Welcome to the

9th Molecular Biology of Hearing and Deafness

Conference

June 22 – 25, 2013

Li Ka Shing Center for Learning and Knowledge,
Palo Alto, CA • Stanford University

STANFORD
SCHOOL OF MEDICINE

Acknowledgements

We would like to thank the following sponsors for their support of this conference. Their generous financial support makes this conference possible.

NIDCD

march of dimes

Hearing Health Foundation
Prevention | Research | Cure

ACTION ON HEARING LOSS

HEAD AND NECK SURGERY
STANFORD OTOLARYNGOLOGY

INITIATIVE TO CURE HEARING LOSS
STANFORD SCHOOL OF MEDICINE

9th Molecular Biology of Hearing and Deafness Conference

PROGRAM

Saturday, June 22		Page
3:00 - 5:30 PM	Registration	
4:00 PM	Welcome Reception	
5:00 PM	**Opening Keynote: A bright future for research on communication and communication disorders,** James F. Battey, Jr.	1
6:00 PM	Adjourn	

Sunday, June 23		Page
8:00 AM	Registration and Breakfast	
8:55 AM	**Welcome to Stanford School of Medicine**, Lloyd Minor	
9:00 AM	**Keynote: Genomics for personalized medicine and human health**, Michael Snyder	2
9:30 AM	**Genetic testing for deafness: The future is now**, Richard J.H. Smith	3
9:45 AM	**Identification of the gene causing nonsyndromic deafness DFNB86**, Atteeq Rehman	4
10:00 AM	**"A complex strategy for complex traits": identification of new genes involved in hearing function and age-related hearing loss**, Giorgia Girotto	5
10:15 AM	**How much of nonsyndromic deafness is actually syndromic?** Thomas B. Friedman	6
10:30 AM	Break & Posters - Group 1	

A BRIGHT FUTURE FOR RESEARCH ON COMMUNICATION AND COMMUNICATION DISORDERS

JAMES F. BATTEY, JR.

NIDCD

The National Institutes on Deafness and Other Communication Disorders (NIDCD) is one of 27 Institutes and Centers that comprise the National Institutes of Health. This presentation will highlight research progress in the seven mission areas relevant to the Institute: hearing, balance, smell, taste, voice, speech, and language. Even in a constrained Federal budget environment, the research community supported by NIDCD continues to make remarkable progress towards achieving a better understanding of human communication and its disorders.

GENOMICS FOR PERSONALIZED MEDICINE AND HUMAN HEALTH

RAJINI R. HARAKSINGH,[1] FERESHTEH JAHANIANI KENARI,[1] JUAN RODRIGUEZ-PARIS,[2] JOEL GELERNTER,[3] KARI C. NADEAU,[4] JOHN S. OGHALAI,[5] IRIS SCHRIJVER,[2,4] AND MICHAEL P. SNYDER[1]

[1] Department of Genetics, Stanford University School of Medicine, Stanford, California, USA. [2] Department of Pathology, Stanford University School of Medicine, Stanford, California, USA. [3] Department of Psychiatry, Yale University School of Medicine, New Haven, CT, USA. [4] Department of Pediatrics, Stanford University School of Medicine, Stanford, CA 94305, USA. [5] Department of Otolaryngology – Head and Neck Surgery, Stanford University School of Medicine, Stanford, CA 94305, USA.

Whole genome sequencing and exome sequencing have impacted human health in many ways. DNA sequencing of cancer genomes has been used to identify somatic alterations and lead to targeted treatments. Sequencing of families has helped elucidate genetics alteration for unsolved childhood and adult diseases. I will review advances in genome and exome sequencing and its use in human health and medicine as well as our efforts to analyse the genetic basis of hearing loss in patients whose underlying genetic defects are not known. The role of other omics technologies (e.g, DNA methylation and RNA-Sequencing) in genome analysis, health care and understanding the basis of human disease will also be discussed.

GENETIC TESTING FOR DEAFNESS: THE FUTURE IS NOW

RICHARD J.H. SMITH[1,2*], A. ELIOT SHEARER[1,2], ROBERT W. EPPSTEINER[1], E. ANN BLACK-ZIEGELBEIN[3], MICHAEL S. HILDEBRAND[1], JOSÉ GURROLA II[1], KEVIN T. BOOTH[1], HARINI RAVI[4], SWATI JOSHI[4], ANGELICA C. GUIFFRE[4], CHRISTINA M. SLOAN[1,2], SCOTT HAPPE[4], SUSANNA D. HOWARD[1], BARBARA NOVAK[5], ADAM P. DELUCA[3,6], KYLE R. TAYLOR[3,6], TODD E. SCHEETZ[3,6,7], TERRY A. BRAUN[3,6,7], THOMAS L. CASAVANT[3,6,8], HELA AZAIEZ[1,9], YILDIRIM A. BAYAZIT[10], MEHMET EMIN ERDAL[11], JOSE A. LOPEZ-ESCAMEZ[12], IRENE GAZQUEZ[12], MARTA L. TAMAYO[13], NANCY Y. GELVEZ[13], GREIZY LOPEZ LEAL[13], CHAIM JALAS[14], JOSEF EKSTEIN[15], TAO YANG[16], SHIN-ICHI USAMI[17], KIMIA KAHRIZI[18], NILOOFAR BAZAZZADEGHAN[18], HOSSEIN NAJMABADI[18], BILL KIMBERLING[1], EMILY M. LEPROUST[5]

[1] Department of Otolaryngology—Head and Neck Surgery, University of Iowa Hospitals and Clinics, Iowa City, Iowa, USA; [2] Department of Molecular Physiology & Biophysics, University of Iowa College of Medicine, Iowa City, Iowa, USA; [3] Center for Bioinformatics and Computational Biology, University of Iowa, Iowa City, Iowa, USA; [4] Agilent Technologies, Cedar Creek, Texas, USA; [5] Agilent Technologies, Santa Clara, California, USA; [6] Department of Biomedical Engineering, University of Iowa, Iowa City, Iowa, USA; [7]Department of Ophthalmology and Visual Sciences, University of Iowa, Iowa City, Iowa, USA; [8]Interdepartmental PhD Program in Genetics, University of Iowa, Iowa City, Iowa, USA; [9]Institut Pasteur de Tunis, Tunis, Tunisia; [10]Department of Otolaryngology, Gazi University, Ankara, Turkey; [11] Department of Medical Biology and Genetics, University of Mersin, Mersin Turkey; [12] Otology and Neurotology Group CTS495, Center for Genomic and Oncological Research (GENyO), Granada, Spain; [13] Instituto de Genética Humana, Pontificia Universidad Javeriana, Bogotá, Colombia; [14]Bonei Olam, Center for Rare Jewish Genetic Disorders, Brooklyn, New York, USA; [15]Dor Yeshorim, The Committee for Prevention of Jewish Genetic Diseases, Brooklyn NY & Jerusalem, Israel; [16] Department of Otorhinolaryngology—Head and Neck Surgery, Xinhua Hospital, and the Ear Institute, Shanghai Jiaotong University School of Medicine, Shanghai, China; [17] Department of Otorhinolaryngology, School of Medicine, Shinshu University, Matsumoto, Japan; [18] Genetics Research Centre, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran

Non-syndromic hearing loss (NSHL) is the most common sensory impairment in humans. Until recently, its extreme genetic heterogeneity precluded comprehensive genetic testing and population-scale sequencing. In this study, we aimed to establish population-level frequencies of reported deafness-causing variants in 1,000 normal hearing controls from six ethnic populations. We then sought to determine the cause of deafness in 100 patients with presumed genetic hearing loss. We performed targeted genomic enrichment and massively parallel sequencing (TGE+MPS) on control samples in non-barcoded pools of 20 and on patient samples barcoded individually and multiplexed. We developed a local Galaxy framework for bioinformatics analysis on a high performance-computing cluster. Using data from controls we found an aggregate carrier frequency of 3.3% for non-DFNB1 autosomal recessive deafness-causing mutations. We also found that 25 reported deafness-causing mutations are implausible due to high carrier frequencies in different ethnic groups. In 42% of patients screened, we identified causative mutations. Diagnostic rates differed significantly based on inheritance and type of hearing loss. Our results highlight the need for continued gene discovery and improved understanding of the contribution of non-exonic mutations to genetic disease. The unsolved families screened using this platform represent a valuable resource to address both of these needs. Data from normal controls of different ethnicities highlight the importance of population-based surveying in the classification of truly pathogenic variants.

IDENTIFICATION OF THE GENE CAUSING NONSYNDROMIC DEAFNESS DFNB86

***ATTEEQ REHMAN*[1], *KWANGHYUK LEE*[2], *ROBERT MORELL*[1], *TAKU ITO*[3], *MEGHAN DRUMMOND*[1], *SAIMA RIAZUDDIN*[4]; *REGIE SANTOS-CORTEZ*[2], *SHAHEEN KHAN*[5]; *ANDREW GRIFFITH*[3], *SHEIKH RIAZUDDIN*[5], *SUZANNE LEAL*[2], *THOMAS FRIEDMAN*[1]**

[1]Laboratory of Molecular Genetics, NIDCD, National Institutes of Health, Rockville, MD; [2]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas, and the University of Washington Center for Mendelian Genomics; [3]Otolaryngology Branch, NIDCD, National Institutes of Health, Rockville, MD; [4]Division of Pediatric Otolaryngology, Cincinnati Children's Hospital Research Foundation, Cincinnati, OH; [5]Allama Iqbal Medical College/Jinnah Hospital Complex, Lahore, Pakistan

Human hereditary deafness is a genetically heterogeneous disorder. Of the 114 autosomal dominantly (*DFNA*) or autosomal recessively inherited (*DFNB*) loci for presumptive nonsyndromic deafness, approximately one half of the corresponding genes are yet to be reported. DFNB86 profound deafness segregating in a large consanguineous family, PKDF799, was mapped to chromosome 16p (maximum two-point LOD score 8.54; Ali and Rehman et al., 2012). To identify the causative variant co-segregating with deafness, exome sequencing of one affected individual from family PKDF799 was performed. Nine homozygous DNA variants were identified within the *DFNB86* linkage interval that had an allele frequency of <0.5% in public SNP databases. Eight of the nine variants were omitted from further consideration as pathogenic mutations due to high allele frequencies in 358 control chromosomes from an ethnically matched cohort. The remaining variant was a guanine-to-thymine transversion, which is predicted to cause a substitution of a highly conserved aspartate to tyrosine. Homozygosity for this missense mutation co-segregates with deafness in family PKDF799 and is predicted to be pathogenic by a variety of in *silico* prediction programs including MutationTaster, PolyPhen-2, and SIFT. Subsequently, we identified allelic missense mutations in three additional *DFNB86*-linked families. Clinical re-evaluations of the affected subjects of these families indicated that DFNB86 deafness appears to be nonsyndromic, although other alleles of this gene are reported to cause a neurological disorder that does not include hearing loss. We will discuss this new deafness gene, its immunolocalization and potential functions.

"A COMPLEX STRATEGY FOR COMPLEX TRAITS": IDENTIFICATION OF NEW GENES INVOLVED IN HEARING FUNCTION AND AGE-RELATED HEARING LOSS

***GIORGIA GIROTTO*[1], *DRAGANA VUCKOVIC*[1], *ANNALISA BUNIELLO*[2], *MORAG LEWIS*[2], *KAREN P STEEL*[2] *AND PAOLO GASPARINI*[1]**

[1]*Med Genet, IRCCS-Burlo Garofolo Children Hospital, Trieste Univ., Trieste, Italy.*
[2]*MedSci, FRS, Wolfson Centre for Age-Related Diseases, King's College London*

The analysis of complex genetic traits/diseases such as normal hearing function (NHF) and Age-Related Hearing Loss (ARHL) has long been an enigma of genetic biology and medical sciences. Thus, the majority of genes/loci involved in NFS and ARHL still need to be identified. To reach this goal an integrated strategy has been designed based on: A) Genome Wide Association Studies (GWAS) on different quantitative and qualitative hearing traits (low, medium and high Pure Tone Average, the separated thresholds and the Principal components), B) replication of GWAS data C) expression studies in mice using immunohistochemistry of genes replicated and D) genotype-phenotype relationships. A meta-analysis of 3417 samples from European isolated populations was carried out and a list of 12 top genes has been defined (Girotto et al. JMG 2011). 9 genes out of 12 have been replicated in 398 individuals from isolated villages located in Caucasus and Central Asia. Furthermore, these 9 genes have been confirmed at the expression level in wildtype mice (at 4 and 5 days postnatal) using immunohistochemistry microscopy. In particular, 3 of them (Arsg, Slc16a6, Dclk1) show strikingly specific expression in the cochlea (e.g. at the top of sensory hair cells and in the marginal cells of the stria vascularis) while the other 6 (Ptprd, Grm8, GlyBP, Evi5, Rimbp2, Ank2) are located in multiple cell types in the cochlea. Moreover, to look for genotype-phenotype relationship, the audiometric profiles (i.e. mean values at each frequency) of the three genotypes of the most associated gene-variant have been analyzed. In particular, 5 genes (GlyBP, Arsg, Slc16a6, Ank2, Grm8) have a peculiar audiometric pattern showing relevant differences for each genotype. Finally, to define specific gender candidate genes sex-separated analyses are in progress.

These preliminary results highlight the effective combination of GWAS/Meta-analysis, expression studies and genotype/phenotype data to provide new insights into the molecular basis of NHF and ARHL, and may suggest new targets for hearing impairment treatment and prevention.

HOW MUCH OF NONSYNDROMIC DEAFNESS IS ACTUALLY SYNDROMIC?

THOMAS B. FRIEDMAN[1], ATTEEQ U. REHMAN[1], EMMA JENKINSON[2], ROBERT J. MORELL[1], MEGHAN C. DRUMMOND[1], JULIE M. SCHULTZ[1], SUZANNE M LEAL[3], SHEIKH RIAZUDDIN[4], WILLIAM G. NEWMAN[2]

[1]NIDCD, National Institutes of Health, Bethesda, MD; [2]Center for Genetic Medicine University of Manchester, UK; [3]Baylor College of Medicine, Houston, Texas; [4]CEMB, University of the Punjab, Lahore, Pakistan

Many unique and complex structures in the inner ear are required for hearing. It's not surprising that mutations of genes encoding such molecular machinery might cause hearing loss alone and no other associated abnormality. However, most of the macromolecules that comprise the inner ear machinery are also expressed in many other tissue types in the body. Therefore, mutations thought to initially be only associated with deafness might cause problems in other organ systems. About 100 genes have been reported in which mutations can cause dominantly (DFNA) or recessively (DFNB) inherited deafness, presumably with no associated signs or symptoms involving extra-auditory organs. However, after comprehensive clinical evaluations, a few of these deafness phenotypes were later shown to be part of a syndrome. For example, we recently reported the map position of "nonsyndromic deafness" DFNB81 segregating in consanguineous family PKDF291 (Rehman et al., 2011). After analyzing the exome of the DFNB81 critical interval on chromosome 19p13, we discovered that this family is segregating a homozygous missense mutation of CLPP. This gene encodes CLPP, a highly conserved partner of CLPX, which together form a mitochondrial chambered endopeptidase. CLPP is a crucial participant in the unfolded-protein response (UPRmt), a signaling pathway interlinking ATP-dependent unfolding and degradation of particular proteins in mitochondria, along with the up-regulation of a specific subset of genes in the nucleus. A second missense allele of CLPP was identified independently in a family segregating Perrault syndrome, a sex-limited, recessive disorder characterized by sensorineural hearing loss in males and females, and ovarian dysgenesis in females. Clinical re-evaluation of affected females of family PKDF291 revealed that they do have Perrault syndrome (Jenkinson, Rehman, Walsh et al., 2013). The pathogenic mechanism and functional nexus between deafness and ovarian failure due to missense mutations of CLPP are key questions we intend to address using mouse models.

TARGETED EXON CAPTURE AND MASSIVE PARALLEL SEQUENCING IDENTIFIES THE MUTATED GENES IN CHINESE DEAF FAMILIES

***HUIJUN YUAN*[1], *JING CHENG*[1], *YU LU*[1], *XUEYA ZHOU*[2], *MICHAEL ZHANG*[2], *RICHARD CHOY*[3]**

1. Institute of Otolaryngology, Chinese PLA General Hospital, Beijing 100853, China.
2. Bioinformatics Division, Tsinghua National Laboratory of Information Science and Technology, and Department of Automation, Tsinghua University, China.
3. Department of Obstetrics and Gynecology, Chinese University of HongKong, HongKong

Hereditary nonsyndromic hearing loss (NSHL) is extremely heterogeneous. Because of the hereditary features of SNHL, genetic diagnosis has exceptionally high value in the medical practice for detecting the cause of hereditary hearing loss. Giving that there are more than 200 known human genes associated with SNHL, a method with high throughput diagnostic capability but greatly reduced cost such as massively parallel sequencing (MPS) may replace Sanger sequencing and chip array in clinical application to identify the genetic cause of SNHL. In this study, we developed a target enrichment method that enables the capture of known regions of the human genome (252 genes) associated with SNHL on a single reaction for high throughput mutation identification by MPS platform. We designed target intervals with Agilent SureSelect platform and ran 13 samples from 7 Chinese deaf families to identify their genetic defects. We use an in-house built computation pipeline based on GATK to align reads to the human reference genome and call variants from the alignments. For confirming the QC filter, all candidate causal variants were closer examined with IGV software and tested by Sanger sequencing. Although barcode pooling often generates unequal amount of total sequences, we observed per-target read-depths were highly correlated between pooled pairs in the same dilution unit. Mutations in MYO6, CDH23, TECTA, and COCH are identified in 4 families, respectively. Linkage and Sanger sequencing data supported co-segregation of these mutations with the phenotypes in these families. Three DFNA families did not find the causal mutations by this MPS approach. Four possibilities might be account for the failure of mutation identification in these DFNA families: (1) causal variants on known genes were missed due to incomplete coverage; (2) hearing loss within the same family may be caused by more than one causal variants of known genes; (3) patients with phenocopy denied the real positive variants; (4) novel DFNA genes underlying the hearing loss in these families.

SOUND ENCODING: FROM MOLECULAR PHYSIOLOGY TO DISEASE AND RESTORATION

TOBIAS MOSER

InnerEarLab, Department of Otolaryngology, University Medical Center Göttingen, Collaborative Sensory Research Center 889, Center for Nanoscopy and Molecular Physiology of the Brain, Bernstein Center for Computational Neuroscience and Focus for Neurotechnology

Sound is encoded at synapses between cochlear inner hair cells and the auditory nerve. These synapses are molecularly, anatomically and functionally specialized to transmit acoustic information with high fidelity over a lifetime. The hair cell synapse features a single and large ribbon-type active zone and each of the 5-20 active zones drives spiking in one spiral ganglion neuron in the absence and presence of acoustic stimulation. High and sustained rates of synaptic transmission require very efficient means of vesicle cycling and interfering with the hair cell vesicle cycle results in auditory dysfunction. I will report on auditory synaptopathy caused by otoferlin defects. Currently, cochlear implants are employed to enable auditory function in the deaf as no causative treatment is available e.g. for auditory synaptopathy. I will discuss efforts towards optogenetic stimulation of the auditory nerve for improving hearing with cochlear implants.

COMPOSITION AND FUNCTION OF THE SYNAPTIC RIBBON.

ALBENA KANTARDZHIEVA [1], ***M. CHARLES LIBERMAN*** [1], ***AND WILLIAM F. SEWELL*** [1]

[1] Eaton-Peabody Laboratory, Department of Otology and Laryngology, Massachusetts Eye and Ear Infirmary and Harvard Medical School, 243 Charles Street, Boston, MA 02114, USA; [2]Harvard Mass Spectrometry and Proteomics Resource Lab; FAS Center for Systems Biology, Harvard University, Cambridge, Massachusetts 02138.

The synaptic ribbon is an electron-dense structure found in hair cells and photoreceptors. The ribbon is surrounded by neurotransmitter-filled vesicles and considered to play a role in vesicle release. We recently published a quantitative analysis of the protein composition of the ribbon complex from the mouse using mass spectrometry-based proteomics analyses (Kantardzhieva, J Proteomics Res, 2012). The ribbon complex primarily comprises proteins found in conventional synapses and this appears to concentrate proteins dealing with vesicle creation, retention, distribution, and consequent exocytosis. The CtBP isoforms are the major proteins in the ribbon. CtBP protein family members play a critical role in membrane fission at several intracellular transport steps, including transfer from the Golgi to the epithelial basolateral membrane. Tubular membrane enclosures protrude out of the Golgi complex and then partition into vesicles, which migrate to the plasma membrane. The overexpression or inhibition of CtBP can either accelerate the Golgi vesicles formation or block the transport respectively.

The intimate involvement of CtBP in vesicle fission at the Golgi suggests that CtBP may be acting similarly in the ribbon. Prolonged excitation of the synapse depletes the vesicles in its vicinity, and leads to the appearance of intracellular membrane-enclosed structures and membrane invaginations compensating for the vesicular membrane loss (Lenzi, Neuron, 2002). Thus CtBP may initiate vesicle fission to create vesicles near the ribbon from larger membrane-enclosed structures (cisterns). To test this hypothesis we analyzed the distribution of vesicles and cisterns around ribbons from serial sections of inner hair cells in the cat, and compared data from low and high spontaneous rate (SR) synapses. Consistent with the hypothesis, we identified a “sphere of influence” of 350 nm around the ribbon, with fewer cisterns and many more synaptic vesicles. While high- and low-SR ribbons tended to be longer and thinner than high-SR ribbons, the total volume of the two ribbon types was similar. There were almost as many vesicles docked at the active zone as attached to the ribbon. The major SR-related difference was that low-SR ribbons had more synaptic vesicles intimately associated with them. Our data suggests a trend in which low-SR synapses had more vesicles attached to the ribbon (51.3 vs. 42.8), more docked between the ribbon and the membrane (12 vs. 8.2), more docked at the active zone (56.9 vs. 44.2), and more vesicles within the “sphere of influence” (218 vs. 166). These data suggest that the structural differences between high- and low-SR synapses may be more a consequence, than a determinant, of the physiological differences.

