SPGH

Sociedade Portuguesa de Genética Humana

18ª Reunião Anual

19 – 21 de Novembro 2014

18.ª Reunião Anual

Sociedade Portuguesa de Genética Humana

19 a 21 de Novembro de 2014

Edifício Egas Moniz
Faculdade de Medicina da Universidade de Lisboa
Lisboa, Portugal

A Sociedade Portuguesa de Genética Humana (SPGH), www.spgh.net, criada em 1996, tem como missão a promoção, desenvolvimento e divulgação da investigação e da prática em Genética Humana, e, em particular em Genética Médica.

Neste sentido, uma das suas atividades importantes é a organização do congresso anual, que tem como objetivo contribuir para a difusão de conhecimentos especializados na área da Genética Humana, facilitando o contacto dos geneticistas entre si, com outros especialistas e com a sociedade em geral. Este congresso constitui um fórum de discussão científica onde é possível assistir a palestras de investigadores nacionais e internacionais de renome e à apresentação de trabalhos de grupos de investigação de todo o país. A reunião pretende ainda dar um destaque especial ao trabalho de jovens investigadores, com a atribuição de prémios à melhor publicação do ano anterior por um autor português, assim como aos melhores trabalhos em investigação básica e investigação clínica apresentados em comunicação oral. Este ano a 18ª reunião realiza-se na Faculdade de Medicina da Universidade de Lisboa e o programa incide sobre: New challenges in pre-natal diagnosis; Paramyloidosis: advance in care; Cancer breaking news; Hemoglobinopathies in Portugal; New approaches in rare diseases; Genomics and aspects bioethics. Como habitual, teremos conferências de especialistas nacionais e internacionais, assim como comunicações orais e apresentação de posters. Esperamos que, com a sua participação, a reunião seja animada por trabalhos inovadores e discussão científica, e seja uma fonte de inspiração para os progressos científicos dos próximos anos.

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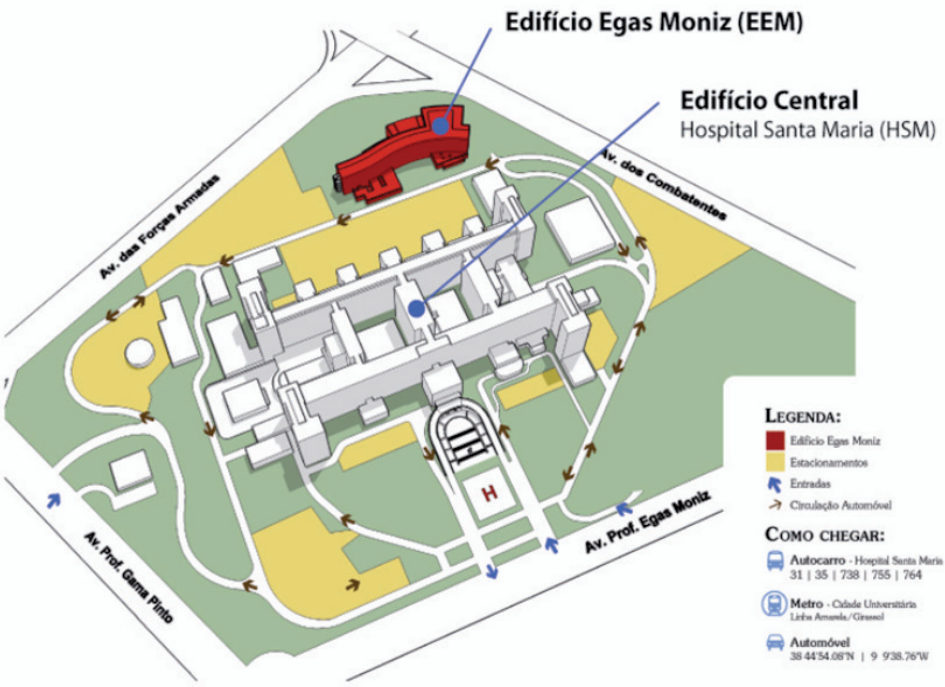

PROGRAMA | *PROGRAM*

18th Annual Reunion | 19 – 21 November 2014

Venue | *Egas Moniz Building, Faculty of Medicine of the University of Lisbon (FMUL) – Portugal*

Day 1 19.Nov.2014 (Wednesday)

Clubs: Parallel Sessions

16:00 – 17:45

Cytogenetics and molecular genetics

Microarray analysis and Next Generation Sequencing(NGS): Strategies of analysis, results and discussion

Chairs | **João Gonçalves**
Filomena Brito

High resolution array: Clinical application
Paula Rendeiro, Laboratório de Citogenética, CGC - Centro de Genética Clínica, Porto

Cytogenetic Laboratory microarray experience: case reports presentation
Bárbara Marques, Departamento de Genética Humana, Instituto Nacional de Saúde Dr. Ricardo Jorge (INSA), Lisboa

Analysis of multi-gene panels using NGS: change of paradigm in hereditary colorectal cancer syndromes
Inês Francisco e Cristina Albuquerque, Unidade de Investigação em Patobiologia Molecular, Instituto Português de Oncologia de Lisboa Francisco Gentil, EPE, Lisboa

Molecular analysis of the CFTR gene by NGS
Susana Fernandes, Departamento de Genética, Faculdade de Medicina da Universidade do Porto Centro Hospitalar de São João, EPE, Porto

Medical genetics and clinical dysmorphology

Rasopathies

Chairs | **Oana Moldovan**
Patrícia Dias

17:45 – 18:15

Target Seq (AmpliSeq™), the example of Colon and Lung Cancer
Presenters| **Juan Barba and João Caldeira, Life Technologies, brand of Thermo Fisher Scientific**

End of Day 1

Day 2	20.Nov.2014 (Thursday)
08:00 – 08:45	*Registration form*
08:45 – 09:00	***Opening Ceremony | SPGH 18th Annual Reunion*** **Hildeberto Correia, Juliette Dupont and José Pereira Miguel**
09:00 – 09:45	**From Pharmacogenetics to Ecogenetics: Past, Present and Future Challenges** **Manuel Bicho,** Laboratório de Genética e Instituto de Saúde Ambiental da Faculdade de Medicina da Universidade de Lisboa (FMUL), Portugal
09:45 – 10:15	*Coffee-break / Poster Session*
	1st. Scientific session – New challenges in Pre-natal Diagnosis *Chairs* | **Paula Caetano and Isabel Marques Carreira**
10:15 – 10:45	**Non-invasive prenatal diagnosis using maternal blood as performed in a clinical genetics department: Over a decade of translational research** **Ana Bustamante,** Servicio de Genética, Hospital Universitario Fundación Jiménez Díaz, Instituto de Investigaciones Sanitarias, FJD, Madrid, Spain
10:45 – 11:15	**From the conception to Prenatal testing: a technical revolution to change the Reproductive Medicine** **Marcus Hausch,** ILLUMINA EMEA RGH Marketing Manager
11:15 – 11:45	**Why Genotype Information Matters in Prenatal SNP Array Diagnostics** **Nicole de Leeuw,** Department of Human Genetics, Radboud University Medical Center, Nijmegen, the Netherlands
11:45 – 12:15	**Dilemmas in prenatal diagnosis** **Ana Berta,** Serviço de Genética Médica, Hospital de Santa Maria – Centro Hospitalar Lisboa Norte (HSM-CHLN), Portugal
	2nd. Scientific session – Paramyloidosis: Advance in Care *Chairs* | **Lina Ramos and Jorge Sequeiros**
12:15 – 12:45	**TTR - FAP: clinical and genetic aspects in non-endemic areas** **David Adams,** French Reference Center for FAP (NNERF), Paris, France
12:45 – 13:00	**TTR - FAP: therapeutic approach** **Isabel Conceição,** Serviço de Neurologia, Hospital de Santa Maria – Centro Hospitalar Lisboa Norte (HSM-CHLN), Portugal
13:00 – 14:00	*Lunch / Poster Session*
	3rd. Scientific session – Cancer breaking news *Chairs* | **Manuel Teixeira and Glória Isidro**
14:00 – 14:30	**Hemato-oncological diseases – New therapies** **Cristina João,** Instituto Português de Oncologia de Lisboa Francisco Gentil (IPOLFG), Portugal
14:30 – 15:00	**Genes and neuroblastoma – hand in hand in therapeutic advances in Europe (developments within SIOPEN)** **Cláudia Constantino and Ana Lacerda,** Instituto Português de Oncologia de Lisboa Francisco Gentil (IPOLFG), Portugal
15:00 – 15:30	**Using genomics for drug discovery in medulloblastoma** **Cláudia Faria,** Serviço de Neurocirurgia, Hospital de Santa Maria – Centro Hospitalar Lisboa Norte (HSM-CHLN), Portugal
15:30 – 16:00	*Coffee-break / Poster Session*
	Oral Presentations *Chairs* | **Sofia Dória and Astrid Vicente**
16:00 – 17:30	**8 selected presentations from submitted abstracts** (8 min for each presentation and 2 min for discussion)
18:00	***SPGH General Assembly***
20:30	*Conference dinner (restaurant)*
	End of Day 2

Day 3	21.Nov.2014 (Friday)
	4th. Scientific session – Hemoglobinopathies in Portugal *Chairs* | **Paula Faustino and Luísa Romão**
09:00 – 09:30	**Epidemiology, pathophysiology and genetic modifiers of morbidity** **João Lavinha,** Departamento de Genética Humana, Instituto Nacional de Saúde Dr. Ricardo Jorge (INSA), Lisboa, Portugal
09:30 – 10:00	**Strategies for prevention: Contribution of the laboratory** **Armandina Miranda and João Gonçalves,** Instituto Nacional de Saúde Dr. Ricardo Jorge (INSA), Lisboa, Portugal
10:00 – 10:30	**Treatment – current clinical management and future prospects** **Alexandra Dias,** Hospital Prof. Doutor Fernando Fonseca (HFF), Amadora, Portugal
10:30 – 11:00	*Coffee-break / Poster Session*
	5th. Scientific session – New approaches in Rare diseases *Chairs* | **Ana Fortuna and Jorge Saraiva**
11:00 – 11:30	**Gene packages and opening the exome – the Nijmegen experience** **Ilse Feenstra ,** Department of Human Genetics, Radboud University Medical Center, Nijmegen, the Netherlands
11:30 – 12:00	**Causes of rare and recurrent disorders unravelled by array** **Nicole de Leeuw,** Department of Human Genetics, Radboud University Medical Center, Nijmegen, the Netherlands
12:30 – 14:00	*Lunch / Poster Session*
	Chairs | **Margarida Reis and Maria Rosário Almeida**
14:00 – 14:45	**Genetic complexity of the inherited cardiomyopathies in the high-throughout sequencing era - from research to clinical practice** **Luís Lopes,** Hospital Garcia de Orta (HGO), Almada, Portugal
	Oral Presentations *Chairs* | **Susana Mendes and Oana Moldovan**
14:45 – 16:00	**6 selected presentations from submitted abstracts** (8 min for each presentation and 2 min for discussion)
16:00 – 16:30	*Coffee-break / Poster Session*
	6th. Scientific session – Bioethics ***SPGH Ethics Commission*** *Chairs* | **Carolino Monteiro and Célia Ventura**
16:30 – 17:30	**Reflexões bioéticas e recomendações sobre os Rastreios ao Recém-Nascido em Portugal** **Luísa Diogo,** Hospital Pediátrico de Coimbra / Faculdade de Medicina da Universidade de Coimbra (FMUC), Coimbra, Portugal **André Pereira,** Faculdade de Direito da Universidade de Coimbra (FDUC),Coimbra, Portugal **Heloísa Santos,** President of SPGH Bioethics Comission, Lisboa, Portugal
	SPGH Awards *Chairs* | **Hildeberto Correia and Juliette Dupont and Maria do Céu Machado**
17:30 – 18:30	**Award Conference** **Basic and Clinical Research Awards**
18:30	***Close of Conference | SPGH 18th Annual Reunion*** **Hildeberto Correia, Juliette Dupont and Maria do Céu Machado**
	End of the Reunion

Índice

PROGRAMA CIENTÍFICO
SCIENTIFIC PROGRAM

PALESTRAS
LECTURES

From Pharmacogenetics to Ecogenetics: Past, Present and Future Challenges

Manuel Bicho
Laboratório de Genética e Instituto de Saúde Ambiental da FMUL
Instituto Rocha Cabral, Lisboa

Fifty seven years ago, an article of Arno Motulsky entitled "Drug reactions, enzymes and biochemical genetics." published in JAMA, summarized the scientific developments in biochemical genetics which explained the inborn errors of metabolism and the chemical individuality of A. Garrod fifty years before. The targets were two adverse drug reactions (ADRs): drug induced hemolytic anemia due to G6PD deficiency and suxamethonium-induced apnea due to pseudocholinesterase deficiency. This report delineated and conceptualized the field of Pharmacogenetics, a word coined two years later by Friedrich Vogel (1959), and was established by Werner Kalow (1962) as a novel discipline that deals with the role of genetic variation affecting drug response or adverse reactions to drugs.

In the sixties, Vesell F. and Page J. (1968) showed in identical compared with fraternal twins the polygenic metabolic disposal of drugs (ex. Phenylbutazone). At the same time, in Portugal, C. Manso and Lesseps Reys described polymorphic variants of G6PD and Reys in 1971 defended a thesis with the title "Pharmacogenetics studies in Mozambique".

Meanwhile, the term ecogenetics was coined by Brewer in 1917 to extend the concept of the role of genetic variation in response to xenobiotics and to environmental agents other than drugs (ex. Nutrients, microorganisms and UVs). Evans, in the last seventies, began to study the role of acetylation polymorphisms using pharmacological probes (sulfamethazine) as a genetic susceptibility factor for several diseases (breast and bladder cancer and SLE) initiating the era of genetic association studies.

However, the development of pharmacogenetics over the years remained low, since relatively few drug responses or adverse drug reactions were under control of single genes and the effects of these were only possible to be studied at the intermediate phenotypic levels at that time.

The increasing availability of DNA Technology and in vitro molecular tests (PCR) advanced extraordinarily the field and the term pharmacogenomics was introduced in the nineties of XX century with the emergence of the Human Genome Project and the development of "omic" sciences in the first decade of XXI century.

At the present, most of the Clinical Biochemistry Laboratories providing molecular tests utilize robust PCR with high capacity instrumentation and the use of mass spectrometry confluent in microarrays. These technologies allowed search for multiple genes and there expression affecting drug responses. As consequence of these high throughput technologies, noticeable advances in identifying new biomarkers, by making use of so called layered approach of molecular technologies (systems biology), provides an integrated multibiological

level dissection including the genomics, epigenomics, proteomics, metabolomics and the organism itself (molecular imaging).

One of the results of this approach was, in epidemiologic terms, the GWAS (genome wide association studies) for discover of new variants and more recently mendelian randomization.

However, some challenges are raised for the future, namely the identification of epigenomic biomarkers, its validation in conjunction with the others in the omic perspective. Another challenge is the development of bioinformatic tools required to be used in personalized identification of risk for diseases and of its subphenotypes and the response to nutrients, xenobiotics, drugs and microorganisms that are some of the environmental causes of diseases.

1ª SESSÃO | *1ST. SCIENTIFIC SESSION*
NEW CHALLENGES IN PRE-NATAL DIAGNOSIS

Non-invasive prenatal diagnosis using maternal blood as performed in a clinical genetics department: Over a decade of translational research

Ana Bustamante
Servicio de Genética, Hospital Universitario Fundación Jiménez Díaz, Instituto de Investigaciones Sanitarias, FJD, Madrid, Spain

Cell-free fetal DNA (ccffDNA) in maternal plasma makes it possible to perform non-invasive prenatal diagnosis (NIPD) without risk of fetal loss. NIPD is a challenge for fetal medicine and for prenatal diagnosis units, and a great deal of effort has been focussed on this field. Although different NIPD tests have been gradually incorporated into clinical practice such as fetal sex and fetal RhD determination, aneuploidy diagnosis of the most common aneuploidies has been the main goal in NIPD. Therefore, a number of large research groups and biotechnology companies have devoted a great deal of work and investment toward this aim. In 2011, and thanks to the incorporation of next-generation sequencing technology, the first commercial test to screen for the most common aneuploidies in maternal blood was launched. Since then, NIPD has become more present in prenatal units.

NIPD for single-gene disorders has attracted less interest because the target diseases affect a smaller population. Moreover, in most cases the need for personalized designs for the familial defect makes methods not easily scalable. In 2013, panels for screening of the most common mutations associated with cystic fibrosis or skeletal dysplasias were developed in the UK. Although NIPD of different single-gene diseases have been widely described in many research studies, a diagnostic (not screening) method has yet to be translated into routine clinical practice.

NIPD in a clinical genetics unit

NIPD research began in our unit in 1997, making it the first research group of its kind in Spain. As a clinical department, the aim of this research is the development and translation of new NIPD studies into routine clinical practice. Experience with routine prenatal diagnosis allows us to evaluate patient needs and aspects lacking from clinical practice. When evaluating new methods, accuracy, ease of handling, and affordability are key criteria.

Our NIPD research was initially aimed at isolating and analyzing circulating fetal cells in maternal blood. Preliminary studies yielded fetal sex determination and aneuploidy detection of chromosomes 13, 18, and 21. However, the tedious handling and the scarcity of circulating fetal cells prompted us to move into analysis of ccffDNA in 2001. In 2008, fetal sex determination was incorporated into clinical practice for pregnancies at risk of sex-linked disorders. One year later (2009), fetal RhD determination was also made routinely available. In 2012, non-invasive prenatal aneuploidy screening was offered in our hospital as an outsourcing study.

As a center of reference for genetic diagnosis of several monogenic disorders, NIPD of single gene diseases has been our main aim. Our experience comprises a total of 51 cases including 25 cases of Huntington disease, 6 cases of cystic fibrosis, and 1 case each of retinitis pigmentosa, propionic acidemia, epidermollisis bullosa, among others. However, our studies have been limited to the analysis of *de novo* or paternally inherited fetal mutations/alleles. Analysis of maternally inherited fetal alleles is more challenging and requires more novel technologies such as digital PCR and NGS. We are currently exploring the use of the digital PCR (recently acquired) to explore its potential for NIPD of monogenic disorders regardless of parental origin.

NIPD is a reality in many prenatal diagnosis units. At present, only a limited number of pregnancies can benefit from the technique, although our goal is to apply translational research in such a way that more patients will have access to the procedure in the near future.

From the conception to Prenatal testing: a technical revolution to change the Reproductive Medicine

Marcus Hausch
ILLUMINA EMEA RGH Marketing Manager

Why Genotype Information Matters in Prenatal SNP Array Diagnostics

Nicole de Leeuw
Department of Human Genetics, Radboud University Medical Center, Nijmegen, The Netherlands

Since 2010, we routinely perform genome wide SNP-based array analysis after a normal QF-PCR test result in prenatal diagnosis in case of structural ultrasound anomalies or intra uterine foetal death. The genotype information from the SNP probes not only significantly improves the diagnostic yield, but it also enhances the quality of the diagnostic laboratory workflow. An overview will be given and various illustrative examples will be shown demonstrating the reliable detection of CNVs, more mosaic imbalances as well as copy neutral changes of homozygosity leading to the subsequent identification of pathogenic mutations in recessive disease genes or uniparental disomies (UPD).

Dilemmas in prenatal diagnosis

Ana Berta Sousa
Serviço de Genética Médica, Hospital de Santa Maria – Centro Hospitalar Lisboa Norte, Portugal

2ª SESSÃO | *2ND. SCIENTIFIC SESSION*
PARAMYLOIDOSIS: ADVANCE IN CARE

TTR - FAP: clinical and genetic aspects in non-endemic area

David Adams
French Reference Center for FAP (NNERF), Paris, France Department of Neurology CHU Bicêtre (APHP) INSERM, david.adams@bct.aphp.fr

Introduction : Transthyretin-Familial Amyloidosis (TTR-FAP) is a progressive, disabling, irreversible and life-threatening neuropathy due to a point mutation of TTR gene with an autosomal dominant transmission. TTR-FAP has been described 60 years ago in Povoa de Varzim, but only 30 years later in France. Contrarily to Portugal with high endemic area in the north due to Val30Met TTR variant, France is a non endemic country. A French reference center for FAP (NNERF) supporting 300 cases has been labelized in Paris in 2005 by the French Health Ministry.

Objectives : Missions of the center were to improve the diagnosis of disease and the care of patients at the national level. A national network for FAP (CORNAMYL) was built in conjunction with 10 other regional reference centers for neuromuscular diseases in 2010.
To report genotypic and phenotypic varieties of FAP in France in 2008-2014 period and the sensitivity of the tools for diagnosis. All patients carried amyloidogenic TTR gene mutations and Congo positive amyloid deposit (CPAD).

Results : In the 2008-2014 period: 180 new TTR-FAP cases were identified, bringing to 81/100 the number of geographic departments affected, and to 41 the number of TTR gene mutations. The most common mutations are Val30Met (60%), Ser77Tyr, Ser77Phe and Ile107Val. Mean age was 60 years (22-89) with a late onset (≥ 50 y) in 69%. Sex ratio: 2.16. A positive family history of FAP was found in 55% and a Portuguese origin in 18.3% of cases. The diagnosis of FAP was delayed by 2.93y years (0.2-13.5) after first symptoms; 69% had a walking disability including 39% requiring aid.
Five phenotypes were identified: Small Fibers Length-Dependent Polyneuropathy (PNP) (43%), All-Fibers PNP (25%), Upper Limbs Onset -NP (17%), Ataxic NP (14%), Motor NP (2%). CPAD were found in Labial Salivary Gland Biopsy in 91/128 pts (71%) and after nerve biopsy in 19/26 pts (73%), 76% required multiple biopsies.

Conclusions : The French reference Center and network for TTR-FAP allowed to identify new TTR-FAP cases in most of geographic departments with varied phenotypes. The larger and earlier use of TTR gene analysis in progressive and idiopathic polyneuropathy cases is useful to accelerate diagnosis of TTR-FAP.
The other goals of National Reference Center and of the national network for FAP are to get access to an anti-amyloid therapy or to clinical trials in most patients, to propose the best genetic counseling and to improve earlier diagnosis in TTR mutated gene carriers.

Varied phenotypes 3 nvx; Age onset; Sporadic; Misleading diagnosis; Genetic heterogeneity

TTR - FAP: therapeutic approach

Isabel Conceição
Department of Neurosciences, CHLN- HSM. Lisbon, Portugal.
Translational and Clinical Physiology Unit. Instituto de Medicina Molecular, Faculty of Medicine. Lisbon, Portugal.

Familial amyloidotic polyneuropathy (FAP) is a fatal, autosomal dominant disease caused by aggregation and organ deposition of mutant and wild-type transthyretin (TTR) amyloid fibrils (ATTR). Almost all circulating TTR is synthesized by hepatocytes, and in FAP this liver-derived TTR is responsible for ATTR accumulation in target organs, including peripheral nerves, heart, kidney and gastrointestinal tract.

Liver transplant is the current standard of care for patients with TTR-FAP through elimination of hepatic production of mutant TTR. Slow disease progression and prolong survival but it is associated with a first-year mortality of 10 % and substantial morbidity due to chronic immunosuppression. Liver transplant does not prevent clinical deterioration (heart, ocular and CNS complications) in all recipients, underscoring the need for new treatment approaches.

