: **J12.28**, P02.126
Mazzei, R.: J12.27
Mazzinghi, B.: P11.077, P11.125
Mazzotta, A.: P09.110
McAllister, M.: **EPL1.6**, EPL5.6
McCarroll, S.: P09.032
McCarthy, N.: C14.6
McClaren, B.: EPL3.6
McCready, E.: P05.04
McDade Walker, D.: **P11.087**
McDermott, M. F.: P12.247
McDonald, K.: EP06.02
McDonald-McGinn, D. M.: P02.006
McDowell, I.: P11.054
McElhinny, T.: P01.25
McElrevaey, K.: P04.27
McEntagart, M. M.: C11.4
McEntee, G.: **P04.13**
McEntee, L.: P02.194
McGee, S.: P11.142
McGettigan, P.: P12.174
McGowan, R.: C03.5
McGowan-Jordan, J.: **P05.04**
McGregor, L. K.: **P02.229**
McGuffin, P.: P09.128
McGuire, E.: P02.178
McHugh, N.: C14.5
McInnes, R.: P11.028
McIntyre, A.: C12.1
McKee, S. A.: **P02.173**
McKenzie, S. E.: P11.109
McLaughlin, N.: S09.1
McManus, R.: C14.5
McNerlan, S.: P02.173
McParland, J.: P03.062
McQuillin, A.: C06.4
Means, D.: P07.15
Medeira, A.: P03.008
Medici, V.: EPL7.3
Medina-Coello, C.: P06.138
Medne, G.: P06.005
Medrano, C.: J12.28, P13.07
Medrano, L. M.: P09.040
Mee, P.: P02.231
Meechan, D.: P02.178
Meesters, C.: P09.078, P10.38
Megarbane, A.: P08.11, P12.055
Megremis, S.: P02.227
Mehdi, M.: P04.49
Mehnert, K.: P02.205, P02.230
Mehraein, Y.: **P02.067**, P03.105
Mehri, S.: **P08.04**, P08.18
Mehta, D.: P11.047
Mei, H.: C06.3
Meienberg, J.: P12.086
Meigs, J. B.: C05.4
Meijers, M.: C11.3
Meindl, A.: P06.117, P07.05
Meinecke, P.: P12.138
Meinke, P.: **C11.6**, P12.204
Meins, M.: EP02.02
Meirosu, M.: J09.04
Meiser, B.: EPL4.1, EPL5.5
Meisinger, C.: C13.2, P13.09
Meissner, T.: P13.19
Meitinger, T.: C12.3, C12.4, C13.2, P03.090, P10.32, P11.047, P14.19
Meixensberger, J.: P07.25
Mejaški-Bošnjak, V.: P10.49
Melamed, Z.: **P11.137**
Melaragno, M. I.: **P03.023**, P03.107, P03.118
Melas, P. A.: **J11.05**
Melegh, B.: J02.02, J09.16, P10.25, P10.30, P10.43, P10.49, P10.52, P12.139, P13.22, J09.16, **P10.25**, P10.30, P10.43, P10.52
Melin, L.: P11.123
Melis, D.: J02.14, J02.52, P02.040, P12.227
Mellert, K.: P14.16
Mellies, N.: P11.048
Mello, C. B.: P03.107
Melnikov, A.: **P03.101**
Melo, J. B.: **P03.017**, P06.169
Melo, S.: P06.063
Meloni, V. A.: P03.023, P03.107
Mencarelli, M. A.: P02.060, P09.030
Mendes, Á.: **EP06.05**
Mendes-Junior, C. T.: P10.03
Mendola, A.: P12.202
Mendonca, B. B.: P04.58
Mendonça, D.: P10.23
Meng, G.: P12.069
Menghini, M.: P09.091
Menke, A.: P08.20
Menko, F. H.: EP05.01, EPL6.2
Mennen, U.: C03.1
Menon, U.: EP05.09, EPL4.2
Mensenkamp, A. R.: EP05.06
Menten, B.: P04.24
Mercado, P.: P06.061
Meresse, B.: P09.115
Merinero, B.: P12.032, P13.01
Merino, C.: P13.42
Merkenschlager, A.: P02.242
Merla, G.: P08.11, P11.103
Merry, C. L. R.: P12.241
Merten, L.: **P11.058**
Mertz, E.: J12.23
Mese, T.: P02.052, P02.071
Meslin, E. M.: EP08.04
Messenger, A. G.: P09.058
Messiaen, L.: P06.158, P06.159, P06.160, P06.176
Mešťánek, J.: P12.251
Métay, C.: P02.161
Metcalfe, S.: EP02.04, EPL1.4, **EPL3.6**
Metin, B.: P15.06
Metrustry, S.: PL2.5
Metsepalu, A.: C17.6, C08.4, P01.02, P06.087, P11.094

Mette, L. A.: **J01.10**
Metz, C. H. D.: P06.212
Metze, D.: P12.068
Meyer, N.: P12.168
Meyer-Kleine, C.: **J02.24**
Mey-Raz, N.: P04.20
Meyron-Holtz, E. G.: P04.22
Mezghani, N.: **P02.260**
Meziane, H.: **P11.045**
Mezzullo, M.: P13.27
Mhamdi, O.: P12.046
Miasnikov, S.: J13.04, P13.15, P13.21
Micale, L.: P11.103
Micalizzi, C.: J07.04
Michalova, K.: P07.02
Michaud, J.: S10.3
Michaud, M.: P14.11
Michel, J.-B.: P02.223
Michelini, S.: P12.203
Michelson, M.: P02.263
Michetti, F.: P09.048
Michiorri, S.: P05.05, P05.12
Michon, A.: C11.1
Michot, C.: **C09.2**, P02.141
Micle, I.: J02.40
Micule, I.: P11.065, P11.066
Middleton, A.: **EP08.03**
Midro, A. T.: **J03.14**
Midyan, S.: P02.009, **P03.052**, P03.055, P03.057
Miehle, K.: P13.24
Miera, O.: P02.215
Mierla, D.: **J03.07**, **P03.024**
Migliavacca, E.: P03.011, P08.11, P11.103
Mignot, B.: P02.011
Mignot, C.: P12.167
Mihaescu, R.: **EP04.01**, **J04.07**, P05.17
Mihalcea, S.: **J14.03**
Mihalová, R.: P03.079, J03.08
Mihaylova, V.: P02.064
Mıhcı, E.: J13.01, **P02.226**, P13.34
Mihova, K.: **P12.116**, P12.117
Mika, J.: P05.44, P05.50
Mikelsaar, A.-V.: **P10.13**
Mikelsaar, I.: P10.13
Mikelsaar, R.: **P03.078**, P10.13
Miklos, M.: P03.040, P05.02, **P05.19**
Mikołajczyk-Stecyna, J.: **P08.01**
Mikstacki, A.: P13.48
Mikulasova, A.: **P07.26**, P07.27
Milá, M.: P03.076, P09.028
Milani, D.: P02.040
Milani, L.: P06.087
Milanova, V.: P11.118
Milanowska, K.: P09.001
Milasin, J.: P09.022
Milia, A.: P03.042
Militaru, A.: J02.05, J02.41
Militaru, M.: P05.17
Militaru-Somogy, A.: J09.33
Millat, G.: P12.237
Miller, F. W.: C04.4
Miller, K.: P03.035, **P15.02**
Mills, J. L.: P12.264
Milly, M.: P02.211
Milovidova, T. B.: **J12.21**
Miltenberger-Miltenyi, G.: **P02.163**
Milz, E.: C03.4
Miñambres, R.: **P11.050**
Minarik, G.: P05.13, P06.060, **P06.102**, P12.209
Minderer, S.: P05.35
Miner Pino, C.: P06.021, P06.023
Miner, C.: P06.065, P06.196
Minetti, C.: P11.063
Mingroni Netto, R. C.: PL2.3
Minguzzi, S.: P04.13
Minichová, L.: **P12.080**
Minniakhmetov, I. R.: **P06.058**
Minopoli, F.: P02.135
Minopoli, G.: J02.14, J02.52
Minot, D.: P12.167
Minzhenkova, M.: J03.06, J05.08, P03.099
Minzhenkova, M. E.: J05.09
Mirab Zadeh, A.: P09.112, J09.08
Miraj, S.: J09.22
Miralles García, J.: P13.18
Miralles, A.: P03.070
Miranda Díaz, M.: P03.005
Mirante, T.: J12.35, P12.081
Mirfakhraie, R.: **J04.11**
Mirlesse, V.: P05.58
Mirmiran, A.: P09.087
Mironova, Z. A.: J09.07
Miroshnikova, V.: **P09.108**
Mirshams Shahshahani, M.: J12.18
Mirtalebi, H.: J09.35
Mirzaee, R.: J09.23
Mirzaei, K.: **P06.211**, P06.215, P09.089
Mirzazadeh, M.: P03.044, **P03.048**
Misceo, D.: P03.007, P03.010, P03.103, P03.145
Miserey-Lenkei, S.: PL2.2
Misiak, B.: P06.071
Missel, A.: P11.013
Missiroli, F.: J09.43
Mitchell, D.: P12.200
Mitchell, G.: EP05.10, EPL4.1, EPL6.5
Mitchison, H. M.: P12.126, P12.200
Mitev, V.: J09.15, P06.044, P06.048, P06.144, P10.56,
  P12.109, P12.116, P12.117, P12.190
Mitic-Milikic, M.: J10.03
Mitkova, A.: P06.044, P06.048, P06.144, P10.56
Mitrohina, L. F.: **J04.02**
Mitsioni, A.: P12.156
Mitsuzuka, K.: P02.196
Mittal, R. D.: P06.019
Mitter, D.: P02.121, P12.253, **P13.24**
Mittlböck, M.: P06.173
Mityushkina, N. V.: P06.036
Miyake, N.: P03.002
Miyamura, H.: P04.41
Mizoguchi, M.: J05.01, P02.196
Mizumoto, S.: P02.223
Mizuno, S.: **P02.048**, P12.131
Mkaouar-Rebai, E.: P02.260
Mkaour, E. R.: P10.31
Mkheidze, M. O.: **J02.28**, P02.042
Mkrtchyan, H.: P02.009, P03.052, **P03.055**, P03.057
Mladenov, E.: P09.060, P12.036
Mlika, A.: P02.053
Mlkva, I.: P02.211
Mni, M.: S01.3
Mnif, M.: J04.16, P02.260
Moazami, N.: P06.150
Moazeni Roodi, A.: P10.54
Moazzeni, H.: **J11.09**
Mochel, F.: C11.2, **P12.120**
Modaber, G.: P02.094
Modarres, H.: C11.4
Modarressi, M.: J04.13
Moebus, S.: C09.4, P08.08, P09.033, P09.078
Moghadasi, S.: **EP05.05**
Moghaddam, M.: P02.085, P02.094, P02.235, P12.075
Moghaddasi, M.: J02.12, P02.094
Moghaddasian, M.: P12.186
Moghbeli, M.: J06.19, P06.091, **P06.119**
Mohaddes Ardebili, M.: J09.02, J09.18, P09.011
Mohamadinejad, P.: P12.208
Mohamed, A. M.: **P03.126**
Mohamed, B. A.: **P11.053**
Mohamed, M.: P13.45
Mohamed, S.: **P14.13**
Mohammad Ganji, S.: **P06.142**
Mohammadi Pargoo, E.: **P10.07**
Mohammadi, A.: P10.22
Mohammadi, H.: P06.043
Mohammadi, M.: P02.119
Mohammadkhani, N.: P03.131
Mohammadnejad, L.: P02.164
Mohammadzadeh, M.: J15.01
Moharrami, T.: **J09.02**
Mohnike, K.: P02.091, P13.19
Mohseni Meybodi, A.: **P03.033**, **P03.039**, P03.077,
  P04.09, **P04.56**, P06.041
Mokhtari, A.: P10.15
Mokhtari, F.: P09.089
Mokhtari, M.: J06.08, J06.09, **P06.193**, **P06.043**, P06.104
Mokrejsova, M.: P09.054
Mokry, J.: C13.3
Molania, R.: **P06.069**, P11.057
Moldagulova, E.: J03.02, J05.04
Moldovan, R.: **EPL4.5**
Molero, M.: P12.005
Molfetta, G. A.: P06.096, P10.46, **P12.212**
Molinatto, C.: P02.010, P02.040, **P02.129**
Molinska-Glura, M.: P09.002
Molla Mohammadi, S.: P11.089
Möller, P.: C10.5
Möller-Krull, M.: P02.153, P02.154
Molnár, M.: J02.02
Momenan, A.: P09.067
Momeni, S.: P09.013
Momozawa, Y.: **S01.3**
Monaco, A. P.: P09.126
Moncini, S.: **J09.46**, P11.008
Moncla, A.: P02.141
Monclair, T.: P12.148
Moncoutier, V.: P11.110
Mondelaers, A.: P01.24
Moneva, Z.: P04.50
Monier, E.: P05.56
Monnerat, C.: EP05.07
Monnier, S.: P05.56
Monserrat, L.: J12.28
Monserrat-Iglesias, L.: P02.126
Montajabi Niat, M.: P02.252
Montalbán, G.: P06.118
Montaldo, C.: P03.042
Montaner, D.: P12.114
Montazer, M.: J06.19
Montazeri, M.: **J02.19**
Monteiro, C.: P11.055
Montenegro, M.: P12.078
Montgomery, S. B.: **C05.6**, PL2.6
Monti, E.: P11.103
Montoliu, L.: P12.177
Montorfano, G.: P12.002
Moog, U.: C13.2, P02.012, **P02.077**, P02.111, P02.189,
  P02.234, P02.246, P03.059
Moore, A.: EPL5.6
Moore-Barton, H. L.: C01.1
Moosa, S.: **P02.208**
Moosavi, A.: **P08.44**
Mora Guijosa, A.: P02.030
Moradi Marjaneh, M.: **P09.024**
Morak, M.: C10.2, P06.154
Moral, P.: P10.28
Moran, C.: P09.024
Moran, J.: P09.032
Morava, E.: C09.6, P12.061, **P13.04**, P13.45
Mordanov, S.: P07.29
Moreira, M. N.: J02.42
Morel, Y.: P02.011
Morel-Kopp, M. C.: P02.107
Morelli, C.: P10.04
Moreno, F.: P12.108
Moreno, L.: P12.237
Moreno, R.: P06.118
Moreno-Igoa, M.: P03.016
Morerio, C.: J07.04
Morgan, J.: C07.3
Morgan, R.: EP06.02
Morgante, M.: P12.225

Morgenthaler, J.: **P02.246**
Morin, G.: P02.088, **P05.18**
Moriya, H.: J05.01
Mork, M.: P02.087
Morle, S.: P12.167
Morlino, S.: P02.065
Morlot, S.: P03.063
Mormile, A.: J02.14, J02.52
Moro Ortiz, A.: P03.005
Mórocz, É.: P06.195
Moroni, I.: C12.3
Morozin Pohovski, L.: **P03.096**, P03.117
Morozov, I. V.: J12.32
Morris, A.: C05.4, **S01.2**
Morris, D. W.: **C06.4**, P11.117
Morris, H. C.: EPL1.5
Morris, M.: P01.24
Morris, M. A.: P15.10
Morris, S.: EPL3.3
Morris-Rosendahl, D. J.: **P02.167**, P12.145
Morsy, H.: P12.027
Mortezazadeh, M.: J05.02, J05.07, P05.28, P05.66, J05.06
Mortier, G.: C13.1, P02.115, P07.22
Mortier, G. R.: C01.3
Mortini, P.: P06.057
Mory, A.: **P03.111**, P05.59
Mosca-Boidron, A.-L.: P02.170, **P03.020**, P05.48, P12.167
Moschese, V.: J12.30
Moschos, M. M.: P12.001
Moschovi, M.: P07.11, P07.18, **P07.33**
Moser, B.: P10.09
Moser, E.: P02.166
Moser, T.: PL2.4
Moshir, S.: **P06.064**
Moshiri, H.: **J12.06**
Mosse, K.: J01.01, P05.03, P12.195
Mossey, P. A.: C14.4
Mössner, R.: P09.111, P09.118
Mostile, G.: J12.34
Mota, E.: P13.10
Mota, M.: P13.10
Motaei, J.: **P09.087**
Mota-Freitas, M.: P03.038
Motahari, B.: **J06.04**
Motamed, N.: P06.193
Motei, G.: P02.031, P03.121
Motovali bashi, m.: J08.01
Motte, J.: P09.026
Mougou Zerelli, S.: P03.066
Mougou, S.: P03.085, P07.31
Moukova, L.: P07.06
Moulton, K.: P11.086
Moura-Nunes, V.: P06.109
Mourits, M.: C10.4
Mousavi Movahedi, A.: P11.133
Mousson, C.: P02.141
Moutafi, M.: P03.112, P11.003
Moutinho, O.: J03.03, P03.072, P03.134
Moutou, C.: C16.3
Mouzarou, A.: J08.02
Movafagh, A.: J04.11
Movahedin, M.: P14.17
Mowla, S. J.: P14.17
Mrad, R.: P02.053, P02.054
Mrasek, K.: **P03.091**, P10.08
Mrizek, N.: P07.31
Mucciolo, M.: P09.030
Mudrunka, J.: P06.182
Mueller, C.: C01.6, P11.010
Mueller, D.: **P03.047**
Mueller, F.: P02.101
Mueller-Malesinska, M.: P02.117, P02.156
Mughal, S. S.: **P09.033**
Muglia, M.: J12.27
Mugneret, F.: P02.170, P03.020, P05.48
Muhammad, S.: P11.060
Mühlbacher, A.: EPL1.1
Mühleisen, T. W.: **P09.047**, P09.069
Mühlfeld, C.: P11.053
Muir, K.: PL2.5
Mulas, A.: C08.3
Mulder, C. J.: P09.115
Mullaney, B.: C15.6
Müller, C. R.: P06.026, P11.021, P12.098, P12.103
Muller, D.: P11.110
Müller, E.: P11.076
Muller, J.: C18.4, J11.10, **P12.046**
Müller, J. S.: P02.064
Müller, K.: **P12.145**
Müller, M.: P12.239
Müller, S.: P02.067, **P03.105**
Müller, T.: C16.5
Müller, U.: P12.232
Müller-Myhsok, B.: C08.5, P08.20, P11.104, P11.122
Mulliken, J. B.: C01.4
Mullikin, J. C.: P10.16
Mullinax, B.: P11.093
Mumoli, L.: J09.45
Munafo, D.: P11.098
Mundlos, S.: C03.1, C03.2, C13.1, P02.046, P02.099, P02.210, P02.223, P11.100, P12.031, P12.061, P12.158, P12.162, **PL1.1**, PL2.3
Munger, J.: P11.049
Muniz, Y. C. N.: P10.03
Munk, R.: P02.112
Munk-Schulenburg, S.: **P02.112**, P12.041
Munnich, A.: C03.5, C09.2, P02.165, P11.123, P12.018, P12.178, P13.32
Muñoz, C.: P07.12, P07.28
Muñoz-Bellvis, L.: P06.131
Muñoz-Calvo, M.: P12.003
Muñoz-Sanz, N.: P12.074
Münster, T.: P09.050
Muntoni, F.: C18.3
Mur, A.: P03.076
Murakami, K.: P09.071
Murakami, N.: **P02.206**
Murakami, Y.: C13.1
Muramatsu, Y.: P12.131
Murday, V.: P02.178
Murdolo, M.: C01.2
Mureau, M. A. M.: EPL4.4
Muresan, B.: P05.17, **P05.24**, P05.69
Muresan, C.: P05.17
Muresan, C. M.: P05.24, **P05.69**
Murgia, A.: J09.46
Muriel Villoria, C.: P09.051
Murphy, H.: C03.5
Murray, A.: EPL4.3
Murray, J. C.: C14.4, P13.43
Muru, K.: P02.231
Murugan, M.: J03.21
Murumets, Ü.: P04.19
Musabaeva, L.: P03.101
Musani, V.: **P06.030**
Musante, L.: P08.37
Muschke, P.: P02.043, P05.32
Musialik-Świetlińska, E.: P02.264
Mussa, A.: **P02.040**, P02.129
Mussaffi, H.: P12.200
Mußotter, T.: **P06.157**, P06.158, P06.160
Mustafin, A.: P06.183
Mustafina, L. J.: **J09.39** ,J09.44
Mustafina, O. E.: P08.22, P09.045
Mutesa, L.: **P12.076**
Mutlu, M.: P12.027
Mutter, M.: P09.092
Mya, H. T.: C10.3
Myers, L.: C01.3
Myhre, A. G.: P02.212
Mymrikov, E. V.: **J09.05**
N
Nabioni, M.: P04.11
Nabuurs, S. B.: P12.124
Nacak, M.: J09.11, P09.082
Nachev, G.: P10.56
Nachury, C.: P12.096
Nadalin, S.: P06.178
Nadarajan, V. S.: C10.3
Nadel, S.: C14.3
Naderi, M.: P10.54
Naderi, N.: J09.35
Nadgornaya, V.: P06.014
Nadir, K.: **J02.38**
Nadyrshina, D.: **P12.180**
Naeem Khan, T.: P02.033
Naeem, T.: P12.020
Naepels, P.: P05.18
Nafei, Z.: J01.05
Nagai, J.-I.: P02.029
Nagai, T.: P02.206
Nagalla, S.: P11.109
Nagel, C.: P02.215
Nagengast, F. M.: EP05.04
Nagimtayeva, A.: **J13.05**, P02.061
Nagl, C.: P09.035, P09.130
Nagorni-Obradovic, L.: J10.03
Nagtegaal, T.: P01.34
Nagy, A.: P12.251, **S02.1**
Nagy, B.: J09.12, P05.47, P05.49, **P05.63**
Naiki, M.: P02.048
Nair, A.: C03.3, C11.4
Najafi, K.: P03.034, P03.048
Najafi, R.: P05.30, P06.125, **P12.090**
Nájera, N.: P02.128, P06.061
Najjar, N.: P12.105
Najm, J.: **P02.107**, P12.041
Najmabadi, H.: C15.3, J09.21, P02.252, P05.30, P06.125, P08.05, **P08.37**, P12.023, P12.024, P12.025, P12.090, P12.168, P12.243
Najmafshar, A.: P09.089
Nakamura, M.: P09.071
Nakamura, T.: P09.071
Nakamura, Y.: P06.171
Nakashima, H.: P09.071
Nakata, T.: P02.110
Nakayama, A.: P09.071
Nanda, I.: P02.254, P12.103
Nandhagopal, R.: P12.205
Nanni, L.: P02.058
Napoletano, S.: P05.05, P05.12
Nardozza, A. P.: P12.222
Naritomi, K.: P02.150, P12.131
Narz, F.: P15.06
Nasedkina, T.: P09.123, P09.127, P09.012
Nasibulina, E. S.: **J09.40**
Nasibullin, T. R.: P08.22, P09.045
Nasr-Esfahani, M.: P12.208
Nasser, E.: C14.4, P08.32
Nastasia, C.: J02.26
Nasykhova, Y. A.: P03.113
Natsume, J.: P02.110
Nauck, M.: P11.047
Naudina, M.: P11.065
Navab Moghadam, F.: J05.02, J05.07, P05.28, J05.06
Navarrete, R.: P12.032
Navarro, A.: P10.51
Navarro-Palou, M.: P04.38
Navas, P.: P13.44
Naveed, M.: PL2.3
Navin, N.: **S02.2**, C02.4
Navvabmoghaddam, F.: P05.66
Nawara, M.: C11.2
Naydenov, C.: P12.264
Naz, G.: P12.153
Nazarenko, L. P.: **P05.61**
Nazari, S.: J09.30
Nazemi, A.: **J06.10**, P05.23, P06.197