Sponsored by a travel award from:
Hearing Health Foundation
Prevention | Research | Cure

LACK OF BRAIN-DERIVED NEUROTROPHIC FACTOR IN THE COCHLEA BUT NOT IN THE BRAIN HAMPERS INNER HAIR CELL SYNAPSE PHYSIOLOGY, BUT PROTECTS AGAINST NOISE INDUCED AFFERENT FIBER LOSS.

***MARLIES KNIPPER*[1], *WIBKE SINGER*[1], *ANNALISA ZUCCOTTI*[1], *STEPHANIE KUHN*[2], *STUART L. JOHNSON*[2], *CHRISTOPH FRANZ*[1], *DIETMAR HECKER*[3], *HYUN-SOON GEISLER*[1], *IRIS KÖPSCHALL*[1], *KARIN ROHBOCK*[1], *KATJA GUTSCHE*[4], *JULIA DLUGAICZYK*[3], *BERNHARD SCHICK*[3], *WALTER MARCOTTI*[2], *LUKAS RÜTTIGER*[1], *THOMAS SCHIMMANG*[4]**

[1]Department of Otolaryngology, Head and Neck Surgery, Hearing Research Centre Tübingen, Molecular Physiology of Hearing, University of Tübingen, Elfriede-Aulhorn-Straße 5, 72076 Tübingen, Germany. [2]Department of Biomedical Science, University of Sheffield, S10 2TN, Sheffield, UK. [3]Department of Otorhinolaryngology, Saarland University Hospital, Kirrberger Straße, 66421 Homburg/Saar, Germany. [4]Instituto de Biología y Genética Molecular, Universidad de Valladolid y Consejo Superior de Investigaciones Científicas, E-47003 Valladolid, Spain

The precision of sound information transmitted to the brain depends on the transfer characteristics of the inner hair cell (IHC) ribbon synapse and its multiple contacting auditory fibers. We found that brain derived neurotrophic factor (BDNF), so far assumed to maintain spiral ganglia neuron survival, differentially influences IHC characteristics in the intact and injured cochlea. Using conditional knockout mice (BDNFPax2 KO) we found that resting membrane potentials, membrane capacitance and resting linear leak conductance of adult BDNFPax2 KO IHCs showed a normal maturation. Likewise, in BDNFPax2 KO membrane capacitance (ΔCm) as a function of inward calcium current (ICa) follows the linear relationship typical for normal adult IHCs. In contrast the maximal ΔCm, but not the maximal size of the calcium current, was significantly reduced in high frequency but not low frequency cochlear turns correlated with a loss of IHC ribbons in these turns and a reduced activity of the auditory nerve (ABR wave I). Remarkably, a noise-induced loss of IHC ribbons, followed by reduced activity of the auditory nerve and reduced centrally generated wave II and III observed in control mice, was prevented in equally noise-exposed BDNFPax2 KO mice. This did not occur when BDNF was deleted in a mice model that lead to deletion of BDNF in central brain neurons. Data describe a differential beneficial and harmful role of BDNF in the intact, respectively injured cochlea with dramatic impact on central sound processing that are discussed in a more widespread context of brain disorders.

COCHLEA SPIRAL GANGLION CELL DEGENERATION AND HEARING LOSS AS A CONSEQUENCE OF SATELLITE CELL DEATH IN SAPOSIN B KO MICE

AKIL, O.[1], SUN, Y.[1], LEE, C.[2], ZHANG, W.[3], KU, T.[1], GRABOWSKI, G.A.[2], LUSTIG, L.R.[1]

1- Department of Otolaryngology- Head & Neck Surgery, University of California San Francisco, San Francisco, CA. 2- Division of Human Genetics and Cincinnati Children's Hospital Medical Center and the Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH. 3- Division of Pathology and Laboratory Medicine University of Cincinnati College of Medicine, Cincinnati, OH

Prosaposin, and its cleaved products Saposins A-D, are multifunctional proteins with both intra- and extracellular functions and are necessary for the activity of specific lysosomal glycosphingolipids. Previously we demonstrated that absence of prosaposin leads to a progressive loss of hearing and balance as a result of abnormal afferent and efferent innervation patterns, suggesting that prosaposin or saposin A-D may be required for normal adult cochlear and vestibular innervation. One of prosaposin's cleaved products, Saposin B (Sap B) serves as an essential activator protein for arylsulfatase A in the hydrolysis of sulfatide, a lipid component of myelin. To gain insight into Sap B's physiological functions in hearing and balance, the inner ear of Sap B knockout mice (B-/-) was studied. Hearing and histology of the cochlea of B-/- mice were tested and analyzed from P30 days to P15months. Through P4mo, B-/- mice had normal hearing and cochlear histology compared to WT littermates, except for the spiral ganglion (SG) cells that showed inclusion bodies in Satellite cells starting at P30. Hearing was stable through P6mo. Beyond P6mo B-/- mice exhibited a significant progressive increase in ABR thresholds for frequency-specific sound stimuli (8, 16, 32 KHz). ABR click thresholds remained normal until P13mo, after which they started to increase. The increased ABR latencies showed decreased conduction velocities in B-/- mice. Outer hair cells in the B(-/-) mice appeared unaffected, as evidenced by normal DPOAE measures and normal counts. At both light and electron micrograph levels, there was evidence of inclusion bodies and vacuoles in Satellite cells, resulting in degeneration and later SG and Scarpa's ganglia degeneration. At the EM level SG myelin sheaths were either reduced or absent in the B-/- mice at P8mo and older. Further, the progressive hearing loss was closely correlated with the increase of inclusion bodies and vacuolization in the SG with subsequent SG and cochlear nerve fiber degeneration. Sulfatide and ceramide analysis and Alcian blue staining of the SG indicated that these inclusion bodies were due to sulfatide accumulation. Together, these results show that in the B-/- mouse cochlea, progressive accumulation of sulfatide in the Satellite cells of the SG and Scarpa's ganglion lead to disruption of sulfatide homeostasis and subsequent Schwann cell degeneration followed by SG and Scarpa's ganglion degeneration, with a resulting hearing loss. These results support a role for Saposin A in the maintenance of normal myelin in spiral ganglion neurons.

THE AUDITORY HAIR BUNDLE: GENETICS AS A GATEWAY TO THE UNDERSTANDING OF ITS MULTIFACETED PROPERTIES

CHRISTINE PETIT

Institut Pasteur, Inserm UMRS 1120, College de France

The hair bundle, the sensory antenna of the hair cells plays a key role in the processing of sounds within the cochlea. Not only does the hair bundle operate the sensory transduction, the conversion of sound-evoked mechanical vibrations into electrical signal (receptor membrane potential), but it also interacts with sound waves to amplify and fine-tune the passive cochlear resonance. These interactions produce conspicuous distortion of sound waveforms, which creates sounds absent from the original acoustic stimulation (Tartini's tones), or suppresses weak signals in the presence of masking competitors. The last two properties are thought to be critical for hearing in noisy environment and speech intelligibility.

The presentation will highlight how the identification of genes underlying monogenic, early-onset forms of deafness in humans combined with pluridisciplinary studies including in-depth analysis of the corresponding mouse models, provides a way to disentangle these various hair bundle properties, to decipher their molecular bases as well as to uncover the interplay of some of these properties. I'll discuss the roles played by the various subsets of hair-bundle links and recent advances regarding the role of the architecture of the outer hair cell hair bundle in the frequency tuning of the cochlear response. Progress in deciphering the structure/function relationships of the molecular complex of the tip-link upper insertion point will also be illustrated. The major impacts of these results on the management of deaf patients will be considered.

THE MECHANOTRANSDUCTION MACHINERY OF HAIR CELLS

ULRICH MUELLER

Dept. Mol.Cell Neuroscience, Dorris Neuroscience Center, The Scripps Research Institute, La Jolla, CA 92037, USA

Hair cells are mechanosensors for the perception of sound, acceleration and fluid motion. Mechanotransduction channels in hair cells are gated by tip links, which connect the stereocilia of a hair cell in the direction of their mechanical sensitivity. To identify components of the mechanotransduction machinery of hair cells, we have carried out forward and reverse genetic screens to generate and identify mouse lines with recessive forms of deafness associated defects in mechanotransduction. Through SNP mapping and exom sequencing, we have identified nearly two dozend gene mutations that cause deafness. More than 90% of the affected genes are expressed within the inner ear specifically in hair cells. Several of the affected genes encode components of the hair cells mechanotransduction machinery including components of the tip link and its associated molecules. Recently, we have complemented these studies by developing a gene transfer system for the perturbation of gene function in hair cells using shRNA approaches. This allows us to screen for components of the mechanotransduction machinery with higher throughput and to functionally characterize them in more detail. We will present our progress on the identification of the molecules of mechano-transduction and how they regulate the transduction process.

HAIR CELL MECHANOTRANSDUCER ADAPTATION IS NOT DRIVEN BY CALCIUM ENTRY IN MAMMALIAN AUDITORY HAIR CELLS.

ANTHONY PENG[1], THOMAS EFFERTZ[1], ANTHONY J. RICCI[1,2]

Department of Otolaryngology[1], Molecular and Cellular Physiology[2], Stanford University

A hallmark of hair cell mechano-electrical-transduction (MET) is an adaptation process posited to underlie several important biological processes including dynamic range, mechanical filtering and amplification. Two described modes of adaptation, termed fast and motor differ based on kinetics, mechanics, dynamic range and pharmacology. To date, both components are hypothesized to be driven by Ca^{2+} entry through the MET channel. Data supporting this hypothesis arises from work in turtle, frog and mammalian vestibular systems (i.e. lower frequency systems), where hair bundles (the mechanosensitive organelle of hair cells) have up to ten rows of stereocilia, channels intimated to be at both the upper and lower ends of the tip-link and adaptation time constants in the millisecond (Fast) and 10-100 ms (motor) component. Experimental evidence includes both steady-state and kinetic sensitivities to intracellular Ca^{2+} buffers, extracellular Ca^{2+} and voltage. In comparison we found that mammalian cochlear hair cells behave drastically different. Mammalian cochlear hair cells have only three rows of stereocilia with the two shorter rows having functional MET channels. Adaptation rates are also much faster with time constants on the sub-millisecond to millisecond range. Longer time constants were only observed with larger, nearly saturating stimulations. The extent of adaptation, as measured from paired pulse stimulations, was unaffected by internal Ca^{2+} buffering or voltage demonstrating that adaptation was not driven by Ca^{2+} entry. Adaptation rates and extent were also unaffected by internal Ca^{2+} concentrations and voltage, though the extent of adaptation and the relative proportion of adaptation components were modulated by these manipulations. These data support a limited role for Ca^{2+}, perhaps as an indirect modulator of adaptation in mammalian cochlear hair cells. Changes in external Ca^{2+} shift the resting probability of opening as previously described, however this effect is independent of adaptation and appears to be due to an extracellular modulation of the hair bundle. A parsimonious explanation for these data is that mammalian cochlear hair cells lose the Ca^{2+}-dependent motor adaptation unmasking the unexpected finding that fast adaptation is not Ca^{2+} dependent. The loss of Ca^{2+} dependence is predicted to result in a constant standing force on the tip link that will result in faster activation and adaptation kinetics, allowing higher frequency responses to be achieved.

THE FUNCTION OF TRANSMEMBRANE CHANNEL-LIKE GENES 1 AND 2 IN MAMMALIAN HAIR CELL MECHANOTRANSDUCTION.

***Jeffrey R. Holt*[1], Bifeng Pan[1], Gwenaelle S. Geleoc[1], Yukako Asai, Geoffrey C. Horwitz, Kiyoto Kurima Kotaro Ishikawa[2], Yoshiyuki Kawashima[2] and Andrew Griffith[2]**

[1]Department of Otolaryngology, F.M. Kirby Neurobiology Center, Boston Children's Hospital, Harvard Medical School, 300 Longwood Avenue, Boston, MA 02115, USA.
[2]Otolaryngology Branch, National Institute on Deafness and Other Communication Disorders, National Institutes of Health, 5 Research Court, Rockville, MD 20850-3320, USA.

Sensory transduction in auditory and vestibular hair cells requires expression of transmembrane channel-like (Tmc) 1 and 2 genes (Kawashima et al., 2011), but the function of these genes is unknown. Possible functions of TMC1 and TMC2 proteins include: 1) trafficking or development of hair cell transduction channels, 2) transduction apparatus components that are mechanically in series with, but not part of, the transduction channels or 3) components of the transduction channels themselves (Kawashima et al., JCI, 2011).

TMC1 and TMC2 have satisfied several important criteria (Christensen and Corey, Nat Rev Neurosci. 2007; Arnadóttir and Chalfie, Annu. Rev. Biophys, 2010) to be considered bona fide mechanotransduction channels: The onset of Tmc2 expression coincides with development of hair cell mechanotransduction; exogenous fluorophore-tagged TMC proteins can be localized to the tips of hair cell stereocilia; genetic deletion of Tmc1 and Tmc2 eliminates hair cell mechanosensitivity; and reintroduction of exogenous Tmc1 or Tmc2 can restore mechanotransduction (Kawashima et al., JCI, 2011). Fulfillment of either of two remaining criteria could demonstrate a direct role for TMC1, TMC2 or both as components of the hair cell transduction channel: Heterologous reconstitution of mechanosensitive currents in cell lines transfected with Tmc1 or Tmc2, or alteration of core biophysical properties of endogenous hair cell transduction using gain- or change-of-function mutations in Tmc1 or Tmc2.

We are actively pursuing these approaches. Thus far, in our laboratories, heterologous expression of Tmc genes has not yielded membrane localization of mammalian TMC proteins, although a recent report concluded that C. elegans tmc-1 expressed in HEK cells can traffic to the plasma membrane and mediate ionic currents (Chatzigeorgiou et al., Nature, 2013). This raises the possibility that the Tmc superfamily may include genes that encode ion channels.

We will present and discuss recent data that focuses on identifying of the function of Tmc1 and Tmc2 in auditory and vestibular hair cells of the mammalian inner ear.

THE ROLE OF TRANSMEMBRANE CHANNEL-LIKE PROTEINS IN COCHLEAR HAIR CELL TRANSDUCTION

ROBERT FETTIPLACE AND KYUNGHEE X. KIM

Department of Neuroscience, University of Wisconsin, Madison, WI, USA

Sound stimuli are detected in the cochlea by sub-micron displacements of the hair bundles of cochlear hair cells, which open mechanotransducer (MT) ion channels in the constituent stereocilia. The molecular composition of the MT channel, a non-selective cation channel with high permeability to Ca2+, is not yet unequivocally established. However, a key role of transmembrane channel-like (Tmc) proteins in hair cell mechanotransduction was recently proposed by Kawashima et al (2011) who discovered that MT currents were totally absent in double knockouts of Tmc1 and Tmc2 isoforms. Tmc1 mutations underlie DFNA36 progressive hearing loss and DFNB7/B11 profound deafness. We have shown (Kim & Fettiplace 2013) that in mice harboring the 'dn' mutation in Tmc1, MT currents in outer hair cells (OHCs) declined after post-natal day (P)6 and were absent by P10, which may account for the 'deafness' phenotype (Steel & Bock 1980). In the absence of Tmc1 during the first neonatal week, Tmc2 is proposed to support mechanotransduction but is down regulated after P6 (Kawashima et al 2011). We have also determined that, prior to P6 when large MT currents still occur, knockout of Tmc1 leads to an increase in the MT channel Ca2+ permeability and a reduction in its single-channel conductance compared to wild-type, the changes being most prominent in basal OHCs. The simplest conclusion is that Tmc1 is a component of the MT channel pore and is more important at the base. We have further found that in double Tmc1/ Tmc2 knockouts, significant MT currents could still be recorded when hair bundles were deflected with a fluid jet (as opposed to an attached glass probe), but these currents were of reversed polarity, being activated by hair bundle displacements towards the shortest edge of the bundle. Such currents usually declined after P7 and might have been missed by applying positive hair bundle deflections with a glass probe. The reversed-polarity currents in the double knockouts resembled wild-type MT currents in their block by micromolar FM1-43 and by extracellular Ca2+, but they were anomalous in persisting after destruction of tip links with BAPTA. Moreover, the channels underlying the reversed-polarity MT currents had a reduced Ca2+ permeability compared to wild-type or single knockouts. We suggest that they may represent another subunit of the MT channel.

FGFR1 SIGNALLING ASSEMBLES A KINOCILIA-SPECIFIC TRANSPORT PARTICLE TO TRAFFIC PROTOCADHERIN-15 DURING INNER EAR HAIR CELL SPECIALIZATION

AKIRA HONDA[1]*, TOMOKO KITA[1]*, YUKO MUTA[1], KAZUYO MISAKI[2], REIKO NAKAGAWA[3], GUY RICHARDSON[4], SHIGENOBU YONEMURA[2], RAJ K. LADHER[1]

[1]Lab for Sensory Development, [2]Electron Microscopy Unit and [3]Proteomics Unit, RIKEN Center for Developmental Biology, 2-2-3 Minatojima-Minamimachi, Chuo-ku, Kobe, Japan. [4]School of Life Sciences, University of Sussex, Falmer, Brighton, UK.

The ciliated mechanosensory cells of the inner ear, the hair cells, are uniquely adapted to their task detecting sound and balance information. The eccentrically located kinocilia and the ordered and elaborate stereocilia register mechanical stimuli when they deflect. The formation of this distinctive apical architecture is considered to be a simple modification of the prototypical epithelial cell: the primary cilium is transformed into the kinocilia, and actin-based microvilli are transformed into stereocilia. While generic mechanisms are responsible for the formation of the cilium, the specialization of the kinocilia must depend on specific pathways. However, the nature of these pathways and the molecular mechanisms that underlie them, are not known. We find that the fibroblast growth factor 1 plays a role in this process in inner ear hair cells. Fgfr1 is localized to the forming kinocilia, where it interacts with IFT components and is responsible for the kinociliary transport of protocadherin-15, important in establishing the morphology of hair cells. FGFR1 leads to the assembly of kinocilliary transport plaques by coordinating the membrane accumulation of clathrin through its adaptor disabled-homologue (DAB) 2. DAB2 also interacts with pcdh15 and in this way ensures that association between FGFR1 and PCDH15. We propose that FGFR1 is part of a kinocilia-specific transporter responsible for the kinociliary distribution of protocadherin-15, and thus the generation of mechanosensory hair cell morphology.

GENOME-WIDE ASSOCIATION STUDY FOR AGE-RELATED HEARING LOSS IN THE MOUSE: A META-ANALYSIS.

RICK FRIEDMAN[1], JEFFREY OHMEN[1], XIN LI[1] EUN YONG KANG[2], FARHAD HORMOZDIARI[2], JONG WHA JOO[2], QING YIN ZHENG[3], A. JAKE LUSIS[4], ELEAZAR ESKIN[2].

Department of Cell and Molecular Biology and Genetics, House Research Institute[1], Computer Science Department, UCLA[2], Department of Otolaryngology, Case Western Reserve University[3], Department of Human Genetics, UCLA[4].

The genetic cross has been the gold standard design for the identification of sequence variants that contribute to phenotypic variation in mice. Utilizing this approach, thousands of quantitative trait loci (QTLs) for a number of traits and disease phenotypes have been characterized. Each QTL region is large, often tens of megabases, and contains hundreds of genes. Thus, only a small fraction of the relevant genes have been identified. In contrast, genome-wide association (GWA) studies have enjoyed tremendous success and have uncovered hundreds of genes involved in common human diseases. The limitations of these human studies, namely, the need for large sample sets and the remaining "missing heritability", have led investigators to develop alternative strategies. Mouse studies reveal that a genetic cross in only a few hundred animals can identify loci that together explain 50% or more of the heritability. It is for these reasons that our collaborators and we have adopted a strategy for doing GWA studies in the mouse, the Hybrid Mouse Diversity Panel, for studying age-related hearing loss.

We have performed the first GWA study of its kind in the mouse by combining several data sets in a meta-analysis to identify loci associated with age-related hearing loss. The result was five genome-wide significant loci ($< 10^{-8}$). One of these loci generated a narrow peak in the region of *Ahl8*. These data confirm the utility of this approach and provide new high-resolution mapping information about variation within the mouse genome associated with this complex trait.

GENETIC VARIATION IN *ESRRG* IS ASSOCIATED WITH ADULT HEARING STATUS IN HUMANS AND ITS TARGETED DELETION CAUSES HEARING LOSS IN MICE.

Lisa S. Nolan[1], Hannes Maier[2], Irm Hermans-Borgmeyer[3], Giorgia Girotto[4], Russell Ecob[1], Nicola Pirastu[4], Barbara A. Cadge[1], Christian Hübner[3], Paolo Gasparini[4], David P. Strachan[5], Adrian Davis[1] and Sally J. Dawson[1].

[1]UCL Ear Institute, London, UK; [2]Institute of Audioneurotechnology (VIANNA) and Department of Experimental Otology, ENT-Clinics, Medical University Hannover, Hannover, Germany; [3]ZMNH, Hamburg, Institute of Human Genetics, Jena, Germany; [4]Institute for Maternal and Child Health- IRCCS "Burlo Garofolo"-DMS, University of Trieste, Trieste, Italy; [5]Division of Community Health Sciences, St George's, University of London, London, UK.