Elucidation of the mechanisms contributing to TTR misfolding and fibril formation identified TTR-tetramer stabilization as a rate-limiting event, leading to the development of several new pharmacologic therapies for patients with TTR-FAP. TTR stabilizing agents (Tafamidis, Diflunisal) can be prescribed at an early stage of disease in anticipation of liver transplantation or, potentially, delaying the need for liver transplant.

Other strategies are emerging (combination doxycycline–tauroursodeoxycholic acid, small interfering RNA, antisense oligonucleotide). Ongoing clinical trials investigating several pharmacotherapies are described.

3ª SESSÃO | *3RD.SCIENTIFIC SESSION*
CANCER BREAKING NEWS

Hemato-oncological diseases − New therapies

Cristina João
Hematologist, Instituto Português de Oncologia de Lisboa Francisco Gentil
Professor, Immunology -Faculdade de Ciências Médicas, Universidade Nova de Lisboa

The last decade handed to onco-hematologists a great number of new drugs helping them to more effectively treat hemato-oncology diseases.

In this conference, I choose some of these new effective drugs and will present theirs different mechanism of action, effect and toxicities. I will present some of the new antibodies approved, immunomodulators, proteossoma inhibitors, small molecules with direct intra-cellular effect as kinase inhibitors and chimeric antigen receptor transduced T-cells (CARTs). These molecules combine high efficacy with a good safety profile.

I will present on a new glycoengineered type II humanized anti-CD20 mAb, obinutuzumab (GA101), that has been developed and demonstrates increased activity against B-cell malignancies by inducing direct cell death and better antibody-dependent cellular cytotoxicity. Immune checkpoint blockade with anti-CTLA4 mAb and anti-PD-1 mAb has also demonstrated clear evidence of objective responses including improved overall survival and tumor shrinkage, driving renewed enthusiasm for cancer immunotherapy in multiple cancer types. In addition, there is a promising novel cancer immunotherapy, CAR therapy—a personalized treatment that involves genetically modifying a patient's T-cells to make them target tumor cells. Also, molecules as lenalidomide and pomalidomide, comprising immunomodulator effects and molecules with anti-proteossoma effect are successfully used to treat B-cell malignancies as multiple myeloma and B cell lymphomas.

We are now facing new era of cancer immunotherapy and in this presentation I will give you a glimpse of what hemato-oncologists may wait from these new and powerful molecules.

Genes and neuroblastoma − hand in hand in therapeutic advances in Europe (developments within SIOPEN)

Cláudia Constantino and Ana Forjaz de Lacerda
Instituto Português de Oncologia de Lisboa Francisco Gentil (IPOLFG), Portugal

Neuroblastoma is the most common solid cancer in children after brain tumors, typically affecting young children and with a varied clinical outcome. It serves as a paradigm for applying tumor genomic data for determining patient prognosis and thus for treatment allocation. According to the International Neuroblastoma Risk Group, patients can be classified as low-, intermediate-, and high-risk for relapse based upon age at diagnosis, disease stage (localized resectable, localized unresectable or metastasized), histo-pathology, tumor cell ploidy (DNA index) and genetic profile. Additionally, genomic information is also essential for biology-based treatment options in high-risk patients.
MYCN status (amplified vs. non- amplified), was one of the very first biomarkers in oncology, discriminating aggressive from less aggressive clinical courses of neuroblastoma. However, it is currently far from being the only genetic change with prognostic value.

Frequently, adjacently located genes, like DDX1, NAG (NBAS) or, more rarely, the proximally located ALK gene (less frequently involved), are co-amplified with MYCN. Information about ALK amplification/mutation may be of interest when looking for alternative treatment strategies such as the use of ALK inhibitors. In addition, genes located on chromosome 12q, like MDM2 and CDK4 (which may be amplified in the presence or absence of the MYCN gene), may indicate a subset of tumors with a unique clinical behavior.

Segmental chromosomal aberrations (SCA), i.e. gains or losses of chromosomal fragments (spanning from at least 3 Mb to a complete arm of a chromosome), can also indicate tumor aggressiveness, having repeatedly been found in tumors with unfavorable behavior even in the absence of MYCN amplification. Deletions at the short arm of chromosome 1, especially of 1p36.3, were among the first genomic aberrations described in neuroblastoma; however, as it is frequently associated with MYCN amplification, it lost importance as a prognostic marker. Conversely, the deletion at 11q has emerged as a powerful outcome biomarker, since it occurs predominantly in tumors without MYCN amplification. Another prominent, frequent and ominous SCA is the unbalanced gain of the long arm of chromosome 17. Additionally, deletions at the chromosomal regions 3p, 4p, 9p, and 12p, which occur at lower frequencies, may also have prognostic impact.

Recently, new types of DNA based aberrations influencing the clinical behavior of neuroblastomas have been described. Deletions or mutations of genes like ATRX and a phenomenon referred to as “chromothripsis” are assumed to correlate with unfavorable outcomes. “Chromothripsis” represents a shredding of single chromosomes or parts thereof and subsequent random reassembly of the fragments; the ensuing breakpoints can affect genes known to be involved in translocation processes and, in addition, a chromothripsis chromosome can also bear amplified regions.

In neuroblastoma patients, information on the genomic profile of the tumor has become an indispensable tool for adequate treatment planning. For on-going and future studies, the application or implementation of technologies that provide a comprehensive picture of the tumor cell genome is essential. The increased use of high resolution SNParrays will allow new insights into the genomic composition of tumors. When combined with other techniques, as targeted sequencing, it is expected it will further uncover neuroblastoma secrets. FISH techniques, which rapidly determine the *MYCN* status at the cellular level and enable the detection of intratumoral *MYCN* heterogeneity, are expected to remain in use.

Using genomics for drug discovery in medulloblastoma

Cláudia Faria
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Medulloblastoma is the most common malignant brain tumor in childhood and accounts for around 10% of all pediatric cancer deaths. Current therapies include surgical resection, craniospinal radiation (in children older than 3 years of age) and high-dose chemotherapy. Despite overall survival rates that can reach 80%, the majority of survivors suffer from long-term side effects induced by cytotoxic therapies to the developing central nervous system. High-throughput genomic studies have recently shown that medulloblastoma comprises four molecular subgroups - wingless (WNT), sonic hedgehog (SHH), Group 3 and Group 4 – with distinct demographics, genetics and clinical outcome. Therefore, there is a need to improve the existing treatment protocols and to develop novel targeted therapeutic approaches.

The hepatocyte growth factor (HGF)/cMET signaling pathway has been associated with tumor aggressiveness and dissemination in several human cancers. In medulloblastoma, cMET activation has been associated with tumor growth and invasion. To determine the subgroup-specific role of cMET in medulloblastoma we analyzed several large non-overlaping cohorts of medulloblastoma patients and determined that cMET is highly expressed, both at the transcriptional and at the protein level, in SHH-driven tumors. In pediatric SHH medulloblastomas, cMET activation correlates with increased tumor relapse and poor survival, thus defining a subset of patients that may benefit from cMET targeted therapy.

In support of this hypothesis, we found that foretinib, an FDA approved cMET inhibitor, could suppress cMET activation, decrease cell proliferation and induce apoptosis in SHH medulloblastomas *in vitro* and *in vivo*. Treatment of mouse intracranial xenografts and of an aggressive transgenic mouse model of metastatic SHH medulloblastoma with foretinib reduced primary tumor growth and invasion, decreased the incidence of metastases and increased survival.

Based on genomic data, our studies identified a novel small molecule inhibitor to treat SHH medulloblastomas thus providing a strong rationale to evaluate foretinib into clinical trials for this subset of patients.

4ª SESSÃO | *4TH. SCIENTIFIC SESSION*
HEMOGLOBINOPATHIES IN PORTUGAL

Hemoglobinopathies in Portugal: Epidemiology, pathophysiology and genetic modifiers

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In the context of this presentation, "hemoglobinopathy" refers to any genetic defect that results in abnormal structure and/or level of one of the globin chains of the hemoglobin molecule throughout development. As clinical entities the hemoglobinopathies are chronic hemolytic anemias usually inherited as autosomal recessive Mendelian traits. The most common hemoglobinopathies in the Portuguese population are sickle cell disease (SCD), beta-thalassemia and alpha-thalassemia. Their epidemiology, including the molecular epidemiology, reflects a long history of gene flow, mainly from Mediterranean and Sub-Saharan African populations, coupled with the persistence of the selective pressure of malaria until the 1950's. The difference in the molecular basis of SCD and the thalassemias explains most of the distinct pathophysiology of the two conditions: whereas HbS polymerization results in sickled, rigid and sticky erythrocytes prone to aggregation, the unbalanced globin chain synthesis in thalassemia originates the intracellular deposition of excess globin chains and ineffective erythropoiesis. The hallmarks of the corresponding clinical course are recurrent vaso-occlusive crises and infection in SCD, and bone marrow hyperplasia and iron overload in the thalassemias. However, both conditions present a marked clinical heterogeneity only partially explained by (physical and social) environmental differences. Thus, there is still a large knowledge gap to be filled in their etiopathogenic architecture, part of which is of genetic or epigenetic nature. In this presentation, I will discuss the current views on the genetic factors proposed to modulate the hemoglobinopathy phenotype, in addition to the major determinant, the mutated globin gene. Proximal and distal genetic modifiers, acting in cis or in trans to the globin gene clusters within their chromatin microenvironment will be considered.

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Hemoglobinopathies in Portugal and strategies for prevention: Contribution of haematology and biochemistry laboratory

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The hemoglobinopathies are inherited monogenic autosomal recessive disorders resulting from mutations affecting genes responsible for synthesis of the globin chains of haemoglobin, or their regulatory regions. They can be classified, into two main groups: the thalassemias resulting from reduced or no synthesis of one or more globin chains and the haemoglobin variants arise from an alteration in the globin protein structure. Sickle-cell disease and β-thalassemia are the most common human monogenic diseases and represent a serious public health problem in many regions of the world.
The most effective way to control this disease is prevention through the detection and identification of carriers of hemoglobinopathies, genetic counselling of couples at risk, and when necessary, the provision of prenatal diagnosis. To be aware the prevalence of hemoglobinopathies in Portugal several studies were conducted. The results indicated that the prevalence is about 2% [1,2,3]. The highest prevalence (more than 5%) was observed in the Mira River Basin and the Western Algarve (Barlavento) for carriers of β-thalassemia, and in Coruche, Alcácer do Sal (lower courses of the rivers Tejo and Sado) for carriers of Hb S [1,2].

The hemoglobinopathies laboratory performs the diagnosis screening and confirmation in collaboration with Public Health Laboratories and other health care entities, in order to prevent the onset of severe forms of hemoglobinopathies.
Hemoglobinopathies are possibly unique amongst all genetic diseases in that which carriers identification is possible (and preferable) by haematological (biochemical) tests rather than DNA analysis. Variant haemoglobins of clinical relevance are Hb S, Hb D^{Punjab}, Hb C, Hb E, Hb Lepore e Hb O^{Arab}, since they confer greater risk when associated with other specific alleles [4]. Presumptive identification of haemoglobin variants requires performing a minimum of two techniques based on different principles. The MCH below 27 pg and the Hb A_2 above 3.5 % make the β thalassemia trait diagnosis.

The screening is directed to detect carriers of thalassemia and haemoglobin variants. To do so, we use first line methods, the red cell indices (with morphology) and isoelectric focusing of haemoglobin. The second line tests involve chromatographic techniques, including the

study of globin chains by reversed-phase high-performance liquid chromatography (HPLC-RP) and functional tests such as the solubility test for Hb S.
HPLC-RP of human globin chains is an important tool in phenotype study of haemoglobin disorders for the detection and presumptive characterization of haemoglobin variants. It was found to be of special value to detect neutral variants.

The cases diagnosed by the laboratory between 2010 and 2013 indicate that β-thalassemia and Hb S trait are the most frequent observed. The regions of Algarve, Alentejo and Lisbon, still are the main origin of hemoglobinopathies. Nevertheless it has been detected several cases in the northern region (including β-thalassemia and Hb Lepore). Furthermore the relatively recent population migration, particularly from Brazil and Eastern Europe (where for example the variant Hb O^{Arab} is frequent [5]) make us expected that the detection of hemoglobinopathies will be a problem not exclusively restricted to certain regions, and should therefore be considered across all country [6].
Also concerning prevention, the INSA laboratory promotes health care professional training/education, bearing in mind the promotion of quality results, improvement in performance and in last direct benefit to the population.

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Hemoglobinopatias em Portugal e estratégias de prevenção: Contributo da genética molecular

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O estudo das hemoglobinopatias em Portugal permitiu estabelecer, há já duas décadas, a prevalência de portadores de beta talassémia e de drepanocitose nas diferentes regiões do país[1]. Ficou a saber-se que as mesmas distribuem-se essencialmente pelo centro e sul do país, apresentando tanto a beta-talassémia como a drepanocitose uma prevalência global de 1-2%. Contudo, existem regiões onde a prevalência pode atingir valores de 5 a 10% (ex. beta-talassémia no barlavento Algarvio, drepanocitose nas bacia dos rios Tejo e Sado e alguns concelhos da região de Lisboa onde existe um maior número de residentes de origem africana). Complementarmente, os estudos de genética molecular da beta-talassémia revelaram que existem quatro mutações mais frequentes[2], as quais também predominam nos países da bacia do mediterrâneo. Contudo, face aos movimentos migratórios globais, ao diagnóstico e investigação contínuos, na nossa população também existem alterações clinicamente relevantes que, sendo relativamente raras em Portugal, são frequentes noutras regiões/países (p. ex. HBB: c.364G>C (HB D Punjab); HBB:c.364G>A (Hb O-Arab). Estes estudos, em conjunto com o aconselhamento genético, contribuem de forma continuada para a prevenção das referidas doenças hereditárias em Portugal, permitem alargar o rastreio hematológico e bioquímico a familiares dos casos índex, identificar portadores assintomáticos e principalmente detetar novos casais em risco de terem descendência afetada com qualquer uma das formas mais graves de hemoglobinopatias (drepanocitose, talassémia major e intermédia e talassodrepanocitose), para as quais está indicado/pode ser oferecido o diagnóstico pré-natal molecular. Dado serem doenças autossómicas recessivas, o risco de cada casal ter, em cada gravidez, descendência afetada é de 25%. Assim, conhecendo-se as alterações moleculares de cada elemento de cada casal, cumpre-se o objetivo primordial do Programa Nacional de Controlo das Hemoglobinopatias (ativo durante vários anos em Portugal), ou seja, pode atuar-se de forma a prevenir o surgimento de novos indivíduos com a doença em causa. A referida prevenção fundamenta-se no diagnóstico clínico junto das populações, no diagnóstico hemotológico e bioquímico, na investigação molecular, no estabelecimento de testes genéticos fiáreis e no aconselhamento genético, culminando estas atividades tanto na identificação da natureza da alteração como na identificação de portadores e de casais em risco.

O diagnóstico molecular de hemoglobinopatias, atualmente tem por base a PCR (amplificação enzimática de DNA) através da pesquisa direta das mutações mais frequentes na população ou identificadas no caso índice ou na família, recorrendo por exemplo a ARMS, restrição enzimática ou através da sequenciação do gene *HBB*, complementadas quando aplicável, com a pesquisa de deleções mais ou menos extensas por GAP-PCR ou por MLPA. A identificação molecular da alteração patogénica no gene beta-globina, beta zero(β^0) (se não há síntese de cadeias beta-globina), β^+ (se ocorre uma redução significativa da síntese de cadeias beta-globina), ou a identificação de alterações que dão origem a cadeias globínicas funcionalmente anormais, constitui a ferramenta indispensável para disponibilizar o DPN a casais e famílias e, para que a prevenção em causa se torne efetiva.

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Treatment − current clinical management and future prospects

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Hemoglobinopathies (genetic diseases of hemoglobin, Hb) are among the most common inherited diseases around the world (the most common monogenic ones) and are a world's major public health issue in today's multiethnic world population - even in many parts of Europe, Hb defects are classified as endemic diseases.
They fall into two main groups: thalassemia syndromes (main types: α- and β thalassemia) and structural Hb variants (main abnormal variants: HbS, HbE and HbC) - subtypes and combined types contribute to a marked clinical heterogeneity. Their highly variable phenotipical presentation include: no clinics and even no laboratory manifestations, mild hypochromic anemia, moderate hematological disease, severe lifelong transfusion-dependent anemia with multiorgan involvement and disfunction.

Adequate and effective care of the affected patients requires a wide variety of diagnostic and therapeutic measures, a health team work, and mostly an individual and qualitative approach (identify and explore influences, vulnerability factors and self-care management resources), prevention focused.
Optimized treatment can give patients a steadily-increasing expected life span, but implies an interest in life quality and health outcomes: events free time, social integration and well being have to be considered as main goals in successful aging. Mortality has steadily decreased in the last 30 years, but morbilities are heavy and severe - even major hemoglobin diseases are becoming a concern of adult medicine, rather than pediatrics alone, and they have to be considered chronic diseases (diabetes like), with all the implications. This health model transition involves sociocultural, behavioural, and health service factors (health care systems with a certain level of functionality - budgets, professionals and technical resources and organization).

Improving nutrition, folic acid supplementation, immunization and antibiotic prophylaxis, physical adequate activities, sleep patterns, educational support, professional guidance and psychological support are main gold standards in medical care of these chronic diseases – most, if not all, severely affected people, should survive, but they should also be well integrated individuals in family and social environment.

A strong investment in anticipatory measures is fundamental: periodic vigilance of organ functions is a heavy burden for health teams and patients – in time and resources - but allows on time interventions, with best results in safeguarding functions and futures.
In all forms, being hemolitic anemias, iron supplements are contraindicated (except in cases of simultaneous and well documented iron deficiency), and colecistectomy and splenectomy may be considered.

For the severe forms of thalassemia, hematopoietic stem-cell transplantation is the prefered curative treatment, if a donor can be found – supportive treatment includes regular red cell transfusions for life, combined with effective iron removal and hemosiderosis-related organ damage specific care.
Sickle-cell disease poses a particular challenge in terms of tractability: drugs to treat the signs and symptoms include analgesics (for pain), antibiotics (for infections), angiotensin conversor enzyme inhibitors (for cardiovascular and renal manifestations) and hydroxycarbamide. Erythrocyte transfusions should be given only when strictly indicated, but iron chelation needs have to be considered. Stem-cell transplantation is a curative therapy, yet this option is seldom used: the large majority of patients will not have an HLA-matched sibling or an available national transplantation unit, or will not be able to tolerate high dose conditioning regimens.
Genetic therapies have be researched for the last 25 years, but face strong technical bias – new drugs and insights have and are regularly researched, but new trends need a regular and sufficient research funding and teams interest in these pathologies.

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5ª SESSÃO | *5TH. SCIENTIFIC SESSION*
NEW APPROACHES IN RARE DISEASES

Gene packages and opening the exome – the Nijmegen experience

Ilse Feenstra
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Intellectual disability, hereditary deafness and movement disorders are examples of diseases with extensive genetic heterogeneity. In order to detect the genetic cause, this requires a genome wide detection of all types of genetic variation.
The implementation of exome sequencing in our diagnostic setting in December 2011 has led to significant changes in our daily clinical genetic practice, including pre-test counselling issues, interpretation of test-results in regard to the patient's phenotype and the approach to unexpected results.
In the past three years over 1000 clinical exomes have been performed in our diagnostic laboratory. Workflow, experiences and pitfalls from a clinician's perspective will be discussed.

Causes of rare and recurrent disorders unravelled by array

Nicole de Leeuw
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Genome wide high resolution SNP-based array analysis has been used in our laboratory for the detection of copy number variations (CNVs) as a first tier diagnostic tool since 2009 for patients with intellectual disability and/or congenital anomalies and prenatally in case of structural ultrasound anomalies or intra uterine foetal death and a normal QF-PCR test result. So far, more than 11,000 patient and 4,000 parental samples have been tested by SNP array in our diagnostic laboratory.

This diagnostic approach allowed us to reliably identify known and new, recurrent microdeletions and – duplications as well as rare, unique genomic imbalances with great accuracy. Moreover, the routine analysis of SNP genotypes revealed one or more significant stretches of homozygosity in 4 to 6 % of patients. Follow-up testing by either gene mutation analysis or patient-parent trio information analysis subsequently led to the respective identification of pathogenic mutations in recessive disease genes or uniparental disomies (UPD), thereby increasing the diagnostic yield with at least 1%.

Using the SNP genotype information also improved the detection of mosaic copy number changes and enabled us to detect clinically relevant, mosaic, copy neutral changes of homozygosity. So far, a mosaic finding (CNV, aneuploidy or allelic imbalance) was detected in over 30 patient samples and at least 12 parental samples, resulting in a dramatically increased recurrence risk for these parents. The percentage of mosaicism often differed between tissues samples of mesodermal, ectodermal or endodermal origin from each of these individuals.

Genome-wide high resolution SNP array analysis is a suitable and particularly effective technique in genome diagnostics to reliably detect various causes of rare and recurrent disorders including CNVs, UPDs and mosaic imbalances as well as pathogenic mutations in recessive disease genes. By using the right follow-up test procedures after initial SNP array analysis, a higher diagnostic yield and more knowledge of the mechanism underlying the genetic disorder are achieved, thereby enabling even more adequate genetic counselling.

Genetic complexity of the inherited cardiomyopathies in the high-throughout sequencing era - from research to clinical practice

Luis Lopes
Hospital Garcia de Orta (HGO), Alamada, Portugal

Cardiomyopathies are characterized by high allelic and locus heterogeneity, highly variable expressivity and age-dependent penetrance. Patients are routinely offered genetic testing in order to provide them with information about the likely impact of disease on their lives and to facilitate lifestyle and medical interventions that could improve prognosis. However, for this strategy to succeed in clinical practice, predictable relationships between specific genotypes and disease expression must be defined. In addition, novel variants with no established clinical phenotype are increasingly common in the literature. High-throughput sequencing facilitates faster and more complete genetic testing, but involves new challenges regarding variant interpretation. Large-scale sequencing studies in the general population, such as the 1000 genomes or UK10K projects, have reported a previously unexpected complexity of variation across the genome. This was also observed in inherited cardiac disease cohorts.

Hypertrophic cardiomyopathy (HCM) is the leading cause of sudden cardiac death in the young. To improve our understanding of the genetic architecture of the disease, we undertook targeted high-throughput sequencing of cardiovascular genes, including non-coding regions, in a large cohort of deeply phenotyped patients. Our calling strategy combined single-nucleotide variants, small indels and larger copy number variants (CNVs). We used whole-exome sequenced control samples to assign a probability of pathogenicity to these variants. Patients with sarcomere protein (SP) variants differed from those without with respect to age, family history, ventricular morphology and prognosis. Novel associations were demonstrated between individual SP genes and several traits and for the first time, associations between non-SP genes and phenotype were described. CNVs in SP genes can contribute to a small proportion of HCM cases.

6ª SESSÃO | *6TH. SCIENTIFIC SESSION*
BIOETHICS

All screening programs do harm. Some do good as well and of theses some do more good than harm at reasonable cost. (Sir Muir Gray, National Committee, UK)

Luísa Diogo
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André Pereira
Faculdade de Direito da Universidade de Coimbra (FDUC),Coimbra, Portugal.
Heloísa Santos
Presidente da Comissão de Bioética da SPGH, Lisboa, Portugal.