Nazir, M. S.: **J11.03**
Nazyrova, R.: P02.061
Ndagire, D.: P09.085
Ndoni, E.: P09.030
Neagos, D.: P05.20
Neagu, E.: P02.031, P03.121
Neagu, L.: P03.024
Neal, B. M.: C07.5
Neale, B.: **S01.1**
Nedd, K.: P14.03
Nedelea, F.: P05.38, **J02.31**
Neerincx, P. B. T.: **P11.134**
Neff, F.: C12.6
Negoro, T.: P02.110
Negru, S.: EP04.01, J04.07
Nehls, B.: P03.143
Neishabury, M.: **P12.243**
Neitzel, H.: P07.32
Nejati, K.: P06.133, P06.134, P10.22
Nejati-Koshki, K.: **P06.121**
Nelen, M.: **C07.2**
Nelis, M.: P03.078, P12.055
Nelkenbrecher, C.: **C16.6**
Nelle, H.: **P03.063**
Nelson, K.: EPL2.6
Nelson, T.: P03.137
Nemati, M.: J12.17
Nematollahi, M.: P12.208
Nemec, P.: P07.26, P07.27
Németh, S.: **P15.01**
Nemethova, M.: P06.168, **P12.161**
Nemtsova, M.: J06.16, P06.099, P06.100, **P11.006**
Nenadic, I.: P09.118, P09.120
Nenasheva, S.: J12.11
Neocleous, V.: P12.107
Neri, G.: C01.2, **P02.058**, P03.116, P11.040, P12.004, P14.07, PL3.3
Neri, I.: P06.110
Nerkizian, N.: P11.086
Nescakova, E.: P09.052
Neshan, M.: J12.09, P04.53
Nesheva, D.: P02.034, P06.093, P06.164, P10.58, P02.063
Ness, A. R.: P10.37
Neto, A.: P05.21
Neto, D. S.: P06.047, P06.216
Neto, J. L.: P09.080, P09.081, P10.20, P10.21
Netz, H.: P02.245
Netzer, C.: C03.3, **S16.1**
Neubauer, D.: P12.138
Neuhann, T.: P02.086, **P02.204**
Neuhaus, C.: P12.211, **P12.253**
Neumann, L.: **P12.036**
Neumann, S.: C13.4
Neuner, M.: P15.14
Neusser, M.: P03.105
Nevado, J.: J02.55, P03.076, **P12.056**, P13.01
Neveling, K.: C01.1, C07.2, **C11.5**, P02.132
Nevinny-Stickel-Hinzpeter, C.: P02.138, P04.43, P05.14, P13.33
Newbury, D.: P09.126
Newman, W. G.: P12.241
Newson, A.: S16.3
Newton, R.: EP06.03
Ng, K. P.: C10.3
Ng, P.: P01.02
Ng, Q. S.: C10.3
Ng, W.: P12.088
Nguyen, H.: P12.187, **P14.11**
NGuyen, K.: P02.007
Nguyen, L.: **C13.3**
Nguyen, R.: P02.179
Nguyen, T.: **J09.42**
Nguyen, T.-M.: P12.120
Niceta, M.: P12.227
Nicholas, S. K.: C15.4
Nicholl, J.: P02.229
Nicholls, W.: P02.172
Nici, C.: P10.58
Nicoara, D.: J13.03
Nicolaides, P.: P11.003
Nicolaou, N.: P11.007, **P12.154**
Nicolaou, P.: **P10.14**
Nicoletti, A.: J12.34
Niculescu, A.-. M.: P06.124
Niedobitek, G.: P02.245
Niedrist, D.: P02.256, P03.067, **P03.138**
Nielsen, M.: **P03.129**, P12.051
Niemeyer, C.: P14.20
Nieratschker, V.: **P09.009**
Niesler, B.: P09.069, **P09.074**
Niessen, R. C.: **P12.095**
Nievelstein, R. A. J.: P02.172
Nigmatullina, N. A.: **P06.151**
Nijman, I. J.: C18.1, P12.259
Nijtmans, L.: P12.061, P13.45
Nikiforova, I.: P13.39
Nikitina, V.: P06.085, **P07.30**
Niklaus, A.: P06.186
Nikolaev, I.: **J08.05**
Nikolaeva, I. A.: P02.035
Nikolaeva, T. Y.: J12.03
Nikolaeva, Y. S.: P03.113
Nikolaidou, M.: P12.145
Nikolic, A.: J10.03
Nikolova, D. N.: **P06.171**
Nikolova, M.: J09.32
Nikzat, N.: P08.05, P12.023, P12.025, P12.168
Nillesen, W. M.: C01.1, P02.192
Nilssen, Ø.: P03.141
Nimzyk, R.: P14.13
Nippert, I.: EPL2.1, EPL2.2
Niranjan, M.: P02.096
Nishi, M. Y.: **P04.58**
Nishiguchi, K. M.: P12.074
Nishikata, J.: P02.196
Nishiyama, S.: P04.41
Nishizawa, H.: P04.41
Nishu, S.: J03.22
Nitsch, L.: J02.14, J02.52, J11.11
Nitschke, P.: C09.2
Nizamutdinov, I. I.: **P09.012**
Nobbs, M.: P12.174
Nobile, H. L.: **P01.03**
Noe, S.: P06.139
Noegel, A. A.: C13.4
Noehammer, C.: P06.070, P11.022
Noethen, M. M.: P09.033, P09.120, P15.12
Nogovicina, A.: P12.261
Noguchi, T.: **P15.13**
Nogueira, R.: P03.134
Nogueira, S.: P09.025
Noke, M.: **EP02.07**
Nolte, D.: **P12.232**
Nolte, J.: **P11.048**
Nomdedeu, B.: P07.28
Noonan, J. P.: P11.113
Noori-Daloii, M. R.: **P08.10**, P12.106
Noori-Dalooii, M.: P06.203
Nordenskjöld, A.: P08.08
Nordheim, V.: P06.004
Nordin, K.: EP04.02
Nordman, E.: C08.2
Noroski, L.: C15.4
Norris, F.: P05.34
North, K. E.: C06.2
Noskova, M.: P12.034
Noss, U.: **P04.43**
Nöthen, M.: P08.08, P09.069, P11.060, C08.5, C09.4, C10.2, C10.3, C14.4, C15.2, P02.026, P02.130, P08.32, P09.004, P09.009, P09.010, P09.047, P09.058, P09.078, P09.118, P11.015
Nothnagel, M.: P04.04
Notini, A.: P06.201, P06.202
Nõukas, M.: P02.009
Nour, V.: J01.03
Nouri Long, M.: **P06.009**
Novak, B.: P11.128
Novakova, I.: P12.134
Novaković, I.: J04.15
Novakovic, S.: P11.072
Novell, R.: P03.041
Novelli, G.: C14.5, J09.43, P02.147, P09.110
Noveski, P.: P04.50, **P04.55**
Novikova, I.: J01.01
Novotná, D.: P02.174, P03.079, P05.37
Nowak, S.: C14.4, P08.32
Nowakowska, B.: J02.55
Noyman, I.: P12.122
Nozima, B.: P06.208
Ntalla, I.: P10.45
Nucaro, A.: **P03.042**
Nuccitelli, A.: P05.05, P05.12
Nunes, A. C.: P11.055
Nunes, J.: P06.109
Nunes, R.: **EP03.01**
Nunes, V.: P12.181
Nuñez, C.: P09.040
Nuñez-Lozano, M.: P06.177
Nuotio, M.-L.: **P09.137**
Nur, B. G.: **J13.01**, P02.226, P13.34
Nürnberg, G.: C13.4, P12.108, P12.201, PL2.4
Nürnberg, P.: C03.3, C13.4, P02.223, P06.200, P12.108, PL2.4
Nursal, A.: P06.040
Nyamsuren, G.: **P11.135**
Nycz-Bochenek, M.: P02.264
Nyman, M.: **P01.32**
Nystad, M.: **P03.141**
Nyström, M.: P02.172

O
Ó Fágáin, C.: P04.13
O´Sullivan, C. K.: P09.042
O'Callaghan, C.: P12.200
O'Donovan, M.: P09.032, **S05.2**
Obata, K.: P02.206
Obenhaus, H. A.: P12.021
Oberkanins, C.: P15.01
Obermayer-Pietsch, B.: P09.103, P13.41
Obersztyn, E.: J02.55, P02.028, P02.185, P03.074, P12.145, P12.172
Oberthür, R.: P06.074
Obici, L.: P12.247, P15.01, P15.07
Obrenovic, M.: J02.06, J03.13
O'Brien, E.: C14.6
O'Brien, K.: P04.13
Öçal, G.: P02.022
Ochiana, D.: P02.031, **P03.121**
Ochoa, C.: EP05.12, EPL2.3
Ochoa, E.: P06.072, P06.082
Ockeloen, C. W.: **P02.055**, PL2.3
Ockhuysen-Vermey, C. F.: EPL6.2
O'Connell, J. M.: **P08.19**
O'Connell, K.: P09.114
Odent, S.: P02.141
Oegema, R.: P12.199
Oehl-Jaschkowitz, B.: P02.234
Oellerich, T.: P12.045
Oertel, R.: P05.34
Oexle, K.: **P02.255**, P03.090
Offenhäuser, N.: PL2.4
Offerhaus, J.: P02.172
Ogilvie, C.: **P03.097**
Oglesbee, D.: **P14.09**
Ogorodnikova, N. A.: **P12.104**
Ogorodova, L. M.: P09.019, P09.062
Ogrizek Pelkic, K.: P05.33
Oguzkan Balci, S.: J09.11, J12.02, **P09.082**, P04.48
Oh, J.: **P09.065**, P09.066
Oh, S.: **P11.113**

Oh, T.: P03.137
Ohadi, M.: **J09.08**, P09.013, P09.112
O’Hanlon, T. P.: C04.4
Ohnaka, K.: P09.057
Ohnuki, Y.: J05.01
Oidovsambuu, O.: P11.135
Õiglane-Slik, E.: P11.059, J03.18
Oikonomakis, V.: P02.261, P03.012, P09.015, **P11.081**
Oji, V.: P12.068
Okamoto, N.: P02.048, **P03.002**
Okan, S.: J09.13
Okoniewski, M.: P12.086
Okur, V.: **J02.07**
Olariu, L.: J02.41
Oldak, M.: P02.117
Oldenburg, J.: P12.103
Oldridge, M.: P12.248
Olesen, J.: J09.25
Oliva, M.: J02.48
Oliva-Teles, N.: **P03.038**
Oliveira, J.: P05.21, P12.043, P02.177, P12.007
Oliveira, M. D. V.: P03.108
Oliveira, M. M.: P03.023
Oliveira, M. V.: P03.140
Oliveira, N. F.: P11.127
Oliveira, R. F.: **P03.050**
Oliver-Bonet, M.: P04.38
Olivier-Faivre, L.: P05.48
Ollert, M.: C12.6
Olszewski, W.: P09.107, P02.084
Omran, H.: P12.200
Omrani, M.: P03.081, P06.192
Omranipour, R.: P06.149
On Behalf of the DDD Study,: EP08.03
Onay, H.: J12.04, P02.052, P02.071, P12.099, P12.165
Oneda, B.: P02.003, P02.162, P02.219, P02.256, **P03.067**, P03.138
O’Neill, F. A.: C06.4
Onesimo, R.: P02.058
Onesto, E.: P10.04
Ong, J.: P11.087, P11.128
Ong, T. S.: C10.3
Ongen, H.: PL2.6
Onofriescu, M.: P05.11
Ooi, L.: P06.116
Oosterveld, M.: P12.154
Oosterwijk, J.: **C10.4**, EPL6.2
Oostra, B. A.: C05.2, P09.076, P10.53
Ophoff, R.: P09.120
Opitz, L.: P06.213
Opitz, M.: P09.092
Oppo, M.: P11.119, P12.125
Orbegozo, J.: J02.43
Ordoñez, E.: **P05.43**
O’Reilly, P. F.: C05.3
Orel-Bixler, D.: P02.182
Orenay Boyacioglu, S.: J06.14
Örenay, S.: P06.012
Orera Clemente, M.: P06.161
Orfao, A.: P06.131
Orlando, L.: C11.2
Ormerod, E.: P03.103
Orosz, Z.: P06.195
O’Rourke, A. W.: **P12.248**
Orr-Ortreger, A.: P02.145, P03.142
Orru, S.: P05.67
Orrú, V.: C08.3, P09.077
Orr-Urtreger, A.: P05.54
Ortega, V.: P07.15
Orteschi, D.: C01.2, P02.058
Ortiz Brüchle, N.: P02.234, **P11.012**, P12.196
Ortiz-Genga, M.: P02.126
Ortiz-Lastra, E.: P03.025, P12.094
Oruc, L.: P09.069
Ösby, U.: J11.05
Oshiro, M.: P02.048
Osinovskaya, N. S.: J04.04
Osnes, L. T.: C15.4
Østergaard, J. R.: P02.144
Ostergaard, P.: **S12.1**
Ostermayer, E.: C16.1
Ostoros, G.: P06.195
O’Sullivan, J.: C15.6
Oswald, G.: C01.3
Oszkinis, G.: P08.01, P09.002
Ota, S.: P04.41
Otasevic, P.: P09.022
Oto, Y.: P02.206
Otsuka, A.: P12.068
Ott, C. E.: **C03.2**
Otte, I. C.: P01.14
Otten, E.: **P01.06**
Oud, M. M.: P12.126
Oudakker, A. R.: P12.124
Oudesluijs, G.: EPL7.4, P05.60, P02.043
Ouertani, I.: P02.054
Õunap, K.: **J03.18**, P02.231, P03.032, **P11.139**
Oury, C.: S01.3
Ousager, L.: P02.152, P02.113
Ouwehand, W.: C17.3
Overbeck, G.: **EP02.03**
Owen, M. J.: P09.032
Oyarzabal, A.: **P12.032**
Oza, N. M.: J05.11
Ozbek, U.: J12.10
Özbek, U.: P12.101
Ozdemir, M.: P06.040, **P07.19**
Ozdemir, O.: **J12.10**, **J14.01**, P06.075, P06.127, P06.152, P06.205, P06.206, P14.05, P14.22
Özdemir, Ö.: P12.101
Ozdemir, S.: P06.205, **P06.206**, P14.22
Ozen, F.: **P06.075**, P14.05
Ozen, M.: J02.35, P02.069, P02.249
Ozeri-Galai, E.: S02.3
Ozgul, H.: P06.180
Özgünen, F. T.: P04.39, P05.65
Ozkan, Y.: P04.48
Ozkinay, F.: J12.04, P02.052, P02.071, P12.099, P12.165
Özpak, L.: **P04.39**
Özpak, L.: P05.65
Ozretić, P.: P06.030
Ozturk, E.: **P04.48**
Ozturk, K.: P06.114
Ozturk, Z.: P12.029
Özütemiz, Ö.: P06.012

P
Pabinger, S.: C18.5
Pabón-Peña, C.: P11.128
Pabst, B.: **P03.035**
Pacini, D.: P02.023
Páclová, D.: P12.152
Paděrová, J.: **P05.37**
Padioleau, I.: PL2.6
Padovani Junior, J. A.: P06.129
Paelinck, B.: P11.130
Paetau, A.: C12.5
Pagani, I. S.: **P06.059**
Pagotto, U.: P13.27
Pahl, L.: P08.29
Paiva, C. L. A.: **P09.139**
Pajarola, S.: P02.256
Pakalapati, G.: C04.4
Pál, E.: P13.22
Palais, B.: P15.04
Palaj, J.: P06.092
Palamara, G.: J09.45
Palanduz, S.: P04.52
Palau, F.: P12.114
Palcevska, S.: J02.11
Palin, K.: P10.45
Palleschi, A.: P12.222
Palma, I.: **P06.061**
Palmeiro, A.: P12.170
Palmejani, M. A.: P06.115
Palomares Bralo, M.: **J02.55**
Palomares, M.: P03.076, P12.056
Palotie, A.: C05.3, P09.086
Palta, M.: P02.130
Pályi, B.: P05.47
Pampukha, V.: J09.19
Panczak, A.: J03.08
Panda, S. S.: P10.42
Pandolfo, M.: P02.062
Panduru, N.: J10.12, P10.10, **P13.10**
Paneque Herrera, M.: **EPL2.5**
Paneque, M.: EP02.06, EP03.01, EP06.05, J01.04
Pangalos, C.: **P12.047**
Pani, A.: P09.077
Pankratov, V.: **J10.14**
Pannes, A.: **P12.140**
Panoutsopoulou, K.: P10.45
Panov, R.: P06.093
Panovski, M.: P06.068, P06.077
Pantel, K.: C10.6
Pantos, D.: J08.04
Pantos, K.: P05.51
Panzaru, M.: J02.21, J02.39, P02.140, P03.125, P03.136, P03.144, **J02.32**
Papa, L.: C07.4
Papageorgiou, E. A.: **C16.2**
Papaioannou, V. E.: P12.221
Papakumari, M.: J03.21
Papasavva, V.: P07.18
Papazachariou, L.: **P12.008**
Pape, L.: P02.201, P12.201
Papoulidis, I.: P02.258, P05.67
Papoyan, A.: P06.183
Papp, C.: P05.47
Paprotka, T.: P11.034
Papuc, S.: **P03.046**
Paquet, C.: C11.1
Pár, A.: J09.16
Par, G.: J09.16
Paraboschi, E. M.: **P09.088**
Paracchini, V.: P11.018
Paradela, E. R.: P09.139
Paradisiotou, A.: P11.003
Parathath, S.: C12.1
Pardo, J.: P12.216
Parenti, I.: P12.058
Pariente, J.: C11.1
Parimi, P.: P12.006
Parivar, K.: J06.02, J06.13
Parker, M.: EP08.03
Parlak, A. H.: P12.123
Parle-McDermott, A.: P04.13
Parmar, A. S.: P09.037
Parra, E. J.: P08.24
Partanen, J.: P09.037, P09.042
Parvathi Kumara Reddy, T.: P09.090
Pascanu, I.: P06.089
Pascual Rodriguez, A.: P06.146, **P07.14**
Pasmanik-Chor, M.: C12.1
Pasquali, F.: P06.059
Pasquin, E.: EP01.02
Passamonti, L.: J12.33
Passarin, M.: J02.53
Passon, N.: P06.163, P12.013, P12.225
Passos-Bueno, M.: P02.020, P02.240
Pasternack, S.: P09.010
Pasternack, S. M.: P12.123, **P12.153**
Pastorino, L.: P06.110
Pasutto, F.: **C14.1**
Paszkowski, J.: P06.162
Patitucci, A.: J12.27
Patkowski, D.: P09.099
Paton, W.: J12.23
Patrignani, A.: P12.086
Patrosso, M.: P12.013