There are clear gender differences in susceptibility to age-related hearing loss (ARHL); it is more common and more severe in men compared to women. Moreover, decline in hearing sensitivity typically manifests within the third decade of life for men, but in women onset is much later and coincides with decreased estrogen levels post-menopause. To investigate the thesis that estrogen plays an important role in maintenance of adult hearing, we examined whether variation in estrogen-signalling genes is linked to adult hearing status in 3,900 participants of the 1958 British Birth Cohort (B58C). This analysis implicated the estrogen related receptor gamma (ESRRG) gene in determining adult hearing function and was investigated further in a total of 6,134 individuals across 3 independent cohorts: (i) the 1958 British Birth Cohort; (ii) a London ARHL case-control cohort and (iii) a cohort formed from isolated populations of Italy and Silk Road countries. Evidence of an association between the minor allele of SNP rs2818964 and hearing status was identified in females, but not in males in 2 of these cohorts: p = 0.0058 (London ARHL cohort) and p = 0.0065 (Carlantino, Italy). In addition, subsequent secondary analysis to examine the effect of noise exposure and family history of ARHL on this association identified the strongest evidence of association in non-noise exposed women who report a family history of ARHL (London ARHL cohort: allelic effect p = 0.0023, ORG/A = 1.61, 95% CI = 1.84-2.20). Furthermore, Esrrg knock-out mice were found to have a 25-dB hearing deficit at 5 weeks of age. By 12 weeks of age, this deficit was 15 dB worse in females(-/-) than males(-/-). Immunofluorescence studies with an anti-ESRRG antibody, show ESRRG is localised to inner hair cells, Reissner's membrane and discrete supporting cell populations of the adult mouse inner ear. Together these data indicate ESRRG plays an important role in maintenance of hearing in both humans and mice.

EXOME SEQUENCING FILLS MISSING PIECES OF THE AUDITORY PUZZLE: IMPLICATIONS FOR DEAFNESS

KAREN B. AVRAHAM

Department of Human Molecular Genetics and Biochemistry, Sackler Faculty of Medicine and Sagol School of Neuroscience, Tel Aviv University, Tel Aviv, Israel

Hundreds of mutations in more than 60 genes are known to be involved in hearing impairment, yet it is estimated that for a majority of individuals with suspected hereditary hearing loss, the etiology remains unknown. In many of these unsolved cases, the causative mutation might be a known or novel mutation in a known gene that is not screened routinely due to practical limitations. Until recently, the large reservoir of known deafness genes, as well as their large size, precluded comprehensive genetic diagnosis. The combination of targeted genomic capture and massive parallel sequencing (MPS) has become a promising tool for detecting novel and known mutations involved in hereditary hearing loss and for solving many deafness cases in a rapid and cost-effective manner. Alternatively, the gene may be an as yet undiscovered one in relation to deafness, which can be solved by whole exome sequencing (WES). In our study, we applied both a targeted gene and whole exome approach to determine the causative genes and mutations in undiagnosed deaf individuals of Israeli Jewish and Palestinian Arab families. New genes and mutations will be described, which are relevant for deafness worldwide. Furthermore, discovery of these new genes will continue to help define compelling mechanisms for deafness.

A Y-LINKED FORM OF HEREDITARY HEARING LOSS IN AN ITALIAN FAMILY

Di Stazio M., Vozzi D., Girotto G., Faletra F., Vuckovic D., Gasparini P.

[1]Med Genet, IRCCS-Burlo Garofolo Children Hospital, Trieste Univ, Trieste, Italy.
FMedSci, FRS, Wolfson Centre for Age-Related Diseases, King's College London

Hereditary hearing loss (HHL) is a common disorder accounting for at least 60% of prelingual deafness. Despite GJB2, GJB6 and A1555G mitochondrial mutations play a major role worldwide, HHL is characterized by a large genetic heterogeneity and clinical variability, which make very difficult to obtain an appropriate molecular diagnosis in a lot of inconclusive cases.

Here, we report the identification of a pedigree resembling a Y-linked form of HHL. A multistep approach using linkage analysis and whole exome sequencing (WES) was carried out. As regards linkage studies, 80 MERLIN linkage simulations were performed to estimate the false positive rate confirming the absence of linkage and supporting the presence of a very rare Y-linked pattern of inheritance. As regards WES, after the enrichment step, library construction, sample sequencing and reads mapping, nucleotide variants were called by Samtools V0.1.18 and filtered comparing with in-house, dbSNP and 1000G. By this approach only a missense mutation (c.A206T:p.D69V) in a gene (YYY) was identified and segregating with the disease. In silico prediction analysis using PolyPhen-2 and MutationTester tools, reported this mutation as disease causing. YYY gene is a homolog of a gene already involve in causing a form of HHL. Preliminary expression studies by immunohistochemistry performed on neonatal human inner ear sections showed a specific staining in multiple cochlea cells type. Functional studies are now in progress to confirm the pathogenetic effect of the variation identified. Transient transfection of two different expression vectors carrying the full-length cDNA (wild-type and mutant) fused to a C-teminal Myc-DDK-tag, will allow to study the mutation effect on protein and mRNA stability. Moreover, since the protein coded by the YYY gene is a member of a co-repressor machineries pathway, this missense mutation might alter the protein folding and stability modifying the proteins interaction and the co-repressor pathway. Very recently, the molecular bases of only two other pedigrees so far described has having an Y-linked HHL have been reported (Wang Q. et al. 2013) but they do not overlap with our findings. These preliminary results suggest an important role of this gene in causing HHL linked to Y chromosome.

MUTATION OF *HOMER2* CAUSES AUTOSOMAL DOMINANT NON-SYNDROMIC HEARING LOSS

***HELA AZAIEZ*[1], *AMANDA R DECKER*[2], *A ELIOT SHEARER*[3], *KEVIN T BOOTH*[1], *SEIJI B SHIBATA*[1], *MICHAEL HILDEBRAND*[4], *ANN TURNER*, *ROBERT A CORNELL*[2], *RICHARD JH SMITH*[1, 5]**

[1]Molecular Otolaryngology and Renal Research Laboratories, Department of Otolaryngology, Departments of Internal Medicine and Pediatrics, University of Iowa, Iowa City, Iowa. [2]Department of Anatomy and Cell Biology, Carver College of Medicine, University of Iowa, Iowa City, Iowa 52242. [3]Department of Molecular Physiology and Biophysics, University of Iowa Carver College of Medicine, Iowa City, Iowa. [4]Austin Health, Department of Medicine, University of Melbourne. [5]Interdepartmental PhD Program in Genetics, University of Iowa, Iowa City, Iowa.

Hereditary hearing loss (HHL) is a clinically and genetically heterogenous disorder. To date, variants in 66 genes have been implicated in this phenotype although comprehensive targeted genomic enrichment with massively parallel sequencing of these genes using the OtoSCOPE® platform identifies causal variants in only ~50% of families segregating HHL. To optimize novel gene discovery, we have developed an analytic pipeline that couples OtoSCOPE® with segregation analysis and whole exome sequencing (WES). Using this approach in a family segregating progressive autosomal dominant non-syndromic hearing loss (ADNSHL), we first excluded plausible variants in known genes using OtoSCOPE® and then completed WES in three hearing-impaired family members. Variants were filtered by using exome data to define regions-of-interest based on segregation analysis thus reducing the number of actionable plausible ADNSHL-causing variants to only five. Sanger sequencing of these variants identified p.R185P in HOMER2 as the only variant that segregated with the hearing loss phenotype in additional family members. R185 is highly conserved in vertebrates and is predicted to be damaging by Polyphen2 and MutationTaster. The encoded protein regulates group 1 metabotrophic glutamate receptor function and couples surface receptors to intracellular calcium release. The identified missense variant is located in exon 6 and is within the coiled-coil domain that mediates homo- and hetero-multimerization. In mice, Homer2 is expressed in the cochlea with enrichment in hair cells. To study its in vivo function, we expressed wild-type or p.P185 Homer2 RNA in the developing Zebrafish and observed that over-expression of mutant mRNA causes anatomical changes and defects in the inner ear and neuromasts as compared to over-expression of wild-type mRNA. In aggregate, these data implicate HOMER2 as a cause of ADNSHL and suggest that Homer2 contributes to inner ear development or maintenance in zebrafish.

SUPPORTING DIAGNOSTICS FOR HEARING LOSS THROUGH NEXT GENERATION SEQUENCING AND COMMUNITY DATA SHARING

HEIDI L. REHM[1,2], SAMI S. AMR[1, 2], SAMUEL ARONSON[1], MARK BOWSER[1], ELIZABETH D. HYNES[1], BIRGIT H. FUNKE[1,2], SIVAKUMAR GOWRISANKAR[1], BRYAN HARRISON[1], KATHERINE A. LAFFERTY[1], MELISSA LANDRUM[3], MATTHEW LEBO[1,2], AMY L. HERNANDEZ[1], DONNA MAGLOTT[3], LISA F. MAHANTA[1], DIANA L. MANDELKER[1], DANIELLE R. METTERVILLE[1], MICHAEL OATES[1], TREVOR J. PUGH[1,2], GEORGE RILEY[3], JUN SHEN[1], AND THE INTERNATIONAL COLLABORATION FOR CLINICAL GENOMICS[4]

1) Laboratory for Molecular Medicine, Partners Healthcare Center for Personalized Genetic Medicine, Cambridge, MA, USA; 2) Department of Pathology, Brigham & Women's Hospital and Harvard Medical School, Boston, MA, USA; 3) National Center for Biotechnology Information, NLM, NIH, Bethesda, MD, USA; 4) https://www.iccg.org

The use of next generation sequencing (NGS) has enabled dramatic improvements in the ability to offer comprehensive diagnostic testing at affordable costs to patients. We developed the OtoGenome Test covering 72 genes for nonsyndromic hearing loss, Usher, Pendred, Branchio-Oto-Renal and Jervell Lange-Nielsen syndromes. The test uses barcoding and hybrid capture followed by NGS on the Illumina HiSeq. A novel tool called VisCap detects CNVs. Sanger sequencing fills in missing data from NGS and confirms variants. Long range PCR and Sanger is used to differentiate *STRC* pseudogene mutations and detect the common STRC gene deletion. Validation showed 100% sensitivity for substitutions (335/335), 97% for indels (63/65), and 100% for CNVs (16/16). The most challenging aspect of the assay is data interpretation. Several hundred variants are identified in each sample. Although most are pre-classified as Benign or Likely Benign using population frequency data and other auto-classification rules, up to 26 novel variants per case are identified. A comprehensive evidence-based variant assessment strategy is used to support variant interpretation and our GeneInsight software auto-drafts patient reports to allow efficiency for the geneticist sign-out process and delivers them in structured form to the EHR enabling automated variant updates as classifications change over time. Our data shows that 4% of reports are updated annually (Aronson *et al.* Genet Med 2012;14(8):713-719).To date, 112 samples have been analyzed using the OtoGenome test with 34% positive for one (n=20) or two (n=18) pathogenic or likely pathogenic variants, using our rigorous evidence-based classification system. An additional 51% of cases had a total of 146 variants of uncertain significance with limited available data in the literature and public databases to infer their potential impact. We recognized that community data sharing would be highly beneficial to enable better understanding of rare variants. To address this need for hearing loss and all diseases, the International Collaboration for Clinical Genomics has been working closely with NCBI to launch the ClinVar database (http://www.ncbi.nlm.nih.gov/clinvar), which went live in April 2013. ClinVar currently contains over 50,000 variant annotations, including our own hearing loss clinical data collected over the last 10 years with detailed evidence-based clinical classifications. This includes 2218 variants in 65 hearing loss genes from 2048 probands. This presentation will include a review of the current state of the database and future plans for ClinVar to enhance community data-sharing of case and variant level data, as well as expert consensus driven curation of these data through a recently funded grant to the International Collaboration for Clinical Genomics.

SCREENING STRATEGY FOR MOLECULAR DIAGNOSIS OF DEAFNESS: FROM SOCIAL HEALTH INSURANCE BASED SCREENING TO MASSIVELY PARALLEL DNA SEQUENCING

***SHIN-ICHI USAMI*[1], *MAIKO MIYAGAWA*[1], *TAKEHIKO NAITO*[1], *HIDEAKI MOTEKI*[1], *SHIN-YA NISHIO*[1]**

[1]*Department of Otorhinolaryngology, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto 390-8621 Japan*

There has been considerable progress in the discovery of deafness genes, but clinical application still entails difficulties due to the genetic heterogeneity of deafness. Approximately one hundred genes are estimated to cause hereditary hearing loss, but a number of these may result in similar phenotypes that entail no abnormalities other than hearing loss. The costs and time required for screening genes one-by-one is prohibitive, but it is now thought that certain mutations are recurrent. An initial screening strategy that focuses on those frequently reported, recurrent mutations expected to be commonly encountered in the clinical setting, might be an appropriate approach for clinical application. We have developed an advanced screening strategy (Invader assay) focusing on frequently recurring mutations that are most likely to be encountered in the clinical setting that identifies approximately 40% of deafness patients. This indicates that 30-40% of patients have deafness due to commonly found mutations, such as in *GJB2* or *SLC26A4*. In Japan, from 2012 genetic testing using Invader assay for deafness can be covered by social health insurance. Currently, we first apply the Invader assay for screening 47 known mutations of 13 known deafness genes followed by direct sequencing as necessary (Usami et al., 2012).

For the remainder of the patients who have unknown etiology, we are now applying Massively Parallel DNA Sequencing (MPS) of target candidate genes to discover rare causative genes. Exome sequencing using MPS is a new powerful strategy for rare Mendelian disorders such as deafness. By the analysis of randomly selected Japanese deafness patients, who had already been evaluated for common genes/mutations by Invader assay, we efficiently identified rare causative mutations and/or mutation candidates. Our data suggests that targeted exon sequencing of selected genes using the MPS technology will be able to identify rare responsible genes including new candidate genes for individual patients with deafness and improve molecular diagnosis in the clinical setting (Miyagawa et al., 2013).

GJB2 TESTING OF INFANTS WITH SENSORINEURAL HEARING LOSS.

***LALAYANTS MARIIA*[1], *MARKOVA TATYANA*[1], *BLIZNEZCH ELENA*[2], *POLAYKOV ALEXANDER*[2], *TAVARTKILADZE GEORGE*[1]**

[1]National Research Centre for Audiology and Hearing Rehabilitation, Moscow, Russia; [2]Medical genetic centre of science of the Russian Academy of Medical Science, Moscow, Russia

Since 2008 264 infants with bilateral sensorineural hearing loss (SNHL), without signs of syndromic SNHL, were investigated for *GJB2* genetic mutations in the Center for Audiology. At least two sets of results of audiological re-examination during the first year of life were available for 113 children. *GJB2* gene testing identified 171 children (64.8%) with biallelic mutation, including 117 patients (44,3%) homozygous for the c.35delG mutation, 11 patients (4,2%) with a heterozygous genotype and 82 children (31%) with a genotype without mutation. A total of 21 different mutations and 30 different genotypes were identified. The most common mutations were c.35delG (79,6% of mutant alleles), c.313del14 (4,8%), IVS1+1G>A (3,4%) and p.Met34Thr (2,0%). Audiological re-examination revealed stable ABR thresholds during the follow-up period in 90% of infants with biallelic mutations in *GJB2* and in 68% of patients without mutations in GJB2. In some patients without mutations in *GJB2* hearing thresholds degreased to normal values during the first year of life. Most of our observations matched the previously published data. 92% of patients with a genotype consisting of such truncating mutations as c.35delG, c.313del14, c.167delT and c.235delC had severe-to-profound HL. All of 7 patients with a genotype consisting of p.Met34Thr or p.Val37Ile in combination with any other mutation had mild HL. At the same time there were cases which were quite difficult to explain by known pathogenesis of *GJB2*-related HL. For example, 13% of patients with biallelic mutation in *GJB2* passed newborn hearing screening at the birth. One patient with c.35delG homozygous genotype had stable mild HL (ABR thresholds 40 dB nHL) from 3 weeks of age to 11 months. Two patients with *GJB2*-related HL had 50 dB interauricular asymmetry in hearing levels. We diagnosed different severity of HL in twins with c.35delG homozygous genotype (moderate in one infant and profound in another one). Genetic testing is the only way to find the real aetiology of congenital SNHL. In most other cases we can just suppose, but not claim the reason of SNHL. Stable ABR thresholds in most cases and slight progression in some cases of *GJB2*-related SNHL are indications for the earliest rehabilitation (hearing aids or cochlear implantation). Infants without mutations in *GJB2*, especially with neurological disorders, require a follow-up period before the final diagnosis and the decision for the exact type of rehabilitation are made. Detection of biallelic mutations in *GJB2* explains the aetiology of HL, but in some cases does not explain the phenotype of *GJB2*-releted HL.

Sponsored by a travel award from:

NIDCD

POPULATION GENETICS IN THE PERSONAL GENOME ERA

CARLOS BUSTAMANTE

Department of Genetics, Stanford University, Palo Alto, CA

Understanding the relative effects of different population genetic forces on the apportionment of human genomic variation is a central focus of medical and population genomics. Much of what we know comes from analyzing patterns of common, and, therefore, ancient genetic polymorphisms via genotyping across diverse human populations. Recent studies have used sequencing approaches to reveal a more complete and genome-wide picture of variation, including lower frequency variants with a more recent evolutionary origin. In this talk, I will present a systematic analysis of 50 human genomes from 11 diverse global populations, sequenced at high coverage. Our sample includes 12 individuals of admixed ancestry that have varying degrees of recent (within the last 500 years) African, Native American, and European ancestry. Using approaches for modeling population history that consider sequence diversity and the length distribution of segments of continuous inferred ancestry, we reconstruct shared and unique aspects of population demographic history based on singly sequenced human genomes that recapitulate extreme bottlenecks at the Out-of-Africa event as well as during the peopling of the Americas.

PROTEINS OF THE HAIR BUNDLE

PETER G. BARR-GILLESPIE[1]

[1]*Oregon Hearing Research Center & Vollum Institute, Oregon Health & Science University, Portland, OR 97239, USA*

Accurately measuring the composition of the vertebrate hair bundle is a necessary first step in determining how the bundle is assembled. We have combined datasets from three different mass spectrometers, representing bundles purified from ~2000 chicken utricles, to identify the principal proteins of the bundle. Control experiments using purified protein standards diluted in complex protein mixtures indicate that quantitation accuracy is similar with all instruments, justifying lumping the data together. Comparison of the chicken dataset with preliminary data for adult mouse utricle hair bundles revealed many similarities but also some significant differences. Future experiments will examine the bundle's composition during development, following pharmacological treatment, or in mouse mutants that lack key bundle proteins.

CLIC5 INTERACTS WITH TAPERIN AT THE BASE OF STEREOCILIA

INNA A. BELYANTSEVA[1], ALEXANDRA K. BOUKHVALOVA[1], EVA MOROZKO[1], ERICH T. BOGER[1], ELIZABETH A. WILSON[1], STACEY M. COLE[1], MARK BERRYMAN[2], ATTEEQ U. REHMAN[1], JONATHAN E. BIRD[1], MEGHAN C. DRUMMOND[1], THOMAS B. FRIEDMAN[1]

[1]Section on Human Genetics, Laboratory of Molecular Genetics, NIDCD/NIH, Rockville, MD 20850,[2]Ohio University Heritage College of Osteopathic Medicine, Department of Biomedical Sciences, Athens, OH 45701

Mutations of TPRN are associated with human nonsyndromic recessive deafness DFNB79. TPRN encodes a protein with unknown function, which we immunolocalized to the tapered region at the base of each stereocilium and named taperin (Rehman et al., 2010). Taperin has limited sequence similarity with phostensin, a minus-end actin-capper, suggesting a possible involvement in stereocilia actin core maintenance. To investigate taperin functions, we sought to identify its binding partners within stereocilia. CLIC5 (chloride intracellular channel 5) has also been shown to localize to stereocilia tapers and the absence of functional CLIC5 in the jitterbug mutant mouse leads to hair cell stereocilia degeneration (Gagnon et al., 2006). Since both taperin and CLIC5 localize to stereocilia tapers and are predicted to be involved in the maintenance of actin filaments in hair cell stereocilia, we hypothesized that these two proteins interact. Using gene gun mediated transfections, we show that both GFP-taperin and GFP-CLIC5 target to the taper region of wild type mouse auditory and vestibular hair cell stereocilia confirming their previously published localizations using antisera. A COS-7 cell based protein-protein interaction assay shows that taperin and CLIC5 are binding partners. Moreover, our yeast two-hybrid screen analyses of a mouse kidney cDNA library also identified CLIC5 as an interacting partner of taperin. Using a variety of internal, N- and C-terminal deletions of taperin, we mapped this interaction site to residues encoded by exon 1. Immunostaining of taperin in the homozygous jitterbug mouse organ of Corti shows that taperin is localized normally in early postnatal hair cells, but is mislocalized in degenerating adult jitterbug hair cell stereocilia. However, mislocalization of taperin in the adult jitterbug mice is likely to be a general consequence of the degeneration process rather than the absence of functional CLIC5 in hair cell stereocilia. Our data indicate that taperin and CLIC5 are interacting partners, but that CLIC5 is not necessary for taperin localization at the base of stereocilia.