The primary purpose of the Bioethics Committee of the Portuguese Society of Human Genetics round table (SPGH) is to evaluate and to discuss the Newborn Screening in Portugal in order to design and to develop some guidelines that could improve the health benefits for children and family of this variety of genetic screening. Our goal is that the Portuguese screening will be able to provide the highest priority to the welfare of persons, maximizing the benefits of the testing (beneficence) and avoiding and preventing harm or, at least, minimizing harms (non-maleficence).

The classical neonatal newborn screening has been introduced in many European countries including Portugal over the last 50 years as a very important public health measure. In that time the principles of screening and the criteria to include new diseases were very different. It would be necessary that the disease should be considered an important health problem for the screened population, to be known their natural story, to be known an accepted treatment and so on (Wilson and Jungner, 1968).

Nowadays, the choices are not fully transparent. We screen in Portugal 25 pathologies and variations (all, with the exception of hypothyroidism, by mass tandem spectrometry), Germany does 15 and in France only 5 disorders are screened. We don't screen important problems of our newborn population (e.g. congenital adrenal hyperplasia, sickle cell disease), in opposition to other countries with less number of newborn tests. We don't have the clinical follow –up of most of positive screened babies. Finally, we don't do informed consent to the parents, even having laws obliging to offer written informed consent in pre symptomatic genetic testing.

So, we believe that, in our country, it is urgent to discuss the inclusion of appropriated ethical and scientific criteria before the addition of new genetic screening tests and before a future offering of next generation sequence screening, which is coming soon...

Clubs: Parallel Session

Lung Cancer Study: DNA and RNA Targeted Sequencing using 10 ng of nucleic acid from FFPE Tissue.

Juan A Barba and João Caldeira
Life Technologies, brand of Thermo Fisher Scientific

For many cancer types, patient tumor samples are tested to ascertain the presence or absence of actionable genetic markers, information that can help physicians choose a course of action best suited for each patient. Since cancer is known to be complex and highly heterogeneous, multiple genetic markers must be interrogated to fully understand the genetic profile of an individual tumor. Current genomic analysis technologies, however, interrogate only one or a few genetic markers at a time, requiring large amounts of sample input and taking weeks to deliver all of the relevant information for therapy selection.

Using the Ion PGM Sequencing Platform combined with Ion AmpliSeq technology, potentially hundreds of genes can be simultaneously analyzed from tumor samples via next-generation sequencing, with high reproducibility and rapid turnaround time. Furthermore, due to AmpliSeq technology's uniquely low DNA and RNA sample input requirements from FFPE tissue (10ng extracted nucleic acid per reaction), the Ion PGM-based sequencing platform can enable comprehensive sequence analysis of a larger range of tumor samples, including small biopsies and fine needle aspirates, in conjunction with other required pathology tests. Ultimately enabling standardization while working within the current pathology workflow, this technology also has the potential to provide more cancer patients the benefit of actionable information afforded through next- generation sequencing for therapy selection and clinical trial matching in the future.

The development of new companion diagnostic tests based on NGS, enables simultaneous testing of single nucleotide variants (SNVs), copy number variants (CNVs), gene fusions, and indels across multiple cancer genes. This approach will help ensure that cancer patients have an opportunity to potentially benefit from a targeted therapy associated with their tumor's genetic profile.

COMUNICAÇÕES ORAIS
ORAL COMMUNICATIONS

1ª SESSÃO | *1ST. SESSION*

Next Generation Sequencing of six iron-metabolism related genes in Portuguese patients with iron overload and a negative Hereditary Hemochromatosis-first level genetic test: a pilot study

Faria R[1], Silva B[1], Silva C[1], Vieira L[1], Loureiro P[1], Gomes S[1], Gonçalves J[1], Fleming R[2], Rivera I[3], Faustino P[1]

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Introduction: Hereditary Hemochromatosis (HH) is an autosomal recessive disorder characterized by excessive intestinal iron absorption and iron deposition in several organs leading to cardiac failure, cirrhosis and hepatocellular carcinoma. Molecular diagnosis of HFE-related HH is typically made by searching for specific genotypes (C282Y homozygosity or C282Y/H63D compound heterozygosity), denominated the "first level genetic test". However, in the Mediterranean area, up to one third of patients with a clinical diagnosis of hemochromatosis do not present those mutations.
This pilot study was designed to develop a "second level genetic test" based on next generation sequencing (NGS) for rapid and simultaneous analysis of 6 HH-related genes (HFE, TfR2, HJV, HAMP, SLC40A1 and FTL). A second objective was to establish genotype/phenotype associations.

Patients and Methods: A TruSeq Custom Amplicon (TSCA, by Illumina) kit was designed in order to generate 97 amplicons covering exons, intron/exon junctions and UTRs of the mentioned genes with a cumulative target sequence of 12115bp. Amplicons were sequenced in the MiSeq instrument (Illumina) using 250bp pared-end reads. Sequences were aligned against human genome reference hg19 using alignment and variant caller algorithms in the MiSeq reporter software. Firstly, some controls presenting known mutations were sequenced in order to validate the test. Subsequently, 88 iron overload patients with a negative first level test were studied according to previous conditions.

Results: We found a total of 55 variants in the 6 selected genes. These include novel missense and splicing variants, a mutation that originates a novel translation initiation codon, among others. Novel potentially pathogenic variants were validated by Sanger sequencing and their functional significance are currently under study. An unusual clinical case will be presented.

Discussion: The merger between TSCA methodology and NGS technology appears to be an appropriate tool for simultaneous and fast analysis of HH-related genes in a large number of samples. However, establishing the clinical relevance of NGS-detected variants for HH development remains a hard-working task, requiring further functional studies.

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Genome-wide association study identifies 4 variants for intracranial aneurysms in the Portuguese population

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Subarachnoid hemorrhage is a life-threatening event that very often leads to severe disability and death. Its most frequent cause is the rupture of an intracranial aneurysm (IA). Genetic contribution to IA is well established but until now, no single gene has been unequivocally identified as responsible for IA formation or rupture.

To identify new susceptibility loci for IA, we performed a genome-wide association study (GWAS) using a DNA pooling approach on a Portuguese dataset.

Two replicate pools of 100 Portuguese IA cases and of 92 age- and gender-matched controls were allelotyped in triplicate on the Affymetrix Human SNP Array 6.0. Of the 101 top markers, 99 were technically validated through individual genotyping.

After replication of validated SNPs in an additional set of 100 Portuguese IA cases and 407 controls, 4 variants (rs4667622, rs6599001, rs3932338 and rs10943471) were associated with IA in both the discovery and replication datasets (individually and in combination). Additionally, we replicated the previously described association with IA of rs1333040 at the 9p21.3 genomic region (Pcombined=1.93E-02, ORT[95%CI]=1.41[1.05-1.89]), thus validating our dataset.

SNP rs4667622 (Pcombined=4.00E-05, ORG[95% CI]=1.75[1.33-2.33]) is located on chromosome 2q31.1 within the regulatory region of the myosin IIIB gene (MYO3B), rs6599001 (Pcombined=2.20E-04, ORC[95% CI]=2.00[1.39-2.88]) maps to chromosome 3p22.2 upstream of the WD repeat domain 48 gene (WDR48) and rs3932338 (Pcombined=1.29E-03, ORA[95% CI]=1.59[1.19-2.08]) is located on chromosome 5p14.2 in a gene desert. SNP rs10943471 (Pcombined=3.20E-04, ORG[95% CI]=1.81[1.31-2.51]) is located on chromosome 6q14.1 in an intergenic region upstream of HTR1B (5-hydroxytryptamine (serotonin) receptor 1B). SNP rs10943471 is of particular interest, since HTR1B is implicated with changes in vascular tone and to mediate vasoconstriction of cranial arteries.

Our novel findings in the Portuguese population warrant further confirmation in other populations to establish their pathogenic role in IA formation.

Improving the genetic diagnosis of congenital myopathies by targeted next-generation sequencing

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Introduction

Congenital myopathies (CM) are a heterogeneous group of diseases, generally characterized by hypotonia and muscle weakness with onset at birth or during infancy. At least 20 different genes have been associated with CM, including three large loci (TTN with 364 exons, NEB with 183 and RYR1 with 106), particularly laborious and expensive to analyze by conventional sequencing. Additionally, although muscle histology is paramount for the diagnostic workup, pathognomonic findings are not gene-specific. Due to these difficulties a significant number (~60%) of CM patients remain genetically unsolved.

Methods

We developed a new targeted resequencing approach based on next-generation sequencing (NGS) technology to simultaneous analyze 20 genes linked to CM. Assay design included all exonic regions, 50 bp into flanking introns and untranslated regions (UTRs). The custom Ampliseq assay covered 92% of the selected regions (~320 Kb) and consisted of 2077 amplicons in two multiplex PCR reactions. Twelve CM patients (2 with known genotypes and 10 undiagnosed) were sequenced on an Ion PGM™ system.

Results

Globally, 6.5 million sequence reads were obtained, generating an average coverage depth of 257x and the detection of 2535 sequence variants. Concerning the experimental controls, all previously identified mutations were successfully detected, but in one of these variants zygosity was not correctly established due to allele dropout.

Eleven disease-causing mutations were successfully identified in 7 of the 10 undiagnosed cases, in RYR1, NEB and TTN. One patient with a NEB-related myopathy had an additional heterozygous mutation in the SEPN1 gene.

Discussion

Considering the patients with structural defects (cores/rods/central nuclei) in muscle biopsy (n=8), the diagnostic yield was an outstanding 87.5%. This work demonstrated not only the clinical utility of this NGS gene panel, but also its particular cost-effectiveness in CM diagnosis given the wide phenotypic heterogeneity and huge size of the candidate genes.

The expression of UPF1 is regulated by a cap-independent translation by initiation mechanism and cryptic promoter within its 5' untranslated region

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Regulation of gene expression occurs at several steps, including translation initiation. Under normal circumstances, translation initiation is mainly cap-dependent; however, several proteins can initiate translation in a cap-independent way, allowing the maintenance of these proteins under conditions that reduce global protein synthesis.

hUPF1(human up-frameshift 1) plays important roles in several key cellular processes such as nonsense-mediated decay, telomere replication and homeostasis, and cell cycle progression, suggesting its expression must be tightly regulated to prevent abnormal cell proliferation. This protein is essential in the G2/M transition, a step known by a reduced overall protein synthesis. Taking these data into account, we hypothesized that UPF1 might initiate translation in a cap-independent way, allowing the cell to maintain its levels under conditions that impair cap-dependent translation initiation.

To test this hypothesis, we cloned the hUPF1 5'UTR in a dicistronic vector and transfected cervical cancer and colorectal cancer cell lines with either this construct or the control counterparts. We observed a 15- to 25-fold increase in relative luciferase activity of the UPF1 5'UTR-containing construct compared to the levels obtained from the empty counterpart in all tested cell lines, suggesting a cap-independent translation initiation. To control whether luciferase activity levels are due to a cryptic promoter within UPF1 5'UTR, we transfected cells with promoterless plasmids and observed the same result, demonstrating that UPF1 5'UTR contains a cryptic promoter. Transfecting cells with in vitro transcribed mRNAs resulted in a 2-fold increase in protein levels, suggesting that translation can occur in a cap-independent way. This is maintained under conditions of global protein synthesis inhibition. Deletional analysis of UPF1 5'UTR revealed that the first 50 nucleotides are essential for cryptic promoter and cap-independent activities.

These results provide new insights on the mechanisms that govern UPF1

ExomeLoupe, a Platform for Exome Analysis and Variant Prioritization. A Type 2 Diabetes case study

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Introduction: Understanding the genetics of common T2D, a complex, polygenic and debilitating disorder is a major challenge. Multiple rare variants with large effect size may be the main determinant of complex disease genetic risk. Under the hypothesis of "common disease, rare variant", exome sequencing is an adequate technique providing the complete study of all low frequency variants with an impact on a disease.

Methods: We studied 51 Type 2 Diabetes diagnosed Portuguese patients with differentiated phenotypes by Whole Exome Sequencing using an optimized AmpliSeq Technology and the Ion Proton Sequencer. In order to select the best analysis approach, several mapping and variant calling software and bioinformatics parameters were tested. The annotation step was performed using the Gemini Framework. A user friendly tool, the ExomeLoupe was developed to allow analysis, visualization and filtering of variants by gene, genotype, structural annotation, variant effect, known polymorphisms, population frequencies, phenotypes or regulatory regions.

Results: An average of 40 million reads were mapped against the reference genome, with 94,2% on target, corresponding to a 116X coverage and 92% uniformity. The average number of variants encountered for each exome was 50,180 (46,337 SNPs and 3,843 Indels). From a list of 184 candidate genes previously associated with T2D we found variants in 22 genes. We also identified 46 rare variants present in 30 T2D related genes with a high deleterious effect as indicated by the CADD score. Three highly deleterious variants, in genes NOTCH2, WWOX and WFS1, were shared by two of the 51 patients.

Conclusion: We have developed an efficient and stand alone platform for large scale Exome Analysis and Variant Prioritization, the ExomeLoupe. Using this platform we identified a set of rare candidate variants. Further studies will elucidate the role of these rare variants in the manifestation or progression of T2D or its complications.

Validation of a next-generation sequencing comprehensive gene panel for the molecular diagnosis of hereditary breast and ovarian cancer

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Hereditary breast and ovarian cancer (HBOC) is caused by mutations in the high penetrance genes BRCA1 and BRCA2, with multiple other genes described as conferring an increased risk of breast and/or ovarian cancer. Molecular diagnosis of HBOC by standard methodologies is usually limited to these two genes, mainly due to their low efficiency, low throughput, high cost and high turnaround times. With the recent development of new sequencing methodologies, the speed and efficiency of DNA testing has dramatically improved. The aim of this work was to validate the use of next-generation sequencing (NGS) for the detection of mutations in the BRCA1 and BRCA2 genes in a diagnostic setting and to study the role of other genes associated with HBOC in Portuguese families. We have selected a total of 59 high-risk families (previously screened for the Portuguese founder mutations) for analysis in parallel by Sanger sequencing and NGS. Sanger sequencing was performed only for the BRCA1 and

BRCA2 genes whereas NGS was performed on a MiSeq using the TruSight Cancer sequencing panel (Illumina) that targets 97 genes suspected to play a role in predisposing to cancer, including 23 genes (containing BRCA1 and BRCA2) that have been described as involved in the predisposition to breast and/or ovarian cancer. A total of 440 variants (431 single nucleotide variants, 6 deletions and 3 insertions) in the BRCA1/2 genes were detected by both methodologies, with a 100% concordance between them. A total of 19 deleterious mutations were detected, eight in BRCA1 (13.6%), six in BRCA2 (10.2%), three in PALB2 (5.1%), one in TP53 (1.7%) and one in ATM (1.7%). Additionally, several variants of unknown significance were detected. These results demonstrate the efficiency of NGS for the detection of mutations in the BRCA1/2 genes and highlight the genetic heterogeneity of HBOC.

Validation of a Next Generation Sequencing pipeline for the molecular diagnosis of multiple inherited cancer predisposition syndromes

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Introduction: One of the Illumina's pre-developed gene panels for next generation sequencing (NGS) is the TruSight Cancer sequencing panel, which includes probes for 97 genes associated with inherited cancer predisposition. In this work we sought to validate the efficacy of this gene panel in the identification of different types of germline variants in positive control samples previously analyzed in our laboratory by routine Sanger sequencing, and also to evaluate its efficacy in identifying NF1 gene variants in Neurofibromatosis type 1 (NF1) patients diagnosed at IPO-Porto and for whom routine molecular testing was not available.

Methods: Thirty-one samples were analyzed, which include 21 positive controls for different types of variants in 21 genes associated with inherited cancer predisposition and ten samples from clinically diagnosed NF1 patients. Paired-end libraries were generated and run in the MiSeq platform. For variant calling three different pipelines were evaluated: MiSeq Reporter and Isaac Enrichment from Illumina and the commercially available NextGENe Software.

Results: Combining the analysis of the different softwares, we were able to identify 100% of the variants from the positive control samples and NF1 variants in all ten NF1 patients. While Isaac Enrichment and NextGENe missed only one indel variant each (different genes), MiSeq Reporter missed four indels (three of which detected by both other softwares). MiSeq Reporter and NextGENe alignments showed the best coverages for SNVs. Nine of the ten NF1 variants were already validated by Sanger sequencing.

Discussion: This work validates the TruSight Cancer workflow as a promising NGS method for the identification of germline mutations associated with multiple inherited cancer predisposition syndromes, allowing to add the NF1 gene to the list of cancer-predisposing genes tested at the Genetics' Department of IPO-Porto. Furthermore, we show that the use of different bioinformatic analyses is necessary to maximize sensitivity.

Detection of somatic mutations in Wilms tumours using gene panel sequencing

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Introduction: Wilms tumour (WT) is an embryonal kidney neoplasia in which the causative mutations are largely unknown. However, approximately one third of patients display somatic mutations in WT1, CTNNB1, TP53 and/or WTX genes, prompting the design of molecular tests to determine the mutational profile of each patient. In this work we describe a novel molecular assay based on next-generation sequencing (NGS) technology which we used to identify mutations in 36 Portuguese WT patients.

Methods: Design Studio (Illumina) was used to create a sequencing panel of 83 PCR amplicons covering 12.306 bases of exonic sequences of WT1, CTNNB1, TP53 and WTX genes. Amplicons were prepared from tumour and matched peripheral blood DNA samples (n=73) using a TruSeq Custom Amplicon kit (Illumina). Libraries were sequenced on a MiSeq instrument using paired-end 250 bp reads. Sequence reads were aligned to hg19 human genome reference sequence using MiSeq Reporter software (Illumina). Variants were annotated using publicly available databases.

Results: Data analysis of the constitutional DNA of WT patients showed the existence of 31 germline variants, including 9 variants not described in the human dbSNP database. Comparison of matched tumour samples revealed the presence of 14 putative mutations in 12 patients. The mutations included WT1 (n=3), CTNNB1 (n=4), WTX (n=5) and TP53 (n=2). In one patient, concomitant WT1 and CTNNB1 mutations were found. Comparison of results with previous Sanger sequencing data for WT1 and CTNNB1 in the same samples confirmed 5 out of 7 mutations detected by NGS in which the mutated allele frequency was above 20%.

Discussion: We conclude that gene panel sequencing is a fast and sensitive molecular assay for identification of recurrent somatic mutations in WT. However, because two thirds of patients lack known mutations, other NGS-based approaches such as exome sequencing may be fruitful to identify novel mutations in WT.

2ª SESSÃO | *2TH. SESSION*

The importance of recognizing the occurrence of anticipation in familial amyloid polyneuropathy (FAP) ATTRV30M

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Introduction: Familial amyloid polyneuropathy (FAP) ATTRV30M is an autosomal dominant systemic amyloidosis, due to a point mutation in the transthyretin (TTR) gene. Early-onset (≤40 years) and later-onset (≥50 years) cases of FAP ATTRV30M are not different entities, often coexisting in the same family, and showing anticipation (earlier age-at-onset (AO) in younger generations, usually associated with more severe phenotype). Our aim was to study anticipation in a very large number of FAP kindreds, removing possible biases, and to gain further insight into parent-of-origin effects.
Methods: We analysed 926 parent-offspring pairs (from the Unidade Corino de Andrade registry, which is the largest worldwide, comprising > 2000 patients, collected over 70 years), where both parent and offspring had been both clinically observed with well-established AO.

Results: Women had a significantly higher AO, either for daughters (mean: 33.70, SD: 6.84) vs sons (29.43, 6.08); or mothers (39.57, 11.75) vs fathers (35.62, 11.62). Also, 291 pairs showed marked anticipation (≥ 10 years); the transmitting parent was the mother in 203 pairs. Mother-son pairs showed larger anticipation (10.43, 9.34), while father-daughter pairs showed only a residual anticipation (1.23, 9.77). Gender of offspring and parents was highly significant (with no interaction). Anticipation was found in all subsamples, with the same trend for a parent-of-origin effect. Noteworthy, parents with AO ≤40 years never had offspring with AO ≥ 50.

Discussion: These findings confirm anticipation as a true biological phenomenon, also in FAP ATTRV30M, occurring in a disease caused by a point mutation, instead of the typical dynamic expansions. These data are of extreme importance for genetic counseling since offspring of patients with later onset have a higher risk of developing the disease earlier. Furthermore, offspring of patients with later onset may benefit from being closely followed-up to allow an early diagnosis and to provide adequate therapeutics.

Early infantile Epileptic Encephalopathies – a Center Audit

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Background: Epileptic encephalopathies (EE) are specific age-related brain conditions where there is a detrimental effect of continuing seizures and electrographic discharges on the normal function of the developing brain. There are overlapping clinic-EEG features and evolutional changes between them. Early Infantile Epileptic Encephalopathies (EIEE) are a group of EE with age of onset before the 3-4 months of age. Etiologies are heterogeneous and new genetic investigation techniques are helping to clarify the different etiopathogeneses of this group of conditions.

Aim: Retrospective study and characterization of a cohort of patients referred to the Epilepsy Clinic between 1995 and 2013 with EIEE.

Patients and Methods: Patients (n=26) were selected based on their clinical and electroencephalographic diagnosis of EIEE, with manifestation before 4 months of age. We excluded patients with brain injury secondary to hypoxic-ischemic encephalopathy, trauma, stroke, infection and intracranial hemorrhage. We performed a descriptive analysis concerning demographics, epileptic phenotype (clinical, electroencephalographic) and investigation (neuroimaging, metabolic and genetic) of all 26 included patients.

Results: Fourteen patients (54%) were male. The median age for first manifestation was 45 days [1day-4months]. Four patients had an Otahara syndrome and one patient an early myoclonic encephalopathy. One had malformation of cortical development. Five (19%) patients died. Nine patients (31%) had a confirmed molecular diagnosis (six metabolic and three genetic syndromes).Eight of the patients without diagnosis are currently awaiting next generation sequencing results.

Discussion/conclusion: Our cohort reflects the impact of a metabolic etiology in this group of EE, their heterogeneity and the difficulty to reach a diagnosis. Children with EIEE have a severe development impairment and elevated mortality and morbidity rates. A confirmed genetic defect was present in only 31% of patients, hence it is worth considering efforts to pursue with next generation sequencing techniques to investigate these patients. An accurate etiologic diagnosis for such disorders is helpful for an effective individual management, prognosis and genetic counseling to the families and in some cases for a personalised therapeutic intervention.

Neurofibromatosis type 1: Clinical and psychological characterization of an adult cohort

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INTRODUCTION: NF1 is a neurocutaneous autosomal dominant syndrome that affects 1/4000 individuals worldwide. The aims of this project were: 1. to characterize the phenotype in terms of visibility and severity of the disease; 2. to study possible correlations between genotype and phenotype; 3. to describe the patients' cognitive profiles; 4. to evaluate the patients' perception about their quality of life and compare our results with the literature.

METHOD: Patients: 32 adult NF1 patients were recruited from over 240 individuals belonging to 160 NF1 families registered at the Genetics Department of Hospital de Santa Maria. Informed consent was obtained.
Measures: A clinical questionnaire including an interview and medical observation was designed to characterize the disease phenotype. The Riccardi and the Ablon scales were used to assess disease's severity and visibility, respectively. The WAIS-III scale and a quality of life (QoL) questionnaire (SF-36V2) were also applied. Blood was drawn for molecular analysis. Statistical analysis was performed using SPSS.