Patsalis, P.: P02.013, P03.112, P11.070
Patsalis, P. C.: C16.2, P11.003, P11.059, P11.061, P11.140, P11.142
Patton, S.: P15.15
Paul, J.: **EP02.04**
Paul, R.: C09.1
Paula, F.: **P12.181**
Paulasová, P.: P04.51
Pauli, S.: P12.041, P12.045
Paulmann, B.: P04.42, P05.45
Pavarino, É. C.: P06.047, P06.090, P06.115, P06.129, P06.216, **P11.030**
Pavel, A.: **P05.38**
Pavlenyk, E. S.: J09.06
Pavlíčková, L.: P06.001
Pavlik, R.: P04.04
Pavlov, V.: P06.016, P06.062, P06.183, P06.189
Pawlaczyk, K.: P08.01, P09.002
Payaró, A.: P05.43
Payet, M.: P03.020, P05.48
Paylakhi, H.: J11.09
Payne, K.: EPL1.6, EPL5.6
Pazarbaşı, A.: P04.39, **P05.65**
Pazhoomand, R.: P03.131
Pazourkova, E.: P09.054
Pchelina, S.: P09.108, P12.189
Pearce, F.: **EP08.01**
Pearson, R. D.: C06.4
Peay, H. L.: **EPL5.5**
Peces, C.: P12.056
Peces, R.: P12.056
Peche, V. S.: C13.4
Pechkurova, O.: P13.21
Pecile, V.: P02.025
Pečiulytė, A.: P03.043
Peculis, R.: P09.098
Pedersen, N. L.: C04.4
Pedrola, L.: J02.03, J02.04, P04.15, P13.42
Pe'er, I.: C17.5
PeerZada, A.: J03.20
Pehlivan, D.: C04.1
Pehlivan, M.: P06.132
Pehlivan, S.: J09.11, J09.29, **J12.02**, P04.48, P06.132, P06.180, P09.082
Peirsman, L.: P12.035
Peitz, M.: C01.6
Pekova, S.: P03.091
Pekrun, A.: P12.175
Pelc, M.: P02.005, P02.041, P02.186, P02.232, P02.262, P03.045, P03.049, P07.24, P13.47
Peleg, S.: P04.20
Pelet, A.: P02.141
Pellegrinelli, J.-M.: P05.58
Pellegrini, A.: P06.110
Pellerin, P.: P09.125
Pelletier, V.: C18.4
Pelte, M.-F. F.: C04.6
Peltecu, G.: J02.31
Pelz-Ackermann, O.: P14.18
Pena, Y.: P06.061
Penco, S.: P12.013
Pendina, A. A.: P03.094, P11.024
Pennachio, L.: P11.123
Pennekamp, P.: P11.001
Pennimpede, T.: C09.4
Penninx, B.: P08.13
Pepermans, X.: P12.184
Pepperkok, R.: C05.1
Percin, F.: C03.4, **P12.193**
Pereda, A.: **P12.003**
Pereira, A.: P12.237, P02.199
Pereira, M. F.: P03.108
Pereira, S. V.: P02.163
Pereira-Monteiro, J.: P09.080, P09.081
Perek-Polnik, M.: P07.24
Perelmuter, V. M.: J06.15
Perera-Molinero, A.: P06.138
Perez Costillas, L.: P14.08
Pérez de Nanclares, G.: P06.191, P11.056, P12.003
Perez Sanchez, M.: **P02.030**
Perez, A. B.: P03.023
Pérez, B.: P13.01, **P13.07**
Pérez, C.: P12.094
Perez, I.: J02.46, J02.47, **J02.48**
Perez, J.: P02.128
Perez, R.: P12.086
Perez, Y.: **P12.072**
Perez-Barbeito, M.: J12.28
Perez-Alonso, M.: P12.005
Perez-Barbeito, M.: P02.126
Pérez-Cabornero, L.: P06.065, **P06.161**, P11.050, P11.091
Pérez-Cerdá, C.: P13.01, P13.07
Pérez-Juana, A.: P03.016
Perez-Jurado, L.: P08.11
Pérez-Ortega, B.: P12.237
Pérez-Segura, P.: P06.118
Peričić Salihović, M.: P10.50
Perin, P.: **P09.017**
Perkova, M.: P10.40
Pernhorst, K.: C08.5
Perola, M.: C05.3, P09.134, P09.137
Perria, C.: P12.125
Perricone, C.: P09.110
Perrin, C.: P12.153
Perron, P.: P11.023
Perrone, M. D.: P02.025
Perrot, A.: C07.4
Perseu, L.: P11.037
Persico, S.: P09.110
Pertile, M. D.: **P05.34**
Pes, V.: P12.125
Peskova, K.: P13.36
Peter, I.: P12.264
Peterlin, B.: J04.15, J05.12, P09.084, P11.079
Peters, D. J. M.: P12.214
Peters, G.: P12.088
Peters, M. J.: C08.4, C17.6, PL2.5
Petersen, A. D.: P02.144
Petersen, M.: P02.258, P05.67, **P09.007**, P12.067
Peterziel, H.: C02.1
Petit, F.: P12.138
Petit, S.: P02.070
Petkovic, I.: P03.117
Petrek, M.: P11.108
Petrie OAM, D. M.: **EP08.02**
Petrie, D.: EP05.11
Petrisor, F. M.: **J09.20**
Petrkova, J.: P11.108
Petrov, D.: P06.164
Petrova, L. I.: P03.094, P03.113
Petrova, N. V.: P09.053, **P10.44**
Petrova, T.: **S12.2**
Petrovic, K.: P09.130
Petrovic-Stanojevic, N.: J10.03
Petrovska, R.: P09.098
Petruzelkova, L.: P09.135
Petterson, A.: P11.025
Pétursson, H.: P12.136
Petychakis, D.: P12.040
Pezzani, L.: J02.53
Pfäffle, R.: P02.091
Pfarr, N.: **P02.215**
Pfeifer, A.: C02.1
Pfenning, P.-N.: C02.1
Pfeufer, A.: C16.4
Pfister, E.-D.: P13.49
Pfister, H.: P11.104
Pfister, M.: P12.253
Pfister, S.: PL2.1
Pflug, S.: P12.041
Pfob, M.: P02.067, **P02.160**, P03.105
Pfundt, R.: C04.5, C15.1, P02.055, P11.078, P12.154
Pham, K. D. K.: C12.2
Phelps, C.: EPL4.2, **EPL4.3**
Philimonenko, V.: P12.134
Philip, N.: P02.007, P02.141
Philippe, C.: P02.161, P12.178
Philips, A. K.: **J12.31**
Phillips, A.: EP06.04
Philtjens, S.: **P12.163**
Phylactou, L. A.: P12.107
Pi, G.: **J02.04**
Piacentini, S.: P10.17
Piard, J.: **P02.011**, P02.120
Picard, V.: P05.18
Picazo, M.: P12.056
Piccinelli, C.: P12.057
Piccoli, C.: J11.11
Pichler, B.: P14.11
Pichler, M.: C10.6
Picinelli, C.: P12.058
Pickart, A.: P02.043
Pickle, L.: P11.009
Pié, J.: P02.066, P03.070
Pieber, T.: P13.41
Pieber, T. R.: P09.103
Piekuse, L.: P06.005, P10.59, P13.02
Piekutowska-Abramczuk, D.: P02.041, P02.186, P02.232, P13.47
Pienar, C.: J02.40
Pierard, G. E.: C09.5
Pierides, A.: P12.008
Pierini, S.: **P06.144**
Pierluigi, M.: P03.069
Pierquin, G.: **P02.190**, P02.238
Pignatti, M.: EPL7.3
Pihko, H.: C12.5
Pilch, J.: **P02.244**
Pilinskaya, M. A.: J03.17, **J07.03**
Pillai, S.: P12.027
Pilu, R.: P11.119
Pimentel, J.: P06.109
Pimentel, T.: P06.109
Pin, D.: C18.2
Pineda, M.: P10.49
Piñeiro-Gallego, T.: **P12.026**
Pinelis, V. G.: J09.10, **P12.112**
Pinelli, L.: P12.203
Pinelli, M.: **P02.001**
Piñero-Fernández, J. A.: P03.019
Piñero-Martos, E.: P04.38
Pingault, V.: J02.43
Pinheiro, D. G.: P11.030
Pinho, T.: P08.45
Pini, G.: P10.49
Pinson, L.: P02.141
Pintaudi, M.: P10.49
Pinto da Costa, E.: **P12.043**
Pinto Leite, R.: J03.03, P03.072, P03.134
Pinto-Basto, J.: P10.20, P10.21
Piotrowski, A.: C04.4
Pippucci, T.: P10.57
Pirags, V.: P09.136
Piras, M.: P09.077
Piras, R.: **P12.060**
Pirasteh, P.: P03.033, P03.039
Pirastu, N.: P08.31
Pires, M.: P11.055
Pires, S.: P03.038
Pirhoushiaran, M.: J09.31
Pirozzi, D.: P02.001
Pirozzi, F.: P12.004, P14.07
Pirrone, C.: P06.059
Pirson, I.: P02.062
Piryaei, F.: **J09.38**, **J14.06**, J14.06
Piryaei, Z.: J09.38
Pirzada, A.: **P09.095**
Pischimarov, J.: C10.5
Piskáčková, T.: P01.11
Pispili, K.: P12.047

Pistocchi, A.: P06.057
Piton, A.: **P13.25**
Pitzalis, M.: P12.060
Pivot, X.: EPL6.4, P01.04
Pizot, C.: J11.10
Plaiasu, V.: J02.31, P03.121
Plaiasu, V. N.: **P02.031**
Planchais, S.: C18.2
Planchon, A.: PL2.6
Plank, L.: P06.102, P12.209
Plaschke, J.: P02.046, P02.243
Plaseska-Karanfilska, D.: P04.05, P04.55, P06.042, P10.19, P12.242, **P04.50**
Plaseski, T.: P04.55
Plasheski, T.: P04.50
Plass, C.: P06.185
Platonova, V. I.: P06.086
Platt, D. E.: P09.046
Platzer, K.: **P02.072**
Platzer, M.: P06.167
Pławski, A.: P06.162, P12.228, **P12.236**
Plaza-Benhumea, L.: P12.164
Plaza-Izurieta, L.: P09.036, P09.038, P09.039, **P09.040**
Plecko, B.: C12.4
Plevova, P.: **P12.009**
Plewniak, F.: J11.10
Pliss, L.: P10.59
Pliushch, G.: C16.5, P11.038
Plong-on, O.: P03.127
Ploos van Amstel, H.: C18.6, P12.259
Ploos van Amstel, J. K.: P12.253
Ploski, R.: P02.117, P02.156
Plotnikova, M. A.: **J11.06**
Plunkett, L.: EP05.10
Poarangan, C.: P12.137
Poch, O.: C18.4
Pocoví, M.: J09.47
Podariu, A.: P02.207
Podralska, M.: **P12.228**
Poelmans, G.: P11.007
Pohl, E.: **PL2.4**
Pohl, M.: P02.136, P12.201
Poisson, S.: P02.141
Pokorska-Bocci, A.: P01.29
Pokudina, I.: P04.44
Polakova, H.: P09.052
Polat, M.: P12.123
Polenakovic, M.: P09.031
Poley, J.-W.: P01.34
Polgár, N.: P10.49
Poliakov, A.: J05.10, J12.11
Polimanti, R.: **P10.17**
Polityko, A.: J01.01
Pollak, A.: **P02.117**, P02.156
Pollice, R.: P09.113
Polster, T.: P02.167
Polucha, A.: P02.066
Polvi, A.: P12.028
Polyak, M.: P12.034
Polyakov, A.: J02.37, J12.26, P12.071, P12.166, P12.166, P12.256, J04.14, J12.15, J12.16, P02.176, P06.076, P12.249
Polyakov, D. S.: J12.07, J14.02
Ponting, C.: **S14.3**
Ponzi, E.: P03.116
Pool, R.: P11.143
Poole, R. L.: P11.056
Poot, M.: **P09.044**
Poot, R.: P12.199
Pop, L.: P13.08, **P13.35**
Pop, M.: J02.05
Popa, C. A.: J02.13, **J03.05**
Popa, I.: P13.08, P13.35
Popa, L. O.: J09.04
Popa, O. M.: **J09.04**
Popa, S.: J02.21, J02.39, **P03.053**
Popa, Z.: P13.08, P13.35
Popelinska, E.: P05.10
Popescu, R.: J02.32, P03.053, P03.125, P03.136, P05.11, P11.043
Popoiu, A. V.: **P02.073**
Popoiu, C.: P02.073, P02.018
Popov, D. V.: J09.44
Poppe, B.: P12.019
Poptodorov, G.: P06.048, P06.144
Populaire, C.: EPL6.4, P01.04
Porcaro, L.: P11.018
Porrmann, J.: P13.24
Porta, F.: P06.059
Porta, G.: P06.059
Portal, E.: P14.11
Portararo, P.: C07.4
Porto, G.: J01.04
Porubsky, D.: P06.102
Pósafalvi, A.: P12.064
Posta-Janicsek, I.: P12.139
Posthumus, M.: P09.114
Posukh, O. L.: **J12.32**, P02.109, P10.11, P10.29
Pothion, S.: P09.056
Potin, J.: P05.18
Potočnik, U.: P09.017, P09.020, **P09.072**, P09.140
Pott, C.: C10.5, P06.098
Pottier, C.: **C11.1**
Poulsen, M. M.: P02.257, **P06.218**
Poulter, N.: C14.6
Poulton, C.: P12.199
Pouresmaeil, F.: **J04.06**
Pouresmaeili, F.: J08.06
Pourfarzad, F.: P12.199
Pourfatemi, F.: P12.023
Pourjahed, A.: **J11.02**
Pourová, R.: **P02.174**
Pousada, G.: **P09.075**
Pouta, A.: C05.3
Pöyhönen, R.: C12.5
Prager, B.: EP02.02, EP02.02
Pranculiene, G.: P09.138
Prange-Kiel, J.: C13.5
Prans, E.: P09.023
Pras, E.: P12.202
Prasilova, S.: P03.051
Prassl, I.: P02.138
Prawitt, D.: **P02.049**, P02.091, P06.111
Prehn, C.: P13.09
Preikšaitienė, E.: **P02.013**, P02.083, P03.043
Preising, M.: P12.211, P12.253
Preisler-Adams, S.: **P12.033**
Prescott, K.: C09.5
Prescott, T.: P02.212, P02.198, P12.148
Preto, P.: P12.078
Prevost, T. A.: EPL1.5
Pricelius, J.: P02.138, P04.43, P05.14
Priebe, L.: **C08.5**, P09.078, P09.118, P11.015
Primignani, P.: P12.169
Prins, J. B.: EP05.06
Prisco, F.: P02.001
Prisco, M.: J11.11
Procaccini, M.: P06.110
Prohászka, Z.: P06.195
Prokisch, H.: C12.3, **C12.4**, P11.047, P14.19
Prokofyeva, D.: P06.038
Prokopenko, I.: C05.4
Pronicka, E.: P13.47
Prontera, P.: C03.4
Proost, D.: **P11.130**
Propping, P.: C14.4
Prossomariti, A.: P12.130, P13.27
Prota, J. R.: P12.244
Prott, E.-C.: P02.021
Provenzano, A.: P11.077, **P11.125**
Prozorenko, E.: P06.145
Prudnicova, T. A.: P12.112
Pruss, D.: P06.214
Pruss, R.: P14.11
Przybyl, J.: **P06.198**
Psaty, B. M.: C08.4, C17.6
Pshennikova, V. G.: P02.109
Psoni, S.: J02.30, P12.055
Puchmajerova, A.: P10.42
Pucillo, C.: P12.225
Pugacheva, I. V.: P13.28
Puglisi, M.: P09.048
Puisac, B.: P02.066, P03.070
Puiu, M.: J02.05, J02.13, J02.40, J02.41, J03.05, J06.12, J09.33, P01.37, P02.018, **P02.139**
Pulverer, W.: P06.002, P06.070, **P06.207**, P11.022
Punab, M.: P03.078
Punina, N. V.: J12.21
Puppin, C.: P06.163
Purcell, S.: P09.032
Purmann, S.: P02.093
Puro, V.: P11.131
Pushkarev, V. P.: **J02.45**, P10.01
Pushkareva, J. E.: J02.45
Puskaric, S.: **P12.016**, P12.221
Pütz, B.: C08.5
Putzová, M.: P05.44, P05.50
Puzuka, A.: **P10.59**
Puzyrev, K. V.: P05.61
Puzyrev, V.: P09.064, P05.61, P09.019, P09.062
Pylypenko, O.: PL2.2
Pyrkosz, A.: P02.103, P02.264

Q
Qoreishi, S.: J09.21
Quaio, C. R. D. C.: J02.42, P02.020, P13.38
Quan Sang, K.: P02.141
Quante, A. S.: **P08.07**
Quast, C.: **P11.104**
Quattrone, A.: J12.33, J12.34, J12.35, P12.081
Queipo, G.: **P02.128**
Quentric, M.: P09.125
Quiezi, R. G.: P02.123, P02.228
Quijano-Roy, S.: C18.3
Quintáns, B.: P12.216
Quintas, M.: **P09.080**
Quintero, C.: P14.08
Qureshi, N.: EPL3.5, P01.01

R
R. Ali, B.: P02.223
Raabe-Meyer, G.: C16.1, P02.234
Rabiee, M.: J11.02
Rabii Gilani, Z.: P08.10
Rabionet, R.: P08.11
Rad, I.: P03.081
Rademacher, N.: P12.042
Radhakrishna, U.: PL2.3
Radhakrishnan, R.: **P02.182**
Radic, K.: J09.36, P02.124
Radivojkov Blagojevic, M.: P10.32
Radka, T.: P02.063, P02.063
Radner, F. P. W.: C18.2
Radoi, V.: J03.07
Radoi, V.: P03.024, **P04.21**
Radojkovic, D.: J10.03
Radovanov, D.: J02.06
Radovic, S.: EP01.02, P12.225
Radovica, I.: J09.09
Radtke, A.: P14.13
Radu, I.: P06.010, P10.10
Radvansky, J.: **J10.06**
Raeisossadati, R.: J06.19, P06.091, P06.119
Raffaldi, F.: P11.010
Rafiei, M.: J09.41
Ragon, C.: P03.020, P05.48
Ragozzino, A.: P12.002
Rahman, B.: **EPL4.1**
Rahmani, S.: J03.09, J04.11, J06.05, J06.07
Raicevic, R.: P09.084
Raime, K.: P06.067

Raitakari, O.: C05.3
Raivio, T.: P04.10
Rajab, A.: PL2.3
Rajaee, A.: **P03.034**, **P03.044**, P03.048
Rajiv, S.: **J03.21**, J03.22
Rajkiewicz, M.: P12.115
Rakhimkulova, A.: **P05.16**
Rakovic, A.: P12.245
Raleigh, S. M.: P09.114
Ralston, S. H.: P09.104
Ramaiah, N.: P11.037
Ramakers, G. J. A.: C13.5
Ramazanova, N.: P09.018
Ramazi, S.: **J08.01**
Rambaldi, A.: P06.059
Rambaud, C.: EPL6.4, P01.04
Ramel, C.: P03.139
Rämet, M.: P09.034
Ramezani, M.: J14.06
Ramezanzadeh, F.: P04.36
Ramic, J.: J09.36, **P02.124**
Ramirez, M.: P11.085
Rammensee, H.-G.: P06.178
Rammer, M.: P06.013
Ramocki, M. B.: C04.1
Ramón y Cajal, T.: P06.027
Ramos, F. J.: **P02.066**, P03.070
Ramos, L.: J02.17, P03.017, P03.050
Ramos, M. A.: P03.118
Ramos, P. Z.: P12.066
Ramos-Arroyo, M. A.: P03.016
Ramsden, S.: C15.6, EPL5.6
Ramsey, D.: P06.071
Ranchich, V.: P06.164
Ranchor, A. V.: P01.06
Randall, J. C.: C06.2
Rantapää-Dahlqvist, S.: P08.15
Ranza, E.: P05.58
Raoofian, R.: P06.203
Raphael, M.: P02.172
Raposo, L. S.: P06.115
Rappold, G.: C13.2, C13.6, P09.069, **P11.039**, P12.016, P12.221, P02.012, P12.021
Rashidi-Nezhad, A.: **P03.082**
Rashkov, R.: J09.32
Rasi, C.: C04.4
Rašková, D.: P03.123, **P05.50**
Rasmussen, A. A.: P03.130
Rasmussen, M.: P02.155, **P03.080**, P03.130
Rasooli, P.: J12.18
Rastaldi, M. P.: P12.002
Rastgar-Jazii, F.: P06.142
Rathkolb, B.: C12.6
Ratiu, A.: P05.39
Rätsep, R.: P03.078, P09.124
Ratti, A.: P10.04, P11.008
Rau, I.: P12.108
Rau, T.: P09.050
Rauch, A.: C03.6, C13.2, P02.003, P02.162, P02.219, P02.237, P02.256, P03.067, P03.068, P03.120, P03.138, P12.053, P12.218, P12.220
Rauen, K.: P02.182
Raum, K.: P12.162
Rauova, K.: P06.092
Rausell, L.: P12.005
Rautenstrauss, B.: P06.108, P09.050, **C01.5**
Ravčuková, B.: P12.233
Ravi, H.: P11.128
Ravn, K.: P10.49
Ravoet, M.: P03.058
Raykova, D.: P12.246
Raymond, K.: P14.09
Raynaud, M.: P02.161, P12.178
Rayner, N. W.: P10.45
Raynova, R.: **P05.62**
Raz, T.: P11.086
Raz, V.: C06.6
Razavi, F.: P12.167
Razzaboni, E.: EP05.02, **EPL7.3**
Razzaghian, H. R.: **C04.4**
Razzaghy-Azar, M.: P03.106
Rebai, T.: P02.039
Rebner, K.: P03.064
Rebouças, M. R. G. Oliveira.: P12.181
Receveur, A.: P02.088
Rechavi, G.: C12.1
Reches, A.: P02.145, **P03.142**
Redensek, A.: P14.11
Redin, C.: P12.046, P13.25
Redler, S.: **P09.010**, P09.058
Reenaers, C.: S01.3
Rees, E.: **P09.032**
Reever, B.: P14.03
Refke, M.: P09.010, P12.153
Regiani, V. R.: P06.047, P06.047
Rehn, M.: P02.254
Reichert, S.: P09.106
Reicherter, K.: P06.111, P06.217, **P12.039**
Reid, S.: P09.016
Reif, A.: P09.028
Reif, S.: P02.046, P02.243
Reilly, M.: C11.4
Reimand, T.: J03.18
Reinhardt, R.: P10.09
Reinier, F.: C08.3, P11.037, P11.119, P12.125
Reinmaa, E.: C08.4, C17.6
Reinscheid, A.: P15.12
Reintjes, N.: **P12.201**
Reis, A.: C03.6, C13.2, C14.1, C14.5, C15.2, P02.158, P02.195, P02.237, P03.068, P09.111, P11.060, P11.090, P12.102, P12.138, P12.218, PL0
Reis, C.: EP02.06, P05.21
Relichová, J.: P01.21
Remely, M.: P13.26
Remenieras, A.: P11.110
Rendeiro, P.: P12.170
Rengaw, T.: P11.134
Renieri, A.: P02.060, P09.030, P10.49
Renkema, K.: P12.154, **P11.007**
Renkens, I.: C18.1, P12.259
Renner, W.: P09.103
Rensen, H.: C15.1
Renwick, P.: C16.3
Repáraz, A.: P12.237
Repiska, G.: **P05.13**, P06.060
Repnik, K.: P09.072
Represas-Represas, C.: P06.122
Restarick, K.: EP02.05, EPL1.5
Rettenberger, G.: P02.224, **P02.230**
Reuter, M.: **P02.195**
Reutter, H.: C09.4, C14.4, P02.026, P02.130, P08.08, P08.32, P09.033
Revencu, N.: P03.058
Revillion, F.: P11.110
Reymond, A.: C08.1, P03.011, P03.082, P11.103
Rezaei, H.: P06.052
Rezazadeh, M.: P09.011
Reznik-Wolf, H.: P12.202
Ribas, N.: P03.041
Ribeiro, I. P.: P06.169
Ribeiro-Bicudo, L. A.: J02.23, P02.123, **P02.228**, P03.095
Ribeiro-Santos, A. K.: P10.46
Ricaño-Ponce, I.: P08.23, **P09.043**, P09.059
Ricci, F.: J09.43
Ricci, M.: P12.203
Ricciardi, S.: P12.042
Riccio, A.: P02.040, P02.129
Richardt, S.: P15.06
Richieri-Costa, A.: J02.23, P02.123, P02.228
Richter, J.: **C10.5**, P04.25, P11.026, P11.120
Richter, K.: C13.5
Richter, U.: C12.5
Richter-Unruh, A.: P02.153, **P02.154**
Richtsmeier, J.: P12.264
Rico, A.: P15.03
Rider, L. G.: C04.4
Rieber, N.: P15.03
Rieck, I.: P12.196
Riedel, S.: P03.139
Riedijk, S.: **EPL7.4**
Riegel, M.: P03.117
Rieger, M.: **P02.046**
Riehmer, V.: **P06.136**
Riepe, F. G.: P11.120
Riera-Baldinger, R.: P03.067
Riesch, E.: P12.082
Riess, A.: C13.2, P02.086, P12.260
Rieß, A.: P02.149
Riess, O.: C13.2, P02.076, P06.178, P12.136, P12.187, P12.260
Rieß, O.: P09.092, P14.11, P14.21
Riethdorf, S.: C10.6
Rietschel, M.: P09.009, P09.069, P09.118, P09.120
Rieu, P. N.: C01.4
Rieubland, C.: **P03.092**
Rigó Jr, J.: P05.47, P05.63, P05.49
Rigo, J.: J09.12
Rigoutsos, I.: P11.109
Rigter, T.: **P01.23**
Rihova, L.: P07.26
Rijavec, M.: P09.021, **P12.110**
Riley, B. P.: C06.4
Rimac, M.: P02.172
Rimoldi, V.: P09.088
Rinaldi, D.: P12.120
Rinaldo, P.: P14.09
Ring, S. M.: P10.37
Ringrose, A.: EPL5.4
Ringshausen, I.: P06.199
Rinne, T. K.: C04.5
Rio, M.: **P02.165**, P13.32
Rios-Visconti, M.: P09.104
Ripatti, S.: C05.3, C08.4, C17.6, P09.086
Rißmann, A.: P02.130
Ristanović, M.: J04.15
Ristic, S.: P09.084
Ritelli, M.: P02.023
Ritter, M.: P02.166
Rittinger, O.: **P03.056**
Rittner, G.: **P09.129**
Rittore, C.: P12.247
Riva, P.: J09.46, P06.057, P11.008
Rivadeneira, F.: C08.4, C17.6, PL2.5
Rivas, F.: P09.069
Riveiro-Alvarez, R.: P12.022, P12.074, P12.177, P12.210
Rivera-Vega, M. D.: P10.18
Rivera-Vega, M. R.: P12.164
Rivera-Vega, R.: **P12.100**
Rivolta, C.: P12.074, P12.114
Rizun, L.: P02.151, P12.104
Rizzo, A. M.: P12.002
Rizzo, G.: P05.05, P05.12
Roa, B. B.: P06.214
Roberti, M.: P02.184
Roberts, D.: P11.028, **P11.128**
Robertson, S.: P02.172, P02.194
Robeva, R.: J09.32
Robinson, J.: P02.217
Robinson, P.: P12.031, **C07.4**, C13.1, P11.100, P11.101
Robinson, R.: C07.3
Robles, C.: P12.032
Robusto, M.: **P12.169**
Robyr, R.: P05.58
Rocca, F.: J12.33
Rocca, M. S.: P02.025
Rocchetti, M.: P09.131
Rocha, C. F.: P09.139
Rocha, J. C.: **EW2**
Rocha, L.: **P02.177**
Roche Martinez, A.: P10.49