ECTOPICALLY EXPRESSED TECTB LOCALISES TO THE DISTAL TIPS OF SENSORY HAIR BUNDLES

***GUY RICHARDSON*[1], *JULIA KORCHAGINA*[1], *RICHARD GOODYEAR*[1], *KEVIN LEGAN*[1], *OMAR RISK*[1,2] *AND ANDY FORGE*[2]**

[1]Sussex Neuroscience and School of Life Sciences, University of Sussex, Falmer, Brighton, BN1 9QG, UK, [2]UCL Ear Institute, University College London, 332 Grays' Inn Road, London, WC1X 8EE, UK

The tectorins, Tecta and Tectb, are non-collagenous glycoproteins that are present in the otoconial and tectorial membranes of the inner ear. Mutations in *Tecta* cause both recessive (DFNB21) and dominant (DFNA8/12) forms of human hereditary hearing loss. Targeted mutagenesis in mice has been used to explore how some of these mutations in *Tecta* cause deafness, and the resultant in vivo models reveal a distinct correlation between phenotype and genotype. A robust in vitro system for studying how tectorin-based matrix is produced and interacts with the apical surfaces of the sensory epithelia in the inner ear would be a useful additional tool for studying the effects of mutations that cause deafness. The tectorins are targeted to and expressed on the apical surfaces of transiently-transfected, polarised epithelial cells (MDCK and CL4) but little evidence is observed for the presence of distinct extracellular matrix in such cells. Although epithelial cell lines derived from MDCK cells stably transfected with tectorin constructs also fail to produce significant amounts of distinct matrix when growing as monolayers, some degree of matrix formation is observed in the hollow centres of epithelial spheres generated from such cells lines in Matrigel-collagen gels. By contrast, discrete patches of matrix are frequently observed when recombinant fluorophore-tagged Tectb constructs are biolistically transfected into the epithelial cells in the outgrowth zones surrounding primary cultures of the early postnatal cochlea. Introducing Tecta ZP domain mutations that are known to cause deafness into conserved residues in the ZP domain of EGFP-tagged Tectb appears to stop the protein from being secreted and prevents matrix formation in these outgrowth zone cells. Biolistic transfection allows one to express tectorin constructs in a number of different cell types in cochlear cultures. When ectopically expressed in sensory hair cells, EGFP-Tectb localises to the distal tip of the hair bundle. Correlative immunofluorescence-immunogold electron microscopy of EGFP-Tectb transfected hair cells in cochlear cultures derived from early postnatal $Tecta^{\Delta ENT/\Delta ENT}/Tectb^{-/-}$ mutant mice reveals the presence of a distinct cap of electron-dense, EGFP-immunoreactive matrix sitting atop of the hair bundle. Stereocilin, a protein product of the DFNB16 locus that may mediate attachment of the hair bundle to the tectorial membrane, is only expressed in the kinocilium at this stage of development. Alternative, as yet unidentified, matrix receptors may therefore localise to the tips of the hair bundle's stereocilia.

DEFECTS IN PROTEIN COMPLEX ASSEMBLY AND ER STRESS AS THE PROXIMAL CAUSE OF USHER SYNDROME

BERNARDO BLANCO-SÀNCHEZ, AURÉLIE CLÉMENT, AND MONTE WESTERFIELD

Institute of Neuroscience, University of Oregon, Eugene, OR, USA

Human Usher syndrome, the most frequent cause of deaf blindness, is a genetically heterogeneous recessive disease. Patients present with congenital deafness due to loss of mechanosensory hair cells and progressive retinal degeneration. Twelve different chromosomal loci have been linked to Usher syndrome, and nine of the genes have been identified to date. Surprisingly, these genes encode a wide range of different kinds of proteins including transmembrane adhesion and signaling molecules, intracellular scaffold proteins, and a myosin motor protein. In vitro binding studies have led to the hypothesis that the scaffold proteins bind the other Usher proteins into a macromolecular complex. Although this model that Usher proteins act together in a complex is an appealing explanation for how the human disease can result from mutation of any one of a number of different genes, it is still controversial. Moreover, the effects of mutations on protein complex formation, subcellular transport, and stability are completely unknown. We have developed an in situ proximity assay to identify if, where, and when Usher proteins form complexes in inner ear hair cells. We find that a subset of Usher proteins preassemble into a complex in the endoplasmic reticulum (ER). In Usher mutants, transport of this complex to the Golgi is disrupted, leading to ER stress and, in some cases, apoptosis. We propose that improper assembly of the Usher complex is the proximal cause of at least some forms of Usher syndrome.

GENOMIC AND PHARMACOLOGICAL STUDIES SUGGEST MULTIPLE ROLES FOR NOTCH SIGNALING IN THE AVIAN INNER EAR

***MARK E. WARCHOL*[1], *MICHAEL LOVETT*[2], *JEFFREY KU*[2], *MATTHEW BARTON*[1], *JENNIFER STONE*[3]**

[1]Dept. of Otolaryngology, Washington University School of Medicine, St. Louis MO, [2]Dept. of Genetics, Washington University School of Medicine, St. Louis MO, [3]Dept. of Otolaryngology, University of Washington School of Medicine, Seattle WA

The avian inner ear is capable of regenerating hair cells after ototoxic injury, largely through a process that involves the proliferation of supporting cell progenitors. As such, understanding the mechanisms that regulate the proliferation of cochlear and vestibular supporting cells is of considerable interest. We have recently characterized the transcriptome of the regenerating chick utricle and cochlea, over a time course beginning immediately after injury and continuing through seven days recovery (e.g., Ku et al., submitted). Data from the utricle indicate that the Notch pathway effectors Hes5 and Hes7 are expressed at moderately high levels in undamaged specimens. We also observed that expression of Hes5 and Hes7 are sharply reduced following ototoxic injury, but that their expression levels begin to recover once supporting cells become post-mitotic and replacement hair cells begin to differentiate. Together, these data indicate that Notch signaling is active in the undamaged utricle, and is disrupted in response to hair cell injury. Such findings suggest that interruption of Notch signaling may play a role in the initiation of regeneration. In order to further characterize the effect of interrupting Notch activity, we treated cultured utricles with the γ-secretase inhibitor DAPT. As expected, DAPT treatment of the uninjured utricle led to a strong reduction in the expression levels of Hes5. Moreover, DAPT treatment also triggered high levels of supporting cell proliferation in undamaged utricles and caused enhanced levels of regenerative proliferation in ototoxically-lesioned utricles. Similar findings were observed when Notch signaling was blocked via treatment with the ADAM/TACE inhibitors TAPI-2 and GM6001.

Additional RNA-Seq studies profiled gene expression in the regenerating chick cochlea. In contrast to the utricle, we observed very low expression levels of Hes5 and Hes7 in the uninjured or regenerating cochlear sensory epithelium. Instead, the regenerating cochlea expressed high levels of the Notch effectors Hey1 and Hey2. Finally, pharmacological block of Notch signaling *did not* evoke proliferation in the undamaged cochlea. These results suggest important differences in Notch signaling in the maintenance and regeneration of the vestibular vs. auditory sensory organs.

NOTCH INHIBITION GENERATES HAIR CELLS IN THE ADULT MOUSE CRISTA

OLIVIA BERMINGHAM-MCDONOGH* AND *AMBER D. SLOWIK

Department of Biological Structure and Institute for Stem Cell and Regenerative Medicine, University of Washington, Seattle WA 98109 USA

Balance disorders caused by hair cell loss in the sensory organs of the vestibular system pose a significant health problem worldwide, particularly in the elderly. Currently, this hair cell loss is permanent as there is no effective treatment. This is in stark contrast to non-mammalian vertebrates who robustly regenerate hair cells after damage. This disparity in regenerative potential highlights the need for further manipulation in order to stimulate more robust hair cell regeneration in mammals.

In previous work we described a robust expression of Hes5 in the adult mouse crista and hypothesized that there was ongoing Notch signaling in this organ. In this study we show that inhibition of Notch resulted in a down-regulation of the Notch effectors *Hes1* and *Hes5.* Hes5, as reported by Hes5-GFP, was down-regulated specifically in peripheral support cells and this down-regulation was accompanied by an increase in the total number of $Gfi1^+$ hair cells. Using lineage tracing with PLP/CreER;mTmG mice, we found that these hair cells arose through transdifferentiation of support cells in cristae. This conversion of support cells to hair cells occurred in crista explants from mice up to ten weeks of age. These transdifferentiated cells arose without proliferation and were capable of developing hair cell morphology, migrating to the correct cell layer, and assembling what appears to be a stereocilia bundle with a long kinocilium. Overall, these data suggest that Notch signaling is active in the mature cristae and important in maintaining the support cell fate in a at least a subset of peripheral support cells.

SINGLE CELL TRANSCRIPTIONAL PROFILING OF NATIVE OTOCYST CELLS AND COMPARISON WITH *IN VITRO* GENERATED MURINE OTIC PROGENITOR CELLS

***ROBERT DURRUTHY-DURRUTHY*[1], *ASSAF GOTTLIEB*[2], *JÖRG WALDHAUS*[1], *RUSS ALTMAN*[2], *STEFAN HELLER*[1]**

Stanford University School of Medicine, Department of Otolaryngology, Head and Neck Surgery[1], Department of Genetics[2], Stanford, USA

The mammalian inner ear originates from a temporarily existing structure called the otic vesicle or otocyst. It comprises committed progenitor cells required for the formation of most cell types of the mature inner ear including sensory hair cells, non-sensory supporting cells and neurons. Individual marker genes are expressed in defined regions of the otocyst suggesting that it comprises progenitor cells with distinct identities. Our goal is to identify and unequivocally define the specific otic progenitor cell (OPC) population that harbors the exclusive capability to give rise to the prosensory domains of the developing inner ear. We call these cells the pre-prosensory population.

To address this question, we selected otic and non-otic marker genes that have been reported previously as well as genes identified with microarray analyses aimed to determine specific otic cell markers. These genes are the basis of a 96 transcript quantitative RT-PCR assay that we conducted with otocyst cells at single cell resolution.

Here, we utilized Pax2(Cre)/Rosa26(mt/mG) mice and fluorescence-activated cell sorting to isolate individual cells from the otocyst and delaminated neuroblasts of E10.5 embryos. Single cell gene expression profiling of 382 cells encompassing 36,672 individual qRT-PCR reactions and subsequent cluster analyses revealed the presence of definite sub-populations and delineates the heterogeneity of otocyst cell populations. Comprehensive bioinformatic analyses further revealed that individual otocyst cell sub-groups can be defined by a minimum set of 9-22 marker genes. Dorsal and ventral, neural, and potentially pre-prosensory identities can be assigned to the established subsets of cells based on pre-existing knowledge of spatial and temporal expression patterns of individual genes. Transcriptional profiles further elucidated Fgf and Notch signaling as key pathways to segregate a pro-neuronal from a pro-otic/sensory domain within the ventral portion of the otocyst.

In addition we profiled 381 individual, putative OPCs derived from Pax2-GFP embryonic stem cells (ESCs) as well as 379 GFP-negative control cells. Correlation analyses between native otocyst cells and ESC-emanated cell populations sorted for Pax2-GFP expression and controls revealed that the adapted guidance protocol enriches for presumptive otic phenotypes. Further tests also disclosed a major limitation of the existing *in vitro* guidance protocol, which fails to maintain presumptive ventral otic progenitor cells in a non-neuroblast stage.

MORPHOGEN GRADIENTS REGULATE TONOTOPIC IDENTITY ALONG THE CHICK BASILAR PAPILLA

***MATTHEW W. KELLEY*[1] *AND ZOE F. MANN*[1]**

[1]*Laboratory of Cochlear Development, National Institute on Deafness and other Communication Disorders, NIH, Bethesda, Maryland 20892*

The auditory systems of vertebrates that perceive sound in air are organized based on the separation of complex sounds into their component frequencies (tonotopy). Tonotopy is present in the sensory epithelia of the mammalian cochlea and the avian/reptilian basilar papilla (BP), as well as, in all higher auditory structures. Manifestations of this organization are present along the longitudinal (frequency) axis of the cochlea/BP but vary between classes with changes in basement membrane characteristics predominating in mammals, while gradients in hair cell phenotype are foremost in birds. Despite the significance of tonotopic organization for auditory function, the molecular and cellular factors underlying its organization remain unknown.

To investigate the mechanisms involved in specifying tonotopy, the chick BP was used because of its more obvious tonotopic metrics. Initial experiments using organotypic cultures indicated that tonotopic identity is established prior to E7. Based on these findings, exome analysis was used to compare gene expression between low and high frequency halves of the BP at E6.5. Results indicated the presence of counter gradients of the morphogen *Bmp7* and a naturally occurring BMP antagonist, *Chordin-like 1*, with *Bmp7* expression highest at the low frequency end of the BP. To investigate potential roles for these gradients, embryonic explants were established and maintained in culture media containing either Bmp7 or one of several known antagonists. In contrast with controls, which developed normal tonotopic gradients, explants maintained in Bmp7 media showed changes in tonotopic phenotypes consistent with conversion to a uniform low frequency identity. Conversely, treatment with Bmp antagonists caused the BP to develop with a uniform high frequency phenotype. *In ovo* electroporation of *Bmp7* induced similar phenotypic changes confirming that tonotopic alterations were not an artifact of the *in vitro* protocol. Subsequent analysis indicated that the effects of Bmp7 are mediated through activation of the Tak1-Jnk signaling pathway. These results demonstrate an instructive role for a gradient of Bmp7 in the specification of tonotopic identity along the chick basilar papilla and suggest that frequency specific hair cell phenotypes are regulated through differences in the level of Tak1-Jnk phosphorylation.

IDENTIFICATION OF FOUR NOVEL GENOME-WIDE SIGNIFICANT LOCI IN SPORADIC MENIERE'S DISEASE

JOSE A LOPEZ-ESCAMEZ[1,*], ELENA SANCHEZ[2,*], IRENE GAZQUEZ[1], SONIA CABRERA[1] BELEN MARTINEZ[3], TERESA REQUENA[1], MANUEL MARTINEZ-BUENO[4], SOFÍA SANTOS-PEREZ[5] JESUS BENITEZ[6], NICOLAS PEREZ[7], GABRIEL TRINIDAD[8], ANDRES SOTO-VARELA[5], EDUARDO MARTIN-SANZ[9], JESUS FRAILE[10], PAZ PEREZ[11], MARTA E ALARCON-RIQUELME[3], ANGEL BATUECAS[12]

[1]Otology & Neurotology Group CTS495, Genyo - Centro de Genómica e Investigación Oncológica – Pfizer/Universidad de Granada/ Junta de Andalucía, Granada, Spain, [2]Department of Medical and Molecular Genetics, Division of Genetics and Molecular Medicine and Immunology, Infection and Inflammatory Disease, School of Medicine, King's College London, UK. [3]Genomic Unit, Genyo, [4]Group of genetic of Complex Diseases, Genyo, [5]Division of Otoneurology, Department of Otorhinolaryngology, Complexo Hospitalario Universitario, Santiago de Compostela, Spain, [6]Department of Otolaryngology, Hospital Universitario de Gran Canaria Dr Negrin, Las Palmas, Spain, [7]Department of Otolaryngology, Clinica Universidad de Navarra, Pamplona, Spain [8]Division of Otoneurology, Department of Otorhinolaryngology, Complejo Hospitalario Badajoz, Spain [9]Department of Otolaryngology, Hospital Universitario de Getafe, Spain, [10]Department of Otolaryngology, Hospital Miguel Servet, Zaragoza, Spain, [11]Department of Otorhinolaryngology, Hospital Cabueñes, Gijón, Spain, [12]Department of Otolaryngology, Hospital Universitario Salamanca, Spain
**Both authors share positions*

Genetic association studies in Meniere's disease (MD) have been limited to case-control studies in small series with low significance and replication studies have failed to confirm previous associations. We have recently described an association between bilateral hearing loss and rs11096955 in *TLR10* gene. Here, we describe the most extensive genetic analysis ever performed for sporadic MD in European population. Four associations with genome-wide significance were found and validated in a replication cohort.

A genotyping case-control study was conducted using a Illumina Immunochip, a high density genotyping array containing 196,524 polymorphisms enriched for autoimmune disease loci. The analyses included data filtering by quality controls and population substructure using PLINK and Structure software. The first set consisted of 247 cases and 607 controls and the replication set included 432 cases and 703 controls.

A meta-analysis of the genotype data from both sets (679 patients and 1310 controls) has identified four novel loci with genome-wide significance. A SNV (Single Nucleotide Variant) in 7p12.2 at *IKZF1* gene (rs61731355, $p= 6.2x10^{-17}$), two strong signals in 10p11.23 downstream the *MAP3K8* gene (rs1034009, $p=3.2x10^{-44}$) and in 11p13 between *PDHX* and *CD44* genes ($p=1.2x10^{-43}$) and an intronic signal at 12p13.33 in *CACNA1C* gene (rs117505557, $p=5.2x10^{-10}$). Imputation of genotypes at 10p11.23 loci also provided additional associations in an intergenic region between *NRP1* and *PARD3* genes, but none with stronger effects than the genotyped variants. Most of these signals are associated with regulatory elements and support the hypothesis that the immune response can modify the clinical course in MD.

SEMA3F-NRP2 INTERACTIONS CONTROL SPIRAL GANGLION NEURON TARGETING TO INNER HAIR CELLS

THOMAS M. COATE AND MATTHEW W. KELLEY

Laboratory of Cochlear Development, NIDCD/NIH, Bethesda, MD, USA

The axon guidance mechanisms controlling connectivity between spiral ganglion neurons (SGNs) and hair cells during cochlear development are not well understood. However, it is known that 95% of SGNs (type I) form synapses with inner hair cells (IHCs) and that the remaining 5% of SGNs (type II) extend past IHCs and pillar cells before innervating the outer hair cells (OHCs). Understanding how these projection patterns manifest during development has proven difficult because the high density of SGNs and lack of unique markers has precluded the resolution of individual neurons. To address this issue, we used a genetic labeling strategy in which sparse numbers of SGNs express a red fluorophore (*Ngn1CreERT2; R26R-tdTomato*) while all hair cells express a green fluorophore (*Atoh1-nGFP*). Using this approach, we observed equal numbers of SGN processes in the IHC and OHC regions at embryonic day 15.5. But, most of these processes progressively segregate to the IHC region at later developmental time points. In addition, between E16.5 and E18.5, putative type I SGNs extend processes into the OHC region that are subsequently retracted. These data are consistent with the idea that repulsive cues from the OHC region promote type I SGN targeting to the IHCs.

Among candidate factors for this potential repulsive cue are members of the Class 3 secreted Semaphorins (Sema3s) which bind Neuropilin/Plexin (Nrp/Plxn) co-receptor complexes to regulate diverse aspects of axon motility and guidance. We have found that the SGNs express Nrp2 while pillar cells and Deiters' cells express *Sema3f*. In *Nrp2* mutant mice, many type I SGN processes fail to stop at the IHCs, and instead project past the pillar cells and into the OHC region. *Nrp2* mutants also have increased numbers of OHC ribbon bodies indicating that exuberant outgrowth leads to ectopic OHC synapse formation. In addition, applying exogenous Sema3F to cochlear explants reduces branching and mobility of type I SGN processes, suggesting that Sema3F normally exerts chemo-repulsive effects. Interestingly, although they express Nrp2, type II SGNs are not affected by exogenous Sema3F, suggesting that an additional level of regulation distinguishes their sensitivity to this factor. Overall, these data support the hypothesis that Sema3F expressed in the pillar cells and Deiters' cells normally activates Nrp2/Plxn receptor complexes to restrict type I SGNs to the IHCs. We are currently investigating the developmental signaling pathways that regulate *Sema3f* expression in the cochlea, and how this signaling pathway ultimately facilitates normal hearing function.

WBP2-DEFICIENT MICE SHOW PROGRESSIVE HIGH-FREQUENCY HEARING LOSS AND ABNORMAL COCHLEAR INNERVATIONS

ANNALISA BUNIELLO, ANDREEA HUMA, RAQUEL MARTINEZ-VEGA, NEIL INGHAM & KAREN P STEEL

Wolfson Centre for Age-Related Diseases, King's College London

The Wellcome Trust Sanger Institute Mouse Genetics Programme generates targeted mouse mutants and screens them for a wide range of disease features, including using Auditory Brainstem Responses (ABR) at 14 weeks to detect new hearing-impaired lines. The *Wbp2* homozygous mutant showed raised thresholds at high frequencies only in this screen. *Wbp2* encodes the WW domain-binding protein 2 that acts as a transcriptional coactivator, binding to the estrogen receptor α (*Esr1*) in the nucleus (Dhananjayan et al. 2006, Mol. Endocrinol. 20:2343).

We found that ABR thresholds were raised as early as 4 weeks of age in the mutants and progressively increased and extended to lower frequencies by 28 and 44 weeks old, indicating progressive hearing loss. Immunocytochemistry revealed widespread expression of Wbp2 in nuclei of multiple cell types in the cochlea. The gross structure of the mutant middle and inner ears appeared normal, and scanning electron microscopy of the organ of Corti showed no obvious damage or degeneration at 4 and 30 weeks old. Therefore, we examined the innervation of the cochlea using antibodies to neurofilament to label unmyelinated nerve fibres, to CtBP2 to label pre-synaptic ribbons, and to GluR2/3 to label post-synaptic densities, viewed using confocal imaging of mutant and control littermates at 4 weeks old. In the mutants, nerve endings below inner hair cells appeared swollen, synapses were more widely-spread around the basolateral hair cell membranes, synapses appeared smaller, and double labeling suggested that pre- and post-synaptic markers were not as well-aligned as in control inner hair cells. We are looking into the possibility that these features result from glutamate excitotoxicity, and also investigating the link between *Wbp2, Esr1* and synaptic defects.