RESULTS AND DISCUSSION: Severity distribution of the sample: 1/32 minimal; 12/32 mild; 16/32 moderate and 3/32 severe. Visibility distribution of the sample: 18/32 minimal; 12/32 mild/moderate; 2/32 severe. Total IQ results: 5/19 patients between 77- 88; 12/19 patients between 91-108 and 2/19 patients with 126. The domains of QoL in which more NF1 patients scored below the normative Portuguese sample were: role physical, bodily pain and role emotional.
Sample size is an obvious limitation of this study and we plan to include more patients in the future. Nonetheless, it is clear that most adult NF1 patients have only mild limitations, as expected from series in the literature. Still, adequate surveillance protocols should be put in place to allow early detection of minor as well as major complications and to help minimize disease impact on QoL. We hope this work will contribute to design such a protocol.

Update of the molecular study of maturity onset diabetes of the young (MODY) in Portugal

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Introduction: Maturity onset diabetes in the young (MODY) is a heterogeneous autosomal dominant form of diabetes mellitus with typical onset before age 25 and a primary defect in pancreatic beta-cell function. Patients with MODY may erroneously be classified as having type1 diabetes or type2 diabetes and MODY is thought to explain about 2% of all diabetes but its true prevalence in many populations is still not clear. It is estimated that Portugal has 600.000 diabetic patients and about 12.000 could be MODY. MODY2 patients have mild, asymptomatic, and stable hyperglycaemia that is present from birth. In contrast, patients with MODY3 have a progressive defect in insulin secretion frequently resulting in severe and progressive hyperglycaemia in adult life. MODY2 and MODY3 are the most common forms in Europe. The different MODY types can only be determined by molecular diagnosis. A genetics diagnosis often changes patient management, since patients with GCK mutations rarely req uire pharmacological treatment and HNF1A/4A mutation carriers are sensitive to sulfonylurea. The aim of this work was to characterize the MODY gene defect associated to each patient to improve patient management.

Methods: A total of 31 index cases with clinical diagnosis of MODY and relatives were received. The molecular studies were performed using direct sequencing and MLPA techniques for GCK (MODY2), HNF1A (MODY3), HNF4A (MODY1) and HNF1B (MODY5) genes.

Results and Discussion: The molecular study is concluded for 21 patients, having identified 40 MODY patients (index and relatives). A total of 15 different mutations were found, 2 of these have not been described before. The mutations are located in GCK, HNF1A and HNF1B genes.
Molecular genetic testing is important because confirms a diagnosis of monogenic diabetes, predicts clinical course, determines treatment according to their condition minimising the effects of the disorder, and defines risk for relatives.

Atypical somatic instability in the FMR1 locus: clinical, molecular and genetic counselling implications

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Fragile X syndrome (FXS) is the most common form of inherited intellectual disability. It is caused by an expansion of a CGG repeat in the 5'UTR region of the FMR1 gene to over 200 triplets. In typical FXS cases, silencing of the FMR1 gene due to methylation of its promoter precludes protein expression. Loss of the FMR1 protein leads to the physical, neurocognitive and behavioral FXS features. Somatic mosaics in the FMR1 locus are uncommon and can be due either to the presence of alleles with various CGG repeat sizes or epigenetic differences in the extent of methylation. Mosaicism for more than two alleles is a particularly rare finding, although it has been previously described. These phenomena hamper prediction of the disease prognosis.

Herein, we report two independent male cases with a phenotype compatible with mosaic FXS who show atypical mosaic patterns for CGG repeat number, one a mosaic for a full mutation/normal allele and the other for a full mutation/premutation/normal allele. Their mothers were carriers of the premutation. We postulate that both boys must have inherited a premutation, and subsequently two opposite postzygotic events occurred in two different cell subsets, a repeat expansion and a repeat contraction. Southern blot analysis, still considered the gold standard for molecular diagnosis of FXS, enabled the characterization of different size mosaics in both cases. Implications for FXS clinical management, molecular diagnosis and genetic counselling are discussed.

COMUNICAÇÕES EM PAINEL
POSTERS

Albright hereditary osteodystrophy-like syndrome [del(2q37)] and 17q25.3 duplication: case report and literature review

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Introduction: A recognizable microdeletion 2q37 syndrome or Albright hereditary osteodystrophy-like syndrome (AHO-like) (OMIM #600430) has been previously described in >100 patients.
The 2q37 locus is one of the most commonly deleted subtelomeric regions (Leroy, 2013). Patients exhibit developmental delay/mental retardation, facial dysmorphism, obesity and skeletal malformations. The trisomy 17q phenotype includes varying degrees of developmental/mental retardation, growth retardation, hypotonia and a large spectrum of dysmorphic features (Lukusa, 2010).

Case report and results: We describe a female aged 23 with short stature, obesity, facial dysmorphism and brachymetaphalangism. Chromosomal studies on peripheral leucocytes showed a normal karyotype. Multiplex ligation-dependent probe amplification (MLPA) and fluorescent in situ hybridization (FISH) subtelomeric evaluations for all chromosomes revealed both monosomy 2q37.3 (chromosome 2q37.3 terminal deletion) and trisomy 17q25.3 (chromosome 17q25.3 terminal duplication). FISH subtelomeric studies in the parents showed a normal karyotype in the father and a balanced translocation t(2;17) in the mother.

Discussion: A literature review for chromosome 2q deletions and 17q duplications was performed; the combination of 2q terminal deletion and 17q terminal duplication is very rare and few patients have been previously described. The most frequently reported features for both syndromes were compared with our patient's phenotype: she has most of the characteristic features previously published for AHO-like syndrome but also shows some 17q terminal duplication clinical signs.
The presentation of this case contributes both to the characterization of 2q37.3 microdeletion and 17q25.3 duplication syndromes. Genetic counseling and prenatal diagnosis are recommended.
The authors reinforce that MLPA and FISH studies for specific subtelomeric regions may become standard tests in patients with unexplained mental retardation and dysmorphic features and apparently normal chromosomes.

Higher Red Blood Cell Distribution Width (RDW) associated with Fanconi anemia. A simple predictive marker for stressed erythropoiesis?

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Fanconi anemia (FA) is a rare genetic disorder clinically characterized by progressive bone marrow failure. Hematologic abnormalities are frequent, but variable among patients. The most consistent features are macrocytosis and increased fetal hemoglobin, always preceding bone marrow failure. At cellular level, FA is characterized by chromosome instability (CI) and hypersensitivity to oxidative stress (OS).

Red cell distribution width (RDW) is a parameter that measures the variability in size of circulating erythrocytes, used in the differential diagnosis of anemia. Increased RDW is related to impaired erythropoiesis and erythrocyte degradation, and it was recently associated to high level of OS.

There are no reports about RDW values in FA patients. Therefore, the purpose of this study was to evaluate its importance as a possible marker of OS-induced stressed erythropoiesis and its correlation with CI.

Standard complete blood cell counts were performed in peripheral blood samples from 15 FA patients. Red blood cell (RBC) parameters (RBC counts, Hb content, mean corpuscular volume and RDW) were selected for comparative analysis. Lymphocyte cultures were performed for evaluation of DEB-induced CI.

Our results show that RDW is increased in peripheral blood from FA patients, suggestive of stressed erythropoiesis. RBC and Hb were significantly correlated with RDW (R^2=0,5814, R^2=0,7522 respectively), and a correlation between RDW and CI (R^2=0,7368) was also shown. As far as we know, it is the first time that RDW is associated with chromosome breakage. We hypothesize that in FA patients the need for an effective defense against OS may lead to the recruitment of immature RBC to the peripheral blood, leading to increased RDW.

In conclusion, a high RDW in FA patients can be a simple predictive marker for stressed erythropoiesis, with the advantage that this value is easily determined as part of the standard cell blood count without additional costs.

Genetic modifiers of chronic haemolysis level in Sickle Cell Anaemia

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Introduction: Sickle cell anaemia (SCA) is a clinically heterogeneous autosomal recessive monogenic anaemia characterised by chronic haemolysis and recurrent episodes of severe vaso-occlusion and infection. Several environmental and genetic determinants have been suggested to modulate the onset, course and outcome of SCA.
The level of chronic haemolysis has been considered a critical measure of SCA severity and a possible proximate cause of some disease complications such as stroke, pulmonary hypertension, priapism, leg ulceration and cholelithiasis. Thus, we proposed to search for genetic modifiers of this sub-phenotype and gain insights into the underlying mechanisms.

Patients and Methods: We studied the association between commonly measured haemolysis biomarkers (LDH, total bilirubin and reticulocyte count) and the inheritance of 41 genetic variants (34 SNP, 6 indel, 1 STR) of 10 candidate genes in a longitudinally observed series of 99 paediatric homozygous SCA patients (median current age of 9.9 yr) followed up in two general hospitals in Greater Lisboa area (median follow-up per patient of 5.0 yr). Candidate gene genotyping was performed by PCR-RFLP, Sanger sequencing, Gene Scan or Gap-PCR. All genotype distributions were tested for adherence to the Hardy-Weinberg equilibrium. When appropriate, haplotypes were inferred by software PHASE, version 2.1.1

Results: Although in a large number of tests seemingly significant association was observed only the following ones were confirmed upon correction for multiple comparisons: i) an increased serum LDH level was associated with haplotype 7 within VCAM1 gene; ii) a lower total bilirubin was associated with the 3.7-kb deletion at HBA gene, rs2070744_T allele at NOS3 gene, and haplotype 9 within VCAM1 promoter; and iii) a diminished reticulocyte count was associated with the 3.7-kb deletion at HBA, whereas an increased count was associated with rs1984112_G allele at CD36 gene.

Conclusion: On the whole, our findings suggest a complex genetic architecture for the SCA haemolysis process involving multiple pathways, namely control of vascular cell adhesion, NO synthesis and erythrocyte volume and haemoglobinisation.

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The physiological role of the soluble HFE isoform – a regulator of dietary iron absorption in the duodenum

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Objective: Dietary iron absorption regulation is a key-step for body iron homeostasis. Once inside the enterocyte, iron is directed to the basolateral membrane being oxidized by hephaestin, which mediates iron efflux towards circulatory transferrin in cooperation with ferroportin. Besides the HFE full-length protein, the HFE gene codes for alternative splicing transcripts responsible for the synthesis of a soluble form of HFE protein (sHFE). The main objective of this work was to assess whether sHFE plays a role in iron absorption regulation in duodenum. In particular, we intended to determine if sHFE transcript levels respond to different iron conditions in duodenal cell models. Also, we aimed to investigate the functional effect of the sHFE protein on the expression of iron metabolism-related genes in duodenal cell models as well as, in ex-vivo, in duodenum biopsy samples.

Methods: The levels of sHFE transcripts were measured in HuTu-80, Caco-2, and HT-29 cells, after holo-Tf stimulus. The expression of iron metabolism-related genes was determined after endogenous and exogenous overexpression of the sHFE protein. Moreover, expression levels of sHFE and HEPH were quantified by RT-qPCR in 6 RNA samples from duodenum biopsies of dyspepsia patients.

Results: Our in vitro obtained results have shown that the sHFE transcripts expression is up-regulated by intracellular iron. Hephaestin and duodenal cytochrome b expressions are down-regulated by endogenous sHFE protein. Exogenous sHFE stimulus also down-regulates hephaestin levels by a clathrin-independent, dynamin-mediated and RhoA-regulated endocytosis mechanism. In agreement, our in ex-vivo studies revealed that HEPH expression correlates negatively with sHFE levels in the human duodenum.

Conclusion: The sHFE is probably an important regulator of iron metabolism. Its levels vary according to the level of intracellular iron. It controls hephaestin expression and most likely the dietary iron absorption in the duodenum.

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Feingold Syndrome type 2: mutation in MIR17HG gene

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INTRODUCTION: Feingold Syndrome type 2 (FS2) is a rare inherited malformation syndrome characterized by skeletal abnormalities of fingers and toes (brachymesophalangy of the second and fifth fingers, clinodactyly, thumb hipoplasia, syndactyly of the second and third toes or the fourth and fifth toes), microcephaly, facial dysmorphisms (micrognathia, short palpebral fissures) and mild to moderate learning disability. FS2 is thought to be caused by a hemizygous deletion in the MIR17HG gene on chromosome 13q31.3. This syndrome is the first example of a syndromic development deficit in humans that is caused by a miRNA gene.

METHODS: The authors report a 31 years-old woman with short stature,microcephaly, abnormalities of fingers and toes, assimetric lower limbs, micrognathia and moderate intelectual disability. Her son, a four months baby, has a similar phenotype.Chromosomal study by array-CGH (180K) was requested.

RESULTS: Array-CGH was performed which revealed a deletion on 13q31.3, with 2,3Mb [46,XX.arr 13q31.3(90,539,056-92,844,733)x1]. This deletion involves 13 genes, 8 genes are describe don OMIM Database (OMIM ID: 609415-MIR17HG, 609416-MIR17, 609417-MIR18A, 609418-MIR19A, 609419-MIR19B1, 609420-MIR20A, 609422-MIR92A1, 602446-GPC5). This results established the molecular diagnosis of FS2.Comparing the phenotype of this two patients with the published cases didn´t reveal additional features possibly atributed to the deletion of the other genes.

DISCUSSION: The diagnosis of Feingold type 2 is of great importance for its autosomal dominant inheritance, allowing the adequate genetic counseling and the possibility of performing molecular prenatal diagnosis and /or preimplantation genetic diagnosis.

Genetic diseases and emergencies: The information available in ORPHANET

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Introduction: There are nearly 8000 rare diseases, with prevalence under 1:2,000, affecting ≈6% of the Portuguese population. About 80% of these diseases have a genetic cause. Information about rare diseases is often scarce, unreliable, and hard to find. ORPHANET - "the portal for rare diseases and orphan drugs" (www.orpha.net), helps overcoming this difficulty, providing credible and validated information to healthcare professionals and researchers, patients and relatives, patient associations, media and the general public. ORPHANET is a free and unique tool, available in 7 languages, including Portuguese. ORPHANET-PT (www.orpha.net/national/PT-PT) is established at CGPP-IBMC, since 2009.

Methodology: ORPHANET offers a variety of services:
• Classification and encyclopaedia of rare diseases, with genes involved
• Assistance-to-diagnosis tool
• Emergency guidelines
• Inventory of orphan drugs
• Directory of expert centres, medical laboratories providing diagnostic tests, patient organisations, research projects, clinical trials and biobanks
• Collection of thematic reports
• Many links to other sources of information

Results: ORPHANET-PT has registered 470 professionals, 144 expert centres, 1228 diagnostic tests, 155 research projects, 24 clinical trials and 70 patients associations, and translated the rare diseases names and synonyms, 1150 abstracts and 18 emergency guidelines. All summaries have an additional detailed information as health care resources and research activities for this disease, and some of them are still associated an emergency, clinical practice and anesthesia guidelines, articles for general public and clinical genetics reviews.Was created the national ORPHANET page and a Facebook page, to spread news on rare diseases in Portugal and facilitate communication among the various stakeholders.

Conclusion: ORPHANET is a large international project, facilitating communication and contributing to improve time to diagnosis, specialized care and/or treatment of patients.ORPHANET website receives more than 25.000 accesses daily from over 200 countries.Lately, ORPHANET-PT managed a very significant increase of the information available in Portuguese on platform, and to provide information to more people.

Importance of conventional cytogenetic analysis in prenatal diagnosis

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Introduction: Translocations are the most frequent structural chromosomal abnormalities and may pass undetected through generations. Miscarriages, infertility or the birth of a child with an unbalanced form of translocation usually reveals the existence of a familial chromosomal translocation. The development of new techniques, such as array Comparative Genomic Hybridization (aCGH), has increased the resolution of novel or rare microdeletions/microduplications, but chromosome analysis remains the gold to delineate chromosomal structural rearrangements.

Material and methods: The authors present a case of a 45-year-old pregnant woman referred to prenatal diagnosis. It has ten miscarriages and five healthy children. Amniotic fluid (two cultures) and parent's blood cultures were performed according to the protocols establish in the laboratory. Oligonucleotide array-CGH was applied. Cytogenetic analysis followed the cytogenetic guidelines (ISCN, 2013).

Results: Cytogenetic analysis of amniotic fluid revealed a 46, XX, t(1;2)(p34.1;p23.),t(1;5)(q10;q10) karyotype in the 20 metaphases analyzed. Chromosome analysis of the parents showed only one balanced translocation t(1;2)(p34.1;p23) in the mother. aCGH analysis and ultrasound parameters were normal. The couple decided to continue the pregnancy.

Discussion: Cases of unrelated double translocations are extremely rare. Currently prenatal diagnosis can benefit from the advances of the molecular techniques that are very useful for the precise characterization of chromosomal anomalies. The aCGH and karyotyping techniques are complementary and a combination of these methods can contribute to provide optimal genetic diagnosis and facilitate comprehensive medical care, as well as accurate recurrence risk counseling for the family.

Modulation of DNA methylation by dietary phytochemicals

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Introduction: In the last two decades there has been an increased use of natural compounds to improve health and to treat chronic diseases as in complementary and alternative medicine. This use has led to the need to understand their mechanisms of action. Several phytochemicals have been described to modulate cellular processes inhibiting or reversing early stages of carcinogenesis, which makes them promising chemopreventive agents. Epigenetic changes, such as DNA methylation, are crucial for the regulation of gene expression. In order to understand if a prolonged exposure to low doses of phytochemicals can influence DNA methylation, we analysed changes in DNA methylation patterns in genes involved in the development of breast cancer, using MCF-7 cells.

Methods: After incubation of breast cancer cell line MCF7 with elemicin, eugenol and genistein for 15 days at a concentration of 10μM, methylation in CPG islands of DAPK1 (involved in apoptosis), GSTP1 (involved in detoxification) and RASSF1 genes (tumour suppressor gene) and miR124-3 (tumour suppressor miR) was assessed by the Methylation-Specific PCR method.

Results: Elemicin and eugenol led to the modification in the methylation pattern of the promoter region of GSTP1 and RASSF1 genes and miR124-3.

Discussion: Our data revealed for the first time that a prolonged exposure to low concentrations of elemicin and eugenol can mediate alteration in DNA methylation, providing additional mechanistic insights into the way eugenol and elemicin can modulate gene expression.

The role of TMPRSS6 gene variants in different types of iron deficiency anaemia - from the rare severe hereditary IRIDA to the common mild acquired IDA

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Objective: Iron-refractory iron-deficiency anaemia (IRIDA) is a rare autosomal-recessive disease characterized by severe hypochromic microcytic anemia, low serum iron and transferrin saturation, normal-high ferritin and inappropriate high levels of the hormone hepcidin. Patients are unresponsive to iron oral treatment and present a slow persistent response to intravenous iron injections. The disease is caused by loss-of-function mutations in the TMPRSS6 gene which encodes the matriptase-2 (MT2), a negative regulator of hepcidin transcription. In those patients, high hepcidin levels prevent iron absorption in the duodenum and iron recycling by macrophages. Furthermore, it has been suggested that common variants in TMPRSS6 might modulate haematological phenotype and iron status. Therefore, the objective of this work was to search for severe genetic variants in TMPRSS6 in order to elucidate IRIDA-like phenotypes in some patients and to evaluate whether the SNP rs855791 influences iron deficiency anaemia (IDA) susceptibility in women.

Patients and Methods: Sequencing analyses of the TMPRSS6 gene were performed in 6 cases presenting IRIDA-like phenotypes. Additionally, the SNP rs855791 (p.V736A) was characterized, using an allele specific amplification approach, in 25 women presenting IDA and in 89 women normal controls.

Results: Sequencing analyses of TMPRSS6 in the IRIDA-suspected cases revealed the presence of a previously described pathogenic variant (c.757A>G, p.K253E), a novel splicing variant (whose functional effect is under study) and 3 other common variants. Concerning SNPs study, the frequency distribution of the rs8855791 genotypes showed a statistically association with women hemoglobin, serum iron level and transferrin saturation, being the proportion of CC homozygotes significantly lower in IDA patients.

Conclusion: Several degrees of iron deficiency anaemia can be attributed to genetic variants of TMPRSS6 gene and a relation genotype/phenotype can be established. Moreover, SNPs within the gene which give rise to a partial impairment of MT2 are able to modulate susceptibility to IDA. This suggests that TMPRSS6 has a role in iron-related common disorders in which it may act as a gene modifier.

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Preliminary results on MGMT gene methylation status by MS-MLPA in patients with Glioblastoma

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Glioblastoma (GBM) is one of the most common and aggressive primary brain tumors with an annual incidence of about three per 100 000. The median survival time is only 14 months after diagnosis and the standard treatment for patients with newly diagnosed glioblastoma consists of surgery followed by temozolomide chemoradiotherapy, an alkylating agent. The DNA repair protein O6-Methylguanine-DNA methyltransferase (MGMT), located on chromosome 10q26, encodes a DNA repair protein that removes alkyl groups, and is an important prognostic factor in glioblastoma since its presence has been associated with decreased survival and resistance to alkylating chemotherapy.

Tissue samples fixed in paraffin collected from resective surgery or biopsy procedures from 80 patients with high-grade gliomas were subject to DNA extraction. The DNA samples were analyzed by methylation-specific multiplex ligation dependent probe amplification (MS-MLPA) to determine the promoter methylation status of the MGMT gene. The panel used for evaluation contains 6 probes specific for the MGMT promoter region.

Of the 80 patients, 4 are not possible to analyze due to insufficient DNA, and 57 have been analyzed by MS-MLPA for MGMT gene methylation status. Of those, 26 presented a non-methylated promoter region, defined by the average methylation value of the 6 probes ≤ 25%; and 31 presented a methylated promoter region, defined by the average methylation value of the 6 probes > 25%. Of these 31 samples, 3 levels of methylation can be considered: low, moderate and extensive methylation.

The next step will be to correlate the methylation levels to the patients' response to the temozolomide alkylating agent. It is expected that patients subject to temozolomide treatment present a favorable prognostic, namely a higher overall survival.

Molecular karyotype of oral squamous cell carcinoma

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Introduction: Oral squamous cell carcinoma (OSCC) is a multistep disease of progressive genomic imbalances, which often occur before a phenotypic manifestation and are not detectable by the diagnostic methodologies currently available. Thus, the identification of the genomic profile of these tumors is a great promise for early diagnosis, prediction of disease progression and response to therapy. Taking this in mind, the main goal of this study was to characterize the genomic profile of OSCC through array-Comparative Genomic Hybridization (aCGH).

Methods: Biopsies of oral tumors were acquired from 75 patients and aCGH was performed using an Agilent oligonucleotide microarray 4x180K. Healthy donors were used as controls.

Results: With this whole genome approach we detected imbalances in almost all chromosomes; however it was possible to verify that the most common losses and gains were observed in specific chromosomal regions. Chromosomes 3, 5, 8 and 11 were the most frequently altered in our cohort. Are example of observed imbalances, losses at 3p26-p11 (17.3%), 5q11-q35 (10.7%), 8p23-p11 (12%) and gains at 3q11-q29 (18.7%), 8q11-q24 (26.7%) and 11q13.3 (42.7%). Apart from these imbalances, the sizes of aberrations detected for the same chromosome were often variable between patients. Additionally, in 5 patients, we verified gain of genetic material all along the long arm of chromosomes 3 and 8 and simultaneously loss in all the short arm of these chromosomes, which seems to be suggestive of the formation of isochromosomes 3q and 8q, respectively.