Roden, M.: P11.047
Rodenburg, R. J.: C12.3
Rodewald, A.: **P10.39**
Rødningen, O.: P02.003, P03.103, P11.096, C15.4, P02.080
Rodrigues, F.: **EP02.06**, P03.050
Rodrigues, M. G.: P02.020
Rodrigues, M. I. G.: **P02.239**
Rodríguez Hernández, I.: P13.18
Rodríguez, L.: **J03.11**, P03.124
Rodriguez, M.: P12.070
Rodriguez-Garcia, M.: J12.28
Rodriguez-Cruz, O.: P11.091
Rodríguez-de Pablos, R.: P11.050, P11.091
Rodríguez-García, M. I.: **P02.126**
Rodríguez-Hernández, I.: **P06.105**
Rodríguez-Pérez, M. C.: P06.138
Rodríguez-Pombo, P.: P12.032, P13.01
Rodríguez-Revenga, L.: P03.076
Rodríguez-Rey, J. C.: J09.47
Roeleveld, N.: P11.007
Roenneberg, T.: P10.32
Roepman, R.: C11.5, P12.126
Roesch, W.: P09.033
Roese, L. L.: **P03.108**, P03.140
Roetzer, K. M.: P03.021
Rogaev, E. I.: P09.012
Rogdaki, M.: J11.05
Rogers, M. T.: EP05.09, EPL4.2
Rogers, R. C.: P03.098
Rogowski, W.: EPL1.1
Rohani, M.: J12.17, J12.24
Röhr, C.: P06.185
Rohrbach, M.: C18.3
Rojas-Martinez, A.: P08.32
Roldán, S.: P02.030
Rolinski, B.: P14.19
Roma, P.: P10.26
Romagnani, P.: P11.125
Romano, C.: P09.030
Romano, L.: PL2.6
Romanos, J.: C17.1, P09.059
Romeo, G.: P02.135, P03.083, P10.57, P11.059, P11.140
Romera, A.: P11.050, P11.091, P12.237
Romero, N.: C18.3
Romero, Y.: P04.15
Romero-Puche, A.: J02.48
Romitti, P. A.: P12.264
Rompola, M. I.: P07.33
Ronaghi, M.: P06.116
Roncarati, R.: C07.4
Rønlund, K.: **P06.135**
Ronowicz, A.: C04.4
Rook, M.: C18.1
Roomere, H.: P06.066, P06.067
Roongpraiwan, R.: P09.029
Rooryck-Thambo, C.: P02.161
Roos, C.: P10.09
Roos, F.: P11.129
Roos, L.: P03.062
Roosipuu, R.: P06.087
Ropers, H.: C15.3, P08.37
Ropers, H.-H.: P12.124
Röpke, A.: P02.043, P03.006, **P04.57**
Roque, L.: **P06.109**
Roquelaure, B.: P12.238
Ros, E.: J09.47
Rosa-Rosa, J.: P06.161, P11.050, P11.091
Rösch, W.: P08.08
Roscioli, T.: C13.1, P12.264
Rose, P.: EPL1.5
Rosell, J.: P04.38
Rosenberger, A.: P06.032
Rosenberger, G.: C13.5
Rosenkötter, H.: P12.145
Rosenstiel, P.: C10.5
Rosenthal, A. N.: EPL4.2
Rosenthal, E.: P06.214
Rosenwald, A.: C10.5
Roski, C.: P09.047
Rosolowski, M.: C10.5
Ross, A.: C18.1
Rossi, C.: P02.187
Rossi, M.: P06.110
Rossi, P.: J12.30
Rossier, E.: P02.169, P02.205
Rossignol, R.: C11.2
Rossignol, S.: P12.178
Rössler, J.: P14.20
Rossodivita, A.: P02.058
Rossokha, Z.: J05.13, J05.14, **J06.20**
Rossow, A. S.: P02.198
Rost, I.: P02.078, P02.245, P11.132, **P12.141**
Rost, S.: P11.021, P12.098, **P12.103**, P13.20
Rostami, M.: P12.090
Rostami, P.: **P05.30**, P06.125
Rostamizadeh, I.: **P06.035**, P10.22
Rostasy, K.: C18.3
Rotenstreich, Y.: C11.5
Roth, C.: P02.200
Roth, M.-P.: P02.216
Röth, R.: P09.069
Rothberg, J.: P06.126, P11.014
Röthlisberger, B.: P06.064
Rötig, A.: P13.32
Rottbauer, W.: P12.016
Rottensteiner, F.: P02.008
Rouleau, E.: P11.110
Rouleau, G. A.: P09.119, **S10.3**
Roume, J.: P02.141
Rousseau, S.: C11.1
Rousseau, T.: P05.48
Rövekamp-Abels, L.: P03.054
Rovelet-Lecrux, A.: C11.1
Roze, V.: P02.011, P12.167
Rozman, D.: P13.43
Rozman, M.: P07.12
Rozowsky, J.: C06.5
Ruan, Y.: C10.3, P12.088
Ruangdaraganon, N.: P09.029
Rubin, M. A.: P06.185, C14.4, P09.125
Rubio-Cabezas, O.: P12.003
Rubtsov, N.: P10.08
Ruczinski, I.: C14.4
Rudaitis, V.: P06.024
Rudd, E.: P12.145
Rudenko, V. V.: **P06.141**, P11.064
Rudenskaya, G.: P12.166, J12.01, J12.21, **P02.019**
Rudež, J.: J02.06, J03.13
Rudkjoebing, L. A.: **J09.25**
Rudko, A.: **P09.064**
Rudko, A. A.: P05.61
Rudloff, C.: P02.049
Rudnik-Schöneborn, S.: P12.041
Rudolf, G.: P09.084
Rueda-Nunez, F.: J12.28
Ruef, P.: P02.205
Ruff, D.: P11.014
Ruffier-Bourdet, M.: P02.265
Rugge, M.: P02.090
Ruggiero, A.: C11.4
Ruivemkamp, C.: P03.062, P03.054, P03.129, P12.051
Ruiz de Garibay, G.: P06.118
Ruiz, C.: P12.005
Ruiz-Espejo, F.: J02.46, J02.47, J02.48, P06.161
Ruíz-Pérez, V. L.: J02.55
Rujescu, D.: P09.118
Rujirabanjerd, S.: **P03.127**
Rukova, B.: P02.034, P06.018, P06.164, P02.063, **P11.118**
Rumiantsava, N.: **J01.01**, P04.08
Rumiantseva, V.: P12.017
Rump, A.: P02.133, P02.203, P06.107, P06.167
Rumpold, H.: P06.013
Rumyantseva, V.: **P02.151**
Rune, G.: C13.5
Runge, C.: P02.093
Runnebaum, I. B.: P06.055
Runz, H.: **C05.1**, P02.241
Rüschendorf, F.: C18.3
Rusconi, D.: **P11.018**
Russo, A.: **P06.115**
Russo, S.: J09.46, P02.040, P02.129, P10.49, **P12.057**, P12.058
Russu, R.: P02.140
Rüstemoğlu, A.: J09.13, P08.25, P08.38, **P08.06**
Rusu, C.: J02.21, J02.32, J02.39, **P02.140**, P02.225, P03.053, P03.125, P03.136, P03.144, P05.11
Rüther, U.: P09.060
Rutkowski, P.: P06.198
Rutsch, F.: P12.060
Rutschow, D.: P12.121
Rutten, J. W.: **P12.173**
Ruvolo, M.: **P03.060**
Ryabchikova, N.: P06.058
Ryan, A. W.: C14.5
Rybarova, A.: **P06.168**, P12.161
Rydzanicz, M.: P11.069
Rydzewski, A.: P02.084
Rygus, T.: **P11.088**
Rytikova, N.: P07.17
Ryzhkova, O. P.: J04.14, **P12.071**
Rzadzinska, A. K.: PL2.4

S

S. Birk, O.: P12.146
Sá, J.: P12.170
Sá, M. J. N.: **P12.007**
Saad, A.: P03.066, P03.085, P04.49, P07.31
Saadatian, H.: J09.02
Saade, S.: P09.046
Saarela, J.: P09.086
Saavalainen, P.: P09.037
Sabagh, F.: P12.023
Sabater Molina, M.: J02.46, **J02.47**, J02.48
Sabbaghian, M.: P04.56
Saber, S.: P02.050
Saberi, A.: P02.119
Sabetta, G.: P12.222
Sablaturova, T.: P07.17
Sabokbar, N.: J05.02, J05.07, P05.28, J05.06
Sabol, M.: P06.030
Sabrautzki, S.: C12.6
Sacara, V.: J13.14
Sacchetti, L.: P02.001
Sadee, W.: **S07.1**
Sadegh, S.: J05.06
Sadeghi Koupaei, M.: **J09.30**
Sadeghi, M.: J04.13
Sadeghizadeh, M.: P06.053, P11.133
Sadhnani, M.: P09.090
Sadighi Gilani, A.: J11.09
Sadighi Gilani, M.: J04.17, P04.56
Sadik, N. A.: P03.094, P03.113
Sadr-Nabavi, A.: **J09.31**
Saeb, F.: J12.09, P04.53
Saenen, J.: P11.130
Sáez-Villaverde, R.: P12.237
Safari, I.: J12.17, J12.24
Safarpour, V.: **J06.06**
Safavi naini, N.: **P09.013**
Saffar, M.: P04.53
Saffari, Z.: P03.033
Safiullina, A.: J09.27
Sahebghadam-Lotfi, A.: P06.142
Sahin, E. M.: P06.152
Sahin, Y.: **P02.097**
Sahun, I.: P09.056
Saifullina, E.: J02.18, P12.113
Sailani, M. R.: C04.6, **P08.11**

Sailani, M. R.: P11.027
Saito, K.: P03.002
Saito, T.: P02.029
Saitoh, S.: C04.3
Saitta, S. C.: P02.006
Sajadi, S.: **J04.03**
Sajjadian, S.: C06.1
Sakalli, E.: P15.06
Sakazume, S.: P02.206
Sakhinia, E.: P06.035, P06.121, P06.133, **P06.134**, **P10.22**
Sakhinia, M.: P06.035, P10.22
Sakız, D.: J02.15
Sakurai, M.: J01.07
Sakurai, Y.: P09.071
Salamian, A.: P12.208
Salamon, M.: P03.110
Salari, S.: P04.53
Salas, A.: C14.3
Salazar, J.: P06.027, P12.070, P12.265
Saleem, S. N.: P02.082
Saleh, A. A. H.: P02.223
Salehi, A.: P08.41
Salek, M.: J02.19
Salekdeh, G. H.: P11.124
Salem, N.: P02.255
Salemink, S.: **P01.35**
Sales, F.: J02.17
Sales-Marques, J.: P03.038
Salgado, D.: P06.109
Salhi, A.: C09.5
Salido, E. C.: P12.201
Salifoglu, H.: **J10.08**
Salih, M. A. M.: C11.2
Salimbaeva, D.: J12.19
Salinas-Riester, G.: P06.213
Salmaso, R.: P02.090
Salomaa, V.: C05.3, P09.134, P09.137
Salowsky, R.: **P11.085**
Salsabili, N.: J04.11
Salsone, M.: J12.33
Saltikova, I. V.: **P09.019**, P09.062
Salvadori, M.: P11.077
Salvemini, H.: P02.229
Salviati, L.: P13.44
Salyukova, O.: P03.083
Samani, N. J.: P09.076, P10.53
Samanian, S.: **J09.23**
Samatkyzy, D.: J03.02
Samei, E.: P06.142
Samocha, K. E.: C07.5
Samoylenko, V. A.: P09.053
Samples, J. R.: C14.1
Samson, I.: P06.198
Samuelsson, L.: P02.102
San Jose, P. F.: **P12.022**, P12.074, P12.210
Sanati, M.: J04.05, J04.10, J11.02, J13.06, P09.087
Sánchez Rovira, P.: P06.161
Sanchez Salido, L.: P14.08
Sánchez Tapia, E.: P06.128
Sánchez Vega, B.: P06.161
Sanchez, A.: P06.061
Sanchez, E.: P02.141, P12.247
Sánchez, J.: P07.28
Sánchez, M.: J03.11, P03.124
Sánchez-Hombre, M.: P06.161
Sanchez-Lara, P.: P12.264
Sancho-de-Salas, M.: P06.177
Sandberg, S.: EP04.02
Sandbothe, M.: P06.113
Sander, G.: P03.056
Sander, M.: P06.032
Sander, T.: C08.5
Sanders, R.: **P11.116**
Sanderson, B.: P11.106
Sandri, M. C. S.: J02.23
Sandri, R. M. C. S.: P03.095
Sangsrakru, D.: P09.029
Sanjari, A.: J05.02, J05.07, P05.28, J05.06
Sanlaville, D.: P02.088, P02.141, P03.062
Sanli, M.: **P06.132**
Sanna, S.: **C08.3**, P09.077, P11.037, P11.119, P12.125
Sansbury, F.: P05.41
Sansbury, F. H.: **P08.21**
Santana, A.: P12.265
Santavy, J.: P06.210
Santen, G.: P03.129
Santen, G. W. E.: C07.1, **P12.051**
Santens, P.: EP01.01, P12.163
Santillán, S.: P06.161, P11.050, P11.091, P12.237
Santini, D.: P06.110
Santoni, F.: P08.11, P09.141, P11.027, P12.055
Santorelli, F.: C11.2
Santoro, A.: P12.130
Santos Simarro, F.: J02.55
Santos, D.: **P10.20**, P10.21
Santos, F.: **J02.54**, P03.076, P13.01
Santos, M.: P06.109
Santos, P. C. J. L.: **P02.199**
Santos, P. T. L.: P04.58
Santos, R.: P12.043, P12.097, P12.250
Santos, S. E.: P10.46
Santos, S. M. B.: P10.03
Santos, S. R.: **P11.055**
Santos-Briz, Á.: P06.105
Santos-Ocaña, C.: P13.44
Sanz, G.: P07.28
Sanz, P.: P12.032
Sarabi, S.: P02.094
Sarabia Meseguer, A.: P06.161
Sarafov, S.: P12.109
Saraiva, J.: EP02.06, P03.017, P03.050, J02.17
Sarangi, S.: EP05.08, **EPL5.1**
Sarbu, O.: J02.41
Sarda, P.: P02.060, P02.141
Sardari, S.: **P06.091**
Sarhadi, S.: P04.36
Sarı, G.: J13.10
Sarin, A.-P.: C05.3
Sarıtaş Yüksel, E.: P06.012, J06.14
Sarkardeh, H.: **J04.17**
Sarkhail, P.: J14.04
Sarkisian, T.: P11.059, P11.140, P02.009, **P02.098**
Sarkozy, A.: P09.131
Sarles, J.: P12.238
Sarnecka, A.: P09.107, **P02.084**
Sarnová, L.: P12.219
Sarova, I.: **P07.02**
Sartorato, E. L.: P02.118, **P12.066**
Sartori, A.: P15.03
Sasannejahad, P.: J09.31
Sasiadek, M. M.: **P06.071**, P09.099
Sassone Corsi, P.: **S03.1**
Satija, S.: P14.06
Satiroglu-Tufan, L. N.: J06.17
Satkin, B.: P02.157, **P03.026**, P05.07, P05.22, P05.29
Sato, S.: J05.01, P02.196
Satta, S.: P11.037
Satti, V.: J06.01
Sauer, H.: P09.118, P09.120
Sauerland, M. C.: C02.3
Saugier-Veber, P.: P02.088
Saunders, C.: EPL4.1
Sauter, G.: P06.185
Sautière, J.-L.: EPL6.4, P01.04
Savaş Erdeve, S.: P02.022
Savenko, L.: P04.08
Savlı, H.: J03.12, P02.014, P06.007, **P06.014**, P06.037, P06.056, P06.175
Savolainen, M. J.: C05.3
Savov, A.: J09.32, P02.079, P03.018, P03.073, P05.62
Savu, L.: J10.05, P05.42
Sawada, K.: C10.3
Sawyer, S.: EP08.01
Sayagues, J.: **P06.131**
Sayar, C.: P03.088
Sayar, E.: P12.238
Saydam, F.: **J13.10**
Saygin, B.: P05.64
Sazhenova, E. A.: **P11.032**
Sbii, L.: P10.15
Scacchi, R.: P10.24
Scalzitti, L.: P12.147
Scalzone, M.: P02.058
Scambler, P.: P12.126, C18.6
Scarano, F.: P02.044
Scarano, G.: P02.044
Scarselli, G.: P02.044
Scerri, C.: P10.36
Schaaf, L.: **P06.139**
Schaafsma, G. C. P.: P11.084
Schaap, M.: **P11.068**
Schackert, G.: P06.107, P06.167
Schackert, H. K.: P06.080
Schäfer, D.: EP02.02
Schäfer, M.: P02.130
Schäferhoff, K.: P02.076
Schäffer, V.: P06.113
Schafmayer, C.: P06.073
Schageman, J.: P11.105, **P11.010**
Schagina, O. A.: J12.21
Schaller, A.: **P11.073**
Schaller, H.-G.: P09.106
Schallert, M.: P09.035, P09.130
Schalling, M.: J11.05
Schallner, J.: P02.203
Schanz, J.: P07.23
Schanze, D.: C18.6, P02.134, P05.32, P11.071, **P12.138**
Schanze, I.: **P02.134**, P02.237, P05.32, P11.071, P12.102, P12.138
Schara, U.: P02.064
Scharf, J.-G.: P06.074
Schauer, S.: **P06.167**
Scheel, C.: **S09.2**
Scheers, S.: P07.22
Scheffer, H.: C01.1, C07.2, C11.5
Scheffold, T.: J02.24, P12.239
Schelhaas, J.: C11.3
Schell-Apacik, C.: P12.211
Schellenberger, E.: P03.143
Schelling, M.: C16.1
Schenck, A.: C01.1, C13.2, P12.102, **S13.3**
Schenck-Kaiser, K.: EPL6.1, P02.112
Schepers, D.: **C01.3**
Scherer, S. W.: P12.021
Schermer, M.: P05.57
Scherthan, H.: C16.6
Schettert, I. T.: P02.199
Schietecatte, P.: P12.019
Schilhabel, M.: C10.5
Schimpf-Linzenbold, S.: P09.109
Schindelhauer-Deutscher, H.-J.: EPL6.1
Schindler, D.: P06.186, P08.14, P11.136, P11.138, P12.091, P12.141
Schindler, M.: P06.181
Schinzel, A.: P03.117
Schioth, H. B.: P09.098
Schiöth, H. B.: P09.136
Schlack, C.: P12.061
Schlade-Bartusiak, K.: P03.071, **P03.137**
Schlawinsky, M.: P04.37
Schlegelberger, B.: P06.022, P06.113, P13.49
Schleicher, T.: C16.1
Schlesner, M.: C10.5
Schlessinger, D.: C08.3, P11.037
Schlitt, A.: P09.106
Schlögel, M.: P12.202
Schlomm, T.: P06.185
Schlötzer-Schrehardt, U.: C14.1
Schmid, M.: P02.254
Schmid, R.: P06.173