COCHLEAR GENE EXPRESSION DIFFERENCES IN *DIAP3* (DIAPHANOUS-HOMOLOG-3) TRANSGENIC MICE

***MARCI M. LESPERANCE*[1], *CYNTHIA J. SCHOEN*[2], *MARGIT BURMEISTER*[2,3,4]**

[1]Division of Pediatric Otolaryngology, Department of Otolaryngology-Head and Neck Surgery, University of Michigan Health System, Ann Arbor, MI, USA; [2]Molecular and Behavioral Neuroscience Institute, University of Michigan Health System, Ann Arbor, MI, USA; [3]Department of Human Genetics, University of Michigan Health System, Ann Arbor, MI, USA; [4]Department of Psychiatry, University of Michigan Health System, Ann Arbor, MI, USA

We have generated transgenic mice overexpressing *Diap3*, the murine ortholog of *DIAPH3*, as a mouse model of human progressive deafness *AUNA1* (auditory neuropathy, nonsyndromic, autosomal dominant, 1). Line 924 shows no hearing loss at 4 or 8 weeks by auditory brainstem response testing, but manifests 35 and 50 dB threshold shifts at 16 and 24 weeks, respectively, at both 12 and 24 kHz. At age 24 weeks, mice retain distortion product otoacoustic emissions, indicative of normal cochlear outer hair cell (OHC) function, despite elevation of auditory thresholds. Scanning electron microscopy of the organ of Corti demonstrates striking anomalies of the inner hair cell (IHC) stereocilia. Over time, IHCs develop elongated stereocilia that appear fused with neighboring stereocilia, in parallel to the time course of hearing loss. A significant reduction in IHC ribbon synapses was noted.

To investigate the downstream effects of *Diap3* overexpression, we compared samples from 4 transgenic 24-week-old mice and 4 gender-matched wild-type littermates. Organs of Corti were removed, submerged in Trizol (Invitrogen), and total RNA was isolated. Biotinylated cRNA was prepared from RNA (TotalPrep RNA Amplification Kit, Ambion) and hybridized to a MouseRef-8 v2.0 Expression BeadChip (Illumina, Inc., San Diego, CA). The chip was scanned on an Illumina BeadStation using Bioinformatics software, and data were downloaded to Illumina BeadStudio. Data were filtered for expression in R, and paired t-tests and false discovery rate (FDR) corrections were done using Significance Analysis of Microarray, with FDR set at 30%. Ingenuity Pathway Analysis (IPA) was also performed (Ingenuity Systems).

The 4 most highly dysregulated genes were *Diap3*, *Aqp1* (aquaporin 1), *Pjvk* (pejvakin), and *Kncn* (kinocilin). 62 probes representing 57 unique genes were identified as differentially expressed, including 8 (*Diap3*, *Pjvk*, *Accn3*, *Grhl2*, *Myo7a*, *Tmprss3*, *Slc17a8*, *Otof)* associated with deafness in humans and/or mice. Interestingly, several of these genes when mutated are associated with a deafness phenotype in which OHC remain functional (eg, auditory neuropathy). IPA analysis generated a network (Lipid Metabolism, Molecular Transport, and Small Molecule Biochemistry) that included 13/57 of the most dysregulated genes. These findings will help elucidate the mechanisms of deafness resulting from *Diap3* overexpression.

ERBB SIGNALING WITHIN SCHWANN CELLS CONTROLS QUIESCENCE OF ZEBRAFISH MECHANOSENSORY PROGENITOR CELLS THROUGH REGULATION OF WNT AND FGF SIGNALING.

MARK E. LUSH*[1] *AND TATJANA PIOTROWSKI*[1]*.

[1]*Stowers Institute for Medical Research, Kansas City, MO 64110.*

The zebrafish lateral line is a mechanosensory system consisting of neuromasts containing support cells and sensory hair cells. The lateral line develops from a migrating primordium that deposits neuromasts and interneuromast cells from anterior to posterior along the trunk of the fish. Interneuromast cells function as lateral line stem cells and give rise to fully differentiated intercalary neuromasts as development proceeds. Previous work from our laboratory revealed a role for Schwann cells in negatively regulating the timing of proliferation and differentiation of these lateral line stem cells. Mutants for *erbb2*, *erbb3b* or *nrg1-3* have increased intercalary neuromast numbers. We show by transplantation experiments and overexpression of a dominant negative ErbB (DN-ErbB) that ErbB is required in Schwann cells while Nrg1-3 is required in neurons. Treating embryos with the ErbB inhibitor AG1478 or overexpression of DN-ErbB after Schwann cell migration also leads to increased neuromast number suggesting that ErbBs are continuously required within Schwann cells to inhibit precocious differentiation of neuromasts. Wnt and Fgf signaling pathways, required for early sensory genesis in the lateral line, are upregulated in *erbb* mutants. Time course expression analysis shows that increased *wnt* expression proceeds increased *fgf* expression. We find that activating Wnt signaling is sufficient to induce proliferation of interneuromast cells. Conversely, interneuromast cell proliferation and extra neuromasts are blocked by inhibition of Wnt signaling. Therefore Wnt signaling plays a crucial role in activating dormant lateral line stem cells. Even though Fgf signaling is required for proliferation in the migrating primordium, inhibition of Fgf signaling in DN-ErbB transgenics has no effect on interneuromast proliferation. However, loss of Fgf inhibits neuromast formation and hair cell specification as shown by loss of *atoh1* expression. In addition to *wnts* and *fgfs* the prosensory genes *sox2*, *eya1*, *six2,* and *notch1* are also upregulated in *erbb* mutants. These results show that ErbB, functioning within Schwann cells, are inhibiting progenitor proliferation and differentiation non-cell autonomously by negatively regulating Wnt and Fgf pathways. So that in the absence or reduction of Schwann cells an early developmental pathway is reinitiated leading to precocious formation of intercalary neuromasts.

ILDR1 IS REQUIRED FOR INNER EAR AND NEUROMAST DEVELOPMENT IN ZEBRAFISH

Qing Sang*[1], Huan Wang[1], Junyu Zhang[1], Ruizhi Feng[1], Haojue Wang[1], Yao Xu[1], Duan Ma[1], Qinghe Xing[1], Xinzhi Zhao[1], Lin He[1], Lei Wang[1#]

1. Institute of Biomedical Sciences, Fudan University, No 138 Yixueyuan Road, Shanghai, 200032, PR China.

Immunoglobulin-like domain containing receptor 1 (ILDR1) was a less characterized gene which was first identified in a lymphoma cells. Recently, ILDR1 was found to be causative for a familial deafness, DFNB42. However, the etiology and mechanism of DFNB42 caused by dysfunction of ILDR1 was not well elucidated. Here, we characterized the function of ILDR1 in zebrafish. ILDR1 morphant zebrafish had reduced number of lateral line neuromasts. The development of semicircular canals were also delayed which resulted in the fusion of otolith. Injection of human ILDR1 mRNA to ILDR1 morphant zebrafish can rescue the phenotype of abnormal inner ear and reduced number of lateral line neuromasts. The subsequent zebrafish genome-wide gene expression profile in 48h and 72h revealed that compared with control, ILDR1 morphant zebrafish have 130 genes decreased and 102 genes increased with at least two fold changes. Among changed genes, the expression of atp1b2b was reduced by three fold in ILDR1 morphant fish compared with the controls. In addition, injection of zebrafish ILDR1 mRNA to ILDR1 morphant fish can enhance atp1b2b expression. Furthermore, injection of atp1b2b mRNA can partly rescue the phenotype resulted from the knockdown of ILDR1. Finally, we concluded that ILDR1 is crucial for the development of semicircular canals and otolith and for the migration of lateral line neuromasts in zebrafish.

Sponsored by a travel award from:
march of dimes

EPIGENETIC REGULATION OF *ATOH1* EXPRESSION DURING DEVELOPMENT AND REGENERATION OF THE MOUSE ORGAN OF CORTI

ZLATKA P. STOJANOVA, TAO KWAN, AND NEIL SEGIL

House Research Institute, Los Angeles, CA, USA

Hearing loss is predominantly caused by death or damage to the auditory hair cells. The *Atoh1* gene is necessary and sufficient for sensory hair cell formation and is a highly desirable therapeutic target for hearing regeneration. The goal of our research is to analyze the modes of *Atoh1* gene regulation in the mammalian organ of Corti.

An important mode of transcriptional regulation is through a variety of reversible post-translational modifications of histone N-terminal tails, such as methylation, acetylation, phosphorylation, ubiquitilation etc., some of which are permissive for expression and some are not. To decode the epigenetic contribution to Atoh1 regulation, we have developed tools for studying the epigenetic status of genes in the purified cell types of the perinatal organ of Corti. These techniques include a small-scale “micro”-chromatin immunoprecipitation (µChIP) from Fluorescence Activated Cell Sorting (FACS) of hair cells and supporting cells, the latter is a technique previously pioneered in our lab (White et al., 2006).

We analyzed some of the major, well-studied histone modifications at the *Atoh1* locus in purified populations of organ of Corti cells and we found that the Atoh1 expression in supporting cells is actively suppressed by epigenetic mechanisms at the *Atoh1* locus. Specifically, histone H3 acetylation at lysine K9 (H3K9ac) is a hallmark of active gene expression, and we have established a positive correlation between H3K9ac at the *Atoh1* locus and *Atoh1* expression in the organ of Corti. Currently, we are investigating the mechanistic link between H3K9ac at the *Atoh1* locus and *Atoh1* gene regulation during development, which will not only confirm our findings but will contribute to better understanding how *Atoh1* regulation could be epigenetically manipulated in order to cure hearing impairment.

FGF SIGNALING IN MUENKE SYNDROME MODEL HEARING LOSS AND RESCUE

***SUZANNE L. MANSOUR*[1], *CHAOYING LI*[1], *LISA D. URNESS*[1]**

[1]*University of Utah, Salt Lake City, UT, USA*

Fibroblast growth factor (FGF) signaling plays dosage-sensitive roles at multiple stages of inner ear development. Mammalian genomes encode 22 FGF ligands and 4 FGF receptors. Three of the receptor genes are alternatively spliced within the extracellular domain to generate “b” and “c” isoforms that differ with respect to FGF binding specificity. FGFs are grouped on the basis of homology and phylogeny into subfamilies that typically bind to and activate to the same extent the same subset of FGF receptor isoforms. Loss-of-function genetic studies show that inner hair cell-expressed FGF8 signals through support cell-expressed FGFR3 to promote differentiation of support cells nearest to the inner hair cell as pillar cells. We studied auditory function in a mouse model of Muenke syndrome (FGFR3 P250R in humans, FGFR3 P244R in mice). This gain-of-function mutation affects a residue located in an extracellular, IgIII-like domain that is common to both the “b” and “c” FGFR3 isoforms, leading to increased ligand-dependent receptor activation. Heterozygous Muenke model mice have hearing loss (HL) that shows relative sparing of the high frequencies, similar to, but more severe than the HL of Muenke syndrome subjects. The basis for Muenke model HL is a 2-to-2 cochlear supporting cell fate transformation from Deiters’ to pillar cells. Unexpectedly, attempts to reduce signaling through FGFR3 P244R by removing one copy of FGF8 had no effect on Muenke model HL, suggesting that other FGFs might play a role in activating the mutant receptor. However, removing one copy of FGF9 or FGF20, other cochlear-expressed FGFs that, like FGF8, can also signal through FGFR3c, had no effect on Muenke model HL, even when combined with loss of one copy of FGF8 and/or loss of the normal copy of FGFR3. Surprisingly, we found that removal of one copy of FGF10, which normally signals through FGFR2b, effected highly efficient functional and structural rescue of the Muenke model inner ear phenotypes. Interestingly, the Muenke phenotype developed between E17.5 and P3, but rescue was not initiated until P5 and not completed until after P14, suggesting a great deal of FGF-dependent plasticity in the identity of supporting cells. Since neither *Fgfr1* nor *Fgfr2* expression was altered in Muenke model mice, and published studies show that FGF10 does not interact with wild type or Muenke mutant versions of FGFR3c, we hypothesize that Muenke model HL is caused by inappropriate activation of mutant FGFR3b by FGF10, which is strongly expressed in the developing Kolliker’s organ, immediately adjacent to the developing inner hair cell. Progress in testing this hypothesis and determining the normal function of cochlear *Fgf10* will be presented.

CONCOMITANT DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS INTO NEURON-LIKE CELLS AND SCHWANN CELL-LIKE CELLS IN A MICROFLUIDIC DEVICE TO DEVELOP APPROACHES FOR FUNCTIONAL REPAIR OF THE INNER EAR

***KATE F BARALD*[1,2], *POORNAPRIYA RAMAMURTHY*[1,2] *FUMI EBISU*[2,3] *JOSH WHITE*[1], *JOONG YULL PARK*[1,4], , *SHUICHI TAKAYAMA*[1,4] *THERESE M. ROTH*[5]**

1. Department of Biomedical Engineering, College of Engineering, University of Michigan, 2. Department of Cell and Development Biology, Medical School, University of Michigan 3. Present address: Stem Cell Centre, Osaka University, Osaka Japan, 4. School of Mechanical Engineering, College of Engineering, Chung-Ang University, Seoul 156-756, Republic of Korea 5. Center for Stem Cell Biology, Life Sciences Institute, University Of Michigan, Ann Arbor, Michigan; Present Address: Department of Human Genetics, University of Michigan Medical School, Ann Arbor, MI.

Regeneration of most central and all inner ear sensory neurons as well as the inner ear's sensory cells does not occur in mammals, including humans. Cochlear prostheses/ cochlear implants (CIs) can restore hearing to patients with extensive sensorineural hearing loss at the level of the cochlea (Hendricks, 2008). However, in order for the CI to send meaningful acoustic information to the brain/CNS it must become closely coupled to the processes of as large and as healthy a population of remaining neurons as possible (Hendricks, 2008). Fully or partially differentiated stem cells differentiated into spiral ganglion-like neurons (SGN) have been introduced into the inner ear (e.g. Chen et al., 2012). We have found that a precise combination of the specific inner ear's neurotrophic cytokine, macrophage migration inhibitory factor (MIF) (Bank et al., 2012, Shen et al., 2012; Holmes et al., 2011) and docasahexaeonic acid (DHA) (Ramamurthy et al, submitted) regulate mouse embryonic stem cell (mESC) differentiation into neurons that resemble inner ear neurons of the spiral ganglion (SGN). Furthermore, Schwann Cells (SCs) are known to influence the growth of neurons and control axon guidance by providing an environment that is favourable for directional outgrowth and growth (Pettingill, 2008). This laboratory produced the first ES-derived myelinating and non-myelinating Schwann cell models (Roth et al., 2007, 2008). By exposing a common population of mouse ESC to MIF and DHA to produce SGN-like cells and to neuregulin to produce SC-like cells in the same microfluidic device we can follow interactions between the two populations during neuronal differentiation, process extension, axon guidance and development of mature synaptic properties as well as early events in myelination by the mESC-derived SC at the cellular and molecular levels. We have now also "coated" a cochlear implant with mESC-derived Schwann Cells and found that both primary inner ear ganglionic neurons (SGN) or ES-derived MIF or DHA-induced SGN-like "neurons" can interact with the CI. In order to more closely mimic the inner ear environment, our in vitro study conditions provide a microscale environment in a microfluidic device: an "ear on a chip", thus providing the ideal paradigm to test functional CI-SGN connections and to devise repair mechanisms for a damaged (deaf) inner ear.

PHAGE DISPLAY DISCOVERY OF RARE PEPTIDES TO SUPPORT DRUG TRANSPORT THROUGH THE TYMPANIC MEMBRANE

ALLEN F. RYAN[1,2], ARWA KURABI[1,2], MARLEN BERNHARDT[1], KWANG PAK[1], ANDREW BAIRD[2]

Departments of [1]Surgery/Otolaryngology, [2]Surgery/Trauma and [3]Neurosciences, UCSD School of Medicine and VA Medical Center, 9500 Gilman Drive, La Jolla, CA 92093-0666, USA

The tympanic membrane (TM) represents an impermeable barrier that protects the middle ear (ME) space from infection. However, it also prevents the local application of drugs to treat ME and inner ear disorders, unless its integrity is surgically breached. For this reason, many disorders of the ear are treated by systemic delivery of drugs, leading to off-target side effects. In the case of otitis media, this includes induction of antibiotic resistance in bacteria throughout the body and potentially serious gastrointestinal distress in infants. We hypothesized that the TM might harbor receptors that would mediate transport through the structure, and that these receptors could be used to deliver cargo from the external to the ME.

We explored whether such transport mechanisms exist using bacteriophage display of peptides and sequential biopanning. Otitis media was induced in rats by injection of nontypeable *Haemophilus influenzae* through the bone of the middle ear bulla, leaving the TM intact. 48 hours after inoculation, a phage library displaying 10^9 random 12-mer peptides was applied to the ME at approximately 1000 copies of each peptide phage, for 2 hours. The ME contents were then harvested and applied to *E. Coli* to amplify any phage that might be present. This process was repeated twice with the resulting amplified ME phage. Samples of phage recovered from the ME were sequenced after round 2 and round 3, and the sequences were aligned.

In 22 phage isolate sequences, four unique peptides that displayed similarity of amino acid structure were represented. To assess the degree of trans-TM transport, each phage isolate was appied to the TM for varying periods of time and ME recovery was compared to that for wild-type (WT) phage, without a peptide on the surface, as a control. All four peptide-bearing phage exhibited ME recovery significantly greater than that of WT phage, with one phage exhibitting recovery 10^5-10^6 times greater. Trans-TM phage transit was dependent upon temperature and ceased after death, indicating an active mechanism. Phage transit was inhibited by free peptide, demonstrating that the petide was the determinant of trans-TM migration. The ability of peptides to transport bacteriophage particles across the TM indicates that this mechanism has considerable cargo-carrying capability that can be harnessed for drug delivery.

GENE EXPRESSION IN THE POSTNATAL COCHLEA IN HEARING AND DEAFENED RATS

STEVEN GREEN, ERIN BAILEY

Department of Biology, University of Iowa, Iowa City, IA, 52242 United States

In deafness models in which hair cells are destroyed by aminoglycoside, the deafferented spiral ganglion neurons (SGNs) gradually die, with type I SGNs dying more rapidly than type II SGNs. It has been often assumed that this is a consequence of loss of neurotrophic support normally provided by the hair cells. However, our data do not support the premise that SGNs are deprived of neurotrophic factors (NTFs) after loss of hair cells. Following daily kanamycin injections in rats postnatal day 8 (P8) through P16, a normal postnatal increase in NT-3 expression in the organ of Corti fails to occur, resulting in low levels of NT-3 transcripts thereafter in deafened rats. Similarly, deafening adult rats results in a sustained decline in NT-3 expression in the organ of Corti. In contrast, expression of other NTFs in the organ of Corti and/or the spiral ganglion persists after hair cells are gone. These include CNTF and GDNF family members known to be trophic for SGNs. Observation of maintained NTF expression in the cochlea can explain why SGNs can survive for months to years after hair cell loss (and may not die at all in some hair cell death paradigms) but it does beg the question of why do SGNs die at all, as they do when hair cells are destroyed by antibiotics or after noise damage. As an approach to determining why SGNs die after hair cell loss we used microarrays to profile gene expression in spiral ganglia from neonatally deafened and from control hearing rats at two ages P32 and P60. P32 is the earliest time at which there is a significant reduction in the number of SGNs, although >80% of SGNs are still alive. By P60 about 50% of the SGNs have died. Comparison of gene expression between hearing and deaf spiral ganglia from P32 or from P60 rats reveals gene expression associated with deafening and genes that may be involved in SGN death. We identified 53 genes significantly up-regulated after deafening at P32 and 165 at P60. We identified 22 genes significantly down-regulated after deafening at P32 and 45 at P60. However, few, if any, of these are changes in expression of genes associated with apoptosis, NTF responses, or NTF deprivation. This does not support a hypothesis that SGNs die because of loss of hair cell-derived NTFs. Rather, the largest number of changes in gene expression post-deafening, evident by P32 and increasing by P60, are associated with inflammation and the innate immune system. Among other changes, these indicate entry of macrophages and NK cells into the spiral ganglion post-deafening. Possibly, SGN death is due to these cellular changes in the environment in the ganglion, brought about by hair cell death, rather than being due directly to hair cell death.

PROTEOME BIOLOGY OF NOISE INDUCED HEARING LOSS

***JEFFREY SAVAS*[1], *KWANG PAK*[2], *ULRICH MUELLER*[1], *JOHN YATES*[1], *ALLEN RYAN*[2]**

[1]*The Scripps Research Institute, La Jolla, CA, USA;* [2]*Univeristy of California San Diego, La Jolla, CA, USA*

Noise induced hearing loss (NIHL) represents one of the most common and ubiquitous occupational and environmental health risk. While the causes of NIHL are well known the molecular underpinnings and sequence of events are just now starting to be revealed and represent key step towards the development of effective treatments and preventative measures. NIHL can be temporary or permanent and occurs from excess sound being transmitted into the auditory nervous system. It is generally agreed that excessive auditory stimulation damages fragile inner ear sensory hair cells which lack regenerative potential. The mammalian “hair cell bundles” -composed of ten to hundreds of stereocilia are the cornerstone sound detection organelles through which mechanoelectrical transduction facilitates hearing. Key studies with animal models have shown that NIHL is temporary up to a critical threshold which when surpassed, becomes permanent. Currently available data regarding the mechanism responsible for noise damage is conflicting and includes mechanical damage, excitotoxicity, ischemia, metabolic exhaustion and ionic imbalance. A critical first step towards the effective treatment and prevention of hearing loss after acoustic over stimulation is the identification of the primary proteins and pathways perturbed both within the hair cells and beyond. Inner ear proteins are likely perturbed by loud sounds through multiple mechanisms such as loss of protein – protein interactions, targeted degradation, aberrant accumulation, and structural perturbations.