Conclusion: With this high-throughput approach we identified the most prevalent chromosomal regions reported in literature as altered in oral cancer and also in other chromosomal regions that might contain important genes related to disease initiation and progression. The correlation between molecular and clinic-pathological data has the power to identify putative biomarkers with possible diagnostic and prognostic value.

Array-CGH detection of 15q11.2 genomic imbalances. Challenges in interpretation and association with intellectual disorders

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Array-Comparative Genomic Hybridization has led to the knowledge that some copy number variants (CNVs) correspond to susceptibility loci for neuropsychiatric, intellectual and developmental disorders. CNVs at chromosomal region 15q11.2 involving four known genes, TUBGCP5, CYFIP1, NIPA2 and NIPA1, are of challenging interpretation due to their presence both in normal populations and in individuals with diverse developmental disorders. In a cohort of 1000 patients analyzed by Agilent 180K oligonucleotide array-CGH we identified 12 patients with 15q11.2 genomic imbalances, 9 deletions and 3 duplications, 7 females and 5 males. Four of the 12 patients had additional genomic imbalances. The patients presented with global developmental delay, dysmorphisms, intellectual disability (ID), epilepsy, microcephaly, amongst others. To date, we were only able to determine inheritance in 4 patients, 2 deletions of maternal origin, 1 paternal, and a de novo duplication. The proximal breakpoint was common in 11 of the 12 patients, while the distal breakpoint was variable, but similar in some patients. The four previously mentioned genes were involved in the genomic imbalances of all the patients, except in the patient with the distinct proximal breakpoint, where TUBGCP5 gene was in normal copy number. Functional data have revealed that TUBGCP5, CYFIP1 and NIPA1 genes are expressed in developing mammalian brain and are involved in processes such as microtubule nucleation, interaction with other proteins and nervous system development and regulation, respectively. To date, there is still no straight interpretation when a 15q11.2 genomic imbalance is detected, but in this cohort of 1000 patients further evidence was given that this region is associated with neuropsychiatric disorders.

The Challenge of X- Chromosome imbalances detected by array-CGH

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Array-Comparative Genomic Hybridization (array-CGH) has increased the diagnostic yield in patients with intellectual disability (ID), autism spectrum disorders and multiple congenital anomalies due to its improved resolution. X-chromosome has been focus of attention due to the bias in the affected male-to-female ratio and to the knowledge of X-linked genes associated with ID. With array-CGH we can either detect single gene imbalances, chromosomal region imbalances and even aneuploidies. In a cohort of 1000 patients studied by Agilent 180K oligonucleotide array-CGH we have detected several X-chromosome imbalances. Single gene deletions involving ZNF41 or IL1RAPL1 genes were equitably observed in 8 patients; DMD imbalances in 3 females and SHOX gene duplications in 1 female and 9 males. We also detected an intragenic deletion in SLC9A6 gene associated with Christianson syndrome that segregated in the family.

In 6 patients we identified Xp22.31 duplications, 3 females, 1 male with maternal inheritance and 2 males whose inheritance was not yet determined. We identified chromosome Xq27.1q28 interstitial duplications in 2 males, 1 maternally inherited and the other not yet determined. We also found other genomic imbalances but in single cases: a complex rearrangement with multiple imbalances at Xp22.33p22.2 in a male patient, maternally inherited; an Xp11.3p11.23 duplication in a female with ID whose mother is also affected and a case of triple X in an autistic female.

The challenge with X-chromosome imbalances is to interpret their impact on the phenotype, due to the presence of some alterations in the normal population and to X-chromosome inactivation in females.

MACRODACTYLY IN TUBEROUS SCLEROSIS COMPLEX AS A CAUSE OF FUNCTIONAL LIMITATION: CASE REPORT AND REVIEW OF THE LITERATURE

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Introduction: Macrodactyly in the context of TSC is a known but rare manifestation, usually thought to be a finding without clinical significance.

Methods: We report the case of a boy diagnosed with TSC at 2 years and 4 months of age, presenting with bilateral macrodactyly of the first three fingers of both hands, with underlying radiographic changes. Molecular analysis identified a frameshift mutation in the TSC1 gene encoding hamartin, causing a premature stop codon. We also conducted a review of the literature for reported cases of TSC patients with macrodactyly.

Results: Previously reported patients show some common features. Age at presentation was at birth or during infancy. In most, the digits affected were the first, second and/or third fingers of the hand. On X-ray, common features were an increase in volume and/or width of the affected digits and an irregular thickening of the periosteum, with or without erosive lesions or cortical cysts. Biopsies were conducted in only two patients; one had a fibrous hamartoma and the other had a skin collagenoma and an epidermal cyst. Most importantly, three of nine patients, including ours, had some type of joint limitation or flexion deformity. Our patient is, to our knowledge, the first reported to have clear bilateral involvement and whose genotype is known.

Discussion: The findings in our patient, corroborated by similar findings in other previously described patients, illustrate the fact that macrodactyly in TSC is often associated with deformity and functional limitation; the clinician must bear this in mind, with appropriate referral to specialist care when necessary.

DNA METHYLATION STATUS IN BONE MARROW VS PERIPHERAL BLOOD – A COMPARATIVE STUDY IN MYELODYSPLASTIC SYNDROME

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DNA methylation status is one of the epigenetic regulators of gene expression and aberrant methylation of gene promoter region is responsible for inappropriate gene silencing, mainly tumor suppressor genes, and it has been associated with cancer. Blood-based specimens may be a potential source of non-invasive DNA methylation cancer biomarkers. Peripheral blood leukocytes from patients with solid tumors exhibit complex and distinct cancer-associated DNA methylation patterns, which might be seen as epigenetic biomarkers with significant clinical potential. However, peripheral blood cell methylation profiles are largely unknown in hematopoietic cancers. Our aim was to compare DNA methylation status in bone marrow (BM) aspirate and peripheral blood (PB) of Myelodysplastic Syndrome (MDS) patients. We compare DNA methylation status of the tumor suppressor genes, p15, p16, p53, DAPK and MGMT, and of TRAIL (TNF-Related Apoptotic Inducing Ligand) receptor genes, TRAIL-DcR1, -DcR2, -DR4 and -DR5, in 68 MDS patients at diagnosis, in genomic DNA obtained from BM aspirate and PB samples, after informed consent. Genomic DNA was isolated by standard protocols and modified by sodium bissulphite. The MS-PCR was performed using two sets of primers, one for methylated and other for unmethylated DNA. $\chi 2$ Test was used to analyses association between groups and Kappa statistics to evaluate concordance, results were considered statistically significant when $p<0.05$.We observed a good concordant results between BM and PB samples in 69,1% of patients for p16, 70,6% for p15 (p=0,005), 57,4% for DAPK, 76,5% for TRAIL-DcR1 (p=0,041), 69,1% for TRAIL-DcR2, 72,1% for TRAIL-DR4 and a discordant results for TRAIL-DR5 gene, since only 48,5% of the tested samples were concordant. In some cases discrepancies were also bidirectional, with cases presenting demethylated PB and methylated BM aspirate and vice versa. No patient presented p53 and MGMT genes methylated.Our results show a correlation between gene methylation patterns in PB and BM aspirate in MDS patients. Although DNA methylation patterns measured in PB may have great potential as informative biomarkers of cancer risk and prognosis, large systematic and prospective studies will be needed.

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WHOLE EXOME SEQUENCING FOR IDENTIFICATION OF CANDIDATE GENES ASSOCIATED WITH PRIMARY CONGENITAL GLAUCOMA

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Introduction: Primary congenital glaucoma (PCG) is an inherited genetic disease characterized by developmental defects in the trabecular meshwork and anterior chamber angle, leading to the elevation of intraocular pressure with optic nerve damage. The major gene associated with PCG is CYP1B1. However, there is a group of patients who do not have mutations in this gene. The purpose of the study is to identify novel genes that may be causative of PCG in these patients, using whole exome sequencing.

Methods: DNA from two unrelated patients and two trios (proband and parents) were sequenced using SureSelect Human All Exon V4+UTR capture kit on the Illumina HiSeq2000 or AmpliSeq kit on Ion Proton. After annotation, several filtration steps were applied according to the autosomal recessive disease model in order to select exonic or splice site variants, with a pathogenic functional impact, which were rare (MAF<1%), homozygous or compound heterozygous and absent in our in-house database of genomes and exomes.

Results: On average 47,419 variants were found per exome. After filtering, the four samples had 5, 12, 8 and 11 altered genes, with 8, 17, 11 and 14 variants, respectively. A common mutated gene was not found when comparing the four patient samples. Four of the genes (FOXD1, KRT10, KRT4 and KRTAP9-1) were shared by some samples; however, current analyzes are not demonstrating a clear involvement of these genes in the PCG pathogenesis.

Conclusion: These results suggest that PCG may be a polygenic disease, since no common mutated gene was found. Additional analyzes must be performed in order to identify the genes associated with PCG, such as the study of the private variants of each patient, including de novo mutations or the UTR variants.

TRANSLATIONAL CONTROL OF THE HUMAN ERYTHROPOIETIN VIA AN UPSTREAM OPEN READING FRAME IN CARDIAC TISSUE

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Erythropoietin (EPO) is the main hormone that regulates erythropoiesis. Beyond its well-known hematopoietic action, EPO has diverse cellular effects in non-hematopoietic tissues, including cardioprotection. Indeed, in cases of tissue injury, the EPO expression increases locally providing a cardioprotective effect supported by numerous experimental data in animal models of ischemia and acute myocardial infarct. Cellular stress activates an integrated stress response, which includes rapid changes in global and gene-specific translation. Translational regulation of specific transcripts mostly occurs at translation initiation and is mediated via different cis-acting elements present in the mRNA 5' untranslated region (5'UTR), which include the upstream open reading frames (uORFs). These uORFs modulate translation of the main ORF by decreasing the number and/or efficiency of scanning ribosomes to reinitiate at the start codon of the main ORF. However, in response to abnormal stimuli, they mediate translational derepression of stress-responsive proteins.

The 5'UTR of the human EPO mRNA has one uORF with 14 codons that is conserved among different species, indicating its potential regulatory role. In the present work, we aimed to test whether EPO expression is translationally regulated in response to ischemia in cardiac tissue. Reporter constructs containing the normal or mutant EPO 5'UTR fused to the Firefly luciferase cistron were tested in H9C2 (rat heart/myocardium myoblasts) and C2C12 (mouse muscle myoblasts) cell lines. Luciferase activity was measured by luminometry assays and normalized to the corresponding mRNA levels quantified by real-time RT-PCR. Results have revealed that the EPO uORF represses translation of the main ORF at about 60-70%, in both cell lines, and recognition of the main AUG occurs by translation reinitiation after uORF translation. Nevertheless, in cell lines under chemical ischemia, EPO uORF-mediated translation repression seems to be released. These findings show that EPO cardioprotection effects might be regulated at the translational level.

Microdeletion syndromes - a rare case of 16q22.1 microdeletion

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Introduction: The 16q22.1 microdeletion syndrome is characterized by failure to thrive, psychomotor developmental delay, dysmorphisms and congenital anomalies. Very few cases have been reported.

Methods: We report a case of polymalformative syndrome (postnatal microcephaly, facial dysmorphisms, agenesis of the left external auditory canal, left preauricular tag, congenital heart defects, left single palmar crease, and renal asymmetry) with failure to thrive and developmental delay. DNA was extracted from patient's blood and chromosomal microarray analysis was performed.

Results: Array CGH identified a de novo 838 Kb deletion at 16q22.1 and a de novo 130 Kb duplication at 4q21.21 of unknown clinical significance.

Discussion: Our patient shows some clinical features common to individuals with a 16q22.1 microdeletion. Currently, chromosomal microarray analysis has an important role in the diagnosis of microdeletion/microduplication syndromes and allows a more accurate genetic counselling of patients and their families.

GENOME-WIDE ASSOCIATION STUDY IMPLICATES SLC6A1 AND AN INTERGENIC REGION AT 8q24.21 IN PRIMARY SPONTANEOUS PNEUMOTHORAX RISK

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Primary Spontaneous Pneumothorax (PSP) is characterised by presence of air in the pleural cavity that occurs without preceding trauma or known cause in individuals with no lung disease. Despite elevated incidence and recurrence rates, little is known about its aetiology and the genetics of idiopathic PSP remains unexplored.

To identify genetic variants contributing to sporadic PSP risk, we conducted the first PSP genome-wide association study. Two replicate pools of 92 Portuguese PSP cases and of 129 age- and sex-matched controls were allelotyped in triplicate on the Affymetrix Human SNP Array 6.0 arrays. Markers passing quality control were ranked by relative allele score difference between cases and controls (|RASdiff|), by a novel cluster method and by a combined Z-test. 101 single nucleotide polymorphisms (SNPs) were selected using these three approaches for technical validation by individual genotyping in the discovery dataset. 87 out of 94 successfully tested SNPs were nominally associated in the discovery dataset. Replication of the 87 technically validated SNPs was then carried out in an independent replication dataset of 100 Portuguese cases and 425 controls.

The intronic rs11708202 polymorphism in SLC6A1 and the intergenic rs4733649 SNP in chromosome 8 were associated with PSP in the discovery (P=4.19E-03, ORG[95% CI]=2.08[1.25-3.49] and P=3.39E-03, ORC[95% CI]=1.88[1.22-2.89], respectively), replication (P=3.43E-02, ORG[95% CI]=1.48[0.95-2.31] and P=1.54E-02, ORC[95% CI]=1.50[1.08-2.09], respectively) and combined datasets (P=5.47E-04, ORG[95% CI]=1.77[1.29-2.44] and P=8.18E-05, ORC[95% CI]=1.65[1.29-2.13], respectively).

This study identified for the first time two genetic risk factors for sporadic PSP, but future studies are warranted to further confirm these findings in other populations and uncover their functional role in PSP pathogenesis.

Accreditation under the International Standard ISO 15189: Experience of a Genetics Laboratory in DNA Sequencing

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Introduction: Health care is to some extent influenced by the results of laboratory tests. In order to provide the best care for the patient, laboratories must seek to achieve high levels of quality and competence. International Standard ISO 15189 specifies these requirements and may be used by laboratories to perform accredited genetic tests of materials derived from the human body. Here we describe the procedures to establish Accreditation of DNA sequencing in our laboratory and the first Accreditation of its kind in Portugal.

Methods: Our laboratory started to prepare to comply with ISO 15189 Accreditation requirements for DNA sequencing in 2010. Documents describing administrative and technical procedures of the sequencing workflow including sample registries, laboratory protocols, operation and maintenance of equipments, as well as preparation and use of reagents were produced. Regular examination of laboratory equipments by an external entity was implemented to confirm compliance with working requirements. Requisites for personnel training and demonstration of competence were also implemented. The laboratory participated regularly in the DNA sequencing scheme organized by the European Molecular Genetics Quality Network (EMQN).

Results: The laboratory obtained formal recognition by Instituto Português de Acreditação (IPAC) in May 2014. A maximum genotyping score for DNA sequencing has been obtained in the external quality assessment scheme since 2010. Sequencing quality measured in terms of the quality read overlap metrics is currently of approximately 96% according to the EMQN scheme. The laboratory processes and analyzes an average of 28.750 samples per year.

Discussion: Accreditation of a genetic test under ISO 15189 is a highly demanding and laborious task for a genetic laboratory. However, it is an important step in order to guarantee the highest quality and reproducibility of genetic test results.

miR-200c and miR-203 misexpression in a Portuguese breast cancer population and their association with clinicopathological characteristics.

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MicroRNAs represent an abundant class of non-coding RNAs that are involved in regulation of gene expression by targeting mRNAs. Emerging evidence has indicated that microRNAs are involved in the development of many human cancers, including breast cancer (BC), which is the prevailing cancer among women in industrialized countries. Since microRNAs have several potential targets, their function may depend on the cellular environment, and alteration of their regulatory roles may have broad consequences.

The aim of this work was to analyse miR-203 and miR-200c expression in BC patients and associate this expression with clinical and clinicopathological characteristics. Thus, we purified total RNA from 65 formalin-fixed, paraffin-embedded tumour and normal tissue samples from BC patients and analysed miR-203 and miR-200c expression by RT-qPCR.

Among several associations between studied microRNAs and clinicopathological characteristics, we highlight the fact that miR-203 presented a higher expression in tumour tissue compared to normal tissue in obese patients (fold change=2,99; p=0,048) and in nulliparous patients (fold change=3,37; p=0,026), which are known risk conditions for BC; regarding miR-200c, patients with stage IIB BC have a higher expression in tumour tissue than normal tissue (fold change=2,66; p=0,028).

Taking into account this data, we conclude that miR-203 and mir-200c misexpression is overt between tumour and normal tissue of breast cancer, and that we can correlate some clinicopathological characteristics with microRNA expression. This might be important in order to understand the influence of microRNAs in breast cancer and in which cellular pathways these microRNAs are involved. These data may enable the identification of new biomarkers of disease and indicators of molecular subtypes of breast cancer.

Immunohistochemistry detection of putative miR-200c and miR-203 Targets in Breast Cancer Patients

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Introduction: The morphological variability and clinical evolution of breast cancer have prompted researchers to find new strategies to classify the disease and to possibly define supportive prognostic and predictive indicators. Recently, some studies have focused on the putative utility of miRNA as a novel class of cancer markers. Parallel to the quantification of the expression of miRNAs in tumor tissue, it is necessary to observe the expression of their targets described bioinformatically, in order to infer if in vivo when a miRNA is overexpressed its targets are downregulated and subsequently the respective protein is underexpressed, and vice-versa. Hence, we aimed to analyze the expression of miR-200c putative targets – SIX1 and SOX2 – in breast cancer samples by immunohistochemistry.

Methods: 45 tumor tissue samples from 43 patients with breast cancer from Central Lisbon Hospital were analyzed. miR-200c expression were quantified and immunohistochemistry was performed for SIX1 and SOX2 detection. Clinicopathological parameters were characterized by the Pathology Department of Central Lisbon Hospital.

Results: The most common tumor type was invasive carcinoma NOS (71,1%) followed by invasive lobular carcinoma (8,9%). 86,4% of samples were ER positive, 79,1% PR positive, 13,6% HER2 positive and 45,5% high ki67. miR-200c was downregulated in 12,8% of samples and upregulated in 23,1%. Regarding to SIX1 and SOX2, only 13.3% and 8.9% of tumors were positive, respectively.

Discussion: A statistically significant association between the expression of both proteins and various clinicopathological parameters was not found, except for the number of pregnancies that seems to be associated with SIX1 positivity ($p = 0.034$). Regarding the relationship between levels of miRNAs and expression of their putative targets, no statistically significant association was found.

Defining a profile of FAP age-at-onset based on a TTR haplotype

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Introduction: Early and late-onset cases of familial amyloid polyneuropathy (FAP) ATTRV30M are not separate entities: they often coexist in the same family, with offspring showing a much earlier age-of-onset (AO) than their affected parent, i.e., anticipation. The protection seemingly existing in late-onset cases may be lost in just one generation, raising the hypothesis of a closely linked modifier. Our aim was, thus, to identify modifiers closely linked to the TTR locus that may in part explain the observed AO variability.

Methods: A haplotype analysis was carried out by genotyping eight SNPs at the TTR locus, in a sample of 722 individuals, comprising 588 V30M carriers.

Results: Haplotype A was the most common in our population, with the highest frequency both in FAP patients and the control group. Importantly, however, haplotype C was more frequent in early-onset carriers than in late patients ($p=0.012$). When we compared allelic frequencies of each SNP of haplotype C between very early-onset (≤30yrs) and late-onset cases (≥50yrs), we found a significant association of the A allele of rs72922947 with early-onset FAP ($p=0.009$), which remained significant after a permutation-based correction. Also, the GA genotype was associated with a significant decrease in mean AO of 8.6 yrs ($p=0.014$). An in silico analysis showed promising results that are being further explored.

Discussion: We identified the most common haplotype (in patients and controls), in which the mutation arose. Importantly, we unravelled a possible modulatory effect on AO exerted by a trans-acting factor, more frequent in early than in late-onset cases. We are now currently searching for rare variants at the TTR locus, in order to identify additional variants that may explain AO variation. Those results might have important future clinical implications.

Whole exome sequencing identifies a rare case of muscular dystrophy linked to choline kinase beta (CHKB) gene

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Introduction: Muscular dystrophies (MD) are a group of hereditary muscle disorders that include two particularly heterogeneous subgroups: limb-girdle MD and congenital MD, which are caused by mutations in 52 different genes (7 loci shared by the two). In patients without suggestive clinical or histological findings, the orientation of the genetic study is difficult. Considering the developments in sequencing technology, we are currently evaluating different approaches to solve the complexity of these interconnected myopathies.

Methods: We report whole exome sequencing (WES) analysis of a patient with childhood-onset progressive MD, also presenting mental retardation and dilated cardiomyopathy. Clinical follow-up and molecular tests carried-out over the past two decades failed to provide a differential diagnosis. Conventional sequencing had excluded eight candidate genes. WES of the trio (patient and parents) was performed using the Ion Proton sequencing system. Data analysis resorted to exploratory filtering steps using GEMINI software and restricted to genes linked to the hereditary myopathies.

Results: A novel silent homozygous variant narrowed down our analysis to a sole candidate gene that codes for choline kinase beta (CHKB). Visual inspection of sequence alignments led to the identification of the disease-causing mutation (CHKB:c.1031+3G>C). Its omission from the candidate list was due to incorrect zygosity calling. Sanger sequencing confirmed this to be homozygous in the patient and heterozygous in both parents. Expression analysis demonstrated its pathogenicity as no normal CHKB transcripts were detected in the patient.

Discussion: Mutations in CHKB have been shown to cause phosphatidylcholine deficiency in myofibers, causing an extremely rare form of MD, with only 21 patients reported worldwide. Notwithstanding interpretative difficulties that need to be overcome prior to the integration of WES in the diagnostic workflow, this work corroborates its utility in solving cases from highly heterogeneous groups of diseases, where conventional diagnostic approaches fail to achieve a definitive diagnosis.

A comparison of two different methods to identify IDH1 mutations

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IDH1 gene encodes an isocitrate desidrogenase which is responsible for convert isocitrate into α-ketoglutarate and NADPH. Somatic mutations in this gene have been associated with several types of cancer namely gliomas, which represent the most common primary brain tumour that arise in the central nervous system. Several alterations have been described in the past years, however the most frequent IDH1 mutation (>95%) is R132H. This missense mutation results simultaneously in a loss of IDH1 normal catalytic activity and α-ketoglutarate production. Currently, IDH1 mutation status is considered a strong diagnostic and prognostic marker and thereby is assessed routinely in our Neurology department. Here we compare the immunohistochemistry assay with Sanger sequencing to identify IDH1 mutations in gliomas patients in a routine clinical setting. One hundred patients assisted in the Centro Hospitalar e Universitário de Coimbra were screened for the presence of soma tic mutations in IDH1 gene using both techniques to determine the reliability of both methods. Gathering the results, despite both methods were extremely accurate and feasible it is important the combination of the two techniques to increase the specificity and sensitivity of mutation detection. Furthermore, in a daily practice, we should use as a first line method, immunohistochemistry specific for IDH1 R132H, the most common mutation whereas sequencing is recommended as a second-step test for IHC-negative or dubious cases.