Schmidt, D.: C09.4, P02.026, P02.130, P08.08, P09.033
Schmidt, G.: EP02.02
Schmidt, H.: P09.035, P09.130
Schmidt, H. H. J.: J13.14
Schmidt, J.: **P14.21**
Schmidt, M.: P06.182
Schmidt, M. K.: P06.031
Schmidt, R.: P09.035, P09.130
Schmidt, S.: **P06.188**
Schmidt, T.: C02.5, J14.08, **P06.179**, P09.092, **P12.136**, P14.21
Schmidtke, J.: EPL1.1, P01.07, P03.035, P04.22, P09.027, P12.226, P12.258
Schmidts, M.: P12.126, **P12.200**
Schmidt-von Kegler, M.: P12.031
Schmiedeke, E.: C09.4, P02.026, P02.130, P08.08, P09.033
Schmit, G.: P09.026
Schmitt, B.: P02.237
Schmitt, W.: P05.70
Schmitz, G.: P09.076
Schmutzler, A.: P04.25
Schmutzler, R. K.: P06.117, P07.05
Schneegans, S.: P06.032
Schneider, A.: P06.055
Schneider, E.: C16.5, P11.033, P11.038
Schneider, F.: C02.3
Schneider, K. U.: P12.016, P12.221
Schneider, M.: P03.108, P03.140
Schneider, R.: P12.121
Schneider, S.: C02.3, P10.26
Schneider, V.: **P05.35**
Schneiderova, P.: **P11.108**
Schober, R.: P07.25
Schoenau, E.: C03.3
Schoener, A.: **P12.258**
Schoeters, G.: P10.26
Schölkopf, B.: C08.5
Schols, L.: C11.2
Scholtysik, R.: C10.5, P06.098
Scholz, M.: P11.132
Schön, U.: P04.42
Schöner, D.: C16.1
Schoner, K.: P05.70
Schönherr, S.: **P15.14**
Schönléber, J.: P05.63
Schopen, T.: P12.176
Schorsch, M.: P04.03, P11.033
Schoser, B.: P12.069
Schossig, A. S.: **C18.5**
Schot, R.: P12.199
Schott, J.-J.: PL2.2
Schrader, J.: P13.03
Schramm, C.: C09.4, P02.026
Schramm, J.: C08.5
Schramm, T.: P05.35
Schrander Stumpel, C.: P02.140
Schranz, D.: P02.215
Schratt, G.: P12.021
Schreiber, E.: P11.115
Schreiber, G.: C18.3
Schreiber, R. D.: S09.1
Schreiber, S.: C10.5, P09.138
Schreml, J.: C01.6
Schröck, E.: C13.2
Schrock, E.: P02.133, P02.203, P06.107, P06.167
Schröder, C.: P12.260
Schröder, J.: P12.213
Schröder, M.: P02.201
Schröder, W.: C11.6, P12.041, P12.204
Schröder-Heurich, B.: **P06.156**
Schroeder, C.: **P06.178**
Schroeder, I.: P14.18
Schroeder, J.: P12.136
Schu, M.: C14.2
Schubert, S.: **P04.22**
Schuberth, C.: C05.1
Schuelke, M.: C12.4, P14.19
Schueller, V.: P08.39
Schuierer, G.: P02.101, P02.203
Schule, R.: C11.2
Schüler, H.: P03.081
Schüller, V.: C17.4, P08.12
Schulte, H. M.: P02.153, P02.154
Schulten, H.: J03.20
Schulz, H. L.: **P12.235**
Schulz, B.: C09.5
Schulz, S.: **P09.106**
Schulz, Y.: P12.045
Schulze, B.: C16.1, P02.234, P12.253
Schumacher, J.: C15.2, P09.004, P09.078, P11.044, P11.060
Schunkert, H.: C05.5, **PL1.3**
Schurmann, C.: C08.4, C17.6, P11.047
Schürmann, M.: P12.211
Schuster, A.: P02.021
Schuster, B.: P08.14, P11.136, **P11.138**, P12.091
Schuster, J.: **P12.246**
Schveigert, D.: **P06.148**
Schwab, M.: **S07.2**
Schwabe, G. C.: P02.181
Schwanitz, G.: P03.081
Schwartz, C. E.: P11.142
Schwartz, P. J.: C16.4
Schwarzmayr, T.: C13.2
Schweiger, B.: P02.021
Schweiger, M. R.: **P06.185**
Schweiger, S.: P12.121
Schweighofer, N.: **P09.103**, P13.41
Schweyer, S.: P06.213
Sciot, R.: P06.198
Sciurba, F. C.: C09.5
Scotcher, D.: **EP06.01**
Scott, A. F.: C14.4
Scott, T. S.: C14.1
Scully, P.: P02.217
Scurr, I. J.: C18.1
Scvortova, E.: **J13.14**
Searle, S. M.: C08.1
Sebesta, I.: **P13.17**
Seckiner, I.: P06.180
Secrieru, V.: J01.03
Seda, O.: P10.42
Sedaghati khayat, B.: P02.085, P12.075, **J02.12**, P02.094, P02.235
Sedláček, R.: P12.219
Sedlacek, Z.: P02.168, P02.213, P03.004, P03.089, P11.059, P11.140, P03.102
Sedláčková, J.: P12.152, P05.13, **P06.060**
Sedneva, I.: P04.31
Seeger, K.: P02.004
Seelow, D.: P02.223
Seeman, P.: P05.50, P12.219
Seemann, H.: P06.074
Seemann, P.: P12.031
Seemanova, E.: P12.138
Seet, J.-E.: C10.3
Sefiani, A.: P12.055
Sega Jazbec, S.: P09.084
Segers, K.: P02.238
Seia, M.: P11.018, P12.002
Seibler, P.: P12.245
Seidahmed, M. Z.: C11.2
Seifert, B.: P04.42, P05.45
Seifert, G.: C02.1
Seifert, M.: P06.199
Seifert, T.: P09.106
Seifert, W.: C09.3
Seifi, S.: P06.133
Seishima, M.: P02.048
Seissler, J.: P13.09
Seitz, A.: P02.111
Seixas, A. I.: P12.216, P12.250
Sejnova, D.: P06.083
Sel, S. K.: P04.39, P05.65
Selgas, R.: P12.056
Selicorni, A.: P02.040, P02.060, P02.129, P12.058
Seliger, B.: P06.188
Sell, H.: P13.03
Sellami, A.: P04.16, P04.27
Seller, A.: P12.248
Semernin, E. N.: J02.44
Semino, O.: P10.58
Semler, O.: C03.3
Sen, A.: P09.035, **P09.130**
Sen, G.: J06.14
Sendelbach, K.: P04.42
Senderek, J.: P02.012
Senders, C.: P12.264
Seneca, S.: P04.24, **P13.23**
Senezuk, R.: P09.026
Sengupta, S.: C16.3
SenGupta, S. B.: P04.30
Sepcic, J.: P09.084
Sequeiros, J.: EP02.06, EP03.01, EPL2.5, J01.04, P08.45, P09.080, P09.081, P10.20, P10.21, P12.216, P12.250
Serafim, S.: P03.008, P03.013
Serafinceanu, C.: J10.12, P10.10
Serban, C.: EP04.01, J04.07
Serban, M.: J03.05, J06.12
Serban-Sosoi, S.: P13.10
Sergeeva, T. V.: J13.13
Seri, M.: P02.023, P02.044, P02.135, P12.130
Sermyagina, I.: **J05.10**
Serna, E.: P12.114
Serpentini, S.: **EP01.02**
Serra, G.: P12.125
Serra-Juhe, C.: P08.11
Serrani, R.: P12.203
Serrano Blanch, R.: P06.161
Serre, V.: C03.5
Seruca, R.: P06.063
Serwanska-Swietek, M.: P02.084
Sessa, A.: P12.042
Sessa, M.: P05.05, P05.12
Sesta, M.: P12.081
Sestigiani, E.: P12.130
Seto, J.: P12.162
Setterquist, R.: P11.105, P11.010
Seufert, K.: P12.145
Sevastidou, S.: P12.047
Seven, M.: J02.35, P02.069, P02.249
Sevenet, N.: P11.110
Sever, P.: C14.6
Sever, T.: J09.29, J12.02, P06.180
Severin, E.: J10.05, P02.037, P02.074, P05.42
Severin, F.: **EPL1.1**
Sevestre, H.: P05.18
Sevilla, T.: P12.114
Seyedhassani, M.: J12.09
Seyedhassani, S.: **P04.53**
Seyedi, Z.: J03.09
Seyrantepe, V.: P11.080
Seyyed Hoseinzadeh, H.: J09.21
Sezer, O.: **P02.191**
Sezer, S.: P08.09
Sezgin, O.: P06.075, P12.027
Shabalov, N. P.: P13.28
Shabani, S.: **P06.049**
Shabanova, E. S.: J02.28
Shackleton, S.: C11.6
Shafeghati, Y.: J02.08, J14.04, P03.131, **P12.208**, **P13.37**
Shaffer, L.: C13.3
Shafigina, A.: P06.062, P06.189
Shago, M.: **P07.01**
Shah, B.: P11.042
Shah, H.: P02.059, PL2.3
Shah, K.: P12.068
Shah, S.: P08.27

Shahhoseini, M.: P04.11, **P11.089**, P11.124
Shahidi, G.: J11.08, J12.17, J12.24
Shahrokhshahi, N.: P03.033, P03.077
Shakeshaft, D.: P04.42
Shakirova, R.: **P09.102**
Shalev, H.: P12.122
Shalev, S.: P12.146
Shamalov, N.: P09.127
Shamanin, V. A.: P06.076
Shamgina, L.: P04.08
Shamir, R.: C12.1
Shammas, C.: P12.107
Shankar, S.: P14.03, P02.182
Shannon, M.: P11.014
Shaposhnikov, A.: P13.39
Shaptyrenko, M.: J09.03
Sharda, S.: **P13.06**
Shargh, S. A. S.: **P06.112**
Shariati, G.: **P02.119**
Sharifi, Z.: P12.144
Sharifizadeh, M.: **J06.03**
Sharkey, F. H.: J02.55
Sharkia, R. A.: **P01.18**
Sharma, A.: P09.120
Sharon, D.: C11.5
Sharp, A.: P08.11, P11.027
Shatokhin, Y.: P07.29
Shavlovsky, M. M.: J02.44, J12.07, J14.02
Shaw-Smith, C. J.: P08.21
Shaykholeslam Esfahani, M.: J08.03
Shearer, W. T.: C15.4
Shears, D.: C18.1
Sheikhi, M.: P06.003
Sheikhol Eslami, S.: J09.41
Shelef, I.: P12.122
Shemetun, O. W.: **J03.17**
Shendure, J.: P10.51, **S13.1**
Sheng, Y. Y.: P02.080
Shepherd, S.: C07.3
Shestak, A.: **P12.017**
Shetova, I.: P09.127
Shi, L.: P12.264
Shi, M.: C06.5
Shields, D.: C14.6
Shilnikova, E.: **P04.54**, P11.024
Shilova, N.: J03.06, J05.08, P03.099, **J05.09**
Shimada, T.: P12.240
Shimizu, T.: P09.071
Shinar, Y.: P15.07
Shinomiya, N.: P09.071
Shinwari, Z.: **P12.128**
Shiomi, M.: P02.029
Shipman, H. E.: **EP05.08**
Shirneshan, K.: **P07.23**
Shkurat, T.: P04.33, P04.44
Shliakhto, E. V.: J02.44
Shoaraye-Nejati, A.: P12.208
Shoda, H.: P09.117
Shoerman, O.: C12.1
Shohat, M.: C12.1, P02.017, P02.132
Shojaei, A.: **P03.106**
Shokrgozar, M.: P06.193
Shomrat, R.: P12.179
Shorer, Z.: P12.122
Shorina, A. R.: **J03.15**, J04.02
Short, J.: P02.217
Shoukier, M.: **P06.032**
Shriver, T.: P08.07
Shunkina, K.: J10.04
Shyamala Sesikeran, B.: P09.090
Siala, O.: P12.194
Siam, R.: P12.105
Sidharta, G.: P01.34, EPL6.3
Sidore, C.: C08.3, P11.037
Sie, A. S.: **EP05.06**
Sieben, A.: P08.03
Siebers-Renelt, U.: **P03.006**
Siebert, R.: C10.5, P04.25, P06.073, P06.098, P06.199, P07.03, P11.026, P11.069, P11.120
Siedek, F. B.: P09.047
Siedlecki, J. A.: P06.198
Sielska-Rotblum, D.: P03.114
Sieron, A. L.: P02.103
Sievers, H.: P14.13
Sifakis, S.: J02.34
Sigaudy, S.: P12.018
Sigua, C. L.: P12.093
Sijbrands, E. J.: P12.206
Sijmons, R.: P01.34, EP05.01
Sikkema-Raddatz, B.: P12.064, **P12.111**
Şıklar, Z.: P02.022
Sikora, P.: P12.201
Silan, C.: P06.152, P06.205, P14.05, **P14.22**
Silan, F.: P06.127, P06.152, P06.205, P06.206, P14.05, P14.22
Silani, V.: P10.04
Šilar, M.: P09.021, P12.110
Silengo, M.: P02.010, P02.040, P02.100, P02.129, P02.187, P12.227
Silhanova, E.: P12.009
Sill, H.: C10.6
Silva Jr, W. A.: P06.029, P06.096, P10.46, P12.212
Silva, B.: P09.063
Silva, J.: P12.043, P06.090
Silva, L. M. R. Batista da.: P06.090
Silva, W. A.: P11.030
Silvanovich, A. P.: P03.115
Silveira, I.: P12.216, P12.250
Silveira-Santos, R.: P03.008, **P03.013**
Simandlová, M.: **P02.168**, P02.213, P03.102
Simeonov, E.: P02.079
Simeonov, V.: P09.031
Simian, C.: PL2.2
Simion, R.: P05.38
Simioni, M.: **P12.244**
Simma, B.: P02.116
Simoes, A. L.: **P10.03**
Simoes-Correia, J.: P06.063
Simon, A.: C12.1
Simon, M.: C03.5
Simon, R.: P06.185
Simone, I. L.: J12.27
Simoneau, J.: P11.086
Simonic, I.: P02.169
Simonova, K. V.: J09.06
Simonova, O. A.: **P11.064**
Simons, M.: P12.153
Šimová, J.: P06.165
Simpson, A.: EP07.03
Simpson, M.: P12.254
Simpson, N. H.: **P09.126**
Şimşek Kiper, P.: P02.097
Simsek, T.: P06.037
Sinclair-Bourque, E.: P05.04
Singer, A.: **P02.075**
Singh, V.: P06.019
Siniauskaya, M.: **J09.03**
Sinicina, I.: P02.245
Sinico, M.: P12.178
Sinilnikova, O.: P11.110
Sinke, R.: C11.3
Sinke, R. J.: **P12.064**, P12.095, P12.111, P12.259
Sinkus, A.: P06.106
Sintas, C.: **P12.083**
Siomou, E.: **P02.258**
Sipek jr., A.: **J03.08**
Sipeky, C.: P10.25, P10.30, P10.43, **P10.52**, P12.139
Sipolatti, V.: P12.181
Sipos, S.: P02.108
Sirchia, S.: P12.058
Sirén, A.: P12.028
Sireteanu, A.: P03.125
Sirotkina, O.: P09.108
Sismani, C.: P03.112, P11.061, P11.140, **P11.142**
Siswara, P.: P10.16
Sitek, J. C.: P02.198
Siu, L. L. P.: **P07.07**, P07.10
Sivalingam, S.: P12.239
Sivan, S.: P12.122, P12.262
Skabar, A.: P02.025
Skachkova, I.: J02.18, **P12.113**
Skalická, V.: P01.11
Skalidakis, I.: P09.007
Skalíková, R.: P06.165
Škarić-Jurić, T.: P08.26
Skarzynski, H.: P02.117, P02.156
Skawran, B.: **P06.113**
Skerbinjek Kavalar, M.: P09.021
Skinner, C.: P11.142
Skinner, S. A.: P03.098
Skiriute, D.: P06.106
Skirton, H.: EP06.02, EP06.06, EP07.01, EPL2.5, EPL3.2, EPL4.6, **EPL5.2**, P01.08, P01.13, P01.31
Sklar, P.: P09.032
Sklyarenko, L.: P06.014
Skokowa, J.: P06.022
Skórka, A.: P02.117, P02.186
Skryabin, N. A.: **J06.18**, P04.32
Skrypnyk, C.: P02.137
Skrzypczak-Zielinska, M.: P13.48, **P14.12**
Skurk, T.: P13.09
Skvortsova, V.: P09.127
Skytte, A.-B.: P03.080, P06.135
Slamova, I.: **P03.051**, P07.06
Slater, H. R.: P05.34
Slavik, P.: P12.209
Sleegers, K.: P08.03, P12.073
Slepičková, I.: P06.001
Slim, R.: P12.252
Sloan-Béna, F.: P05.58, P12.055
Slominsky, P.: P09.127
Słomski, R.: P06.084, P06.204, P08.01, P12.228, P06.162, P09.001, P09.002, P13.48, P14.12
Slunga-Tallberg, A. M.: P05.52
Smeets, D. F. C. M.: C04.5
Smeitink, J.: C12.3
Smet, J.: P13.23
Smetana, J.: P07.26, **P07.27**
Smets, E. M.: EP05.01, P01.34
Smickova, E.: P06.042
Śmigiel, R.: P02.244, P03.074, P02.028, P02.251, **P09.099**
Smirnikhina, S.: P06.085, **P06.086**
Smirnova, S.: J10.04
Smit, L.: P03.054, P03.054
Smith, C.: **P11.141**
Smith, F.: P12.254
Smith, J.: J11.03
Smith, K.: C05.6
Smith, R. J.: P08.05
Smith, R. J. H.: P12.168
Smithson, S. F.: C18.1
Smogorzewska, A.: P11.036
Smolej Narančić, N.: P08.26
Smolin, A.: P06.145
Smolnikova, E.: P03.101
Smolyaninov, A.: J10.04
Smouter, F. A. S.: P11.122
Smyth, L.: P08.21
Smyth, M. J.: S09.1
Snadden, L.: P02.178
Snyder, M.: C06.5, J11.12
Soares Thiele de Aguiar, R.: PL2.3
Soares, R. A. G.: P02.199
Sobczyk-Kopciol, A.: P02.117
Sobczyńska-Tomaszewska, A.: J12.25, P12.127
Sobhani, M.: P08.10
Sobiak, B.: P06.204
Soblet, J.: **C01.4**
Sobol, H.: P01.20, P11.110, P15.13
Sobol, M.: P12.246