In order to probe potential NIHL mechanisms in discovery mode, we have applied several recently developed proteomic technologies to this challenge. We use metabolic labeling of mice with the stable isotope nitrogen-15 to achieve proteome wide quantitation. Stable isotope labeling of mice facilitates the identification of proteins whose abundances are acutely influenced by noise exposure in the inner ear. To cover a broad range of acoustic stimulation we expose mice to noise at 0, 70, 100 (temporary hearing loss), or 105 dB (permanent hearing loss). We are using bioinformatic pathway analysis to probe datasets to identify cellular pathways most relevant to NIHL and those potentially specific to temporary or permanent hearing loss. Further, by comparing our novel candidate NIHL target proteins against the human heredity hearing loss database may reveal key proteins. We are probing the inner ear biology at the whole proteome, organ of Corti, and hair cell bundle levels. We are isolating the organ of Corti with the recently reported “Cell and Tissue Acquisition System (CTAS)” micro dissection approach with GFP expressing hair cells from POU4F3-GFP mouse, followed by proteomic analysis by mass spectrometry. Further we are using antibodies to affinity purify candidate stereocillia protein complexes such as “tip-links” and MET channel protein complexes as they relate to NIHL. Proteomic data is being validated by immunohistochemical approaches. Through these efforts we will also contribute a novel proteomic data to serve as a resource to the inner ear community.

NEUROTROPHIN GENE THERAPY VIA ELECTROPORATION ENHANCES THE COCHLEAR IMPLANT NEURAL INTERFACE

***GARY D. HOUSLEY*, JEREMY L. PINYON, SHERIF F. TADROS, KRISTINA E. FROUD, ANN C. Y. WONG, ISABELLA T. TOMPSON, EDWARD N. CRAWFORD, MYUNGSEO KO, RENEE MORRIS, MATTHIAS KLUGMANN**

Translational Neuroscience Facility, School of Medical Sciences, The University of New South Wales (UNSW), Sydney, Australia

We have developed a derivative of electroporation gene delivery which provides a direct translational application to enhance cochlear implant performance. We utilize a cochlear implant microarray as the means to electroporate mesenchymal cells which line the perilymphatic chambers of the guinea pig cochlea. This new mode of electroporation delivered transfection efficiencies approaching 50% using a circularized plasmid including a bicystronic expression cassette comprising Flag-tagged brain-derived neurotrophic factor (BDNF) and green fluorescence protein (GFP) reporter elements. Cell transformation was site-specific, with GFP nuclear labeling occurring within both scala tympani and scala vestibuli mesenchymal cells in the basal turn region. Control experiments where DNA was perfused into the cochlea, but electroporation was omitted, failed to exhibit significant cell transfection. Electroporation-driven BDNF expression in the deafened cochlea drove regeneration of the peripheral neurites of the primary afferent spiral ganglion neurons, as well as increase in somata size. The regenerated neurites extended through the osseous spiral lamina to the habenula perforata, and exhibited branching into the inner and outer suclus regions, as well as projecting beyond the basilar membrane into the scala tympani compartment. The restoration of neural structure to the deafened cochlea resulted in functional improvement in cochlear implant performance. This was established using electrically-evoked auditory brainstem response (eABR) analysis. The average current stimulus levels required to evoke eABR threshold responses in cochleae treated by BDNF electroporation gene therapy were half that required for cochleae treated with a gutted control plasmid (GFP expression only). Complementing this, the dynamic range (growth function) of the eABR was significantly increased in the BDNF gene therapy treatment group. This study paves the way for safe therapeutic delivery of naked DNA gene constructs to the cochlea utilizing cochlear implants, with the demonstrated potential to transform the efficiency of the bionic interface.

IN VIVO REPROGRAMMING OF INNER BORDER AND PHALANGEAL CELLS TO THE INNER HAIR CELL FATE BY ECTOPIC ATOH1 EXPRESSION IN THE POSTNATAL MOUSE COCHLEA

***JIAN ZUO*, ZHIYONG LIU[*], JIE FANG[*], JENNIFER DEARMAN**

Department of Developmental Neurobiology, St. Jude Children's Research Hospital, Memphis, TN 38105. [*] These authors contribute equally to this work.

Regenerating auditory hair cells (HCs) is a promising approach to hearing restoration. Recent studies have demonstrated that iPS/ES cells or supporting cells (SCs) adjacent to HCs can be reprogrammed to adopt the HC fate. However, little is known whether the new HCs are auditory (outer or inner) HCs or vestibular HCs. Here we showed *in vivo* reprogramming of Inner Border cells (IBs) and Inner Phalangeal cells (IPhs), two subtypes of SCs, to the inner HC (IHC) fate. We achieved this by ectopically activating Atoh1, a transcription factor necessary for the HC fate, in IBs/IPhs at postnatal day 0 (P0) and P1. Starting at P6, we observed new HCs expressing more than 10 generic HC markers and the conversion rate gradually increased to ~17% of Atoh1-expressing IBs/IPhs at P60. Interestingly the new HCs exhibited IHC characteristics: typical straight-line stereocilia shape, expression of Fgf8 and otoferlin, and presence of potassium outward currents with larger amplitudes than those of endogenous outer HCs. Unfortunately even at P130, the new IHCs still lacked the terminal differentiation IHC marker vGLUT3. These new IHCs also maintained α9AChR expression, exhibited reduced density of Cbtp2 and displayed immature outward currents with smaller amplitudes than those of endogenous IHCs, suggesting their immature state. Our results demonstrate that IBs/IPhs can be reprogrammed to the IHC fate by Atoh1 ectopic expression *in vivo*; however, these new IHCs are arrested before terminal differentiation. We propose that IBs/IPhs are good candidates to regenerate IHCs *in vivo*.

THE TETRASPAN TMHS BINDS PCDH15 AND REGULATES MECHANOTRANSDUCTION IN COCHLEAR HAIR CELLS

***WEI XIONG*[1], *NICOLAS GRILLET*[1], *HEATHER M. ELLEDGE*[1], *THOMAS F. J. WAGNER*[1], *BO ZHAO*[1], *KENNETH R. JOHNSON*[2], *PIOTR KAZMIERCZAK*[1] *AND ULRICH MUELLER*[1]**

[1]The Dorris Neuroscience Center, Department of Cell Biology, The Scripps Research Institute, 10550 N. Torrey Pines Road, La Jolla, CA 92037, USA; [2]The Jackson Laboratory, Bar Harbor, ME 04609, USA

Hair cells are mechanoreceptors for the perception of sound. Mechanotransduction channels in hair cells are gated by tip links, which connect the stereocilia of a hair cell in the direction of their mechanical sensitivity. The molecular constituents of the mechanotransduction channels of hair cells are not known. Here we show that mechanotransduction is impaired in mice lacking the tetraspan TMHS. TMHS binds to the tip-link component PCDH15 and regulates tip-link assembly, a process that is disrupted by deafness-causing *Tmhs* mutations. TMHS also regulates transducer channel conductance and is required for fast channel adaptation. TMHS therefore resembles other ion channel regulatory subunits such as the TARPs of AMPA receptors that facilitate channel transport and regulate the properties of pore-forming channel subunits. We conclude that TMHS is an integral component of the hair cells mechanotransduction machinery that functionally couples PCDH15 to the transduction channel.

ACF7 IS A HAIR-BUNDLE ANTECEDENT, POSITIONED TO INTEGRATE CUTICULAR PLATE ACTIN AND SOMATIC TUBULIN

BRIAN MCDERMOTT JR. [1,2,3,4], ***PATRICK ANTONELLIS*** [1,2], ***SHIH-WEI CHOU*** [1,2], ***LANA POLLOCK*** [1,3], ***AHMED HASSAN*** [5], ***RUISHUANG GENG*** [1], ***XI CHEN*** [1,2], ***ELAINE FUCHS*** [6], ***KUMAR ALAGRAMAM*** [1], ***MANFRED AUER*** [5]

[1] Department of Otolaryngology–Head and Neck Surgery, [2] Department of Biology, [3] Department of Genetics and Genome Sciences, and [4] Department of Neurosciences, Case Western Reserve University, Cleveland, OH 44016; [5] Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720; [6] Howard Hughes Medical Institute, The Rockefeller University, New York, NY 10065

The precise morphology of the mechanosensitive hair bundle requires seamless integration of actin and microtubule networks. Here, using RNA-seq and *in situ* hybridizations, we identify *macf1* (encoding Acf7) as a gene whose cognate protein is positioned to bridge these distinct cytoskeletal networks in hair cells. By imaging an Acf7-Citrine fusion protein in zebrafish and immunolabeling of vestibular and cochlear mouse hair cells, we show that Acf7 circumscribes, underlies, and is interwoven into the cuticular plate (CP), and it also encircles the basal body. In cochlear hair cells, ACF7 localization is graded, with the highest concentration near each fonticulus. During development and hair-cell regeneration, Acf7 precedes formation of the hair bundle and CP. Finally, electron tomography demonstrates that the ends of microtubules insert into the CP and are decorated with filamentous linkers connecting microtubules to the CP. These observations are consistent with Acf7 being a linker protein, which may shape the hair cell's cytoskeleton early during ensemble genesis.

LOCALIZATION OF STEREOCILIA PROTEINS BY STRUCTURED ILLUMINATION MICROSCOPY (SIM)

***MATTHEW AVENARIUS*[1], *PETER BARR-GILLESPIE*[1]**

[1]*Oregon Hearing Research Center, Oregon Health & Science University; Portland, Oregon*

The ability to localize proteins within a cellular structure by fluorescence microscopy has revolutionized the field of cell biology. Determining the spatial and temporal localization of a protein is an important element in elucidating its function. The resolving power of conventional fluorescence microscopy is dictated by the diffraction limit of light (200 nm laterally [xy] and 500 nm axially [z]) and thereby poses a significant hindrance in localizing proteins within a small cellular structure. One technique that has overcome this barrier is structured illumination microscopy (SIM), a technique that collects images of a sample illuminated with defined patterns of light and utilizes a sophisticated algorithm to reconstruct a high-resolution image. SIM enhances the spatial resolution of a given image by at least two fold in all imaging planes (x, y, and z), increasing volume resolution by a factor of ten; this improved resolution improves discrimination of cellular structures and subcellular localization of proteins. Stereocilia, the mechanosensory structures on the apical surfaces of hair cells, are excellent candidates for SIM because of their small dimensions and their proximity to the coverslip in the context of a whole mount preparation. Here we demonstrate the use of SIM to image cochlear stereocilia and show resolution that is substantially better than conventional confocal microscopy. Moreover, we demonstrate our ability to localize deafness-associated proteins to various subcellular compartments of the stereocilia. In particular, we are able to show that radixin localizes to stereocilia shafts but is excluded from tips and tapers from early postnatal mice.

GENETIC DISSECTION OF THE VIBRATORY MODES OF THE ORGAN OF CORTI USING *IN VIVO* IMAGING

***JOHN S. OGHALAI*[1], *SIMON S. GAO*[1,2], *PATRICK D. RAPHAEL*[1], *ROSALIE WANG*[1], *BRIAN E. APPLEGATE*[3]**

[1]*Department of Otolaryngology–Head and Neck Surgery, Stanford University, CA, USA*
[2]*Department of Bioengineering, Rice University, Houston, TX, USA 77005,*
[3]*Department of Biomedical Engineering, Texas A&M University, College Station, TX, USA*

In non-mammalian vertebrates, each hair cell is tuned by the electrical resonance properties of its cohort of voltage-gated ion channels. In contrast, mammalian hair cells have only minimal intrinsic tuning. Instead, they are tuned primarily by their position along the vibratory basilar membrane that varies in mass and stiffness, thus creating a tonotopic distribution of resonances. However, basilar membrane tuning alone cannot account for the enhanced sensitivity and sharper frequency tuning of mammalian hearing. The other structures of the organ of Corti are involved in this process but controversy exists regarding their roles. Here we show how these structures function in transgenic mice with altered tectorial membrane anatomy and outer hair cell function to dissect their functional roles. We used spectral-domain optical coherence tomography (OCT) to image the mouse cochlea in vivo and measure the vibratory patterns across the organ of Corti in response to auditory stimuli. In wild type mice at the resonance frequency, the tectorial membrane vibrated with a larger magnitude than the basilar membrane, there was a traveling wave within the tectorial membrane, and the Hensen cells vibrated out of phase with the hair cells. Post-mortem, vibratory magnitudes declined, frequency tuning broadened, the basilar membrane vibrated with the highest relative magnitude, and phase differences within the organ of Corti were reduced. Transgenic mice, including TectaC1509G mutants, Prestin null, and Prestin 499 mutants had altered vibratory patterns that stemmed from their biomechanical defects. Thus, our genetic dissection of cochlear mechanics in vivo reveals that there are multiple resonances within the living mouse cochlea and demonstrate how the resultant modes function to produce exquisite hearing sensitivity and frequency selectivity, commonly referred to as cochlear amplification.

ENSEMBLE AND SINGLE MOLECULE KINETICS OF PURIFIED MYOSIN-15 DEMONSTRATE IT IS A *BONA-FIDE* MOLECULAR MOTOR SPECIALIZED FOR TRANSPORT WITHIN STEREOCILIA

***JONATHAN E. BIRD*[1], *YASUHARU TAKAGI*[2], *NEIL BILLINGTON*[2], *SARAH HEISSLER*[2], *JAMES R. SELLERS*[2], *THOMAS B. FRIEDMAN*[1]**

[1]Laboratory of Molecular Genetics, NIDCD/NIH, Rockville, MD 20850. [2]Laboratory of Molecular Physiology, NHLBI/NIH, Bethesda, MD, 20892.

Recessive mutations in *MYO15A* cause DFNB3 deafness in humans. Studies of mice carrying the *Myo15*^{sh2} or *Myo15*^{sh2J} alleles show that an essential role of myosin-15 is to regulate the developmental elongation of stereocilia. Myosin-15 is an ATPase hypothesized to move on actin filaments and transport proteins to the distal tips of stereocilia; whirlin and EPS8 are two interacting proteins that require myosin-15 for correct targeting within stereocilia. How myosin-15 transports these molecules remains an open question, and little is known regarding its activity, structure and regulation within the highly specialized stereociliar compartment.

We purified and characterized the biochemical kinetics of a subfragment-1 (S1) like truncation of mouse myosin-15 comprising the catalytic ATPase/actin binding domain plus IQ light chain binding sites. Expression of the recombinant S1 fragment in Sf9 insect cells required co-expression of multiple molecular chaperones in order to recover significant quantities of active protein. Unlike most other unconventional myosin classes, the IQ regions of myosin-15 did not bind calmodulin with high affinity, instead they preferentially bind both essential (MYL6) and regulatory (MYL12A) light chains that are typically associated with myosin-2 isoforms. Single molecule TEM confirmed that the purified S1 was correctly folded and monomeric. The S1 fragment was able to move actin filaments in an *in vitro* gliding motility assay and single molecule optical trapping was used to measure the power stroke and dwell time. A full characterization of the pre-steady state kinetics was performed to estimate rate constants for binding to ATP, ADP and actin, as well as ATP hydrolysis and phosphate release. Of note was a slow rate constant for ATP binding that may help limit saturation of the ATPase within the high-[ATP] environment of stereocilia. We also identified ADP release as the likely rate-limiting (slowest) step within the ATPase cycle. These data indicate that the predominant steady-state intermediates for myosin-15 would either be with ADP, or no nucleotide bound. Both of these states bind strongly to actin, suggesting that myosin-15 spends a significant percentage of its ATPase cycle attached to actin, and therefore could be capable of longer-range processive motility as an oligomer.

These data demonstrate that myosin-15 is a *bona-fide* molecular motor and provide the basis for further kinetic and structural studies, including understanding how DFNB3 mutations targeted within the ATPase disrupt motor activity. We will further speculate how kinetic specializations of myosin-15 are involved in both stereocilia transport and function at the stereocilia tips themselves.

TRICELLULIN INTEGRATES BICELLULAR AND TRICELLULAR TIGHT JUNCTIONS INTO A FUNCTIONAL COMPLEX ESSENTIAL FOR COCHLEAR HAIR CELL SURVIVAL

Saima Riazuddin[1], Gowri Nayak[1], Sue I. Lee,[2] Rizwan Yousaf,[1] Stephanie Edelmann,[3] Claire Trincot,[1] Christina M. Van Itallie,[4] Ghanshyam P. Sinha,[3] Maria Rafeeq,[5] Sherri M. Jones,[6] Inna A. Belyantseva,[2] James M. Anderson,[4] Andrew Forge,[7] Gregory I. Frolenkov,[3] Zubair Ahmed,[1]

[1]Divisions of Pediatric Otolaryngology and Ophthalmology, Cincinnati Children's Hospital Research Foundation, Cincinnati, Ohio, USA; [2]Laboratory of Molecular Genetics, National Institute on Deafness and Other Communication Disorders, NIH, Rockville, Maryland, USA; [3]Department of Physiology, University of Kentucky, Lexington, Kentucky, USA; [4] National Heart, Lung, and Blood Institute, NIH, Bethesda, Maryland, USA; [5]National Center of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan; [6]Department of Special Education and Communication Disorders, University of Nebraska, Omaha, Nebraska, USA; [7]Centre for Auditory Research, University College London, London, UK.

The apical side of the cochlear sensory epithelium faces the endolymph, a K^+-rich fluid that carries a large positive endocochlear potential. In contrast, the hair cell bodies are bathed in a Na^+-rich fluid, the perilymph. The endolymph plays a major role in the transmission of mechanical stimuli to hair cell stereocilia and initiation of the mechanosensory transduction. Thus, maintaining proper ionic composition of the cochlear fluids is essential for the normal function of hair cells. The tight junctions in the cochlea facilitate this by providing a seal between the two distinct fluid compartments. We and others have reported that mutations in tricellulin, a tight junction protein that shows wide tissue expression, cause a nonsydromic deafness phenotype (DFNB49). To study the effects of tricellulin deficiency *in vivo*, we generated a mouse model of DFNB49 that carries a *Tric* mutation p.R497X, orthologous to a previously identified mutation p.R500X in humans. The *Tric* knock-in mice display early onset rapidly progressing hearing loss and are profoundly deaf across all frequencies by P30. Morphological examination of the organ of Corti at various time points reveals severe outer hair cell loss in these mice by P16 and a nearly complete loss of hair cells by P30. However, the endocochlear potential and the vestibular evoked potentials were unaltered in *Tric* knock-in mice despite the loss of tricellulin from the tight junctions. Tricellulin deficiency affected the ultrastructure of tricellular tight junctions, preventing the coalition of the bicellular tight junctions strands with the central elements of the tricellular junctions in inner ear epithelia. We hypothesize that these ultrastructural changes may affect selectively the paracellular permeability of ions or small molecules resulting in a toxic microenvironment for cochlear hair cells. In agreement with this hypothesis, the hair cell loss in *Tric* knock-in mice was rescued by deleting the Pou3f4 transcription factor, necessary for generation of normal endolymph. Lastly, a comprehensive phenotypic screening of the *Tric* knock-in mice exhibited a broader pathological phenotype, revealing the requirement of tricellulin in other tissues. This study helps us elucidate not only the mechanism of tricellulin function in the inner ear but also highlights the importance of the protein outside of the inner ear.

EXPRESSION PROFILING OF FACS-SORTED HAIR CELLS WITH DEEP SEQUENCING LEADS TO THE IDENTIFICATION OF A STEREOCILIARY ACTIN-BINDING PROTEIN

***DÉBORAH SCHEFFER*[1,2], *JUN SHEN*[1], *DUAN-SUN ZHANG*[1], *ZHENG-YI CHEN*[2], *DAVID COREY*[1]**

[1]Howard Hughes Medical Institute and Department of Neurobiology, Harvard Medical School, Boston, MA 02115, USA, [2] Eaton-Peabody Laboratory - Massachusetts Eye and Ear Infirmary, Boston, MA 02114, USA

To identify genes important for the development and function of hair cells, it is useful to know the complete transcriptome of hair cells at various times during their differentiation and maturation. We have previously used mutant animals lacking hair cells (Atoh1), or mutants with hair cells but no bundles (Pou4f3) to approach a hair-cell transcriptome (Scheffer *et al.*, 2007a; 2007b), but these are not perfect models and cannot be used for later developmental stages.

We have now developed a new model for expression profiling in hair cells. We generated a mouse expressing green fluorescent protein (GFP) under the Pou4f3 promoter, and used fluorescence-activated cell sorting (FACS) to purify dissociated hair cells and non-hair cells. Cells were purified from cochlear and utricular epithelia at E16, P0, P4, P7 and P16 and submitted to deep sequencing.

FACS-sorted hair cells are apparently very pure: more than 50 genes (such as Gfi1, oncomodulin, otoferlin, Ptprq, Atoh1, Grxcr1 and Tmc1) are represented highly in the GFP^+ sample but at least 100-fold less in GFP^- cells. Expression data were assembled into a sortable database accessible online: the Shared Harvard Inner-Ear Laboratory Database (SHIELD; https://shield.hms.harvard.edu). This public database includes additional annotation, such as domain architecture and chromosomal location relative to deafness loci. By one sort criterion, for instance, a third of all known deafness genes occur in the top 3% of the 20,000 genes represented.

This database allowed us to identify new hair-cell-specific genes. Their expression was confirmed by *in situ* hybridization and immunostaining. One in particular was enriched 26-fold in hair cells compared to the surrounding cells. The gene product, an actin-binding protein, was localized at the cuticular plate and stereocilia of hair cells. We studied the consequence of the gene disruption in a mouse knock-out strain and found that these animals exhibit a unique phenotype in hair cells.

Based on its chromosomal location, this gene is a good candidate for human deafness and illustrates the power of deep sequencing FACS-sorted cell populations. Similar FACS-based approaches might be used for identifying genes expressed in subsets of hair cells, for instance outer-hair-cell-specific genes that might participate in electromotility.

PITCH: A MODEL OF SENSORINEURAL DEAFNESS IDENTIFIES NEUROPLASTIN AS ESSENTIAL FOR INNER HAIR CELL MATURATION AND FUNCTION.