The role of apoptosis polymorphisms in individual susceptibility to Philadelphia-negative Myeloproliferative Neoplasms

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Background: Although somatic mutations in the Janus kinase 2 gene (JAK2) occur in many Philadelphia-chromosome negative chronic myeloproliferative neoplasms (PN-MPNs), disease evolution, distinct phenotypes and the continuous clinical evidence of an increasing number of cases, with younger patients affected, have been pointing to a growing involvement of environmental factors in the pathogenesis of these diseases.
Several single nucleotide polymorphisms (SNPs), influencing DNA repair capacity and apoptotic status, confer genetic predisposition to disease and determine therapeutic response. Genetic polymorphisms encoding apoptotic proteins are candidates for association with PN-MPNs, since apoptosis is a high regulated process in cancerigenesis.

Objectives: Evaluate the role of apoptotic SNPs in PN-MPNs susceptibility and therapy response.

Methods: Case-control study in 121 Caucasian Portuguese PN-MPNs patients and 280 matched controls. rs1045485 and rs1035142 (CASP8), rs1052576, rs2308950, rs1820204 and rs1052571 (CASP9), rs2227309 and rs2227310 (CASP7) and rs13006529 (CASP10) were genotyped using real-time PCR (RT-PCR 7300 Applied Biosystem), through TaqMan® SNP genotyping assays (Life Technology), according to manufacturer instructions. Differences in genotype frequency, smoking status, age class, gender, therapeutic and pathology distributions between patients and controls were evaluated using SPSS 22.0 (SPSS Inc.).

Results: Considering all PN-MPNs cases, rs1035142 (CASP8) was associated with a consistent increasein overall PN-MPNs protective role, observed for the presence of at least one variant allele carriers (OR=0,6, 95% CI=0,4-0,9).Concerning pathology stratification, both polycythemia vera and essential thrombocytopenia present the same protective influence for the presence of at least one variant allele carriers (OR=0,5, 95%CI=0,2-0,9 and OR=0,5, 95%CI=0,3-0,9, respectively).Studies related with therapeutic response are still ongoing.

Conclusions: Our results suggest that apoptotic polymorphisms such as rs1035142 (CASP8) may influence PN-MPNs susceptibility, playing a protective role.However, larger studies are required to confirm these results and to provide conclusive evidence of association between these and other apoptosis variants and PN-MPNs and therapeutic response.

From Sanger to Next-Generation Sequencing in Neurogenetics

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The worldwide success of next-generation sequencing (NGS) in research and clinical laboratories, the advent of affordable bench-top NGS machines and an increased knowledge on the genetics of disease, have revolutionized the molecular diagnosis field. The validation process of NGS protocols comprises several technically challenging steps. So far, few guidelines have been established in an effort to harmonize the validation methodology however, a general consensus has not been achieved yet. Therefore, each laboratory must tailor their approach to tackle their specific challenges. CGPP is a leading expert laboratory in the field of genetic diagnosis of neurological disorders and the only one accredited to ISO15189 in Portugal. In this context, we are dedicated to upgrade our methodologies to NGS. Here we describe the strategy chosen to assess the validity of this technology to be implemented in the diagnostics routine.

We selected and sequenced amplicons harbouring disease-causing mutations identified in the lab during 2013. A panel of 71 unique mutations from 40 different genes was screened. All amplicons were amplified with conventional primers, pooled and sheared during library preparation, to be sequenced on Ion-PGM, with a minimum coverage of 40x.

Sequencing data was analysed from FASTQ files, screened using four software programs (JSI/DNASTAR/TSS/Ion Reporter) and compared in terms of capability to detect the selected mutations.

The analysis with JSI proved to be the most reliable in identifying all the mutations and the most effective in integrating all the steps for data analysis. Our results showed that duplications or deletions in homopolymeric regions are challenging mutations to be detected, as they are frequently associated with the generation of false positives. Detection of larger deletions is also a challenging aspect.

With this panel we have tested and established a workflow to implement NGS into our molecular diagnostic routine and designed a pipeline for bioinformatics analysis.

The role of ApoE polymorphisms in age-at-onset variation in Familial Amyloid Polyneuropathy

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Introduction: Familial amyloid polyneuropathy (FAP ATTRV30M) is 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 [19-82 yrs] and this variation is also often observed between generations. Only a few studies have searched for genetic modifiers involved in AO variability.
Several studies described that APOE genotype influence AO of some neurodegenerative diseases such as Alzheimer's disease and the rate of progression in others such as multiple sclerosis. The liver synthesizes both APOE and TTR, which may influence the aggregation and deposition of TTR fibrils. Therefore, our aim was to assess if APOE gene has a modifier effect in AO variation in FAP ATTRV30M Portuguese families.

Methods: We collected a sample of 89 FAP families with 144 patients. Variants were screening by direct automatic DNA sequencing of the APOE gene.

Results: We found some frequent polymorphisms in our sample that were never associated with FAP. In the preliminary statistical analysis significant differences were observed in mean AO when we compared patients with ε2/ε3 and ε3/ε3 genotypes against ε2/ε4, ε3/ε4 and ε4/ε4 genotypes ($p=0.004$). However, this difference was no longer significant ($p>0.05$) after increasing our sample.

Discussion: We hypothesize that APOE gene does not seem to be responsible for the early AO observed. Therefore, variants of the APOE gene do not seem to have a clear modifier effect in AO variation in our group of FAP ATTRV30M patients studied. Further studies are necessary to confirm these results and a better understanding the involvement of this protein in the pathogenesis of FAP can also help to unravel the mechanisms of other neurodegenerative diseases with implications in treatment strategies.

THE ROLE OF MOLECULAR DIAGNOSIS IN OPHTHALMOLOGY

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Introduction: Knowledge of the molecular basis of ocular genetic disorders has increased significantly in the last thirty years. Mutations in nearly 300 genes are known to cause various forms of inherited retinal diseases. In multifactorial common disorders like age-related macular degeneration and late-onset primary open-angle glaucoma, the role of genetics begins to be recognized.

Methods and Results: The authors present three families with different ocular genetic diseases: congenital aniridia, X-linked retinoschisis and congenital glaucoma. All cases have molecular diagnosis with analysis of PAX6, RS1 e CYP1B1 genes, respectively.

Discussion: The molecular diagnosis, as shown in the presented families has an important role in the optimization of the diagnosis, management and prognosis of the patients. The pattern of hereditarity can be defined. Additionally it makes possible presymptomatic testing in at-risk individuals prospecting preventive therapy before clinically detectable damage to tissues. Affected individuals or carriers of familial mutation can take informed decisions with access to reproductive options. It is also possible to participate in clinical trials. With the expansion of knowledge it is important to recognize the importance of a multidisciplinary team approach integrating ophthalmic and medical genetics services.

CONGENITAL ANIRIDIA

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Introduction: Abnormal development of the anterior segment of the eye is one of the most common groups of ocular developmental abnormalities. One of these disorders is congenital aniridia.

Congenital aniridia is a rare disorder with bilateral and panocular involvement in which the most prominent abnormality is the near-total absence of the iris.

Methods: We report the clinical characterization of a family with several ocular abnormalities with high phenotypic variability ranging from total near-total absence of iris to mild abnormalities of the iris. Some systemic manifestations including central nervous system are also present.

Results: The molecular analysis of PAX6 gene revealed a heterozygous mutation c.1047_1090del (p.Gln350Glufs*6). This result has confirmed the diagnosis of congenital aniridia.

Discussion: The clinical presentation and molecular analysis of this family allows us to characterize the spectrum of abnormalities related to mutations in the PAX6 gene associated with congenital aniridia.

CHROMOSOME 20q11.2 DUPLICATION SYNDROME – A CASE REPORT

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Introduction: In the past years many new chromosome syndromes were described. Recently Avila et al have presented four patients with some common clinical features like metopic ridging/trigonocephaly, developmental delay, epicanthal folds, and short hands. The four patients had a duplication of chromosome 20q11.2.

Case Report: We report three patients from the same family: one female adult and her two sons. Both have a microduplication of chromosome 20q11.2 and one has that microduplication and also a microdeletion of chromosome 1q21.1.
The index case was the older boy with ten years-old. He had intellectual disability, attention deficit disorder, epicanthus, riding of the metopic suture and short extremities. The molecular cytogenetics analysis (arrayCGH) revealed a duplication of 20q11.2 with 2,7Mb containing 55 genes, 34 described in OMIM and 7 reported in OMIM Morbid Map. Analysis of mother sample was done and the duplication was shown to be inherited. She had history of learning difficulties.
The youngest boy with fourteen months-old had developmental and growth delay, microcephaly, epicanthus and riding of the metopic suture. The molecular cytogenetics analysis (arrayCGH) revealed a duplication of 20q11.2 with 2,7Mb and a deletion of 1q21.1 with 1,3Mb inherited from the mother and from the father, respectively.

Comment: These three patients share some clinical features with the patients reported by Avila et al. ASXL1 gene was recognized as the explanation of part of the phenotype by those authors, however our patients have a smaller microduplication (2,7Mb) that do not involve this gene.Clinical and molecular characterization of additional patients will improve the delineation of this new syndrome.

LEGIUS SYNDROME, SHOULD WE THINK ABOUT IT?

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Introduction: Legius Syndrome (LS) is a relatively newly described autossomal dominant RASophathy, also known as Neurofibromatosis type 1 like syndrome. Its cardinal feature is pigmentary dysplasia consisting of café au lait macules (CALM) with or without intertriginous freckling, without neurofibromas or other tumor manifestations of neurofibromatosis type 1 (NF1). The prevalence of this disorder is unknown however, almost 200 patients have been molecularly diagnosed with LS. The ARUP Scientific Resource for Research and Education of the University of Utah has created a SPRED1 database with 99 cases at present, with the description of SPRED1 mutations and gene variants and with their phenotypes. SPRED1 gene is the only gene known to be associated to LS and the detection of a mutation in this gene is necessary to confirm a LS diagnose.

Methods and Results: We have implemented the sequence analysis and MLPA technique to study SPRED1 gene mutations and/or deletions/duplications at the haematology laboratory of our hospital, in order to study four cases identified in our service with CALM with or without freckling, without other NF1 criteria and with negative NF1 molecular test. None of the 4 cases have LS molecular confirmation, but since then we have studied and identified two families with LS with molecular confirmation.

Discussion: We would like to report our cases and compare their features to those described in the ARUP SPRED1 database and we would like to discuss the pros and cons of consider the LS diagnose and its respective genetic counseling.

FAMILIAL BREAST/OVARIAN CANCER RISK: CLINICAL PRACTICES IN PORTUGAL

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Introduction: We aimed to characterize current national practices pertaining familial breast/ovarian risk clinics, including models used for risk stratification, criteria for molecular study of BRCA1/BRCA2 genes and surveillance protocols for healthy probands at moderate or high risk or with a BRCA1/2 mutation.

Methods: We conducted a cross-sectional, descriptive study involving all known public national cancer risk clinics. Questionnaires were distributed and answers were collected via e-mail. Data analysis was performed with IBM®SPSS® software.

Results: Answers were obtained from seven departments of different medical centers. The majority (4/7) always uses models for breast/ovarian cancer risk calculation. The most used models were BRCAPRO to calculate probability of BRCA1/2 mutations and IBIS to calculate lifetime probability of breast cancer. Mean values to consider a proband's lifetime breast cancer probability as moderate or elevated were 16.5% [range 10-25%] and 27.5% [range 20-35%]. A combined probability of 10% for a BRCA1/2 mutation was unanimously considered the threshold for molecular testing. Five centers answered on surveillance protocols for women with BRCA1/2 mutations. All recommend clinical breast exam, mammogram and breast MRI in all cases; most recommend initiating screening at 25 years, every 12 months. Four also recommend endovaginal pelvic ultrasound and CA-125 determination in all cases; most recommend initiating screening at 35 years, every 6 months. Recommendations regarding rectal exam and PSA determination differed for BRCA1 and BRCA2 mutation male carriers. Six departments would discuss bilateral mastectomy and oophorosalpingectomy with healthy BRCA1/2 mutation female carriers, but only three with healthy high risk women in families with no BRCA1/2 mutation.

Discussion: Although the sample is relatively small, we found that recommendations for genetic testing and surveillance of patients at moderate to high risk of breast/ovarian cancer, including BRCA1/2 mutation carriers, are not uniform across national medical centers. There is a need for consensus and national practice guidelines.

Genetics of Familial Paget's Disease of Bone

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Paget's disease of bone (PDB) is a systemic disease characterized by increased bone resorption and formation, causing gradual destruction of parts of the skeleton and subsequent reconstruction of a more fragile bone. PDB has an overall incidence of 2% in the population over 55 years. PDB is a complex disease with multiple genes implicated in its pathogenesis, but in its monogenic form, only one gene (SQSTM1) has been linked to PDB.

To identify novel genes causing familial PDB, we performed whole exome sequencing (WES) in six individuals from a Portuguese multiplex family composed of five PDB cases, two unaffected individuals and one individual with unclear diagnosis. Given the uncertain diagnosis for one family member, we conducted two analyses: model 1, in which this individual is considered affected and model 2 where he is unaffected. DNA was captured using the SureSelect Target Enrichment System kit and sequenced using Hiseq2000 (Illumina'a Solexa). We identified three variants (c.C4786T (KIAA1875), c.C53T (NLRC3) and c.T566C (SRL)) in model 1 and one variant (c.G180A (SERINC2)) in model 2 that were present in all affected and absent from the unaffected in next-generation sequencing (NGS) data. Validation of these mutations by Sanger sequencing in all family members revealed that all model 1 mutations were present in all individuals, while the model 2 mutation was present in all family members except the individual with unclear diagnosis. None of these variants were present in a second Portuguese PDB multiplex family.

In conclusion, our findings support the notion that bioinformatics analyses of NGS data is a process requiring optimization. We found four novel variants which may cause PDB in this family with an autosomal dominant pattern of inheritance and incomplete penetrance. Further studies in other PDB families are warranted to determine the pathogenic potential of these genes/variants.

A MICRODELETION INCLUDING MBD5 IN A PATIENT WITH INTELLECTUAL DISABILITY, DYSMORPHIC FEATURES AND EPILEPSY

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Introduction: The 2q23.1 deletion syndrome is recognized as a neurodevelopmental disorder associated with severe developmental and cognitive delays, minimal speech, autism spectrum disorders (ASD), behavioral problems, seizures, microcephaly, craniofacial dysmorphism and short stature. The size of deletions is highly variable ranging from >19 Mb to 38 kb. Recently, methyl-CpG-binding domain 5 gene (MBD5), located in this region, has been considered the single causative gene for this syndrome. MBD5 is a member of the methyl binding gene family and appears to be responsible for regulating DNA methylation in the central nervous system.

Case report: We report a 17-year old caucasian female patient first evaluated in the Genetics Department when she was 18 months because of concerns regarding hypotonia, dysmorphisms and developmental delay. She was the second child of healthy, nonconsanguineous parents. Her gestation and birth were uneventful. She had feeding difficulties and by 16 months of age global developmental delay and mild craniofacial dysmorphisms were evident. Her karyotype was 46,XX and the metabolic profile was normal. She had a generalized tonic-clonic seizure at 14 years and her EEG showed spike and wave epileptiform discharges. Brain MRI was normal. Seizure control was obtained with valproic acid and levetiracetam. At 17 years of age she presented moderate intellectual disability, truncal obesity, small hands and feet, peripheral hypertonia, postural and intentional tremor. Chromosomal microarray testing revealed a cryptic de novo 43.13 kb microdeletion located in the 2q23.1 region that includes MBD5.

Discussion: This case highlights the need to analyse the gene content of even small cryptic deletions as these can be associated with haploinsufficiency of critical genes involved in brain development. It calls attention to a possibly underdiagnosed etiology of developmental delay associated with epilepsy, ASD and specific dysmorphic features. Understanding how epigenetic regulator genes act in brain development should help finding new therapeutics.

6q terminal deletion: a new contribution to genotype-phenotype correlation

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Deletion of chromosome 6q is a relatively rare clinical entity associated to a considerable variability of the phenotypic spectrum. Mental retardation, facial dimorphisms, seizures, and brain abnormalities are typical features of this syndrome but until recently genotype-phenotype correlations have been scarce. We report a 15-year-old boy with slight developmental delay, intellectual disability, hypotonia, bilateral eye cataracts, mycrocephaly, agenesis of the corpus callosum, ventriculomegaly, paroxysmal attacks, kyphoscoliosis and trigonocephaly. Cytogenetic analysis revealed a de novo karyotype 46,XY,del(6)(q25.3). Microarrays genomic analysis with Cytoscan 750K allowed the refinement of the breakpoint region to 6q26q27, spanning approximately 7.76 Mb. The variation of the features attributed to 6q deletion syndrome is due primarily to differences in size and location of the segmental aneuploidy. Several studies suggest that deletions of 6q25 region can cause more severe anomalies that those including 6q26-27. Absence of IUGR, ear anomalies, ear loss, cleft palate, cardiac defects and genital hypoplasia in our patient are compatible with studies that generally correlate those features with deletions of 6q25 region. In addition, our patient presents retinal abnormalities, which has been associated to 6q26-q27 deletion. Some new candidate genes, localized at 6qter, have recently been described as being associated with some clinical features; an example is the candidate gene DLL1 and holoprosencephaly. Analysis of the breakpoints in most cases revealed a potential common breakpoint region at 8.0-9.0Mb from the chromosome 6q terminus where a fragile site exists (FRA6E). This suggests the breakage at the FRA6E may be the mechanism behind chromosome 6q subtelomeric deletions in some of the cases.Once the genotype-phenotype correlations have been scarce until now, with this study we aim to contribute to a better knowledge of the genotype-phenotype correlation of 6q terminal deletion and help to identify critical regions for several clinical features and developmental relevant genes.

Molecular analysis of the NR0B1 in three Portuguese families with X-linked Adrenal Hypoplasia Congenita

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X-linked Adrenal Hypoplasia Congenita (X-linked AHC) is a rare disorder associated with acute adrenal insufficiency in the newborn age that typically cause vomiting, feeding difficulty, dehydration, and shock due to a salt-wasting episode. Hypoglycemia, frequently presenting with seizures, may be the first symptom. If untreated, adrenal insufficiency is lethal.
Affected males, despite hormonal treatment, typically have delayed puberty (onset after age 14) caused by hypogonadotropic hypogonadism and most of them are infertile at adult age. Carrier females may occasionally have symptoms of adrenal insufficiency or hypogonadotropic hypogonadism, possibly caused by skewed X-chromosome inactivation.
X-linked AHC is caused by mutations in NR0B1 gene, a critical gene involved in the development of adrenals and hypothalamic-pituitary-gonadal axis. Since the identification of the NR0B1 gene, numerous mutations have been discovered including deletions, alterations of splice-sites, missense, nonsense and frameshift mutations.
Here we present the molecular results obtained in three Portuguese families with NR0B1 mutations. Mutation analysis was performed by PCR followed by SSCP analysis and sequencing of DNA fragments showing abnormal patterns on a second PCR product, or by direct DNA cycle sequencing of PCR products.
Molecular analysis of the NR0B1 gene in proband A revealed a nonsense mutation, c.1084A>T, p.Lys362*, in exon 1, not previously described. His mother and sister were asymptomatic carriers; in family B a nonsense mutation, c.243C>G; p.Tyr81*, also in exon 1, was identified in two affected males and their mother and sister were also asymptomatic carriers; in family C a frameshift mutation, c.1292delG, p.Ser431Ilefs*6, in exon 2, was detected in a 7 years old affected male and his mother.
The maternal origin of mutations was confirmed in the three families studied.
The identification of a NR0B1 mutation in a family has important implications: a correct clinical diagnosis can be established, appropriate clinical management of affected members and suitable genetic counselling can be offered, female carriers can be identified and disease can be prevented.

Classification of the dup 15q13.3 CNV: A National data collection

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Introduction: The proximal region 15q11q14 is one of the most unstable regions in the human genome, with six recognizable break points (BP1-BP6). In 15q13.3 there is a recurrent small CNV (BP4-BP5) consisting of a 350-680 Kb duplication, encompassing the CHRNA7 gene, which encodes the alpha 7 subunit of the neuronal nicotinic acetylcholine receptor.
Although microdeletions of CHRNA7 are known to cause intellectual disability and neuropsychiatric phenotypes with high penetrance, the patogenicity of CHRNA7 duplications remains unclear. Microduplication 15q13.3 seems to be associated with a phenotypic spectrum of cognitive impairment and neuropsychiatric/neurobehavioral disorders. However, the penetrance of this CNV is considered incomplete since it is present in clinically unaffected individuals in the general population and it is frequently inherited from apparently clinically normal parents. Nonetheless, some pedigree studies have found a history of neuropsychiatric problems among carrier family members.
This study aimed at re-evaluating the dup 15q13.3 CNV in national laboratories.

Materials and Methods: Our study collected data on 15q13.3 microduplications in eight Portuguese genetics laboratories, among subjects referred for microarray.

Results: Here we present a total of seventeen cases with dup 15q13.3. The subjects had somewhat variable phenotypes, with a bias towards developmental delay and autism spectrum disorders. Inheritance was established for eight of the subjects, and the majority originated from the father. We had no access to clinical data on carrier parents. No de novo CNV was found. All laboratories involved classified this variant as of uncertain significance.

Discussion/Conclusion: To better determine whether this CNV is benign or pathogenic, careful characterization of patient and control cohorts must be performed, including detailed patient phenotyping, inheritance, clinical evaluation of carrier parents, prevalence in controls, as well as genetic functional studies.
We strongly support the creation of a national database for uncertain CNVs in order to clarify the relevance of these recurrent findings, allowing a definitive classification in either pathogenic or benign.

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Oligonucleotide array-CGH in Prenatal Diagnosis: Challenges and Benefits

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Array-CGH was performed in 67 prenatal samples. The indications for the analysis were diverse, ranging from ultrasound anomalies/major abnormalities (37), medical interruptions due to major anomalies (13), carrier progenitors of genomic imbalances (4) and even to clarify conventional cytogenetic findings (13).

Array-CGH was performed using Agilent oligonucleotide 180K in DNA obtained in the majority of the samples from amniotic fluid and chorionic villus, but also from fetal blood and skin biopsy of the death fetus. Each sample was hybridized against a sex-matched commercial control and the analysis was performed to detect imbalances above 400Kb in size, except in the cases where a specific familiar imbalance was being evaluated.

Of the 13 samples analyzed due to cytogenetic findings, we were able to: characterize 2 marker and 4 derivative chromosomes, exclude genomic imbalances in 2 translocations and 2 inversions, detect genomic imbalances in 2 apparently balanced translocations and detect mosaic trisomy for chromosomes 13 and 21 in a 13;21 Robertsonian translocation. Of the 4 samples tested due to carrier progenitors of genomic imbalances, 1 fetus was normal for the imbalance observed in the progenitor, while the other 3 were carriers of the same imbalance. In the remaining 50 samples, 10 imbalances were observed, 3 maternal, 4 paternal, 1 de novo, 1 imbalance resulting from a maternal balanced translocation and 1 sample where inheritance was not available.

Array-CGH is valuable not only to establish a diagnosis in samples with ultrasound anomalies but also to characterize cytogenetic findings, showing to be a good toll for genetic counseling.

Use of machine leaning approaches to explore genetic and phenotypic associations for Autism Spectrum Disorder

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Autism spectrum disorder (ASD) is a neurodevelopmental disorder of well known complexity. ASD is characterized by impaired social interaction and communication and by stereotyped behaviors, and a high heterogeneity in clinical and genetic presentation. It is hypothesized that such complex heterogeneous phenotypic behaviors are associated with genetic factors.