Sobreira, C.: P12.231
Sobrido, M. J.: P12.216
Soda, M.: C10.3
Soehn, A. S.: **P12.187**
Soellner, L.: P02.092
Sofia Kitsiou-Tzeli, S.: P02.261
Sofocleous, C.: J02.34, P05.51
Sogati, S.: J03.20
Sohrabi, E.: **P12.024**
Soininen, P.: C05.3
Sokolenko, A. P.: P06.036
Sokolowska, J.: P11.110
Solanas, M.: J09.47
Solanes, A.: EPL2.3
Solberg, J.: P14.09
Soldà, G.: P09.088, P11.018, P12.169
Soldatova, I.: **P05.44**
Solé, F.: P07.28
Sole, G.: C08.3
Soleimani, M.: J04.03, P14.15
Soler, S.: P12.247
Solfrank, B.: P05.14
Solimanpour, A.: J01.05
Sollacher, D.: P03.090
Solomon, B. D.: P02.130
Soloviev, A. V.: P02.109, P10.11
Soloviev, I. V.: P03.014
Soloviov, O.: P11.070, P03.084
Solovyov, K.: P03.027, **J02.44**, J12.07, J14.02
Soltanghoraee, H.: J04.13
Soltani, M.: P06.095, P06.174
Soltysova, A.: **P12.139**
Somboontham, T.: P09.029
Somer, M.: J12.31, P12.192
Somova, R. S.: P08.22
Somuncu, M. N.: P07.20
Sondore, V.: P13.02
Sönmez, F.: P02.131
Sonnenberg, A.: P12.154
Sonntagbauer, M.: P06.070, P11.022
Soo, R. A.: C10.3
Sood, A.: P09.059
Soplepmann, J.: P06.067
Soranzo, N.: C17.3
Sorokina, T. M.: J04.14
Sorte, H. S.: C15.4, **P02.080**
Sosnina, K.: **P04.46**
Sota, J.: **P12.127**
Soto, J. L.: P06.072, **P06.082**
Soubrier, F.: P11.110
Soukalova, J.: P12.009
Soukaseum, C.: PL2.2
Soukup, J.: P06.182
Soukupova, M.: P05.68
Soulaymani, A.: P10.15
Soulier, A.: **EPL7.2**, P01.27
Soumit, S.: J03.22
Sousa, A.: P03.008, P08.45, P09.080, P09.081, P10.20, P10.21, **P10.23**, P03.013
Sousa, A. C.: P03.013
Sousa, I. R.: P10.03
Sousa, L.: EP06.05
Southam, L.: P10.45
Souto, M.: J03.03, P03.072, **P03.134**
Souza, A. P.: P11.127
Sowa, A. S.: **P09.092**
Sowinska, A.: P02.193
Sowiński, J.: P06.084, P06.204
Soyah, N.: P03.066, P03.085
Soylemez, M. A.: **P02.220**
Soysal, Y.: **P02.038**
Spadafora, P.: J12.29
Spaeth, J. M.: P02.192
Spaich, C.: P02.234
Span, M.: **P15.11**
Spandole, S.: **J10.12**, P06.010, P10.10
Spangenberg, C.: P06.111
Spanik, S.: P06.092
Spanou Aristidou, E.: EP06.07
Spanou, E.: J02.16, P12.107
Spatz, J.: P14.16
Specht, G.: P15.14
Specker, C.: C09.3
Speck-Martins, C. E.: P03.108, P03.140
Spector, T.: C17.3, PL2.5
Speicher, M. R.: C10.6, P03.021, P06.051
Spencer, C. C.: C06.4
Spengler, S.: C04.2, P02.092, **P02.234**, P03.081
Sperl, W.: C12.3, C12.4, P14.19
Sperling, K.: P07.32
Spiden, S.: P02.217
Spiegler, S.: **P12.041**
Spiekermann, K.: C02.3
Spielmann, M.: **C03.1**
Spier, I.: C04.2, C04.2, **C10.2**
Spinelli, O.: P06.059
Spinner, N. B.: P03.118
Spirovski, Z.: P06.042
Spizzichino, L.: P05.05, P05.12
Splichal, Z.: P06.004, **P09.005**, P10.55
Sporns, P.: P11.069
Spranger, S.: P12.175
Sprengel, R.: P12.021
Sprenger, S.: P12.061
Sprincean, M. L.: **J01.03**
Spruijt, L.: EP05.06, P01.05
Spyrou, C.: C16.2
Sram, J.: **P11.086**
Srebniak, M. I.: P05.60
Srinivasan, M.: P03.060, P11.093
Sripo, T.: P03.127, P09.029
Srivastava, P.: P06.019
Srour, W.: P03.135
Staab, T.: P12.141
Stadheim, B. F.: **P02.198**
Stadler, P.: C10.5
Staehli, N.: P06.176
Stafova, D.: P10.55
Stahl, M.: P12.201
Stalke, A.: **P13.49**
Stalker, J.: P12.126
Stamatin, M.: P05.11
Stamatoyannopoulos, J. A.: P11.028
Stambouli, D.: P02.031, P05.38
Stamenkovic Radak, M.: P06.170
Stan, A. M.: J10.05, **P05.42**
Stancheva, G.: **P06.048**, P06.144
Stanclova, A.: J09.12
Staneva, R.: P11.118, **P09.031**
Stange, J.: P11.100, P11.101
Stangier, K. A.: **P11.034**
Stangler Herodež, Š.: J05.03, **P04.07**
Staniszewski, R.: P09.001
Stankova, K.: P04.05
Stankovic, A.: P09.084
Stankovic, M.: **J10.03**
Stanton, A.: C14.6
Stanulla, M.: C02.3, P06.022
Stara, M.: P05.68
Starcevic Cizmarevic, N.: P09.084
Stark, K.: C05.5
Stark, Z.: EPL7.5
Starke, S.: P12.204
Stastny, J.: P10.55
Stathaki, E.: P12.055
Staurenghi, G.: J09.43
Stavarachi, M.: J10.12, P06.010, P10.10, P13.10
Stavropoulou, A.: P06.028
Stavrou, C.: P12.008
Stavusis, J.: **J09.09**, P11.065, P11.066
Stecher, M.: P04.43, P05.14
Stechova, K.: **P09.135**
Steegers-Theunissen, R.: P08.32, C14.4, P11.029
Steel, K. P.: P08.31, PL2.4
Steenpass, L.: **P11.111**
Stefanadis, C.: P02.227
Stefanelli, G.: P12.042
Stefanescu, R.: **J02.13**
Stefanidis, C. J.: P12.156
Stefaniotou, M.: P09.007
Stefanova, I.: **P02.093**, P12.041
Stefanova, M.: **P02.102**
Stefanovic, V.: P09.031
Stefansdottir, V.: **EPL4.6**
Steffann, J.: P13.32
Stefoni, S.: P12.130
Stegel, V.: P11.072
Stegerer, A.: P02.086, P04.42
Stegle, O.: C08.5
Stein, J.: P02.142
Stein, R.: P08.08, P09.033
Steinbeisser, H.: P12.016
Steinberga, Z.: P13.02
Steinemann, D.: **P06.022**, P09.027
Steiner, B.: C18.5
Steinlein, O.: P02.067, P02.160, P03.105
Steinmann, B.: C12.3, C18.3, P12.086
Steinraths, M.: P03.137
Steinwender, C.: **P06.111**, P06.217, P12.039
Stejskal, D.: P02.259, P03.123, P05.44, P05.50
Stella, L.: P12.222, P12.227
Stellmer, F.: **P05.70**
Stemper, C.: **P10.47**
Stepanov, V.: P04.40, J09.06, **P10.34**
Stepanova, A.: **J02.37**
Stephani, U.: P11.069
Stepniak, I.: P12.115
Sterba, J.: P06.004
Steri, M.: C08.3
Stevanin, G.: **C11.2**
Stevanovic, S.: P06.178
Stevenson, D. A.: P12.158
Stewart, F.: P11.098
Stiburkova, B.: P13.17
Sticht, H.: C14.1, P12.218
Stiege, A.: P12.031
Stienen, D.: C10.2
Stirling, L.: EP02.04
Stöbe, P.: P12.150, **P12.176**
Stochholm, K.: P10.35
Stock, F.: **P02.242**, P13.24
Stocker, G.: C18.5
Stoeckli, E.: C13.6
Stoetzel, C.: C18.4, P12.046
Stoian, A.: EP04.01, J04.07
Stoian, D.: P05.17
Stoian, M.: J02.20, P02.127, **P03.007**, P03.010, P03.145, P05.39, P06.034
Stoicanescu, D.: J14.03, P02.125, **P02.127**, P02.207, P06.034
Stoicea, M. C.: **P06.123**
Stokes, P.: P11.086
Stoklasova, M.: **P07.17**
Stoler, J.: P12.264
Stolk, L.: **P11.029**
Stoll, C.: **P02.216**
Stoller, I.: P12.041
Stolnaya, L.: P13.36
Stolte- Dijkstra, I.: C03.5
Stoneking, M.: P10.41, P11.074
Stoppa Lyonnet, D.: P11.110
Stouffs, K.: P04.24
Stoyanov, S.: P10.32
Stoycheva, M.: J09.32
St-Pierre, J.: P11.023
Strachan, E.: C04.4
Strahle, U.: C18.4
Strain, L.: P08.21
Strand, L.: P02.056, P03.128
Strand, S.: P11.121
Stranecky, V.: P13.30

Strange, H.: **S16.3**
Strasser, U.: P02.172
Stratilă, M.: J01.03, J10.02
Stratis, Y.: P04.57
Straub, V.: C18.3
Strauss, E.: **P09.001**
Strauss, G.: P02.081
Strauss, J.-P.: C18.4
Strautmanis, J.: P11.065
Stray-Pedersen, A.: **C15.4**, P02.003, P02.080
Strecker, V.: P14.19
Streinu, V.: **P06.177**
Strelnikov, V.: P06.145, P06.141, P11.064
Stricker, S.: C03.2, P02.223, **P12.158**, PL2.3
Strnkova, A.: P07.17
Strobl-Wildemann, G.: P02.092
Strohmaier, J.: P09.009, P09.069, P09.118
Strohmayer, D.: P13.33
Strom, T.: C15.2, C12.3, C12.4, C12.6, C13.2, P12.138, P13.12
Strömbom, L.: P09.042
Strong, L. C.: C02.4
Struengmann, S.: PL3.2
Strullu, M.: P06.166
Stückler, F.: P13.09
Studnickova, M.: P05.09
Stuhrmann, M.: P08.29, P12.258
Stuhrmann-Spangenberg, M.: P12.226
Stumm, M.: C16.1, **P04.37**
Sturm, J.: C02.3
Sturm, M.: P06.178, P12.260
Su, C.-T.: C09.5
Su, M.: **P04.45**
Su, Y.: **P01.14**, P14.16
Su, Y. N.: P11.102
Suarez, L.: P11.029, P11.056
Suárez-Hernández, J.: P06.138
Subrt, I.: P07.08
Suc, A.-L.: P05.18
Succi, M.: P06.090
Suchy-Dicey, A.: C08.4, C17.6
Sudholt, I.: **P02.219**
Sudmant, P. H.: C06.1
Suess-Burghart, H.: P02.138
Sufliarsky, J.: P06.102
Sugahara, K.: P02.223
Suhomyasova, A. L.: P02.035
Suhre, K.: P13.09
Sukalo, M.: **P02.225**
Sukarova Stefanovska, E.: **P10.19**
Sukarova-Angelovska, E.: **J02.11**
Sukenik-Halevy, R.: **P02.145**, P03.142
Sukhomyasova, A. L.: J12.03
Suleimanova, E.: P09.122
Sulek, A.: P12.115
Süleymanlar, G.: P12.143
Suleymanova, Y.: **P09.014**
Sullivan, P.: P10.06
Sultanov, I.: **J04.04**, P06.183
Sültmann, H.: P06.185
Sumalla, E.: EP05.12, EPL2.3
Sumner, C.: P11.098
Sun, K.: P10.02
Sun, X.: P11.025
Sun, Y.: C07.1, P12.051
Sundquist, A.: P11.141
Sünnetçi, D.: J03.12, P02.014, P06.007, P06.014, **P06.037**, P06.056, P06.175
Sünter, A.: P10.13
Suomalainen, A.: C12.5
Suomalainen, M.: **P11.131**
Suomalainen, S.: P11.131
Superti-Furga, A.: C12.3, P02.090
Supic, G.: P06.170
Supunpong-Hernandez, N.: P11.106
Surace, C.: P02.184
Surani, A.: C08.2
Sure, U.: P12.041
Surendran, P.: **C14.6**
Sušac, I.: P06.030
Suskovic, S.: P09.021
Suspitsin, E. N.: **P06.036**
Sutter, C.: P02.077
Sutter, F.: P09.091
Suttner, R.: P04.42
Sutton, S.: EPL1.5
Suzuki, A.: **P09.117**
Suzuki, M.: P02.150, P04.41
Suzuki, H.: P09.071
Svarovskaya, M. G.: J09.06
Svecova, I.: J09.12
Svendsen, M.: P03.128
Sventoraityte-Skieceviciene, J.: **P09.138**
Svidnicki, M. C.: P12.066
Svingou, M.: **P12.040**
Svishcheva, G. R.: **P08.34**
Svobodova, E.: P02.259
Svrcek, M.: P06.078
Svyatova, G.: J12.19
Swagemakers, S.: P12.004
Swaminathan, K.: P11.093
Swertz, M.: C07.5, P08.23, P11.134
Świąder, A.: P02.233
Swinkels, D. W.: P09.096
Swinkels, M. E.: C18.1
Swinnen, E.: EPL7.6, **P10.48**
Switzeny, O. J.: **P11.076**
Syczewska, M.: P02.233
Syed, S.: C01.4
Symoens, S.: P12.048, P12.183
Syrbe, S.: P02.121
Syrmou, A.: J02.30, J02.34, P02.261, P03.012, **P09.015**
Syrrou, M.: P11.142
Syx, D.: **P12.048**
Szabo, G.: J09.12, **P05.49**, P05.63
Szabo, I.: P10.52
Szabó, T.: P12.015
Szafranski, K.: P06.167
Szalata, M.: P14.12
Szamalek-Hoegel, J.: P02.215
Szarras-Czapnik, M.: P02.233
Szczałuba, K.: **P02.028**, P02.185, P03.074, P12.172
Szczepanik, E.: P12.217
Szczepanowski, M.: C10.5, P06.098
Szczepański, T.: C02.2
Szemes, T.: P06.102
Szepe, P.: P06.102
Szilágyi, Á.: P06.195 ,P13.11
Szmida, E.: P06.071
Sznajer, Y.: P12.184
Szpecht-Potocka, A.: P12.172
Szperl, A.: P09.043, P09.059
Sztriha, L.: P13.22

T
't Hoen, P. A. C.: C06.6
Tabaglio, T.: P09.088
Tabarroni, C.: P02.135
Tabatabaei Panah, A.: **P08.36**
Tabatabaiefar, M. A.: P12.106
Tabibi, A.: P09.116
Tabka, F.: P07.31
Tabolacci, E.: P11.040, **P14.07**
Tachmazidou, I.: P10.06, **P10.45**
Tadini, G.: J02.53
Tafazzoli, A.: P02.220
Taghavi Basmanj, M.: J05.02, J05.07, P05.28
Taghavi, M.: **J05.06**, J06.08, P05.66
Taghavi, M. S.: **P06.104**
Taghizade Mortezai, F.: **J09.22**
Taghizadeh, M.: P09.116
Tagi Aliyev, R.: P12.144
Tagizade, M.: P14.23
Taglia, F.: J09.43
Taheri, M.: P06.052, P06.149, **P10.54**
Tahsili, M.: P03.033, P03.039
Taíra, I.: P02.123
Tajara, E. H.: P06.216, P11.030
Tajik, A.: J13.06
Tajnik, M.: **P11.052**
Tajsic, J.: P12.049
Takac, I.: P04.07
Takács, B.: P06.195
Takács, M.: P05.47
Takada, T.: P09.071
Takada, Y.: P09.071
Takahashi, N.: C10.3
Takanari, H.: C18.1
Takayanagi, R.: P09.057
Takeno, S. S.: P03.023, P03.107
Takhauov R.M., L. G. M. Karpov A.B..: **J03.19**
Talantova, L. I.: P03.113
Talvik, I.: J03.18
Tamas, L.: P13.08, P13.35
Tamasauskas, A.: P06.106
Tamási, L.: P06.195
Tamburrini, G.: P09.048
Tamhankar, P. M.: P02.059
Tamowicz, B.: P13.48
Tan, A. S. C.: P10.02
Tan, C. W.: P02.107
Tan, D. S. W.: C10.3
Tan, E. H.: C10.3
Tan, P.: C10.3
Tanabe, H.: C04.3, **P03.036**
Tanas, A. S.: P06.141
Tanev, D.: J09.32
Tang, F.: C08.2
Tang, W.: P12.021
Tangen, I.: P11.069
Tangphatsornruang, S.: P09.029
Taniai, H.: P12.131
Tańska, A.: P02.041, P02.186, **P02.232**, P02.233, P07.24, P13.47
Tanteles, G.: EP06.07, P12.107
Tanteles, G. A.: **J02.16**
Tantra, M.: P12.142
Tanzer, A.: C08.1
Tanzi, R.: C08.2
Tapias, I.: P12.210
Tapscott, S. J.: P11.042
Tarani, L.: P02.040
Tarantino, P.: **J12.33**, J12.34, J12.35, P12.081
Tarasenko, N. V.: **J09.34**
Tarasenko, O. A.: P03.113
Tarasenko, T.: J02.06
Taraskina, A.: P09.108
Tarasova, L.: P09.098, P09.136
Tariq, M.: P12.020
Tariverdian, G.: P02.205
Tarko, A. M.: **P02.264**
Tarkun, I.: P06.175
Tarlak, B.: P06.097
Tarlos, G.: P10.52
Tarnev, I.: P12.116, P12.117
Tarrío, R.: P12.216
Tartaglia, M.: P02.100, P02.175, P02.184, P02.187, P02.197, P12.222, P12.227
Taruscio, D.: J15.02, J15.03
Taschner, P. E. M.: **P11.084**
Tascón, M.: P06.065
Tashireva, L. A.: J06.15
Taskinen, M.-R.: C05.3
Taşlıyurt, T.: P08.06
Tassano, E.: **J07.04**
Tataj, R.: P12.217
Tatu, S. D.: **P12.210**
Tatum, Z.: P11.084
Taube, R.: P12.262
Taushanova, M.: P06.044
Tavakkoly-Bazzaz, J.: **J13.02**, J13.11, P03.106

Tavakol zadeh, T.: P03.033, P03.039, P03.077
Tavares, P.: **P12.170**
Tavassoli, A.: J06.19, P06.091
Tavazzi, D.: P12.118
Tavella, E.: J07.04
Tavokina, L. V.: P03.084
Tawamie, H.: C15.2
Tawil, R.: P11.042
Tay, K. Y.: P02.146
Tayaranian Marvian, A.: **J11.01**
Taylor, A.: P11.054
Taylor, G.: C07.3
Taylor, J. M.: **P09.016**
Taylor, L.: **EP05.11**
Taylor, R.: C12.3, P02.217
Tazi-Ahnini, R.: P09.058
Tazzioli, G.: EPL7.3
te Loo, M. W. M.: P06.080
te Meerman, G.: C08.4, C08.6, C10.1, C17.6
Technau, M.: C13.4
Tedeschi, P.: J02.14
Teek, R.: J03.18, P02.231
Teixeira, C.: P02.163
Teixeira, M. C. T. V.: J02.42
Teixeira, N.: C10.4
Teixidó, I.: P05.43
Teleky, B.: P06.173
Télez, M.: P03.025, P12.094
Telkova, M.: J09.12
Temel, S. G.: **P02.036**
Temple, I.: P11.005
Temple, K.: P11.123
Temtamy, S.: P12.055
Tenconi, R.: P02.060
Tenés, A.: P06.118
Teng, M. W.: **S09.1**
Tennant, J.: EPL3.1
Teo, A. S. M.: C10.3
Teo, C. R. L.: P10.02
Teo, J.: P02.107
Teran, N.: J04.15
Terczynska, I.: P12.217
Teresa-Rodrigo, E.: P02.066
Terhal, P.: P12.154
Terhal, P. A.: C18.1, **P02.115**, P12.259
Ternby, E.: EPL1.3
Terpylyak, O.: P04.46, P13.16
Terry, M.: P08.07
Terwindt, G. M.: P12.173
Teryutin, F. M.: **P02.109**, P10.11
Tesar, V.: P09.054
Tesarova, M.: C12.4, **P13.30**
Tesi, B.: P11.125
Tesio, C.: P10.39
Teske, A.: P06.199
Tesner, P.: **P03.079**
Tesson, C.: C11.2
Tester, D. J.: C16.4
Tetreault, M.: **P12.087**
Teumer, A.: C08.4, C17.6
Textor, B.: **P11.098**
Tezel, G.: P02.226
Thalassinos, C.: C09.2
Thaler, C.: P04.04
Thalhammer, M.: P06.051
Than, H.: C10.3
Thatjana Gardeitchik, T.: C09.6
Thauvin- Robinet, C.: P02.265
Thauvin, C.: C11.1, P03.020
Thauvin-Robinet, C.: P02.170, P05.48, P12.167, **P12.178**
Théâtre, E.: S01.3
Theil, L.: P06.098
Thelen, P.: P06.213
Themmen, A.: P02.154
Then, C.: P13.09
Theuns, J.: P12.188
Thevenon, J.: P02.265, **P12.167**
Thiel, C.: P02.195, P12.218, **C03.6**, C13.2, P03.120, P12.220
Thiel, G.: P05.31
Thiele, H.: C03.3, C13.4, P12.108
Thierry, P.: P02.043
Thijssen, P. E.: P11.042
Thilbeault, K. S.: C04.4
't Hoen, P. A. C.: P08.13
Thom, S.: C14.6
Thomaidis, L.: P02.258
Thomale, J.: P09.060
Thomas, A.: C18.2
Thomas, S.: **P03.037**, P12.178
Thomas-Anterion, C.: C11.1
Thompson, M. D.: C13.1
Thomsen, C.: P02.257
Thomson, K.: P09.016
Thomson, P. C.: P09.024
Thongthip, S.: P11.036
Thormar, H. G.: P11.036
Thuresson, A. C.: J02.55
Thys, C.: J02.49
Thys, K.: P01.03
Tian, F.: **P06.155**
Tibben, A.: EPL2.1, EPL2.2, EPL4.4, EPL5.5, EPL7.4
Ticozzi, N.: P10.04
Tienari, P. J.: P09.086
Tiidema, A.: P03.032
Tikhonov, A. V.: **P03.094**, P11.024
Tikkanen, E.: C05.3
Till, M.: P02.141
Tiloca, C.: **P10.04**
Timasheva, Y. R.: **P09.045**
Timcheva, C.: P06.044
Timkovskaya, E. E.: P10.44
Timman, R.: EPL4.4
Timmermann, B.: P12.031
Timmermans, D. R. M.: EPL6.2
Timmermans, I.: P12.035
Timmermans, J.: C01.3
Timshina, Y. I.: J09.39
Timucin, M.: J14.01
Tincheva, R.: P02.034
Tinschert, S.: P02.210, P12.162
Tinto, N.: P02.001
Tirado, I.: **P15.09**
Tita, R.: **P02.060**
Titgemeyer, C.: P12.175
Tiwari, H. K.: C04.4
Tizazu, B.: C02.3
Tizzano, E. F.: P12.265
Tkach, I.: **P04.31**
Tobias, E.: P02.178
Todd, C.: EPL1.6
Todoran Butila, A.: P09.093
Todoran, A.: P06.089
Todorov, T.: J13.14, P12.109, P12.190
Todorova, A.: **P12.109**, P12.190
Toenshoff, B.: P12.201
Toes, R.: P08.15
Togha, M.: J12.13
Togni, R.: P06.176
Toh, H.: P06.116
Toh, S.: P06.116
Tohma, T.: P12.131
Toksoy, G.: P03.088, P05.22, **P05.36**, P12.030
Toliat, M. R.: P06.200
Tollenaar, R. A. E. M.: P06.031
Tolmacheva, E.: P03.083, **P04.32**
Tolmie, J.: C03.5
Toma, C.: P09.028, P12.083
Toma, M.: J10.12, P10.10, **P06.010**
Tomandl, J.: P06.004, P09.005, P10.55
Tomandlova, M.: P09.005, P10.55
Tomas, Ž.: **P08.26**
Tomassini, A.: P09.113
Tomek, A.: P02.174
Tominaga, M.: P02.029
Tomkova, M.: P10.42
Tommiska, J.: **P04.10**
Tomova, A.: J09.32
Toncheva, D.: P02.034, P02.063, P06.018, P06.054, P06.093, P06.164, P06.171, P09.031, P10.58, P11.118
Tonder, S. L.: C12.2
Tonekaboni, S.: P02.094, P02.171
Tongsima, S.: P09.029
Toni, S.: P11.077
Tõnisson, N.: **P06.087**
Tonkin, E.: EP06.02, EP07.03, **P01.08**
Tonkovic Djurisevic, I.: P03.040, P03.110, **P05.02**, P05.19
Tønne, E.: **P02.212**
Tönz, O.: C18.5
Toome, K.: **P06.066**, P06.067
Toomik, P.: P10.13
Tootee, A.: P09.089
Topçu, V.: **P02.022**
Toprak, M.: P08.09
Toraman, B.: P12.027
Torreri, P.: P12.222
Torres, L. C.: P13.38
Torrico, B.: **P09.028**
Torrioli, M.: PL3.3
Torroni, A.: P10.58
Tortelli, R.: J12.27
Tortora, G.: P02.023, **P06.110**
Tortorella, C.: J12.27
Tortorella, G.: P12.081
Tortorelli, S.: P14.09
Torun, B.: P15.06
Tory, K.: P12.015
Tosi, M.: P11.110
Tosto, F.: J15.02, J15.03
Tothova Tarova, E.: **P09.052**
Totomoch, A.: P12.100
Totonchi, M.: J04.17, P03.039, P04.56
Touboul, C.: P02.104
Toufan Tabrizi, M.: J09.02, J09.18
Touitou, I.: P12.247, P15.01, **P15.07**
Touraine, R.: P02.161, P08.11, P12.178
Tournaye, H.: P04.24
Tournev, I.: P12.109
Tousi, P.: P02.085, P02.235, P12.075
Toutain, A.: C01.1, P02.141, P05.18
Tovar Zapata, I.: P06.161
Tovar, I.: J02.46
Towne, J. E.: S09.1
Toylu, A.: **P06.130**, P13.34
Trabacca, M.: P02.147
Trabelsi, M.: **P02.054**
Tradowsky, D. C.: P08.32
Traeger Synodinos, J.: P12.156, C16.3, P02.227, P05.51
Trainor, P. A.: S04.2
Trajanoski, Z.: C18.5
Trakadis, Y. J.: **P11.046**
Traks, T.: P09.124
Tranchant, C.: J11.10
Traupe, H.: C14.5, P09.111, P12.068
Trautmann, H.: C10.5, P06.098
Trautmann, U.: C03.6, P03.120
Trecroci, F.: J12.29
Treslova, H.: P13.36
Tretyak, B.: **P04.18**
Trevino, M.: P12.021
Trevisson, E.: **P13.44**
Trifa, A.: P05.17
Trifonov, V.: P03.063, P10.08
Trifonova, E.: **P04.40**
Trigo Sanchez, I.: P06.161
Trimborn, M.: P02.099
Triviño, J.: P11.050, P11.091, P12.237
Trkova, M.: P02.259, P03.089, **P03.123**
Trofimov, V. I.: J09.07