***LEA CARROTT*[1], *MICHAEL R BOWL*[1], *CARLOS AGUILAR*[1], *MELISSA WEST*[1], *JOANNE DORNING*[1], *SUE MORSE*[1], *RACHEL HARDISTY-HUGHES*[1], *GREG BALL*[1], *STUART L JOHNSON*[3], *ANDY PARKER*[1], *SARA WELLS*[2], *WALTER MARCOTTI*[3], *STEVE BROWN*[1].**

[1]Mammalian Genetics Unit and [2]Mary Lyon Centre MRC Harwell, Harwell Oxford, Oxfordshire, OX11 0RD, UK, [3]Department of Biomedical Science, The University of Sheffield, Western Bank, Sheffield, S10 2TN, UK.

At MRC Harwell n-ethyl-n-nitrosourea (ENU) mutagenesis coupled with auditory phenotyping is utilised to generate new mouse models of hearing loss. This approach led to the discovery of *pitch*, an autosomal recessive model of early-onset, profound sensorineural deafness (+90dB) with a late-onset head tilt. Mapping studies identified a 1.6Mb critical region comprising a novel deafness locus on chromosome 9 and prioritised candidate gene sequencing identified a coding mutation in *Neuroplastin* (*Nptn*). *Nptn* encodes two protein isoforms, both of which are highly glycosylated cell adhesion molecules and are believed to be involved in synaptic stabilisation and maintenance. The *pitch* mutation causes substitution of a highly conserved structural cysteine residue, which is common to both isoforms. To confirm *Nptn* as the causative gene a second allele was sought and identified from the Harwell ENU archive, encoding a nonsense mutation (Y219X) causing a severely truncated protein. Like $Nptn^{pitch/pitch}$ animals, $Nptn^{Y219X/Y219X}$ and $Nptn^{pitch/Y219X}$ are also profoundly deaf with apparent vestibular defects. Transient transfection studies using wild-type (WT) and *pitch* EGFP and cMyc tagged constructs suggest that whilst WT protein is fully-glycosylated and trafficked to the cell membrane, *pitch* protein is not fully-glycosylated and is retained in the endoplasmic reticulum (ER). Histological assessment of inner ear sections from postnatal day (P)90 WT, heterozygote and homozygote *pitch* mice indicate pathological changes including abnormal organ of Corti and absence of otoconia in the sacculus of *pitch* homozyotes. Ultra-structural studies using scanning electron microscopy (SEM) indicate that *pitch* homozygotes appear to have normal inner ear physiology at P27. However, by P90 degeneration of the inner and outer hair cells is evident in homozygous *pitch* mice. Electrophysiological studies suggest that inner hair cells (IHCs) are able to initially develop normally at least up to just after the onset of hearing. However, the biophysical properties of IHCs fail to fully mature, thus preventing them from becoming adult sensory receptors. Immunolabeling studies demonstrate expression of Nptn at the basolateral membrane of the IHCs from P14 to P22, consistent with our hypothesis that Nptn is required for synapse formation/stability at IHCs. Further functional characterisation of *pitch* will elucidate the essential molecular requirement of *Nptn* within the auditory system.

GENE EXPRESSION PROFILING OF YOUNG AND ADULT MOUSE COCHLEA BY RNA-SEQ IN STRAINS WITH NORMAL AND AGE-RELATED HEARING LOSS

***ANNE B.S. GIERSCH*[1,2,3], *JUN SHEN*[2,3], *NAHID G. ROBERTSON*[1] , *CYNTHIA C. MORTON*[1,2,3]**

[1]Brigham and Women's Hospital, [2]Harvard Medical School, [3]Harvard Medical School Center for Hereditary Deafness, Boston, Massachusetts, USA

Age-related hearing loss is the most common sensory deficit, reducing the quality of life in the aged population. Its social impact will become more pronounced as life expectancy continues to increase. Despite the discovery of many deafness genes, pathophysiology of progressive hearing impairment due to the aging process remains elusive. We hypothesize that gene expression profiling of the cochlea from mouse models with various degrees of age-related hearing loss will reveal the molecular mechanism and inform potential target selection for prevention and treatment.

We performed gene expression profiling of mouse cochlea using next-generation sequencing (RNA-Seq). Cochleae from mouse strains with good hearing past one year of age (CBA/CaJ and B6.CAST-*Cdh23*$^{Ahl+)}$ or with documented age-related hearing loss (*Coch*$^{G88E/G88E}$ and *Coch*$^{-/-}$ in a CBA background and C57BL/6J) were dissected at discrete ages ranging from one week through late adulthood. PolyA selected mRNAs were extracted and the derived cDNA samples were fragmented, indexed, pooled, and sequenced by Illumina HiSeq. Biological replicates were used for all conditions. Expression levels of all transcripts were analyzed and differential gene expression analyses were performed.

We have obtained gene expression profiles of mammalian cochlea at various ages by RNA-Seq. With total reads of at least 30 million, more than 16,000 genes were detectable in each sample, and the expression levels were highly reproducible. We detect significant systematic differences in gene expression profiles between C57BL/6J and CBA/CaJ strain backgrounds, regardless of age. Comparing mouse models with or without age-related hearing loss of the same genetic background, we have found that few genes show statistically significant differential expression at young ages before the onset of hearing impairment, but the number of genes with differential expression dramatically increases to hundreds at later stages between good hearing and age-related hearing loss strains.

Conclusions: We surveyed gene expression in mammalian cochlea by RNA-Seq and identified genes that show age-related differential expression. Systematic differences exist between different genetic backgrounds. Temporal gene expression profiles in the cochlea may suggest candidate targets for prevention and treatment of age-related hearing loss.

MET CHANNEL-INDEPENDENT UPTAKE OF AMINOGLYCOSIDES BY COCHLEAR HAIR CELLS

***HONGZHE LI*[1], *JAWON KOO*[2,3], *DOUGLAS VETTER*[4], *PETER STEYGER*[1]**

[1]Oregon Hearing Research Center, Oregon Health & Science University, Portland, OR 97239, U.S.A.; [2]Seoul National University Bundang Hospital, [3]Seoul National University College of Medicine, Seongnam, Kyunggi-Do 463-707, Korea; [4]Neurobiology and Anatomical Sciences, University of Mississippi Medical Center, Jackson, MS, 39216, U.S.A.

The mechanoelectrical transduction (MET) channel located at the apical surface of cochlear hair cells is highly permeant to aminoglycosides. In mice, the cochlea becomes fully functional approximately 2 weeks after birth. During this postnatal maturation period, tight junctions between adjacent epithelial and marginal cells, and between adjacent endothelial cells within the strial vascularis continue to develop. This completes the morphogenesis of the blood-labyrinth barrier (BLB), and permits enrichment of endolymph with potassium, thus generating the endolymphatic potential. We have taken advantage of the neonatal maturation period to study how ototoxic drugs enter hair cells in the absence of a functional BLB *in vivo*.

Neonatal wildtype C57Bl/6 or protocadherin 15 knockout mice were intraperitoneally injected with fluorescently-conjugated gentamicin (GTTR) or fluorescent dye AM1-43 for up to 30 minutes at various postnatal ages prior to *in situ* fixation. Cochlear tissues were excised and processed for confocal microscopy. The emission intensity and distribution of GTTR or AM1-43 were acquired at the same laser power in all cochlear tissues.

In wildtype neonatal C57Bl/6 mice, the intensity of GTTR fluorescence in hair cells or strial tissues exhibited a bell shape curve that peaked at P7 during postnatal development, suggestive of paracellular permeation of GTTR through the immature BLB. Furthermore, fluorescence intensities within cell types in the sensory epithelium exhibited a heterogeneous pattern of GTTR uptake between p7 to p10. Thus, a subset of sensory and supporting cells presented overtly elevated GTTR fluorescence compared to adjacent cells of the same cell type. This heterogeneity of GTTR fluorescence was also observed in protocadherin 15 knockout mice with dysfunctional MET channels, but not with the MET channel-permeant fluorophore AM1-43 in either wild type mice or protocadherin-15 knockout mice.

In summary, prior to complete maturation of the cochlear BLB, GTTR readily permeates into strial cells, fibrocytes and sensory hair cells. The heterogeneous pattern of GTTR uptake in the organ of Corti from p7 to p10 corresponds with a known wave of purinergic activity that precedes the onset of functional hearing. This suggests one or more yet-to-be-identified aminoglycoside entry route(s) into hair cells in addition to, and independent of, the MET channel. This may include channels associated with olivocochlear (efferent) system, activity such as BK channels or the nAChR itself.

FOLLOWING INTRACELLULAR CALCIUM DURING AMINOGLYCOSIDE-INDUCED HAIR CELL DEATH

DAVID W. RAIBLE, ROBERT ESTERBERG, DALE W. HAILEY, EDWIN W RUBEL

University of Washington, Seattle, WA 98195

Although intracellular Ca2+ is considered pivotal to life or death decisions, defining the role of Ca2+ during *in vivo* cell death remains nebulous. By generating transgenic zebrafish lines expressing the genetically encoded Ca2+ indicators targeted to distinct subcellular compartments in mechanosensory hair cells of the lateral line, we have succeeded in monitoring intracellular Ca2+ dynamics in real time during aminoglycoside-induced hair cell death. We find that intracellular Ca2+ homeostasis is disrupted in dying cells following aminoglycoside exposure, and manipulating Ca2+ alters aminoglycoside susceptibility. Aminoglycoside-induced hair cell death stimulates the mobilization of Ca2+ from the ER shortly following aminoglycoside exposure, triggering downstream changes in mitochondrial and cytoplasmic Ca2+. Pharmacological agents that disrupt Ca2+ transfer between ER and mitochondria mitigate the toxic effect of aminoglycoside exposure and suggest that intracellular Ca2+ flow at microdomains where ER and mitochondria associate is critical for cell death. We propose that mechanisms used by hair cells to control mitochondrial energetics underlie their susceptibility to aminoglycoside exposure.

Poster Abstracts

Sunday,
June 23

A COMPARATIVE TRANSCRIPTOME ANALYSIS OF REGENERATING AVIAN COCHLEAR AND UTRICLE SENSORY EPITHELIA.

***Michael Lovett*[1,2], *Nicole Renaud*[1], *Yuan-Chieh Ku*[1], *Rose Veile*[1], *Cindy Helms*[1] *and Mark Warchol*[1]**

[1]*Washington University School of Medicine.* [2]*Imperial College, London*

Lower vertebrates are capable of inner ear hair cell (HC) regeneration, but mammals have little capacity to replace these essential mechano-electrical transducers. We employed next generation nucleic acid sequencing (RNA-seq) to derive complete transcriptome information from regenerating avian utricle and cochlea organotypic cultures. Pure sensory epithelia (SE) were collected at 24 hour intervals over a 168 hour time course following aminoglycoside ablation of HCs. Transcriptomes were computationally assembled from biological replicate samples and were compared to untreated control samples cultured in parallel. The differentially expressed (DE) transcriptome of regenerating utricle SE (~3,600 genes) can be partitioned into distinct expression patterns that are highly diagnostic of particular processes and pathways. For example, new HC-specific markers can be identified in this way, as can cell cycle and phenotypic conversion candidate genes. Many of these show evidence of participating in interactive networks. The situation in the cochlea is more complex, perhaps due in part to differential regional sensitivities to aminoglycoside damage within the cochlea SE. However, the overall genetic toolbox of cochlear regeneration is also much more complex. Approximately 4,500 genes are DE during cochlea SE regeneration, of which only ~1,700 overlap with the utricle time course. This cochlear complexity is also reflected in the numbers of DE transcription factor (TF) genes. The utricle data contains ~200 DE TF genes, whereas the cochlea data contains ~400 with ~100 overlapping. Nevertheless, the cochlea data can also be partitioned into expression patterns that are enriched for specific functions, networks and processes such as HC function. There are many surprising differences between the two time courses, which include the use of different components of NOTCH signaling. Even within the “shared” set of differentially expressed genes there are many interesting differences in transcript abundance and timing of gene expression. For example, both the utricle and cochlea show DE of the same 8 WNT genes. However, in most cases the gene expression patterns and abundance levels of these vary greatly between the two SE. We will discuss these two datasets in the context of gene networks and pathways and their utility for dissecting the exact cascade of gene expression changes that result in new HC production in each organ.

MOLECULAR BIOLOGY OF ATOH1 REGULATION BY HES/HEY TRANSCRIPTIONAL REPRESSORS

***YASSAN ABDOLAZIMI*[1], *NEIL SEGIL*[1,2]**

[1]University of Southern California, Los Angeles, California, USA; [2]House Research Institute, Los Angeles, California, USA

Background: The organ of Corti is characterized by a mosaic of hair cells and supporting cells. Genetic experiments indicate that this mosaic arises partly through Notch-mediated lateral inhibition leading to regulation of Atoh1 gene expression. During embryonic development, Atoh1 up-regulation is required for hair cell differentiation of selected sensory progenitors, and coincident inhibition in the surrounding progenitors, which subsequently differentiate as supporting cells. A requirement for Notch signaling continues to be required during perinatal maturation of the organ of Corti to maintain the fate of supporting cells. The molecular basis of Atoh1 gene regulation during these processes remains to be studied.

We have investigated the role of the Hes/Hey family of transcriptional repressors in the suppression of Atoh1 expression, and thus the maintenance of supporting cell fate. Hes/Hey expression in most perinatal supporting cells depends on the continued activation of the Notch signaling pathway. Blocking Notch signaling leads to Atoh1 activation and supporting cell transdifferentiation. Our working hypothesis is that Atoh1 is actively suppressed in supporting cells by Hes/Hey factors. In response to the loss of Notch activity in supporting cells, Atoh1 up-regulation is mediated by a process of dis-inhibition caused by the loss of Hes/Hey activity. Experiments supporting this hypothesis will be described.

Methods: Immunohistochemistry, Chromatin immunoprecipitation (ChIP), organ culture, FACS purification of hair cells and supporting cells, *in vitro* transfection, site-directed mutagenesis, and reporter assays were used to investigate the role of Hes/Hey factors in regulation of Atoh1 expression.

Results: Hes/Hey gene expression is rapidly downregulated when Notch signaling is blocked, and Atoh1 is rapidly up-regulated. ChIP assays provide evidence of direct binding of Hes/Hey proteins to the Atoh1 gene in a model cell line. Transfection assays indicate that Hes/Hey are sufficient to bring about Atoh1 transcriptional down-regulation, and that this likely occurs through direct binding to Atoh1 gene regulatory regions, and the changes in epigenetic regulation that accompany this binding.

Conclusion: Hes/Hey factors contribute to the regulation of Atoh1 through a mechanism that involves direct binding of these transcription factors to Atoh1 gene regulatory elements. Regulation of Atoh1, Hes/Hey genes and the importance of these observations to both the maintenance of the differentiated state, and regeneration, will be discussed.

ESTABLISHING A TONOTOPIC GRADIENT IN THE AVIAN AND MAMMALIAN AUDITORY SYSTEM.

***MOHI AHMED*[1], *GABRIELLE PARKINSON*[1], *ANDREA STREIT*[1]**

[1]*King's College London, London, UK*

Mechanosensory hair cells in the cochlear transform sound waves into electrical signals and relay the information to the central nervous system via the auditory nerve. The centrally projecting axons of the spiral ganglion neurons (SGNs) form the auditory nerve, which branches to innervate three subdivisions of the cochlear nucleus in the hindbrain: the anteroventral (AVCN), posteroventral (PVCN) and dorsal cochlear nucleus (DCN). The peripherally projecting SGN axons innervate hair cells that are arranged along the cochlea according to frequency (frequency tuning), thereby generating the cochleotopic map, with basal cochlear regions tuned to high and apical regions tuned to low frequencies. This is topographically organised in each of the subdivisions of the cochlear nucleus, giving rise to the tonotopic gradient (map) of sound frequency along a particular axis within each subdivision. The tonotopic map is maintained in the entire auditory pathway, and is necessary for the brain to process and interpret the sound signal.

Despite the many studies on tonotopy, there is still a lack of understanding in how cellular and molecular mechanisms drive tonotopic innervation. Here, we map the axonal projections of SGNs in chick and mouse, using immunohistochemistry and axon tracing after dye labelling and in transgenic lines. While the timing of tonotopic innervation is well established in mouse, this is not the case in chick. We therefore conducted a detailed analysis of SGN projections during chick development to identify the precise stages when central and peripheral connections are established. Our aim is to combine experimental and imaging techniques, using both chick and mouse models, to advance our understanding of the cellular and molecular mechanisms that establish tonotopy.

Sponsored by a travel award from:
ACTION ON HEARING LOSS

ULTRASTRUCTURAL 3D MODELING OF WOUND HEALING IN THE ORGAN OF CORTI POWERED BY SBF-SEM

***TOMMI ANTTONEN*[1], *ILYA BELEVICH*[2], *EIJA JOKITALO*[2], *ULLA PIRVOLA*[1]**

[1]Institute of Biotechnology, University of Helsinki, Finland; [2]Electron microscopy unit, Institute of Biotechnology, University of Helsinki, Finland

Serial block-face scanning electron microscopy (SBF-SEM) can be used to generate three-dimensional models from biological samples in high resolution. In SBF-SEM, the surface of the resin-embedded fixed tissue block is imaged, not the sections, by detecting back-scattered electrons. After each 20-200nm section, cutted by an ultramicrotome mounted inside the scanning electron microscope, the freshly cut block face is raised to the plane of focus and imaged. With this setup, one can automatically acquire thousands of images resembling TEM images of thin sections. As the images are in optimal orientation, ultrastructural 3D reconstruction of the biological sample can be easily done with different segmentation methods.

This method suits perfectly the research on the organ of Corti, characterized by a complex cytoarchitecture. The distinct cellular patterning as well as the prominent cytoskeletal structures and extracellular lumens facilitate 3D reconstruction. By using SBEM as a powerful tool to characterize cytoskeletal changes in different mutant mouse models, our aim is to model different stages of wound healing response and repair following an ototoxic lesion in vivo. Our focus is to provide fine detailed characterization of cytoskeletal changes of supporting cells and apoptotic hair cell clearance, deepening our understanding of the traumatized organ of Corti, the target of future regenerative therapies.

HEAD-TO-TAIL INTERACTION IN THE SCAFFOLD PROTEIN HARMONIN, UNDERLYING USHER SYNDROME TYPE 1C (USH1C)

***AMEL BAHLOUL*[1], *ELISE PEPERMANS*[1], *DOMINIQUE DURAND*[2], *BERTRAND RAYNAL*[3], *NICOLAS*[4] *WOLFF, PATRICK ENGLAND*[3], *AHMED HAOUZ*[5], *FRÉDÉRIC SAUL*[5], *PATRICE VACHETTE*[2] *AND CHRISTINE PETIT*[1,6]**

[1]Institut Pasteur, Génétique et Physiologie de l'Audition, INSERM UMRS587; 25 rue Dr Roux, 75724 Paris, France; [2]Institut de Biochimie et Biophysique Moléculaire et Cellulaire, Université Paris-Sud, UMR CNRS 8619, F-91405 Orsay, France ; [3]Institut Pasteur, Plate-Forme de Biophysique des Macromolecules et de leurs Interactions, 75015 Paris, France ; [4]Institut Pasteur, Unité de Résonance Magnétique Nucléaire des Biomolécules, Département de Biologie Structurale et Chimie, 75724 Paris, France ; [5]Institut Pasteur, Plate-forme 6, CNRS-UMR3528, 25 rue du Dr. Roux, 75724 Paris, France ; [6]Collège de France, 75005 Paris, France.

Harmonin, a scaffold protein containing three PDZ domains (PDZ1-3), is considered to be a key organizer of the USH1 protein complex in the inner ear hair cells. This protein complex is critically involved in the formation of the lateral hair bundle early links and the tip-links, that gate the mechanoelectrical-transduction channels. Three harmonin subclasses have been detected (a, b, c) in hair cells. Harmonin-a consists of an N-terminal globular domain followed by PDZ1, PDZ2, a coiled coil domain, PDZ3 and a C-terminal PDZ-binding motif. Harmonin-b also contains a second coiled coil domain and a PST-region located after the first coiled coil domain. Finally, harmonin-c only contains the Nter domain, PDZ1 and PDZ2.

We studied the role of the C-terminal PDZ-binding motif in harmonin's conformation and structure by using different biochemical (Surface Plasmon Resonance, Analytical Ultracentrifugation) and structural (Nuclear Magnetic Resonance, Small-angle X-Ray scattering, X-Ray crystallography) approaches. Our results led us to conclude that harmonin can display an open or a closed conformation which affects its binding to the other USH1 proteins.

IDENTIFICATION AND CHARACTERIZATION OF ENU-INDUCED MOUSE MODELS OF AGE-RELATED HEARING LOSS

***MICHAEL R BOWL*[1], *CARLOS AGUILAR*[1], *SUE MORSE*[1], *JOANNE DORNING*[1], *PRASHANTHINI SHANTHAKUMAR*[1], *RUAIRIDH KING*[1], *LAUREN CHESSUM*[1], *LAURA WISBY*[1], *MICHELLE SIMON*[1], *ANDY PARKER*[1], *SARA WELLS*[2], *PAUL POTTER*[1], *STEVE DM BROWN*[1]**

[1]Mammalian Genetics Unit and [2]Mary Lyon Centre, MRC Harwell, OX11 0RD, UK.

Age-related hearing loss (ARHL) is a significant health and social burden on the population and is one of the four most common chronic health conditions experienced by the elderly. Greater than 25% of adults aged 50 and over have a hearing loss of 30 dB or more (increasing to 70-80% of people aged 75 and over). At MRC Harwell we are utilizing a large-scale *N*-ethyl-*N*-nitrosourea (ENU) mutagenesis screen to identify mouse models of aging. G3 pedigrees, of ~100 mice, are bred and enter a phenotyping pipeline comprising recurrent assessment across a wide range of disease areas including, diabetes, metabolism, neurobehaviour, bone, renal, cardiac, and sensorineural. The Deafness Models and Mechanisms team is taking advantage of this screen to identify models of ARHL. We conduct recurrent auditory phenotyping, consisting of Clickbox at 3, 6, 9 and 12 months of age, and Auditory-Evoked Brainstem Response at 3 and 9 months.