To further dissect the complex correlations between phenotype and genotype in ASD, in the current study we used powerful machine learning algorithms, like decision trees, to integrate clinical information (from diagnostic instruments ADI-R and ADOS as well as adaptive behavior and cognitive scales VABS and WISC) and genetic data (Copy Number Variants, CNVs) of 334 ASD individuals.

The relationship between phenotypic and genetic data was tested using interpretable decision tree algorithms (J48, REP and Random tree), and designing a 10 fold cross validation model. We obtained an overall model that classified the ASD subjects with a good accuracy of 67.66%. The model identified a common behavioral signature, including socialization dysfunction, social interaction problems and communication dysfunction, associated with inherited CNVs in males with slight intellectual disability (in 123 male subjects). Moreover, we found that the same behavior signature was associated with CNV deletions in females with more severe intellectual disability (in 10 females). This result validates previous separate observations of deletions being more pathogenic than duplications in the same genomic regions, and of a more severe clinical presentation, with lower cognitive levels, in females with ASD.

Enhanced understanding of genetic and behavioral data association will be useful to assist in ASD diagnosis. However, for that we need more data sets to increase the power to detect true effects and to improve accuracy and its soundness (statistical significance). Therefore we expect to expand our analysis to a larger dataset comprising 980 individuals with ASD.

Expression Profile of Circulating miRNAs in Autism Spectrum Disorders

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Autism Spectrum Disorder (ASD) is a common complex disorder, highly heterogeneous and with unclear etiology. Common genetic factors have not yet been identified. The possible contribution of epigenetic factors, such as deregulated miRNAs expression, should be addressed.

miRNAs are small noncoding RNA molecules that negatively regulate gene expression via degradation or translational repression of their target messenger RNAs. Recent studies have demonstrated that miRNAs play critical roles in several biological processes, and are associated with human pathology. Recently, studies have suggested that miRNAs in plasma and serum might be derived from circulating blood cells under healthy conditions, but might be released from pathological tissues during an illness. The strong correlation between circulating and tissue miRNAs indicates that circulating miRNAs might serve as biomarkers for various diseases, including central nervous system diseases.

In a preliminary in silico analysis, we observed that ~0.5% of the potentially pathogenic CNVs identified in the genomic screening of a large population sample of ASD subjects by the Autism Genome Project contain 95 miRNA genes, with 17 targeting 19 genes implicated/candidate for ASD. Since brain tissue is not easily accessible, the validity of using blood samples to study the miRNA profile in neuropsychiatric disorders is a new focus in the search of biomarkers.

We are currently comparing the miRNA profiles in plasma collected from ASD patients with patients with other neurodevelopmental disabilities (eg. psychomotor developmental delay, intellectual disability, etc). All patients selected are male, aged 2-4 years old, with an average developmental coefficient of 51, no dysmorphisms and no family history of neurodevelopmental disorders. Exiqon human miRNome PCR panels (for 752 miRNAs) were used for miRNA profiling. Preliminary results identified 15 miRNAs that are differentially expressed in ASD. Further studies and analysis of miRNA targets are ongoing.

NEXT GENERATION SEQUENCING (NGS) FOR THE GENETIC DIAGNOSIS OF HEREDITARY BREAST CANCER

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Introduction: Patients with family history or diagnosis of breast cancer at a young age have an increased risk for monogenic origin of this disease. The use of new molecular diagnosis approaches such as next generation sequencing (NGS) allows the screening of several genes in a single assay for a faster molecular diagnosis.

Objectives and Methods: Establishing a next generation sequencing panel for the molecular diagnosis of hereditary breast cancer. This protocol has reduced turnaround time and cost comparing to Sanger sequencing while maintaining sensitivity, specificity and detection rate. Different panels are available according to the clinical history of the patient. DNA library is prepared by target capture, which does not have the limitations of an amplicon-based sequencing approach, and then sequenced on an Illumina NGS platform. The full panel includes probes for the complete coding region, including intron/exon boundaries of 18 genes (ATM, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, ERCC4, MLH1, MSH2, MSH6, MUTYH, PALB2, PMS2, PTEN, RAD51C, RAD51D, STK11 and TP53). Quality control of the data is performed to assure an average coverage of over 100X and 100% coverage of the target regions. Mutations and regions that do not fulfill these criteria are tested by Sanger sequencing.

Results and Discussion: The validation of the NGS panel protocol was done and all mutations and variants identified by Sanger were also detected by NGS. Comparing to Sanger sequencing, the new NGS protocol reduced significantly the turn-around time. Testing cost was also dramatically reduced since the full NGS panel of 18 genes has a similar cost of 1 or 2 genes by Sanger sequencing. The use of NGS for genetic diagnosis on everyday clinical practice has great advantages to patients, families and health institutions.

16p11.2 Microdeletions

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Introduction: Recurrent microdeletion on chromosome 16 is a well-recognized syndrome that causes different clinical features including autism spectrum disorder (ASD) and severe early-onset obesity. 16p11.2 deletions are detectable by current clinical oligonucleotide arrays comparative genomic hybridization (aCGH) platforms and are classified according to the OMIM database in: (1) ~593Kb (Chr16:29.6-30.1Mb) and (2) a small microdeletion located distal to the typical microdeletion ~220Kb (chr16:28.82-29.0Mb). Other CNVs detected with genomic intervals between 30.0–34.0 Mb are normally considered as uncertain.

Material and Methods: 443 patients were analyzed using Agilent 4x180K microarrays and cytogenomics 2.7.22 software. The main clinical indications were intellectual disability, ASD, epilepsy, and multiple congenital abnormalities.

Results: 16p11.2 deletions were found in 13 (2,9%) of the 443 patients analysed by aCGH. Among these 13, 4 were classified as pathogenic and the remaining 9 as uncertain.
Pathogenic CNVs sizes ranged nearly 217Kb-1064Kb (including 9 to 44 genes) with genomic intervals between 28,824,794 and 30,198,600. Clinical features included mental retardation, malformations, global development delay and obesity. In the 9 cases classified as uncertain, sizes and genomic intervals ranges between 1015Kb-1154Kb and 32,471,625-33,625,989, respectively.

Conclusions: Since high resolution aCGH techniques have become increasingly available in the diagnostic procedures, several recurrent microdeletion/microduplication syndromes have been identified. The 16p11.2 deletion (~593Kb) impacts in behaviour, cognitive disabilities and body mass index, possibility through direct influences on neuronal circuits. The higher frequency of this deletion associated with obesity and development delay, suggests that these two phenotypes may be fundamentally interrelated. Distal 16p11.2 deletions (~220Kb) involve at least 9 genes. SH2B1 is the most likely obesity candidate gene taking into account his role in the regulation of body weight and glucose homeostasis in mouse. This study highlights the relevance of rare CNVs which have been associated with a wide range of neurological outcomes.

The role of targeted BRCA1/BRCA2 mutation analysis in hereditary breast/ovarian cancer families of Portuguese ancestry

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Purpose: The BRCA1/BRCA2 mutation pattern in hereditary breast/ovarian cancer (HBOC) families varies widely among different populations, some of which show highly prevalent founder mutations. Here we present the influence of founder mutations in genetic testing criteria and strategy of hereditary breast/ovarian cancer families of Portuguese origin or ancestry.

Methods and Results: We report the analysis of altogether 1050 suspected HBOC families, 524 fully screened for BRCA1/BRCA2 mutations and 526 tested only for the most common mutations. Of the 119 families with pathogenic mutations, 40 (33.6%) had the BRCA2 c.156_157insAlu rearrangement and 15 (12.6%) the BRCA1 c.3331_3334del mutation,the former being specific of Portuguese ancestry and the latter showing a founder effect in Portugal. Interestingly, the two most common mutations were found in a significant proportion of the HBOC families with an a priori BRCAPRO mutation probability <10%.

Conclusion: We recommend that all suspected HBOC families from Portugal or with Portuguese ancestry, even those fulfilling moderately stringent clinical criteria for genetic testing, should be specifically analyzed for the two most common BRCA1/BRCA2 founder mutations, and we here present a simple method for this first tier test. Screening of the entire coding regions of BRCA1 and BRCA2 should subsequently be offered to those families with a mutation probability ≥10% if none of those founder mutations are found.

Filaggrin gene and Atopic Dermatitis: A polymorphism Pro478Ser relates with the disease severity and increased Staphylococcal aureus colonization

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Introduction: The filaggrin (FLG) is responsible for the integrity of the cutaneous barrier. Therefore, atopic dermatitis (AD) severity and predisposition to cutaneous infections is associated to the filaggrin mutations in the European population. However, the relation between filaggrin mutations and inflammatory allergic biomarkers is still controversy. The polymorphism p.Pro478Ser was recently associated to a high risk for AD in the Asiatic population, affecting the filaggrin aggregation to the keratin segments.
The aim of this study was to assess the impact of filaggrin (FLG) gene mutations p.Arg501Ter, c.2282del4 and the polymorphism p.Pro478Ser on disease severity, skin bacterial colonization, and allergy in Portuguese subjects with long term atopic dermatitis (AD).

Methods: In this cross sectional study, data from 73 patients, (30±13 years, 61% female, 77% atopic) with AD for 16±10 years was analyzed. Mutations were analyzed by PCR amplification of exon 3 of FLG gene followed by Sanger sequencing, disease severity through SCORAD, allergy by serum levels of IgE, Phadiatop, eosinophil cationic protein and specific IgE to Staphylococcus aureus (SA) enterotoxin A, B, C, TSST and Malassezia spp. Number of colony forming units of staphylococci and SA species in 25cm2 of poplitea, brachial ceases, interscapular regions were determined. Non-parametric statistical analyses and chi-square were used.

Results: FLG mutations p.Arg501Ter (n=9) and c.2282del4 (n=2) were identified in 14.8% patients and were not associated with AD severity, allergic or microbiological parameters; p.Pro478Ser polymorphism was present in 38% (n=28) of participants and was associated with more severe disease (p=.005), higher colonization with SA (brachial right cease p=.01, popliteal right cease, p=.04, left popliteal cease, p= .02) and had relation with allergy in the subgroup of atopic patients.

Discussion: This study further emphasizes the role of the p.Pro478Ser FLG polymorphism on AD pathogenesis and the IgE response in allergic adult subjects.

Distinct spectrum of apc germline mutations in familial adenomatous polyposis at the center-south of portugal: identification of a mutational hotspot and suggestion of a founder effect

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Introduction: Familial adenomatous polyposis (FAP) is caused by APC germline mutations. These have been reported in classic and attenuated FAP (AFAP) but only two hotspots were described (codons 1309 and 1061-range:0-15%). We aimed to characterize the APC mutation spectrum in a FAP/AFAP population from the familial polyposis registry of the Portuguese Oncology Institute in Lisbon.

Methods: We performed mutation analysis in 95 index patients from our FAP/AFAP cohort (61 FAP; 34 AFAP) using PTT, DGGE, sequencing and MLPA. Haplotype analysis was performed using 3 microsatellite markers flanking APC and 2 intragenic SNPs in 12 families with an intron 9 mutation (6 from our registry, 2 from INSA and 4 from IHG), occasionally detected in the literature, in order to evaluate a possible founder effect. All samples were anonymized. Statistics: Fisher's exact and X2.

Results: APC mutations were found in 47/61(77%) FAP and in 12/34(35%) AFAP families. The 1309del and 1061d el contributed for 6/59(10%) and 2/59(4%) of the families, respectively. Exon 15 mutations were more frequent in FAP than in AFAP [30/47(64%) vs 1/12(8%),P<0.001]. A high mutation frequency was also found in exon 9 and flanking regions (9/59;15%), contributing for the majority of AFAP with APC mutation (8/12;67%). An intron 9 mutation (c.1312+3A>G) was highly represented (6/59,10%), exclusively in AFAP (6/12;50%). Segregating with this mutation, we detected a common haplotype apparently shared by 6 families. For D5S346, the common allele segregating with this haplotype was more frequent in the index patients (11/20;46%) than in a control population (20/90;22%).

Discussion: We identified a specific distribution of APC mutations and a mutational hotspot in our population. The higher frequency of the c.1312+3A>G mutation in Center-South Portugal suggest a non-uniform distribution which may be explained by a founder effect. Further studies using SNPs flanking intron 9 and the analysis of more families/relatives are needed.

Polymalformative genetic syndromes: Could exome sequencing give new clues in the diagnostic of undiagnosed diseases?"

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Introduction: Exome sequencing for clinical applications is now arising with the goal of establishing diagnosis of rare, clinically unrecognizable or puzzling disorders. Emerging as a genome-based technology for demystifying undiagnosed illnesses, the clinical exome sequencing has the potential to make real contributions to these unsolved cases. We report a case of polymalformative genetic syndrome that is under investigation since 2008, with unknown diagnosis up to date.

Methods: The DNA sample was extracted with QIAamp DNA Blood Mini Kit, according to the supplier's instructions and quality was confirmed by fluorimeter and agarose gel. Library preparation was done with the Exome Enrichment kit - Nextera Rapid Capture Exome v1.2 and sequencing was performed on a HiSeq 2500 Instrument.
The bioinformatics analysis of the obtained raw data was performed using three pipelines. Two in-house pipelines developed by different bioinformatics companies, and CLC Genomics Workbench pipeline by ®CLC bio, a QIAGEN Company.

Results: After sequencing and analysing the exome we were able to determine two suspect pathological mutations with clinical relevance. The different pipelines ended up highlighting different variants. The found mutation pointed in the directions of the Char Syndrome and Marfan Syndrome. The most important variants are now being validated with sanger sequencing, the gold standart.

Discussion: We hereby describe the case of a female child with several pathological characteristics, with emphasis on characteristics like "delay", "dysmorphism", "hydronephrosis", "craniosynostosis" and "hypotonia". In the past, several attempts to get to a diagnosis were made, but no significant result, and therefore no conclusion was found.
As such, due to this doubtfulness, an approach using exome sequencing was performed. After selecting the significant variants, we could observe that even if the same raw data was under analysis, through different pipelines, different variants with pathological relevance, which had not been reported by any of the previously performed tests, were found.

Escobar syndrome diagnosed in a young woman

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Introduction: Multiple pterygium syndrome is a phenotypically and genetically heterogeneous disease featuring joint contractures, pterygia, scoliosis, and facial dysmorphisms. It has a prenatally lethal form and a non-lethal form, the Escobar syndrome. Multiple pterygium syndrome is most commonly inherited as an autosomal recessive trait, although autosomal dominant and X-linked recessive inheritance have been described. Biallelic mutations in the CHRNG gene cause near 30% of Escobar syndrome cases.

Methods: We report a 27-year-old woman with congenital arthrogryposis, pterygia, facial asymmetry, and a severe kyphoscoliosis. Family history was irrelevant. CHRNG gene analysis was requested.

Results: CHRNG gene sequencing revealed a homozygous mutation c.459dupA (p.Val154SerfsX24), previously described in the lethal and non-lethal forms of multiple pterygium syndrome.

Discussion: Our patient showed clinical evidence of a neuromuscular disease. The combination of clinical and molecular analysis not only allowed a diagnosis and proper referral, but also permitted a prognosis and an adequate genetic counseling of the patient and her family.

Estudo cromossómico em array (array CGH) em Diagnóstico Pré-natal

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Introdução: O estudo cromossómico em array ou array CGH é uma técnica de elevada sensibilidade que permite detectar ganhos e perdas de material genético, não detectáveis por cariótipo convencional, ao longo de todo o genoma. Esta técnica é já aplicada como teste de primeira linha no diagnóstico citogenético do atraso de desenvolvimento, e cada vez mais tem sido aplicada aos estudos pré-natais, com maior incidência nos casos de alto risco de cromossomopatia e cariótipo normal. A sua elevada sensibilidade permite aumentar a taxa de detecção em mais cerca de 10% face ao cariótipo convencional (Wapner et al, 2012).

Objectivo: Aplicação da metodologia de array CGH a uma população de grávidas de alto risco para cromossomopatia, com cariótipo prévio normal (85% dos casos) ou sem cariótipo (15% dos casos). Análise dos resultados obtidos e avaliação da performance do teste.

Metodologia: Para o array CGH foi utilizada a plataforma da Affymetrix, CytoScan 750K, tendo sido analisadas 133 amostras fetais (105 líquidos amnióticos e 28 CVS). As principais indicações para o estudo foram a translucência da nuca aumentada e as anomalias ecográficas.

Resultados e conclusões: Foram detectados 15 casos com anomalias (11%), sendo que destas 14 eram anomalias não detectáveis pelo cariótipo. Estes resultados permitem demonstrar a utilidade e beneficio desta metodologia, no aumento da capacidade diagnóstica do teste pré-natal. É expectável que a técnica venha a substituir no futuro o cariótipo convencional, no entanto e por agora é consensualmente aceite que a aplicação do array CGH na rotina pré-natal aumente consideravelmente a sensibilidade do diagnóstico pré-natal e permite a detecção de patologias genéticas clinicamente relevantes que práticas convencionais, por métodos tradicionais não o conseguiriam.

CGPP-IBMC: First Portuguese laboratory accredited for neurogenetic diseases

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Quality requirements are particularly high in genetic testing, as tests are normally performed once in a patient's lifetime, increasing the potential harm of errors, their results having major implications for patients and their relatives.

The OECD Guidelines for Quality Assurance in Molecular Genetic Testing aim to improve quality, recommending that "laboratories reporting molecular genetic testing results for clinical care purposes should be accredited or hold an equivalent recognition".

The ISO15189 is the preferred and most demanding standard for genetic testing laboratories, as it emphasizes the quality of contributions to patient care, in addition to laboratory and management procedures.

CGPP implemented a quality management system, complying with the requirements of ISO15189, and only performs tests with proven analytical and clinical validity and utility, in accordance with international recommendations (OCDE, Council of Europe).

In 2014, our competence was officially recognized, through Accreditation by IPAC, the only organism in Portugal authorized to grant Accreditation, in laboratory testing and clinical activities (ISO15189), and is the first Portuguese laboratory accredited for neurological diseases. Among the tests accredited, we highlight the FAP ATTRV30M (the Portuguese type of amyloidosis), Charcot-Marie-Tooth disease, Huntington disease, Wilson disease, Friedreich ataxia, Machado-Joseph and the other dominant ataxias, together with the DNA extraction and blood sampling procedures.

Accreditation is instrumental to improve a laboratory's quality, including the reduction of turn-around-time (TAT), that reduces anxiety of patients, allows a quicker confirmation of the clinical diagnosis and the timely implementation of the appropriate clinical measures, including genetic counselling.

Accreditation allows also to covey a greater confidence in the reported results, as well as in their analytical and clinical interpretation; accreditation (formally and officially) recognizes technical and scientific competence, facilitates exchange of services, provides a valuable management tool and enhances the confidence that the needs and requirements of clinicians, patients and families are met.

Genetic characterization of a Portuguese patient with fibrodysplasia ossificans progressiva

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Fibrodysplasia ossificans progressiva (FOP) is a rare autosomal dominant disease with a prevalence of approximately 1 in 2 million worldwide.
FOP is characterized by the presence of malformations of the big toes and of postnatal progressive heterotopic endochondral osteogenesis, especially in the presence of exacerbating factors such as trauma, surgical intervention, lesion biopsy, and intramuscular injection.
FOP has been associated with a specific mutation on ACVR1 (c.617G>A; p.Arg206His), which encodes a receptor for bone morphogenetic proteins (BMPs).
Our aim was to establish the molecular diagnosis by mutation screening of ACVR1.
We report a male patient with progressive ossificans since childhood, showing calcification of the axial line with inability to perform flexion and extension of the scapular and pelvic girdle with neck stiffness. Surgical intervention in adolescence resulted in disease progression. At the moment, patient is bedridden and with partial jaw fixation.
Mutation screening was performed by PCR amplification of all coding and flanking regions, followed by bidirectional direct sequencing.
We have found one missense mutation in exon 6 (c.617G>A; p.Arg206His), previously described as a FOP disease-causing mutation.
The codon 206 is at the end of the highly conserved glycine-serine rich (GS) activation domain at the junction with the kinase domain.
To our knowledge, this is the first genetic study of FOP in a Portuguese patient. The mutation screening of ACVR1 distinguishing FOP from other disorders allows the correct clinical management of the patient. Molecular diagnosis will also allow appropriate genetic counseling to this patient and at-risk relatives.

Differential diagnosis of Autism Spectrum Disorder (ASD) by CNV detection – can early diagnosis be improved?

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Copy Number Variants (CNV) are collectively responsible for a substantial fraction of etiological diagnosis in Autism Spectrum Disorders (ASD). However, there is a large heterogeneity in the genes disrupted by these CNVs, and an important overlap with other neurodevelopmental disabilities. In this study we are characterizing CNVs present in ASD children that are rare or absent in typically developing controls and may be specific to ASD.

We have analyzed 84 ASD subjects (74 males, 10 females, age range from one to four years). In this cohort we identified 446 genes disrupted by CNVs (53.78% deletions and 46.22% duplications), of which 35 were absent or very rare in the Database of Genomic Variation (DGV) or other control datasets (which total 4964 controls). We identified multiple CNVs containing genes commonly described in ASD, including NRXN3, DLX2, ASMT, ANXA1 and others. No clear demographic or clinical patterns were defined in patients presenting these variants. In addition, we identified a number of deleted or duplicated genes there have been more rarely described in ASD individuals, such as FOXC1 (in 5 patients) or ASMTL (in 3 subjects).

Identified CNVs were further analyzed in DECIPHER (DatabasE of Genomic variants and Phenotype in Humans Using Ensembl Resources), to discover exclusive ASD variants. However, thus far none of the CNVs found in ASD children contained genes that were not present also in CNVs presented by patients diagnosed with intellectual disability, language impairment or global developmental delay. A larger dataset, including patients with other neurodevelopmental disabilities, is currently under study.

The eventual identification of a panel of CNVs specific for ASD might contribute towards a differential diagnosis, to assist clinicians in early detection of ASD cases for earlier and more specific intervention, improving patient prognosis.

Molecular diagnosis of Fahr's disease

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Introduction: Fahr disease is most commonly transmitted as an autossomal dominant trait and is characterized by abnormal calcified deposits being the most frequently affected areas the basal ganglia, while cerebellum, brain stem, centrum semiovale and subcortical white matter may also affected. Commonly affects young to middle aged adults. Clinically, manifestations of the disease incorporate a wide variety of symptoms, ranging from extrapiramidal symtoms, cerebellar dysfunction, dementia and neuropsychiatric symtoms.
It is caused by mutations in the SLC20A2 gene, located on chromosome 8p11.21, which encodes a member of the inorganic phosphate transporter family. The encoded protein is a type 3 sodium-dependent phosphate symporter that plays an important role in phosphate homeostasis by mediating cellular phosphate uptake. Mutations in SLC20A2 have been reported in about 40 families worldwide.

Methods: We have clinically ascertained 2 patients with a clinical diagnosis of Fahr disease and performed SLC20A2 mutation analysis, by PCR amplification of all exons and flanking intronic regions, followed by direct bi-directional sequencing.
The first case, a 58yo male, with multiple calcium deposits on the lenticular nucleus, caudate, thalamus and dentate nuclei on brain CT and MRI; normal calcium metabolism.
The second case, also a 58yo male patient, presenting with dementia with frontal symptoms and parkinsonism; brain CT with multiple calcifications; calcium metabolism was normal.