Trofimova, I.: **P03.027**
Tronstad, R. R.: C12.2
Tropea, D.: C06.4
Trost, D.: C02.1
Trovero, F.: P09.056
Trubicka, J.: P02.041, P02.186, P02.232, **P07.24**, P13.47
Trujillano, D.: PL2.2
Trujillo-Tiebas, M.: P02.248, P12.177
Trummer, O.: P13.41
Trümper, L.: C10.5
Tryggvadottir, L.: EPL4.6
Trynka, G.: C17.1, P08.15, P09.004, P09.059
Trzeciak, W. H.: P02.251
Tsai, F.-J.: P12.223, P12.234
Tsai, Y.-C.: P12.187
Tsaliki, E.: C16.2
Tsang, P.: P03.060
Tselousova, O.: P09.041
Tsipi, M.: **P12.001**
Tsitlaidou, M.: P06.028
Tsoleridis, T.: P12.136
Tsuji, K.: J05.01
Tsukerman, J. V.: **P12.195**
Tsyganov, M. M.: J06.15
Tuba Eminoglu, F.: P12.060
Tubelis, L.: P08.17
Tucci, S.: P13.03
Tuch, B. B.: C08.2
Tucker, K.: EPL4.1
Tucker, T.: P03.137
Tufan, T.: P04.39
Tug, E.: P12.123
Tukiainen, T.: **C05.3**
Tuktarova, I. A.: P08.22
Tükün, A.: P06.097
Tükün, F. A.: P02.022
Tulay, P.: **P04.30**
Tulbah, S.: P12.135
Tulić, C.: J04.15
Tulinius, H.: EPL4.6
Tuluc, E.: P02.018
Tumiene, B.: P02.013
Tunc, E.: P05.65
Tunca, Y.: J10.08, P02.096
Tuncer, F. N.: J12.10, P12.101
Tuncez, E.: P03.109, **P03.132**
Tuncozgur, B.: P06.132
Turchetti, D.: EP05.02, EP06.06, P06.110
Turculet, L.: J02.31
Turgut, B.: P06.206
Turkeri, L.: P06.020
Türkmen, S.: **P12.198**
Turley, H.: P04.03
Turner, C. L. S.: C18.1
Turnpenny, P.: P05.41, P08.21
Turyk, S.: **J01.07**
Tüttelmann, F.: **P04.04**, P04.51, P04.57
Tutulan-Cunita, A.-C.: P03.046
Tüysüz, B.: C09.3, **P02.051**
Tuzun, A.: J10.08
Tyabut, T.: J09.03
Tyler-Smith, C.: P10.06
Tyrkus, M.: P04.18, P04.46
Tyynismaa, H.: **C12.5**
Tzankova, M.: P05.62
Tzetis, M.: J02.34, P02.261, **P03.012**, P09.015, P11.081, P12.001
Tziperman, B.: P02.263
Tzschach, A.: C13.2, C15.3, **P02.076**, P08.37, P12.260
Tzveova, R.: **P10.56**

U
Uda, M.: P11.037
Udagawa, Y.: P04.41
Udilova, A.: **P06.100**
Uebe, S.: C14.5, C15.2, P03.068, P09.111, P11.060, P11.090, P12.218
Ugarov, I. V.: P11.107
Ugarte, M.: P12.032, P13.01, P13.07
Ugocsai, P.: P09.076
Uğur İşeri, S.: P12.101
Ugur, M. G.: P04.48
Uhlhaas, S.: C10.2
Uitterlinden, A. G.: C08.4, C17.6, P11.029, P12.206, PL2.5
Uktverytė, I.: **P10.41**
Ulasoglu, C.: P06.075
Ule, J.: P11.052
Uliana, V.: P02.147, P03.069, P03.093, **P09.113**
Ulitina, A. S.: **J09.07**
Ullmann, U.: P12.035, P13.23
Ulph, F.: EP02.07, **EPL3.5**
Ulucan, H.: J02.35, P02.069, P02.249
Ulucan, K.: P06.020, **P09.132**, P12.037
Uludag, A.: P06.127, P06.152, **P06.205**, P06.205, P06.206, P14.05, P14.22
Ulz, P.: C10.6
Unal, N.: **P02.071**
Undlien, D. E.: C15.4
Ungaro, C.: J12.27
Ungaro, F.: P12.042
Unger, S.: C09.5, EP05.07
Ünsal, B.: J06.14, P06.012
Ural, O.: P07.20
Urazova, L.: P03.101
Urban, A.: C06.5, **J11.12**
Urban, Z.: C09.5
Urbankova, M.: P07.17
Urbanovská, I.: P06.165
Urcelay, E.: P09.059
Urgard, E.: P06.087
Urlaub, H.: P12.045
Urmantsev, M.: P06.016
Urquhart, J.: P12.241
Urru, M. F.: C08.3, P11.119
Urru, S. A. M.: **P09.077**
Urueta-Cuellar, H.: P12.100, P12.164
Usala, L.: P11.037
Usami, S.-I.: P02.150
Useche, F.: P11.028, P11.128
Usenko, T.: **P12.189**
Uslu, &. N.: P04.39
Ustaeva, O.: P07.29
Uster, T.: P12.202
Utine, E.: P02.097
Utkus, A.: J02.25, P03.043
Utsch, B.: P08.08
Utsiyeva, M.: P02.061
Utsumi, Y.: P09.071
Uudag, A.: **P06.152**
Uvírová, M.: P06.165
Uwineza, A.: **P02.238**
Uyanik, G.: P02.093, **P02.131**, P02.179, P12.145
Uyguner, O.: PL2.4, **P12.030**
Uyguner, Z.: P05.36, P02.157
Uzulmez, N.: P06.007, P06.056
Üzümcü, A.: PL2.4
Uzunalic, N.: P12.060

V
Vaidla, K.: P06.067
Vaitsiakhovich, T.: C17.4, **P08.12**, P08.39
Vaivade, I.: P09.098, P09.136
Vakili, S.: P02.252
Vakyla, S.: J09.03
Valabregue, R.: P12.120
Valaei, A.: J05.02, **J05.07**, P05.28, P05.66, J05.06
Valášková, I.: P01.21, **P06.190**, P12.159
Valdes, A. M.: PL2.5
Valdes, M.: J02.46, J02.47
Valdes-Miranda, J. M.: **P02.106**
Valduga, M.: P03.020, P09.026
Vale, J.: P12.250
Valentini, M.: P11.119, P12.125
Valev, S.: P06.044
Valiullina, A.: P09.128, **P09.049**
Valizadegan, S.: P05.30
Välk, K.: P06.087
Valladares-Salgado, A.: P08.24
Vallespí, T.: P07.28
Vallespín, E.: J02.55, **P03.076**, P12.056, P12.210, P13.01
Vallian Broojeni, S.: **J08.03**
Vals, M.-A.: P02.231
Valsesia, A.: P08.11
Valtorta, E.: P06.176
Valverde, D.: P06.122, P09.075, P12.026
Valverde, Y.: EPL2.3
Vamre, T. B.: **J02.09**
van 't Veer, L. J.: P06.031
Van Alfen, N.: P02.055
Van Allen, M.: P03.137
van Asperen, C.: EPL2.1, EP05.05, EPL2.2, EPL6.2
Van Assche, E.: P07.22
van Beek, R.: C04.5
van Belzen, M.: P12.051, **P12.214**
van Bokhoven, H.: C15.1, P02.192, P12.124
Van Bon, B. W.: P02.226, P02.192, P11.019, P11.035
Van Broeckhoven, C.: P08.03, P12.073, P12.163, P12.188
Van Camp, G.: P02.023
Van Campenhout, K.: P13.23
Van Casteren, V.: P10.48
Van Cauwenberghe, C.: **P08.03**, P12.073
Van Coster, R.: P13.23
Van Damme, T.: C09.5
van de Warrenburg, B.: C11.3
van den Berg, M. P.: P12.064
van den Bergh, T.: P11.097
van den Boogaard, M. J. H.: P12.259, **P02.253**
van den Broek, A. J.: **P06.031**
Van den Bruel, A.: J02.49
van den Meiracker, A. H.: P12.206
Van der Aa, N.: C03.5
van der Eerden, B.: P12.004
van der Heyden, M.: C18.1
van der Hout, A.: C10.4, P02.253
van der Kolk, D.: C10.4
van der Lans, C. A. C.: P12.214
van der Looij, E.: P01.05, P01.35
van der Maarel, S. M.: C06.6, P11.042, P11.068
van der Meer, L. B.: **EW1**
van der Post, R.: P06.063
van der Smagt, J. J.: C18.1
van der Spek, P. J.: P12.004
van der Velden, V. H. J.: C02.2
van der Vleuten, C.: P01.22, C01.4
van der Vliet, P. J.: P11.068
van der Weijden, T.: EP05.04
van der Zanden, L. F.: P11.007
van der Zee, J.: P12.163
van der Zwan, Y.: P06.201, P06.202
van Diemen, C. C.: P09.043
van Dijk, A. H. A.: C02.2
van Dijk, F.: C06.3, C07.5, P11.134
van Dijk, H. K.: P11.068
van Dijk, K. W.: P10.53
van Dommelen, P.: P02.115
Van Dongen, J. J. M.: C02.2
Van Dooren, S.: P13.23, **P12.035**
van Duijn, C. M.: C05.2, P08.35, P09.076, P10.53, P11.122, P12.073
van Duinen, S. G.: P12.173
van Dullemen, H. M.: P01.34
Van Dyke, D. L.: C16.4
Van Eden, M.: P11.025
van Eerde, A.: P12.154
van El, C. G.: EP08.06
van Engelen, B. G. M.: P11.042
van Essen, T.: C03.5, P12.124
Van Eyck, J.: C01.3

van Galen, E.: C13.5
van Gassen, K. L. I.: P12.095
Van Geet, C.: J02.49
van Gessel, S. L. J.: C04.5
van Haaften, G.: C18.1, P12.259
van Haelst, M.: P02.172, C18.1, **C18.6**, P12.259
van Haeringen, A.: C07.1, P12.051
van Haren, N.: P09.120
van Harssel, J. J. T.: **C18.1**
van Heel, D.: C17.1, P09.059
van Hooft, J. E.: P01.34
van Huet, R. A. C.: C11.5
van Kessel, I.: P01.17
van Krieken, J. H. J. M.: EP05.04
Van Laer, L.: C01.3, P02.023, P11.130
van Langen, I. M.: P01.06
Van Langenhove, T.: P12.163
van Leeuwen, E. M.: **P11.122**
van Leeuwen, F. E.: P06.031
van Leeuwen, F. N.: P06.080
van Leeuwen, J. P. T. M.: P12.004
van Lieshout, S.: C18.1, P02.095, P12.259
van Lishout, F.: P08.28
van Luijk, S. J.: P01.22
Van Maldergem, L.: C09.5, J02.43, P02.011, **P02.104**, P02.120, P12.178
Van Meurs, J. B.: PL2.5, C08.4, C17.6
van Mil, N. H.: P11.029
van Ommen, G. B.: P08.13
van Ommen, G.-J. B.: C07.1, P12.051
Van Opstal, D.: P05.60
van Ravenswaaij-Arts, C. M. A.: P02.006
Van Reijmersdal, S. V.: C02.2
van Rooij, I.: P06.063, P11.007
van Rooij, J.: P12.206
van Schrojenstein Lantman-de Valk, H.: C15.1
van Spaendonck-Zwarts, K. Y.: P12.064, P12.111
Van Steen, K.: P08.28
van 't Slot, R.: P02.095
van Tintelen, J. P.: P12.111
van Tintelen, P.: P12.064
Van Tongerloo, A.: **EP01.01**
van Tuil, M.: P12.259, C18.6
van Tuyl, L. M. C.: EP08.06
van Wanrooij, R. L. J.: P09.115
van Zelst-Stams, W.: P01.35, EP05.06, EP05.04
van Zon, P.: C18.6
Vanakker, O.: C09.5, **P02.045**, P12.207
Vaňášková, M.: P06.190, P12.159
Vanasse, M.: P12.087
Vanbellinghen, J.: P12.076
Vandenberghe, R.: P08.03, P12.163
Vandermaelen, D.: P04.24
Vandersickel, V.: P12.019
Vandyck, G.: P04.24
Vangjeli, C.: C14.6
Vanlerberghe, C.: **P02.070**
Vannier, A.: P12.055
Vanspauwen, V.: P06.198
Vanwijck, R.: P09.125
Vargas de los Monteros, M.: P06.161
Varlamova, M. A.: **P02.035**
Vasantha Srinivasan, P.: J03.22
Vasileva, E.: J08.05, J12.07, **J14.02**
Vasilica, V.: P03.053
Vasilieva, L. M.: P02.109
Vasiljeva, I.: P11.065, P11.066
Vasilyev, S.: P03.101
Vasilyev, V.: P03.027
Vasilyeva, T. A.: **P09.053**, P10.44
Vasin, A. V.: J11.06
Vasku, A.: P06.004, P09.005, P10.55
Vasli, N.: **J11.10**
Vasovcak, P.: P02.213
Vast, M.: P12.184
Vater, I.: P02.131
Vaula, G.: P12.147
Vavrova, L.: **P02.211**
Vávrová, V.: P01.11
Vazharova, R.: P03.073, P05.62, P02.079, **P03.018**
Vaziri Nasab, H.: P03.039, P03.077
Vazna, A.: **P03.089**
Vaz-Osório, S.: **P09.081**
Vázquez, C.: P12.265
Vazquez-Alvarez, M.: J12.28
Vcelakova, J.: P09.135
Vega, A.: C14.3
Vega-Cabrera, C.: P12.056
Vejvalková, Š.: **P02.068**, P03.089
Vekemans, M.: P12.178
Velagaleti, G. V. N.: **P07.15**
Velasco Sampedro, E. A.: P06.021
Velasco, E.: P06.065, **P06.196**, C16.4
Velea, I.: P13.08
Vélez, C.: P12.177
Velikaya, V.: P03.101
Velinov, N.: P06.048, P06.144
Velissari, A.: P09.007
Velissariou, V.: C16.2
Velkova, L.: P06.018
Vella, J.: **P10.36**
Veltman, J.: C07.2, P02.132, C01.1, C11.5, C15.1, P11.019, P11.035, P12.126
Veltrop, M. H. A. M..: P11.020
Venâncio, M.: EP02.06, P03.050
Venanzi, S.: P12.222
Venchikova, N.: J01.01
Venema, A.: C06.6
Veneselli, E.: P10.49
Venghaus, A.: P11.012, **P12.196**
Venselaar, H.: C18.1, P06.080
Ventruba, P.: P09.005
Ventura, M.: C06.1, P10.16, **P10.51**
Venturin, M.: J09.46, P11.008
Verbeek, D. S.: **C11.3**
Verbeek, E.: P12.199
Verbeek, S.: P11.020
Verbiest, M. M. P. J.: P11.029
Verblakt, M. A. W.: P15.11
Verde, F.: P10.04
Verdin, H.: **P12.191**
Verdura, E.: P12.070
Verhaeghe, C.: P12.076
Verheijen, F.: P12.199
Verhoef, S.: P06.031
Verhoef-Post, M.: P02.154
Verkerk, A. J.: P12.206
Verloes, A.: C03.5, P06.166, **S11.2**
Vermaat, M.: **P11.074**, P11.084
Vermeesch, J. R.: P02.053, P02.221, P05.15
Vermeulen, E.: P01.03
Vermeulen, S. H.: P09.096
Vermont, C. L.: P03.129
Veronique, D.: P12.076, P12.076
Versacci, P.: P02.184
Verschuuren-Bemelmans, C.: C11.3
Verstraeten, A.: **P12.188**
Vert, J.: P11.110
Verwiel, E. T. P.: C01.1, P06.080, **P11.078**
Vesela, K.: P13.30
Veselská, R.: P01.21
Vesely, S.: P06.182
Vessely, M. D.: S09.1
Via, M.: P10.28
Viaggi, C.: P03.069
Viaggi, S.: P11.063
Viana, H.: P02.163
Viassolo, V.: P02.147, P03.069
Vibe, A.: **J03.02**
Vicaire, S.: J11.10
Vicari, S.: P08.11
Vicente, M.: P06.191
Vicente-Gabas, A.: P02.066
Victorova, T.: P09.041
Vidailhet, M.: P02.007
Vidal-Petiot, E.: PL2.2
Vidone, M.: P02.135
Vieira, D. N.: P09.063
Viellieber, M.: P02.205
Vierlinger, K.: P06.207, P11.022
Vigneron, J.: P12.167
Vignoli, A.: P10.49
Viikari, J.: C05.3
Vikkula, M.: C01.4, P09.125, P12.184, P12.202
Viktorova, E.: P09.041
Viktorova, T.: J09.14, P06.016
Vila, M.: P12.083
Vilariño, C.: P09.075
Vílchez, J. J.: P12.114
Villacieros-Hernández, L.: P03.019
Villamor, N.: P07.12
Villard, L.: P10.49
Villatoro, S.: P11.062
Villavicencio Lorini, P.: **P02.099**, C03.2
Vilo, J.: P11.094
Viloria, M.: P03.005
Vimaleswaran, K. S.: **P10.12**
Viñas, M.: P03.041
Vinault, S.: P02.141
Vincent, M.-C.: P12.046
Vincent-Delorme, C.: P02.070, P02.141
Vincenzi, O. C.: **P06.096**
Vinci, M.: P09.030
Vinkler, C.: **P02.263**
Vinogradova, O. L.: J09.44, P09.083
Vinsova, K.: P13.30
Vinuela, A.: P12.108
Virdis, F.: C08.3
Visel, A.: P11.123
Vishnev, V.: J02.45, P10.01
Visitacion, M.: P11.128
Vissers, L.: C07.2, C15.1, C01.1, C02.2, P06.080, P11.019, P11.035
Visweswariah, S. S.: C12.2
Vitoria, J. C.: P09.036, P09.038, P09.039
Vives, L.: C06.1
Viviani Anselmi, C.: C07.4
Vlachopoulos, C.: P02.227
Vlad, C.: P02.108, P11.043
Vlad, D.: P02.108, **P11.043**
Vlahova, A.: P06.044
Vlaisavljević, V.: P04.07
Vlasak, I.: P03.056
Vlasin, P.: P05.10
Vlaskova, H.: P13.36
Vlassov, A.: P11.105
Vlčková, M.: P02.068, P02.168, **P03.004**
Vlčková, R.: P06.001
Vlckova, Z.: **P12.150**
Vlizadegan, S.: **P06.125**
Vlummens, P.: C09.5
Vodicka, R.: P05.09, **P06.210**
Vodopiutz, J.: P02.166
Voelckel, M.-A.: EPL5.2, P01.20
Voesenek, K.: P12.124
Vogel, J.-H.: C08.1
Vogel, M. J.: C18.6
Vogelaar, I.: **P06.063**
Vogiatzakis, N.: **P01.30**, P11.081
Vogl, I.: P11.132, P12.038
Vogt, J.: P06.157, P06.158, **P06.159**, P06.160
Vogt, M.: P12.021
Vogt, S.: C10.2, P02.169
Voigt, M.: **P03.061**, P10.08
Voinova, V. Y.: P03.115
Voisin, G.: P11.049
Voldgorn, Y. I.: P11.011
Volk, A.: **P12.011**, P12.012
Volk, M.: **J05.12**
Völker, U.: P11.047
Vollebregt, E. J.: P12.173

Volleth, M.: **P05.32**
Volodarsky, M.: **P02.202**
Volosciuc, M.: J02.21, J02.32, J02.39, P02.140, P03.125, P03.136, P05.11
Voltes-Cobo, A.: P04.38
von Bothmer, J.: P11.012, P12.196
von der Goltz, C.: P09.009
von der Hagen, M.: P02.064, P02.133
von Hardenberg, S.: C02.5, P06.213
von Kalle, C.: C18.5
von Kampen, O.: P06.073
von Kanel, T.: P06.064
von Kodolitsch, Y.: C07.4, P12.239
von Neuhoff, N.: P13.49
von Raison, F.: P14.06
Vondeling, H.: EPL7.6
Vondrackova, A.: P13.30
Vooder, T.: P06.087, P11.094
Vorobieva, E.: P08.16, J08.05
Vorobyeva, E. V.: P09.083
Voronina, E.: P06.085, P06.086
Vorsanova, S. G.: P03.009, P03.014, P03.022, **P03.115**
Võsa, U.: P06.087, **P11.094**
Voskarides, K.: P12.008
Vossen, R. H.: P06.033
Votava, F.: P01.11
Vovk, S. L.: P05.61
Vozzi, D.: P12.105
Vral, A.: P12.019
Vranova, V.: P03.051, P06.101, **P07.06**, P07.27
Vreeswijk, M. P. G.: EP05.05
Vrettou, C.: P05.51
Vriend, G.: C18.1
Vrints, C.: P11.130
Vroling, B.: **P11.097**
Vrtel, R.: P05.09, P06.210
Vucinic, N.: P09.022
Vulliamy, T.: P11.078
Vulto-van Silfhout, A. T.: **P02.192**, P11.019
Vuree, S.: **J06.01**
Vydra, J.: P07.02
Vydyborets, S.: J06.20

W
Waanders, E.: C02.2
Wachter, O.: P06.079
Wacklin, P.: P09.037
Wada, T.: C04.3, P03.036
Wadia, P. P.: P04.12
Wagener, R.: C10.5, P06.136
Wagman, I.: P04.20
Wagner, A.: P01.17, P01.34
Wagner, C.: P11.132
Wagner, F.: P05.31, P12.031, P12.108
Wagner, K.: P03.021
Wagner, K.-H.: P11.076
Wagner, W.: P02.188
Waha, A.: C02.1
Wahl, C.: P12.049
Wahl, D.: **P02.078**, P02.180
Wahl, S.: **P13.09**
Wahle, P.: P08.32
Waite, L.: C04.4
Wakamatsu, N.: P02.048
Wakui, K.: C04.3
Walden, U.: P02.136
Waldispuehl-Geigl, J.: C10.6
Waldman, I.: P09.105
Waldmüller, S.: P12.239
Waldstrøm, M.: P06.135
Wales, J.: P02.173
Walia, M.: P13.06
Waliszewski, K.: P09.001
Wall, S. A.: P12.264
Wallentin, M.: P02.144
Wallon, D.: C11.1
Walrafen, P.: P12.098
Walsh, T.: P06.028
Walter, C.: **EPL6.1**
Walter, F.: EP02.05, EPL1.5
Walter, L.: P10.09
Walter, M.: P06.071
Waltereit, R.: C13.6
Walters, N.: C08.1
Walther, J. U.: J07.01
Walzer, M.: P06.178
Wand, D.: EP02.02, P02.057
Wang Jabs, E.: P12.264
Wang, F.: P11.115
Wang, J.: C12.1, P06.116, P12.064, P12.169
Wang, N.: P14.10
Wang, S.-Y.: P11.010
Wang, W.: P08.14
Wang, X.: C08.2
Wang, Z.: **J14.08**, P09.092
Wanherdrick, K.: P06.078
Wanker, E.: P12.121
Wappenschmidt, B.: P06.117, P07.05
Ward, C. M.: P02.107
Warman, M.: PL2.3, **S15.1**
Wary, C.: P12.120
Waskiewicz, A.: P02.117
Waszak, S.: C06.5
Watanabe, A.: **P12.240**
Watanabe, H.: P12.240
Watanabe, K.: P02.110
Watson, C.: C07.3
Watson, E. K.: EPL4.2
Watson, R.: C01.4
Watt, C. M.: **P02.178**
Watts, K.: EPL4.1
Watzinger, N.: P09.035
Wauters, J.: P07.22
Weber, B. H.: P12.235, P09.008
Weber, J.: P09.092, P12.136
Weber, P.: P11.104
Weber, R. G.: **C02.1**, P06.136, P07.05
Weber, S.: P02.201, P12.155
Weber, Y.: P12.082
Weber-Ferro, W.: P02.004
Weberova, L.: P07.08
Webersinke, G.: P03.047, P06.013
Weersma, R.: P08.15
Wegner, F.: P13.13
Wegner, R.: P04.37
Wehnert, M.: C11.6, **P12.204**
Weichselbaum, A.: P02.149
Weidemann, J.: P03.035
Weih, S.: P02.130
Weimer, J.: P07.05
Weinhaeusel, A.: P06.070, **P11.022**, P06.002, P06.207
Weinreb, N.: P14.03
Weis, E.: **P12.137**, P12.226
Weis, M.: J12.22, J12.23
Weisbrod, M.: P09.120
Weise, A.: P03.063, P03.091, **P10.08**
Weishaupt, J.: P12.011
Weismanova, E.: P06.092
Weisschuh, N.: C14.1, **P11.112**
Weißensteiner, H.: P15.14
Weissman, S.: C06.5
Weissmann, R.: P10.09
Weitzman, S.: P07.01
Welte, K.: P06.022
Welting, T. J. M.: P12.039
Wen, Y.-Y.: C02.6
Wenstrup, R. J.: P06.214
Wenz, M.: **P11.075**, P15.03
Werdan, K.: P09.106
Wernstedt, A.: P02.172, **P06.176**
Wertheim-Tysarowska, K.: **J12.25**, P12.127
Wessels, M. W.: P12.051
Westerman, M. P.: C04.4
Western, P.: P06.202
Westerneng, M.: P01.22
Westra, H.-J.: C08.4, C08.6, C10.1, **C17.6**, P08.13, P08.23, P09.059, P11.126
Wevers, R.: P13.04
Whalen, S.: P02.007
Whatley, S.: P11.054
Wheeler, M.: PL2.5
White, S.: C08.1, P06.201, **P06.202**
White, W. M.: C16.4
Whiteford, M.: P02.178
Whittaker, J. C.: P10.12
Whittemore, A. S.: P08.07
Wibbeler, E.: P02.064
Wichmann, H.-E.: P13.09
Wick, J.: C16.4
Wick, W.: C02.1
Wickert, J.: **P02.179**
Wicki, R.: **P06.126**
Wicklein, E. M.: P12.108
Widomska, J.: P14.12
Wieacker, P.: C13.2, P02.043, P03.006, P04.02, P04.57, P12.033
Wiechmann, H.-E.: C14.5
Wieczorek, D.: C03.4, C13.2, P02.021, P02.043, P02.222, P03.120, **S04.3**
Wieland, B.: P06.156
Wieland, I.: P11.071, **P13.19**
Wieland, K.: P02.255
Wieland, T.: C12.6, **C13.2**
Wielscher, M.: **P06.002**, P11.022
Wienker, T. F.: C14.4, P15.12
Wierzba, J.: P02.066
Wiesener, A.: C13.2
Wieskamp, N.: C07.2
Wiezel, C. E. V.: P10.03
Wijatyk, A.: P14.03
Wijmenga, C.: C08.4, C08.6, C10.1, C17.1, C17.6, P08.13, P08.15, P08.23, P09.004, P09.036, P09.043, P09.059, P09.115, P11.126, P11.134
Wijnen, J. T.: EP05.05
Wilichowski, E.: C01.5, C12.4, P14.19
Wilimska, M.: P05.68
Wilkens, L.: P06.113
Wilkie, A. O. M.: P12.264
Will, M. L.: C16.4
Willam, M.: P12.137
Willatt, L.: P02.169
Willems, M.: P02.141
Willems, S. M.: C05.2
Willemsen, G.: P11.143
Willemsen, M. H.: **C15.1**, P11.019
Williams, A. G.: P09.068
Williams, D.: P11.106
Williams, J.: **S05.1**
Williams, M.: P05.41, P11.054, P12.050
Williams, S.: **P11.095**
Williamson, G. R.: EP07.01
Williamson, K. A.: P02.228
Willoughby, C.: P09.016
Wilson, A. F.: P12.264
Wilson, C. D.: C02.4
Wilson, L.: C18.1
Wilson, R. K.: C06.1, P10.51
Wimmer, K.: C18.5, **P02.172**, P06.158, P06.159, P06.176
Wimmer, R.: **P05.31**
Wineinger, N. E.: C04.4
Wineker, T.: P08.37
Winkelmann, J.: P10.33
Winkler, J.: P02.101
Winkler, T. W.: **C06.2**
Winnicka, D.: P02.066
Winterpacht, A.: C16.6, **P09.050**
Wirbelauer, J.: P02.254
Wirth, B.: C01.6
Wirth, R.: C03.4
Wirtz, M. K.: C14.1

Wischmeijer, A.: **P02.023**, P02.135
Wissinger, B.: C11.5, P09.109, P11.112
Wissink-Lindhout, W.: C15.1
Withoff, S.: P11.126
Witsch-Baumgartner, M.: P02.236, **P12.229**
Witt, D.: **P06.213**
Witt, J.: **EP05.09**
Witt, S. H.: P09.009, P09.069
Wittevrongel, C.: J02.49
Wittig, I.: C12.4, P14.19
Wittig, V.: P13.49
Wittler, L.: C09.4
Wittmann, S.: P04.29
Wittmann, W.: P12.218
Wittwer, C. T.: P15.04
Wizinska, P.: P09.099
Wlodkowski, T.: P14.11
Woermann, B. J.: C02.3
Wohlleber, D.: C02.1
Wohlleber, E.: C13.2, P02.012, P02.169
Wojcicki, P.: P02.251
Wolf, B.: **P06.172**, P06.173
Wolf, C.: C08.5
Wolf, E.: C12.6
Wolf, L.: **P02.017**
Wolf, M.: P11.038, P11.058, P12.201, P12.140
Wolf, N. I.: C18.5
Wolfer, D.: C13.5
Wolff, D.: P02.237
Wolff, H.: P09.010, P09.058
Wolff, M.: P02.076
Wolfshöfer, S.: C16.6
Wollnik, B.: C03.3, C03.4, P12.061, P12.193, PL2.4, **S11.3**
Wong, C.: P05.57
Wong, H. F.: P07.10
Wong, H. K.: P05.57
Wong, J. W.: C02.4
Wong, K. F.: P07.07, P07.10
Wong, S.: EPL3.6
Wong, T. Y.: C10.3
Wong, W. S.: **P07.10**
Woo, X. Y.: C10.3
Wood, A. M.: EPL1.6
Wood, F.: EP05.09
Woods, C. G.: **S11.1**
Woods, M.: P09.095
Worachotekamjorn, J.: P09.029
Worley, E.: P11.141
Wormley, B.: C06.4
Wouters, M. M.: P09.004
Wouts, J. N.: EP05.05
Woźniak, K.: J12.25, P12.127
Wrba, F.: P06.173
Wright, C.: EP08.03
Wright, G.: EPL5.6
Wright, J.: C11.4
Wright, V. J.: C14.3
Wu, C.-C.: P09.100
Wu, H.-T.: P09.061
Wu, J.-Y.: P12.223, **P12.234**
Wu, L.: P04.45
Wu, V.: P09.142
Wu, W. C.: P06.155
Wüller, D.: P02.230
Wunderink, M.: C04.5
Wunderlich, A.: P06.185
Wünsch, L.: P04.02
Würfel, W.: P04.42
Wüstemann, M.: C16.1
Wyder, S.: **P06.181**

X
Xaidara, A.: P02.261
Xaidara, A.: P03.012
Xavier, J.: P09.139
Xia, B.: P11.141
Xu, L.: **P07.25**

Y
Yaghmaei, P.: P10.07
Yaghmaie, M.: C02.3
Yaghootkar, H.: C08.4, C17.6
Yagi, T.: P12.200
Yagnik, G.: P12.264
Yakıcıer, C.: P02.038
Yakimovskii, A.: P12.189
Yakoreva, M.: J03.18
Yakshembitova, E.: P08.16
Yalçıner, G.: P06.097
Yamacı, S.: J12.04
Yamada, K.: P02.048
Yamada, R.: P09.117
Yamada, Y.: P02.048
Yamagata, Z.: **P01.26**
Yamamoto, K.: P09.057, P09.117
Yamanashi, Y.: P09.071
Yanagi, K.: P02.150, P12.131
Yanagihara, K.: P02.110
Yanberdin, I.: P09.101
Yanchina, E. D.: J09.07
Yáñez, Z.: P12.216
Yang, C.-F.: **P12.223**, P12.234
Yang, L. Y.: C10.3
Yang, M. H.: C01.3
Yang, Y.-F.: C14.1
Yannoukakos, D.: P06.028
Yap, Z.: P10.02, P12.126
Yarar, C.: P12.171
Yaron, Y.: P02.145, P03.142, P04.20, P05.54
Yarram, L.: P11.054, P12.050
Yaspan, B. L.: C14.2
Yatabe, Y.: C10.3
Yatsyk, G. V.: J09.10
Yavuz, D.: **P06.007**, P06.056
Yazdi, H.: P06.112
Ye, F.: P15.04
Ye, K.: C06.3
Ye, X.: P12.264
Yegin, Z.: P06.143
Yeh, F.-C.: **P11.004**
Yekaninejad, M. S.: P08.10
Yepiskoposyan, L.: J10.01, P10.40
Yesil, G.: **P03.088**
Yesilipek, A.: P02.226
Yesilipek, M. A.: P12.029
Yetiskin, M.: P09.132
Yigit, G.: **C03.4**, P12.201, PL2.4
Yiğit, S.: J09.13, P08.09, **P08.25**, P08.38, P08.06
Yildirim, M. S.: **P03.109**, P03.132
Yildirim, S.: P06.137
Yilmaz Gulec, E.: **P12.171**
Yilmaz, B.: P05.65
Yilmaz, D.: P02.131
Yilmaz, K.: P03.026, P05.36
Yılmaz, M. B.: P04.39
Yılmaz, S.: P02.051
Yin, Y.: P12.169
Yip, S.: P09.142, P06.155
Yntema, H. G.: C01.1, C15.1, P02.192, P12.124
Yntema, J.-B. L.: P12.126
Yokomori, K.: P11.042
Yokoyama, H.: J05.01, P02.196
Yonguc, N. G.: J06.17
Yordanov, Y.: P10.58
Yoshiura, K.: P12.131
Yosunkaya, E.: J02.35, P02.069, P02.249
Young, K. C.: **P04.12**
Young, M.-A.: **EP05.10**
Yousefi, M.: P02.235, P12.075
Yousoof, S.: P12.088
Yu, L.: P14.11
Yu, N.: P06.120
Yu, S.: P02.047
Yu, Y.: C14.2
Yücesan, E.: P12.101
Yuceyar, H.: J06.14, P06.012
Yudina, E.: J05.08, J05.09
Yüksel, A.: J02.35, P02.069, P02.249, P05.07, P05.22, P05.29
Yung, T. C.: P02.250
Yuniati, L.: P06.080
Yunusbaev, B.: P09.018
Yurdakök, M.: P05.64
Yurov, Y. B.: P03.009, P03.014, **P03.022**, P03.115
Yurovsky, A.: PL2.6
Yusefi, M.: P02.085

Z
Zabel, B.: P06.111, P06.217, P12.039, P14.20, P02.049
Zabnenkova, V.: J05.10
Zaborovskiy, A. V.: P11.107
Zackai, E. H.: P02.006
Zagidullin, A.: P06.062, P06.189
Zagidullin, S.: P09.018
Zagorac, A.: J05.03, **P05.33**
Zagoras, T.: P02.102
Zagozda, M.: P02.084, **P09.107**
Zagradišnik, B.: J05.03, P04.07, **P06.094**
Zagury, J.-F.: C17.2
Zaharchuk, A.: P09.108
Zaharieva, M.: P10.32
Zahn, S.: **P05.53**
Zahnleiter, D.: C03.6, **P03.120**
Zahrieh, D.: P13.14
Zainali, S.: P02.094
Zainullina, A.: **P09.128**, P09.049
Zajc Petranović, M.: P08.26
Zaker, F.: **P06.003**
Zakerska, O.: P09.002, **P13.48**, P14.12
Zakhama, A.: P02.221
Zakharova, E. Y.: J12.01, P02.019
Zakharova, M. N.: J09.37
Zaki, M.: C11.2, P12.198, **P02.082**, P12.055
Zakirov, D.: P09.121, **P09.122**
Zaklyazminskaya, E.: P02.151, P12.017, **P12.034**, P12.104, EP04.03, J09.05
Zaletaev, D.: J06.16, P06.184, P11.006, P11.064
Zaletayev, D.: P06.145, P06.141
Zali, M.: J09.35
Zalloua, P. A.: P09.046
Zaman, T.: J13.09
Zamani, A.: P06.137, P03.109, P03.132, **P06.137**, P07.20
Zamani, F.: P12.243
Zamani, S.: P12.243
Zamba-Papanicolaou, E.: P10.14
Zampatti, S.: J09.43
Zampieri, B. L.: P11.030
Zandieh, F.: P04.36
Zankl, A.: **C09.1**
Zanni, G.: **J12.30**
Zannoni, G.: P02.058
Zaoralova, R.: P07.27
Zapevalova, D.: P06.183
Zappia, M.: J12.34
Zappu, A.: P10.57
Zara, F.: P11.063
Zara, I.: P11.119, **P12.125**
Zarate, Y. A.: P03.098
Zarbati, N.: P03.131
Zare Abdollahi, D.: P06.192
Zaremba, J.: P12.115
Zargari, P.: J09.31
Zari Moradi, S.: P03.033, P03.077, **P04.09**, P04.56
Zarif Yeganeh, M.: J09.41
Zarina, A.: **P13.02**
Zarindast, M.: J02.01
Zarkesh, M.: **P09.067**
Zarnack, K.: P11.052
Zarrinrad, G.: P09.116

Zarrouk-Mahjoub, S.: **P08.18**
Zasedatelev, A.: P09.127, P09.012
Zastavna, D.: J09.26, P04.18, P04.46, P13.16
Zastera, J.: P12.009
Zatkova, A.: J09.28, P06.168, P12.161
Zavalishina, L.: P06.103, P06.184
Zavelevich, M.: P06.014
Zavodna, K.: P06.101
Zavyalova, M. V.: J06.15
Zayts, O.: EPL5.1
Zaza, A.: P09.131
Zdzienicka, E.: P12.115
Žebráková, I.: P06.165
Zechner, R.: C18.2
Zechner, U.: C16.5, P04.03, P09.129, P11.038, P11.058, P11.121, P12.137, P12.157, P12.213
Zedda, G.: P09.077
Zeggini, E.: P10.06, P10.45
Zeharia, A.: C12.1
Zeinali, S.: J02.12, J05.02, J05.07, **P04.36**, P05.28, P12.144, J05.06, P05.66
Zelante, L.: P02.060
Zelenika, D.: C11.2
Zelenova, M. A.: P03.115
Zeljic, K.: **P06.170**
Zeller, B.: P02.080
Zellmeier, E.: C02.3
Zeltzer, A.: P07.29
Zeman, J.: P13.30
Zemánková, E.: P03.123
Zemanova, Z.: P07.02
Zembutsu, H.: P06.171
Zemkov, V. A.: J14.02
Zemljic, L.: **P09.140**
Zemlyakova, V.: P06.145
Zemni, R.: P02.053
Zenkel, M.: C14.1
Zenker, M.: C18.6, P02.091, P02.134, P02.175, P02.225, P05.32, P06.166, P11.071, P12.138, P12.175, P13.19
Zenz, T.: C10.5
Zenz, W.: C14.3
Zeraati, H.: J04.13
Zerjavic, K.: P06.094
Zerova, T. E.: **P04.28**
Zerres, K.: P02.201, P02.234, P11.012, P12.196
Zeschnigk, C.: P12.145
Zeschnigk, M.: **P06.212**
Zeviani, M.: C12.3, P14.19
Zevit, N.: C12.1
Zhai, G.: P09.095
Zhanataev, A.: P06.085
Zhang, B.: C02.4
Zhang, J.: P12.064, P12.169
Zhang, W.: PL2.5
Zhao, H.: P11.113
Zheleznyakova, G.: P12.230
Zhemkov, V. A.: **J12.07**
Zhernakova, A.: **P08.15**
Zhernakova, D. V.: **P08.13**
Zhernakova, S.: P09.115
Zhou, L.: **P15.04**
Zhuraev, R.: **P14.14**
Zhuravleva, I. V.: P01.10
Zhurkova, N. V.: J09.10
Ziadi, M.: J09.02
Ziamatin, H.: C09.1
Zibert, A.: J13.14
Zidarn, M.: P12.110
Ziegler, M.: P03.063
Ziehe, M.: P09.060
Zieker, D.: P06.178
Ziemnicka, K.: P06.084, P06.204
Žilina, O.: P03.078
Zilles, K.: J03.18, P03.032, P09.047
Zimanová, V.: P03.079
Zimmermann, F.: C12.3
Zimmermann, K.: C02.1
Zimmermann, M.: P12.103
Zimmermann, R.: C16.1, C18.2
Zimmermann, W.-H.: P11.053
Zimovina, T.: P13.21
Zimran, A.: **P13.14**, P14.10
Zinchenko, R. A.: J10.10, **P10.27**, P10.44
Zink, A.: C09.4, P02.012, P02.026, P02.169
Zinovik, A.: **P13.15**, P13.21
Ziv, Y.: P14.04
Zivkovic, M.: P09.084
Zivna, J.: P07.17
Zlatkov, V.: P06.054
Zlotogora, J.: P12.146
Zmítková, Z.: P02.174, P03.004, P05.37
Žmolíková, J.: P06.165
Zoghbi, H. Y.: C04.1
Zoll, B.: P12.041
Zollino, M.: **C01.2**, **P03.116**
Zollner, U.: P02.254
Zolotuhina, T.: J03.06
Zolotukhina, T.: **P03.099**, J05.09
Zoppi, N.: C18.3
Zordania, R.: **P03.032**, P04.19
Zorkoltseva, I. V.: P08.35
Zorn, B.: J04.15
Zou, Y.: C18.3
Zoukovskaya, S.: **P04.08**
Zouvani, I.: P11.072
Zrenner, E.: C11.5
Zribi, N.: P04.16, P04.27
Zrnova-Hladilkova, E.: P03.051
Zrůstová, J.: P06.190
Zschocke, J.: C18.3, C18.5, P02.008, P02.116, P02.172, P06.176, P12.229, P12.232
Zubacova, Z.: P12.134
Zubairov, R.: **P06.183**
Zubor, P.: J09.12
Zuccari, D. A. P. C..: P06.047
Zuccotti, P.: **P11.008**
Zuffardi, O.: P03.065, P09.030, P11.077
Zühlke-Jenisch, R.: P07.03
Zuñiga, A.: J02.03, J02.04, P04.15, **P13.42**
Zúñiga, S.: P11.050, **P11.091**
Zúñiga-Trejos, S.: P06.161, P12.237
Zuzulova, M.: P09.052
Zvirbliene, A.: P09.138
Zweier, C.: C13.2, P02.158, **P02.237**, P12.102, P12.218
Zweier, M.: P02.237, **P03.068**, P12.218
Zweiker, R.: P09.035
Zwienenberg-Lee, M.: P12.264
Zwink, N.: P02.130
Zwolinski, S.: P03.093
Zygulova, I.: P07.17