Results: We have identified two different mutations in SLC20A2. Both were novel frameshift mutations, resulting from 2 different deletions. The first case presents a deletion of one base at position 425, located in exon 3 (p.Lys142Argfs*29).The second case presents a 2bp deletion at position 1952, located in exon 11 (p.Tyr651Cysfs*12).

Discussion: In this study, we have confirmed the diagnosis of both patients and expanded the SLC20A2 mutational spectrum. Molecular confirmation of the clinical diagnosis in patients with Fahr's disease allows proper genetic counselling to patients and their relatives.

Molecular diagnostic of autoinflammation, lipodystrophy and dermatosis syndrome

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Introduction: Autoinflammation, lipodystrophy and dermatosis syndrome (ALDS, #OMIM 256040) is an autosomal recessive disorder with a prevalence less than 1/1 000 000. It is characterized by annular erythematous plaques on the face and extremities with development of partial lipodystrophy and evidence of immune dysregulation. Other features of this disease are recurrent fever, muscle weakness and atrophy, joint contractures, hepatosplenomegaly and microcytic anemia. ALDS has been found to be caused by mutations in PSMB8 gene on chromosome 6p21. This gene encodes a catalytic subunit of the 20S immunoproteasomes called β5i.

Methods: The patient is a 28 years old male with recurrent erythematous lesions since first month of life associated with hepatosplenomegaly and anemia; in childhood he developed lipodistrophy, muscular atrophy and contractures that led to severe motor incapacities. The PSMB8 coding regions and intron-exon boundaries of this patient were tested by PCR- amplification and bidirectional direct sequencing.

Results: We found a homozygous missense mutation in exon2 of PSMB8 gene, c.224C>T, which cause a substitution of threonine for methionine at position 75 of the protein.

Discussion: This mutation has been previously described in ALDS patients, including the two original pedigrees from Mexico and Portugal that allowed the gene identification after homozigosity mapping (Agarwal AK, et al. 2010). This molecular diagnostic allowed the definitive confirmation of the etiology of this very rare syndrome and the possibility of proper genetic counseling to this patient and their at-risk relatives.

Massive Parallel Sequencing Approach for the Analysis of Whole Mitochondrial DNA

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Mitochondrial diseases are thought to be the most common group of inborn errors of metabolism occurring in childhood, with an estimated frequency of 1 in 5,000 live births. Mitochondrial DNA (mtDNA) is highly susceptible to mutation and several mtDNA variants result in a variety of syndromes with neurological, muscular, or metabolic manifestations. Indeed, it has been shown that more than 300 mtDNA point mutations and deletions are linked to human diseases. Lesions in this tiny “genome” account for 15 to 30% of all childhood cases. Whole mtDNA can be routinely resequenced by amplification of 46 fragments followed by Sanger sequencing.

We have successfully applied a Massive Parallel Sequencing (MPS) strategy, together with a dedicated data analysis pipeline, in whole mtDNA analysis. The entire human mitochondrial genome was enriched by a single amplicon long-range PCR followed by MPS to simultaneously detect mtDNA point mutations and large deletions. A training set of samples was used to optimize the entire process. This strategy is a step-forward for the widespread use of MPS in the clinical setting in the complex field of mitochondrial disorders. Additionally it enables the quantification of mtDNA point mutation at low levels of heteroplasmy and detection of deletions in a timely and cost-effective manner.

Small but Significant: 4 cases of Single-Gene Copy Number Variants

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Large copy number variants (CNV) are frequently associated with neurodevelopmental disorders (NDD), dysmorphic features and congenital abnormalities. However, the clinical significance of rare and small sized CNVs (<200 kb), encompassing one or a few genes, is usually less obvious to elucidate. The classification of these CNVs relies on an intensive search of the literature and online data resources and critical comparison with the patient's phenotype.
We screened among our internal database of individuals referred for arrayCGH testing, looking for small (<200 kb), single-gene disruptive CNVs, in order to ascertain the diagnostic relevance of these variants in NDD.
Two oligonucleotide array platforms were used: 135K CGX and 180K CGX-HD, both from PerkinElmer. Analysis was performed with Genoglyphix software (Signature Genomics).
In our study, among 1200 individuals analyzed, 326 (27%) had an abnormal arrayCGH result (≥ 1 relevant CNVs). Among the latter, 87 (7.3%) patients had only CNVs smaller than 200 kb. Four patients had a unique single-gene disruptive CNV clearly correlating with the observed phenotype, namely: two unrelated patients (case 1 and case 2) had small deletions at 22q13.3, with respectively 42.95 kb and 51.63 kb, both involving the SHANK3 gene; one patient (case 3) had a 173 kb deletion at 7q11.22 involving the AUTS2 gene, and one patient (case 4) had a 43.13 kb deletion at 2q23.1 involving the morbid MBD5 gene. In two patients the variants were confirmed to be de novo.
All the four patients had developmental delay and specific dysmorphic features. Additionally, patient 2 had a behavioral disorder and patient 3 suffered from epilepsy.

The increasing genomic resolution of chromosomal microarray enhances detection of small sized benign CNVs which can confound clinical interpretation. Three of our cases had a pathogenic CNV around 50kb, much smaller than the recommended reporting size in international guidelines. Our data shows that this threshold must be carefully set in order not to lose clinically relevant findings.

Mutational analysis of the Portuguese cohort with clinical diagnosis of FH

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Introduction: Familial hypercholesterolemia (FH) is a common autosomal dominant disorder of lipid metabolism (1:500 frequency), caused by mutations in genes involved in cholesterol's clearance. FH patients present high levels of plasma cholesterol since birth, and if untreated, develop premature coronary heart disease (pCHD). The aim of the Portuguese FH Study is to promote the early identification and characterization of FH patients in order to decrease their cardiovascular risk by the implementation of correct/adequate and early counselling/treatment.

Methods: The clinical criteria of FH were adapted from the Simon Broome Register (UK) and genetic diagnosis was performed by the analysis of LDLR, APOB and PCSK9, using PCR plus Sanger sequencing and NGS techniques; MLPA was also performed for LDLR.

Results: A genetic defect was identified in 663 patients: 104 children, 154 adults (index-cases) plus 107 children, 298 adults (relatives), representing 3.32% of the FH cases estimated to exist in Portugal. We also identified 3 true homozygous and 5 compounds heterozygous. Our cohort presented 92 different LDLR mutations, 22 being exclusive of the Portuguese population; 12 FH patients had one of the 4 different APOB mutations, 2 being exclusive of the Portuguese population and recently characterized by our group as pathogenic (p.Arg1164Thr and p.Gln4494del); and 3 patients had the Portuguese PCSK9 mutation (p.Asp374His). CHD was present in 107 adults (index-cases) with clinical diagnosis of FH (1st event: 45.4±11.1 years), however, a genetic defect was only found in 39 of these cases.

Discussion: The genetic diagnosis of FH provides an unequivocal diagnosis and allows early identification of relatives at risk, allowing the implementation of appropriate treatment in early ages to decrease avoidable deaths.

THE ROLE OF OXIDATIVE STRESS GENES POLYMORPHISMS IN MYELOID NEOPLASIAS DEVELOPMENT AND PROGRESSION

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The pathogenesis of myeloid neoplasia (MN) is complex and involves genetic, epigenetic and other molecular alterations. Oxidative stress (OS) can contribute to cell damage, apoptosis and ineffective hematopoiesis. OS has been observed in several hematopoietic malignancies and some evidences suggest a role of OS in etiology and pathogenesis of hematological neoplasias, namely in Myelodysplastic Syndromes (MDS) and Myeloproliferative Neoplasias (MPN). In this context, we investigated the influence of GPX1, SOD1, SOD2, CAT, NADPH oxidase oxidasep22phox, MPO, NRF2, KEAP1, OGG1, XRCC1 and NEIL1 polymorphisms, as a risk factor for myeloid neoplasia development and progression. This study enrolled 160 patients diagnosed with MN (106 MDS, 54 MPN) and 260 controls. The polymorphisms of GPX1 (rs1050450), SOD1 (rs2070424), SOD2 (rs4880), CAT (rs1001179), NADPH oxidasep22phox (rs4673), MPO (rs2333227), NRF2 (rs13001694), KEAP1 (rs11085735), OGG1 (rs1052134), XRCC1 (rs1799782) and NEIL1 (rs4462560) were assessed by RFLP-PCR or tetra-primer-PCR. Disease risk was assessed by odds ratio (OR) with 95% confidence interval (CI95%) and survival by Kaplan-Meier method. Our results show that individuals with TT, GG and AA genotypes of GPX1, MPO and NRF2, respectively, have an increased risk for MN development about 1,9-fold (CI95% 1,1-3,3;p=0,025), 1,6-fold (CI95% 1,0-2,4;p=0,047) and 1,8-fold (CI95% 1,1-2,8;p=0,022). Besides that, individuals with NRF2 AA genotype have an increase risk about 2,0-fold for MDS development (CI95% 1,1-3,5;p=0,017). Moreover, while MPO GG genotype increased the risk for MPN development of 2,4-fold (CI95% 1,2-4,7;p=0,012), the some genotype from NRF2 had shown to be a protective factor for MPN development (OR 0,3-fold; CI95% 0,1-0,7;p=0,008). Furthermore, MDS patients with CG OGG1genotype had 1,9- and 2,5-fold less overall survival than patients with CC and GG genotype (p=0,05). These results show that polymorphisms in oxidative-stress related genes might be related with MN development and may constitute novel genetic markers for MPN and MDS susceptibility and could influence patient´s survival.

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Genetic Etiology of Presbycusis in Portugal

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Presbycusis or age-related hearing loss (ARHL) is the most common sensory impairment in older adults. It affects millions of people worldwide, leading to social isolation and exclusion of valid citizens. ARHL is characterized by bilateral progressive hearing loss predominant in the high frequencies. It is a multifactorial condition with etiologic factors both environmental and genetic, the latter dictating an intrinsic susceptibility to presbycusis.

Previous candidate gene studies in different populations found an association between presbycusis and genes involved in hereditary deafness or in oxidative metabolism. Mitochondrial haplogroups U and K in an Australian population, and the NAT2*6A haplotype in Europe, were found to be significantly associated with ARHL. In addition, three different GWAS have recently been published and a highly significant and replicated SNP in GRM7 gene was identified.

We have audiologically assessed a meaningful sample of Portuguese elder individuals and are establishing genotype-phenotype correlations by analyzing GJB2, GJB6, GRM7, NAT2 and mtDNA genotypes using a variety of methods.

The pattern of variants found in this sample seems to be consistent with those previously described for the general European population. Some associations between presbycusis and certain genotypes are discussed.

In conclusion, this study aims at contributing to the genetic knowledge of ARHL susceptibility factors as well as the audiological characterization of the elderly Portuguese population.

Functional Analysis of GJB2 mutation found in Portuguese population

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Deafness is the most common sensory deficit in humans affecting the social development of each individual affected by this pathology. It is estimated that 50% of hearing impairment can be avoided. About 1/1000 newborns show deafness and 1/3 of the population over 65 is also affected.Mutations in GJB2 gene, which encodes connexin 26, are the main cause for hereditary non syndromic deafness, like GJB6 gene, which encodes connexin 30. Three mutations of the Cx26 – a p.Leu213X, p.Gly160Ser and p.Gly160Cys have been functionally characterised in HeLa cells by immunofluorescence.

A small quantity in the plasmatic membrane of the proteins carrying the p.Leu213X mutation has been verified. They were observed mainly in the cytoplasm, which suggests that they are hold there. The deficit in their course towards the membrane might be connected to the fact that this mutation originates a STOP codon in the C-terminus domain of the protein. The proteins carrying the p.Gly160Ser and the p.Gly160Cys mutation are transported up to the plasmatic membrane, just like the wild Cx26. However, the permeability of the intercellular channels composed by Cx26 presenting these mutations was not yet researched.

This study contributes to deepen the knowledge on hereditary deafness and the range of mutations in the DFNB1 locus. It has also allowed the first functional characterization of this three mutations of connexin 26. The functional studies carried out present a greater importance when one has in mind the future implementation of therapies focused on the recovery of the native function of connexin 26.

PHARMACOGENETICS APPLICATION TO FORENSIC TOXICOLOGY: SEQUENCING CYP2D6 IN POST-MORTEM BLOOD SAMPLES WITH HIGH CONCENTRATION OF TRAMADOL

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Introduction: Tramadol is a centrally acting opioid analgesic used very often in hospital for pain control. Tramadol is bioactivated in the liver by CYP2D6 enzyme to O-desmethyl-tramadol that has approximately 200 times more affinity to opioid receptors and is responsible for the main analgesic effect. CYP2D6 gene is located on the chromosome 22q13.1 and is highly polymorphic. More than 20 allelic variants are related to enzyme inactivation, which may lead to adverse drug effects and even to death. Approximately 5 to 10% of the European population is homozygous for a defective CYP2D6 gene. Some authors have demonstrated that there is a correlation between genotype and phenotype for tramadol.
To aid interpretation of the forensic toxicology results in tramadol positive cases, we have sequenced CYP2D6 in post mortem blood samples to identify some polymorphisms, such as CYP2D6 *3, *4 or *6, that cause absence of enzyme activity, specially the *4 allele (more prevalent in Caucasians). With sequencing methodology is possible to identify more allelic variants already known (*8, *12, *14, *15, *19, *20, *41, *44) or other located in the same fragments that could be identified in the future without costs.

Methods: Blood samples spots were extracted by Chelex 100® method and quantitated with Applied Biosystems (AB) Kit Human Quantifiler® by Real-time PCR. Fragments amplification was done by PCR and verified by PHASTGEL® gradient 10-15 electrophoresis. Sequencing was done with Big Dye v.3 (AB) and analyzed in a Genetic Analyser 3130 (AB). Allelic variants were found comparing the results with a reference sequence (CYP2D6*1, GenBank entry M33388.1).

Results and discussion: The detection of genetic polymorphisms at CYP2D6 described as non-functional allelic variants were usefull to explain some circunstances of death in the study of tramadol positive cases and showed the importance of this sequencing methodology to forensic toxicology and pathology.

FAMILIAL CASE OF NOONAN SYNDROME CAUSED BY A MUTATION IN THE CBL GENE

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Introduction: Germline mutations in the CBL tumor suppressor gene have been reported in patients with a Noonan Syndrome (NS)-like phenotype. CBL encodes a multivalent protein with ubiquitin ligase activity, which promotes ubiquitylation and vesicle-mediated internalization and degradation of the epidermal growth factor receptor (EGFR). Pathogenic CBL mutations result in aberrant EGFR trafficking, which leads to enhanced ERK phosphorylation and hence contributes to augment RAS-MAPK signaling. This effect is similar to that produced by activating mutations in genes encoding components of the Ras-MAPK pathway which are known to cause NS. Thus, EGFR trafficking emerges as another disease-relevant regulatory level in the RASopathy-network.

Case Report: We describe an 8-year-old male patient with mega cisterna magna in antenatal imaging. His birth somatometry was normal. Postnatally he was diagnosed with neonatal hypotonia, tetralogy of Fallot, hip dysplasia, blepharophimosis and von Willebrand's disease type 1. He evolved with growth retardation, global psychomotor delay, and hearing loss. Physical examination revealed characteristic NS features. His mother had learning difficulties, a posterior fossa arachnoid cyst and similar facial dysmorphisms. A NGS gene panel for the RASopathies identified a splice site mutation (c.1288-2A>G) in the CBL gene that was maternally inherited.

Discussion: Somatic mutations in CBL have been identified in about 10-15% of patients with juvenile myelomonocytic leukemia (JMML). Specifically the c.1288-2A>G mutation was previously identified in homozygosity in the bone marrow of two JMML patients not know to be affected with NS. This residue appears to be a hotspot for both somatic and germline mutations. Follow-up of our patient should consider an increased risk for myeloproliferative disorders. Both the proband and his mother have posterior fossa abnormalities, a feature rarely observed in NS patients albeit frequent in other rasopathies. Reports on further patients are needed to establish if the latter are more common in NS patients with pathogenic CBL mutations.

THE MSH2 EXON 5 DELETION (C.792+8_943-450DEL) IS A FOUNDER MUTATION IN PORTUGUESE LYNCH SYNDROME FAMILIES WITH A CENTER-SOUTH ANCESTRY

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Introduction: Lynch syndrome (LS) is a hereditary colorectal cancer syndrome caused by germline mutations in the DNA mismatch repair (MMR) genes. Worldwide, large genomic deletions, particularly in MSH2 gene, account for ~20% of the mutational spectrum. The aim of this study was to evaluate a possible founder effect of a recurrent exon 5 deletion in MSH2 gene, detected in 10% of the families from the LS family registry of the Portuguese Oncology Institute in Lisbon. This mutation was not reported by other Portuguese Oncology Centers and it was described only once in the literature, in a family with Portuguese ancestry[1].

Methods: We analyzed 15 unrelated LS families (11 from our registry, 3 from INSA and one family from LUMC) with the MSH2 exon 5 deletion, detected by MLPA, including a total of 57 individuals (30 carriers and 27 non-carriers, all samples were anonymized). The genomic breakpoint was identified by direct sequencing and haplotype analysis was performed using 6 microsatellite markers flanking MSH2 (from D2S2174 to D2S123, spanning ~6Mb) and three intragenic SNPs.

Results: All families shared the same deletion breakpoints (c.792+8_943-450del) and a common haplotype, extending from D2S391 to D2S2227 microsatellite marker (0.858 Mb). Considering the average of mutation and recombination events in this region, we estimate that this mutation occurred ~400 years ago.

Discussion: Our data suggests that the MSH2 exon 5 deletion (c.792+8_943-450del) is a founder mutation in Portugal, which is reinforced by the fact that, for seven families, it has been possible already to establish a common geographical origin. Moreover, the high frequency of the exon 5 deletion in our LS registry indicates that screening of this mutation, using MLPA, should be considered a first and cost-effective approach in the genetic diagnosis of suspected LS families with a Portuguese ancestry, especially in those with a Center-South origin.

[1] – Soravia et al., Am J Med Genet A. 2003

Dunnigan-type familial partial lipodystrophy in large Portuguese kindred

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The lipodystrophies are a clinically heterogeneous group of acquired or inherited disorders affecting adipose tissue distribution. Dunnigan-type familial partial lipodystrophy (FPLD; OMIM 151660) is a rare autosomal dominant disease, characterized by selective absence of adipose tissue in the extremities and trunk and accumulation of fat in the face, neck and supraclavicular fossa. The patients have a muscular hypertrophic appearance, especially in the lower limbs. Affected children are born with normal fat distribution, may present hyperlipidemia in childhood and after puberty start to progressively lose the subcutaneous fat. Later in life, affected adults may experience some metabolic disorders including hypertriglyceridemia, insulin resistance, diabetes mellitus, hepatic steatosis and high blood pressure. Acanthosis nigricans, hirsutism, menstrual abnormalities and polycystic ovarian disease can also occur in affected women. The phenotype appears more pronounced in females.

In this report we present the clinical and molecular characterization of a large Portuguese kindred with various affected family members of different ages and one premature death at 52 years due to myocardial infarction. The pattern of dyslipidemia present in this family is similar to familial combined hyperlipidemia.

Sequencing of the lamin A/C (LMNA) gene was performed by PCR and direct sequencing of all exons. The index patient revealed a heterozygous R482W missense mutation. All family members were subsequently screened for this mutation, confirming the heterozygous status in 4 additional relatives, including 2 children.

The high prevalence of premature and severe cardiovascular events in these patients makes early diagnosis of this condition essential for treatment strategy, and prevention of disease progression.

Microarray in clinical practice – utility *vs* complexity. Mixed phenotype of duplication 15q11.2q13.1 and deletion 16p11.2

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Introduction: There's a consensus to perform chromosomal microarray technique as first-tier clinical diagnostic test for individuals with developmental disabilities. However, given the complexity of clinical presentations, often several diagnostic methods are held before conducting microarray.

Method: We report the case of a 5 year-old boy referred to Medical Genetics due to short stature, developmental disabilities and facial dysmorphic features.
He was born from eutocic delivery after an uneventful pregnancy. He had psychomotor milestones delayed like sitting at 9 months and walking at 24 months, holding an immature broad-based gait. There was history of learning difficulties from both parents, and the mother has also short stature.
On examination it was noted some facial dysmorphic features like high forehead, conical canines and rarefaction of the distal portion of the eyebrows.
Due to the history of an episode of transient ataxia, and suspicion of an inherited metabolic disorder, he had already performed various analytical and imaging screenings, all normal.

Results: Chromosomal microarray analysis revealed two pathogenic Copy Number Variants (CNV's): 16p11.2 deletion and 15q11.2q13.1 duplication.
The 15q11q13 microduplication syndrome (OMIM # 608636) is a very rare clinical entity with about 30 reported cases with maternal origin, and it is characterized by neurobehavioral disorder, hypotonia, cognitive impairment, epilepsy and short stature.
The 16p11.2 microdeletion syndrome (OMIM # 613444) is also a rare clinical entity, with high penetrance, associated with obesity and developmental disabilities.

Discussion: Despite the unquestionable utility of microarray, the correlation of the CNV's with the phenotype is often difficult by the rarity of these new microdeletion/duplication clinical entities. In this case the interpretation has increased difficulty because of the simultaneous existence of two distinct clinical entities. Segregation studies, which in the first step include parental analysis, are essential for genetic counseling and determining the risk of recurrence but also for a more accurate correlation genotype-phenotype.

A three generation Portuguese family affected with spondyloperipheral dysplasia

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Introdution: The spectrum of diseases associated with type II collagen is related to its presence in articular cartilage, eye humor vitreous and vertebral body nucleus pulposus. The association of spine degenerative changes and brachydactyly type E (shortening of IV and V metacarpals/metatarsals) is distinctive for spondyloperipheral dysplasia (OMIM #271700), a rare type II collagenopathy.

Case report: The index patient is a 39 years old man born with club feet. He has moderated myopia (-3,5 diopters) since 5 years old and developed precocious large joint osteoarthritic disease that required bilateral hip replacement surgery at 29 and 34 years of age. Physical examination demonstrated disproportionate short stature, flat face profile, barrel shaped thorax with mild scoliosis, geno valgo, short hands and feet. Patient's mother is 65 years old, has a similar phenotype with club feet, bilateral hip replacement in the third decade of life followed a decade later by multiple knee joint surgeries, high grade myopia (-20 diopters) associated with cataracts operated at 54 years of age. The third patient is the index patient daughter, who had prenatal diagnosis of club feet at 13 weeks of gestation.
The association of severe osteoarthritic disease at young age with ocular disease and facial gestalt, suggested collagen type II pathology. Sequence analysis of COL2A1 gene in index patient concluded a heterozygous variant c.4300delC in exon 53, causing a stop codon at protein level [Leu1434*]. His daughter was confirmed to have inherited paternal mutation.

Discussion: Spondyloperipheral dysplasia is caused by heterozygous truncating mutations in the last exons, affecting the C-propetide of COL2A1. It is proposed that mutated proa1(II) chains escape non-sense mediated decay and accumulate free within the endoplasmatic reticulum, having a calcification-promoting affect that leads to premature fusion of metacarpal and epiphysis growth plates resulting in brachydactyly. We this work we aim to describe the natural history of this disease in three successive generations.

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