As of February 2013, 112 pedigrees have entered the phenotyping pipeline, of which 82 have completed auditory screening. To date 16 pedigrees (>14%) have confirmed auditory phenotypes. Of these, 10 pedigrees display elevated hearing thresholds by 3 months of age. The remaining 6 pedigrees exhibit ARHL, evident from 6 months (4 pedigrees) or from 9 months (2 pedigrees), which is most pronounced at the highest frequency tested (32kHz). Currently, 3 of the ARHL pedigrees have undergone genome-wide mapping and whole-genome sequencing (WGS). These studies demonstrate that each pedigree maps to a distinct and novel deafness locus, and identify ENU-induced DNA changes causing missense substitutions within proteins for 2 of these pedigrees. Studies to relate mutant protein to phenotype are ongoing. In addition, mapping studies and WGS of the remaining 3 ARHL models is underway.

The Harwell Aging Mutant Screen is producing pedigrees with interesting age-related auditory phenotypes. Investigation of these, and as yet unidentified pedigrees, promises to increase our understanding of the genetics underlying hearing and its age-related decline.

CHARACTERIZATION OF SIX CREER MOUSE LINES IN THE ADULT UTRICLE

Brandon C. Cox[1], Tot Bui Nguyen[2], and Jennifer S. Stone[2]

[1]Southern Illinois University, School of Medicine, Department of Pharmacology, Springfield, IL; [2]University of Washington, Department of Otolaryngology, Seattle, WA.

Cell-type specific control of gene expression can be achieved using the Cre/loxP system. Temporal control of gene expression is achieved by adding a modified estrogen receptor to the Cre enzyme (CreER) which results in sequestration of CreER in the cytoplasm. In the presence of tamoxifen, CreER will translocate to the nucleus to excise DNA flanked by loxP sites. More than 30 Cre or CreER alleles have been published in studies of the cochlea where gene manipulation or fate mapping was performed, resulting in many significant advances for the auditory field. However, few of these lines have been studied in vestibular organs, and none have been studied in adult vestibular organs (Cox et al., 2012 *J Ass Res Otolaryngol* 13:295-322). Here, we characterized the Cre expression pattern of six CreER lines in the adult utricle using the ROSA26/CAG-loxP-stop-loxP-tdTomato reporter line and tamoxifen induction at 6 weeks of age. We also checked for Cre leakiness, which may occur if some CreER molecules enter the nucleus without tamoxifen, by using samples that expressed both CreER and the tdTomato reporter, but did not receive tamoxifen. The following CreER alleles were studied: GFAP-CreER (2 different alleles), Otoferlin-CreER, Plp-CreER, Prox1-CreER and Sox2-CreER. The Otoferlin-CreER allele showed expression of the tdTomato reporter specifically in hair cells and no Cre leakiness was observed in controls. In contrast, the Plp-CreER allele had tdTomato expression in supporting cells and glia of the vestibular ganglia, with only rare hair cells labeled. In controls testing for Cre leakiness, we observed ~100 tdTomato+ supporting cells as well as some tdTomato+ glia. Very few tdTomato+ cells (<40) were detected in Prox1-CreER utricles, and no Cre leakiness was observed. The Sox2-CreER line exhibited tdTomato expression in the vast majority of supporting cells, as well as in all Type II hair cells. Type I hair cells did not express tdTomato and Cre leakiness studies with this allele are underway. The expression pattern of the other CreER lines is currently being analyzed. Future studies will be conducted to examine the expression pattern of these alleles in the saccule and ampullae, as well as at other ages of tamoxifen induction. This work provides a catalog of mouse genetic tools that can be used by researchers in the vestibular field for studies of their gene of interest or for fate mapping of specific cell types.

THE INTRAVESICULAR DOMAIN OF OTOFERLIN IS ESSENTIAL FOR SUSTAINED VESICLE RELEASE AT THE IHC RIBBON SYNAPSE

***JACQUES BOUTET DE MONVEL*[1], *DIDIER DULON*[2], *PHILIPPE VINCENT*[2], *YOHAN BOULEAU*[2], *SYLVIE NOUAILLE*[1], *CHRISTINE PETIT*[1,3] *AND SAAID SAFIEDDINE*[1]**

[1] Pasteur Institute & University of Paris VI, Unité de Génétique et Physiologie de l'Audition / Inserm UMRS1120, Paris, France.[2] University of Bordeaux II, Equipe Neurophysiologie de la Synapse Auditive, Bordeaux, France. [3] Collège de France, Paris, France.

Otoferlin, a six C2-domain synaptic vesicle (SV) transmembrane protein defective in a genetic deafness form, is present at the ribbon synapses of auditory inner hair cells (IHC) where it may function as the major calcium sensor to trigger exocytosis. Deaf mutant mice lacking otoferlin lack both the fast and sustained components of synaptic exocytosis of IHCs, despite normal synaptic structure. To shed further light on the role of otoferlin in the IHC synaptic vesicle cycle, we engineered a knock-in mouse model expressing an otoferlin-GFP fusion gene (Otof-GFP), in which EGFP was fused to the intravesicular carboxy-terminal domain of otoferlin. We found that the expression of Otof-GFP in IHCs occurs at appropriate developmental stages with a subcellular distribution similar to that of the native protein. In addition, IHC ionic currents and ribbon synapse maturation proceed normally in these mice. However, adult homozygote Otof-GFP mice display severe hearing loss with IHCs unable to sustain exocytosis during repetitive stimulations. The mobility of Otof-GFP-containing SVs at IHC presynaptic sites, studied by FRAP experiments, revealed that Otof-GFP has a significant intracellular turnover which increases upon stimulation. We also used time-resolved patch-clamp capacitance recordings to probe SV fusion in individual IHCs. IHCs from GFP-Otof knock-in mice displayed normal Ca^{2+}-dependent exocytosis at postnatal day 6, before SV recruitment in these cells is mature. However, SV exocytosis was significantly impaired in IHCs from P8 mutant mice. Mobilization of the readily releasable pool (RRP) was normal, but the sustained component (SRP) was nearly abolished, and recovery of the RRP was strongly reduced during a paired-pulse protocol. These results indicate that a normal intravesicular otoferlin domain is essential for the maintenance of sustained exocytosis in IHCs.

GRADIENTS IN THE INTERACTION OF THE OTOTOXIC PEPTIDE, D-JNKI1, WITH THE HAIR CELL'S MECHANO-ELECTRICAL TRANSDUCER CHANNEL

TERRI DESMONDS[1], NERISSA KIRKWOOD[1], RICHARD GOODYEAR[1], JONATHAN GALE[2], SIETSE VAN NETTEN[3], SIMON WARD[1], GUY RICHARDSON[1] AND CORNÉ KROS[1,4]

[1]School of Life Sciences, University of Sussex, Falmer, Brighton, BN1 9QG, UK; [2]UCL Ear Institute, 332 Gray's Inn Road, London, WC1X 8EE UK; [3]Artificial Intelligence, University of Groningen, Nijenborgh 9, 9747 AG Groningen, The Netherlands; [4]Department of Otorhinolaryngology, UMCG, Postbus 30001, 9700 RB Groningen, The Netherlands.

We have identified a novel class of mechano-electrical transducer (MET) channel blockers based on the HIV-TAT peptide with dissociation constants (K_D – equal to the half-blocking concentration) in the order of tens of nM, two orders of magnitude lower than that reported for FM1-43, the highest-affinity MET channel blocker reported in the literature (K_D = 1-3 µM depending on the membrane potential: Gale et al, J Neurosci 2001). D-HIV-TAT, a 12-amino acid peptide (MW 1590 Da) containing 8 positively charged basic amino acids, acts as a permeant blocker of MET currents in outer hair cells (OHCs) from the mid-basal coil of mouse cochlear cultures with a K_D of 80 nM at a holding potential of -84 mV. Block is relieved at positive membrane potentials by electrostatic repulsion. At extreme negative membrane potentials the block is also substantially reduced, due to the blocker being dragged through the MET channel pore into the hair cells by the large electrical driving force, strong evidence for permeant block (Gale et al 2001; Marcotti et al, J Physiol 2005). An inhibitor of Jun c N-terminal kinase, D-JNKi1 (MW 3822 Da), comprising 19 amino acids (4 of which are basic) from the human JNK inhibitory protein coupled to D-HIV-TAT (with a total of 12 positive charges) has an even lower K_D of some 16 nM in mid-basal OHCs held at -84 mV, and also acts as a permeant blocker of the MET channel.

Texas Red-conjugated D-HIV-TAT accumulates in OHCs in both the basal and apical cochlear coils. The larger compound, Texas Red-conjugated D-JNKi1, accumulates mostly in basal OHCs, and shows little labelling of OHCs from approximately the middle of the apical coil onwards. Consistent with these findings, D-JNKi1 is an order of magnitude less effective at blocking MET currents in mid-apical OHCs, with an estimated K_D of 151 nM at -84 mV, although it still acts as a permeant blocker. We speculate that this might reflect a gradient in the pore properties of the OHC MET channels along the length of the cochlea (Beurg et al, J Neurosci 2006).

Sponsored by a travel award from:
ACTION ON HEARING LOSS

VANGL2 DIRECTS SUPPORTING CELL MORPHOGENESIS AND IS NOT ESSENTIAL FOR THE POSTNATAL REFINEMENT OF HAIR CELL PLANAR POLARITY

JEREMY S. DUNCAN, CATHERINE O. COPLEY, MICHAEL R. DEANS

Department of Otolaryngology, Johns Hopkins School of Medicine, Baltimore, Maryland, USA

Auditory hair cells have a distinctive planar polarity evident in the polarization and orientation of their stereociliary bundle. Mutations in the core planar cell polarity (PCP) gene *Vangl2* result in hair cells that form polarized bundles but fail to align bundle orientation with the cochlear axis. In addition, there is a gradient of phenotypic severity ranging from the least affected basal to the most affected apical turn of the cochlea. This gradient is similar to the progression of hair cell maturation suggesting that active refinement could correct planar polarity phenotypes in the basal cochlea of *Vangl2* knockout (KO) mice.

Since the *Vangl2* deletion results in perinatal lethality, *Vangl2* conditional knockouts (CKO) were generated to test this hypothesis. When crossed with *Pax2*-Cre, *Vangl2* is deleted from the inner ear yielding planar polarity phenotypes similar to *Vangl2* KOs at late embryonic stages. *Pax2*-Cre; *Vangl2* mice do not have the lethal neural tube defect that occurs in the *Vangl2* KO making the CKO mice viable. Quantification of planar polarity through postnatal development demonstrates the activity of a *Vangl2*-independent refinement process that rescues the planar polarity phenotype of most hair cells within 10 days of birth. In contrast a similar refinement of vestibular hair cell planar polarity in the striola region of the utricular maculae does not occur during this period. In addition the *Pax2-Cre*; *Vangl2* CKO has profound changes in the shape and distribution of the apical surfaces of Outer Pillar Cells and Deiters' Cells. These changes in supporting cell morphology are persistent, and are not corrected during the period of planar polarity refinement. ABR analyses of adult mice show a 10-20 decibel shift in auditory threshold across all tested frequencies and DPOAE measurements indicate that this mild hearing deficit is of cochlear origin. SEM indicates that this shift is not due to loss hair cells.

Together these data support the hypothesis that a *Vangl2*-independent refinement mechanism actively reorients auditory hair cells during the first 10 days of postnatal development. Vangl2 is required during supporting cell development however it is unclear if this reflects a 'conventional' planar polarity activity. Nonetheless since the altered supporting cell morphology is not refined these changes in supporting cell shape likely underlie the hearing deficits measured in *Vangl2* CKOs.

EXAMINING THE EXPRESSION OF POTENTIAL CELL CYCLE REGULATORS IN THE DEVELOPING MOUSE INNER EAR

MARYAM EBRAHIMI [1], ***GILLIAN L. DRURY*** [2], ***GISELLE BOUKHALED*** [2], ***MELISSA A. VOLLRATH*** [1, 2]

[1] *Department of Neurology and Neurosurgery and* [2] *Department of Physiology, McGill University, Montreal, Quebec, Canada*

Sensory epithelia of the mammalian inner ear are composed of hair cells and supporting cells. These cells originate from common precursors, which exit the cell cycle during late embryogenesis. Thereafter, hair cells and supporting cells maintain a non-proliferative state. As a result, hair cell loss in mammals is irreversible. If inner ear organs are to be regenerated, supporting cells must reenter the cell cycle to produce new hair cells and supporting cells.

Little is known about genes that maintain the post-mitotic state of inner ear supporting cells. Previous studies revealed the role of cell cycle inhibitors such as p27kip in regulating the post-mitotic fate of differentiated supporting cells (Lowenheim et al., 1999).

The purpose of our study was to identify other potential regulators of cell cycle in supporting cells and determine their expression at different ages. We used the Shared Harvard Inner-Ear Laboratory data base (SHIELD; shield.hms.harvard.edu) to select 15 candidate genes following these criteria: 1) an established role in cell cycle regulation in other cell types, 2) maintained postnatal expression in mouse utricular supporting cells, and 3) not previously studied in the mouse inner ear.

We used RT-PCR and in situ hybridization to determine the expression of these candidate genes in inner ear sensory epithelia. Our RT-PCR results, from mouse utricles at postnatal day 7, (which contained hair cells, supporting cells, and non-sensory cells) confirmed the expression of all candidate genes. Our in situ hybridization results, which came from mice between embryonic days 13.5 and 18.5, were mixed. In some cases expression was seen mainly in supporting cells, as expected based on the SHIELD data and in other cases in hair cells or non-sensory cells. Further studies are required to test whether these genes play a role in regulating cell cycle in the mouse developing inner ear.

OTOPROTECTIVE EFFECTS OF ERYTHROPOIETIN ON *CDH23*$^{ERL/ERL}$ MICE

FENGCHAN HAN, HEPING YU, TIHUA ZHENG, XIUFANG MA, XIN ZHAO, PING LI, LINDA LE, YIPENG SU, QING YIN ZHENG

Department of Otolaryngology-Head & Neck Surgery, Case Western Reserve University, 11100 Euclid Ave, Cleveland, OH, 44106-4952, USA

The Cdh23(erl/erl) mice are a novel mouse model for DFNB12 and are characterized by progressive hearing loss. In this study, erythropoietin (EPO) was given to the Cdh23(erl/erl) mice by intraperitoneal injection every other day from P7 for 7 weeks. Phosphate-buffered saline-treated or untreated Cdh23(erl/erl) mice were used as controls. Auditory-evoked brainstem response (ABR) thresholds and distortion product oto-acoustic emission (DPOAE) were measured in the mouse groups at the age of 4, 6 and 8 weeks. The results show that EPO can significantly decrease the ABR thresholds in the Cdh23(erl/erl) mice as compared with those of the untreated mice at stimulus frequencies of click, 8-, 16- and 32-kHz at three time points. Meanwhile, DPOAE amplitudes in the EPO-treated Cdh23(erl/erl) mouse group were significantly higher than those of the untreated groups at f2 frequency of 15383 Hz at the three time points. Furthermore, the mean percentage of outer hair cell loss at middle through basal turns of cochleae was significantly lower in EPO-treated Cdh23(erl/erl) mice than in the untreated mice ($P<0.05$). This is the first report that EPO acts as an otoprotectant in a DFNB12 mouse model with progressive hearing loss.

A COMPARATIVE ANALYSIS OF THE REGULATION OF ATOH1 EXPRESSION IN MAMMALS AND AVIANS

***MIRIAM GOMEZ-DORADO*[1], *JONATHAN GALE*[1], *NICOLAS DAUDET*[1], *SALLY DAWSON*[1]**

[1]*UCL Ear Institute, 332 Gray's Inn Road, London WC1X 8EE.*

It is known that adult mammalian cochlear hair cells do not regenerate and the vestibular hair cells have a limited capacity for hair cell regeneration when damage occurs. However, non-mammalian vertebrates including birds can produce new hair cells through trans-differentiation of surviving supporting cells into hair cells.

Atoh1 is a transcription factor known to be necessary and sufficient for hair cell differentiation. Q(RT)-PCR and immunohistochemistry studies have shown that Atoh1 expression is activated in the avian auditory epithelium after damage whereas in mammals there is little or no change in expression. Due to the importance of Atoh1 for hair cell formation and regeneration we have explored the differences in the regulation of this gene in mammalian and avian species. To this end, we have utilised a comparative bioinformatics approach to identify putative transcription factors binding to evolutionary conserved regions located 3' of the Atoh1 gene. Some of these conserved regions correspond to the previously identified Atoh1 enhancers which are known to be necessary for its expression. These regions, known as enhancer A and B are well conserved in mammalian and avian species sharing a high degree of homology (>85%). Bioinformatic analysis identified ~200 transcription factor binding sites in these enhancer regions that potentially could play a role in Atoh1 regulation. The families identified include some of those transcription factors already shown to have an effect on Atoh1 expression including SOX2, Zic1 and Atoh1 itself.

To identify differences in Atoh1 regulation across species, this database of putative candidates was segregated into three groups depending on whether they were predicted to bind exclusively to mammalian or avian enhancers or to enhancers in both species. In addition to enhancers A and B our analysis also identified a 300bp region that is well conserved in avian species but is absent from mammals. Like the previously identified enhancers, this region shares a high degree of homology (>80% conservation) across avian species. Thirty-five transcription factors families were predicted to bind to this region, including Nuclear Factor-KappaB, members of the E2F family and also, as with the other enhancers, Atoh1 itself. These transcription factor candidates are currently being evaluated using EMSA and reporter gene assays.

Identifying and comparing potential candidates binding to conserved regions in mammalian and avian species could lead to a better understanding of the Atoh1 regulatory network and contribute to revealing the molecular mechanisms which are responsible for the different regenerative capabilities of mammals and birds.

Sponsored by a travel award from:
ACTION ON HEARING LOSS

NOTCH INHIBITION INDUCES COCHLEAR HAIR CELL REGENERATION AND RECOVERY OF HEARING AFTER ACOUSTIC TRAUMA

***MAKOTO HOSOYA*[1,2], *KUNIO MIZUTARI*[3], *MASATO FUJIOKA*[1,4], *NAOMI BRAMHALL*[6,7,8], *HIROTAKA JAMES OKANO*[5], *HIDEYUKI OKANO*[2], *ALBERT EDGE*[6,7,8] *AND KAORU OGAWA*[1].**

[1]Department of Otorhinolaryngology Head and Neck surgery, Keio University School of Medicine, Tokyo, Japan; [2]Department of Physiology, Keio University School of Medicine, Tokyo, Japan; [3]Department of Otolaryngology, National Center for Child Health and Development, Tokyo, Japan; [4]Department of Otorhinolaryngology, Keiyu Hospital, Yokohama, Japan; [5]Division of Regenerative Medicine, Jikei University School of Medicine, Tokyo, Japan; [6]Department of Otology and Laryngology, Harvard Medical School, Boston, MA, USA; [7]Tillotson Unit, Eaton-Peabody Laboratory, Massachusetts Eye and Ear Infirmary, Boston, MA, USA; [8]Program in Speech and Hearing Bioscience and Technology, Division of Health Science and Technology, Harvard & MIT, Cambridge, MA, USA

Hair cells produced during development are postmitotic and are not replaced after loss or as part of normal cell turnover in mammals. As a result, deafness due to hair cell loss is irreversible. Hair cell development includes a complex series of fate decisions, in which prosensory epithelial cells acquire different fates, either hair cell or supporting cell, through a process of lateral inhibition that is mediated by Notch signaling. Supporting cells are prevented from differentiating into hair cells by active Notch signaling stimulated by ligands on adjacent hair cells.

We manipulated Notch signaling with a γ-secretase inhibitor, which inhibits Notch signal, to generate new hair cells in ears damaged by noise trauma. First, we identified a potent γ-secretase inhibitor in an assay with utricle derived inner ear stem cells. LY411,575 has the most profound effect on inducing hair cell differentiation among four candidate compounds we used. We also assessed the effect of LY411,575 on organ of Corti explant with or without hair cell ablation revealing LY411,575 increased hair cell numbers. Secondary, to assess whether differentiation of hair cell could be induced in a mature ear, we exposed mice to an acoustic injury, producing widespread outer hair cell death and permanent hearing loss with preservation of supporting cells, and administrated LY411,575 systemically. Oral intakes of LY411,575 decreased noise-induced threshold shift and outer hair cell numbers were increased, while severe side effects limited the maximum dose. Therefore we next tried local application of the drug to the ear by surgical approach and we successfully achieved a significant hearing improvement without side effect. Finally, we determined the source of the new hair cells by performing lineage tracing with a Cre-reporter strain of Sox2-positive cells, which is expressed in supporting cells. Consequently, we showed that new hair cells formed after treatment with the inhibitor arose by trans-differentiation of supporting cells, and that the new hair cells contributed to a partial reversal of hearing loss in mice. Our findings indicates manipulating cell fate of peripheral auditory sensory cells in vivo is a novel, feasible therapeutic target against deafness including trans-differentiation of auditory supporting cells to hair cells by pharmacological inhibition of Notch signal.

ROLE OF THE TRANSCRIPTION FACTOR SOX9 IN THE INNER EAR FORMATION

***AURÉLIA HUYGHE*[1], *LAURENCE DELACROIX*[1], *MORGAN BODSON*[1], *PRISCILLA VAN DEN ACKERVEKEN*[1] *AND BRIGITTE MALGRANGE*[1].**

[1] *GIGA-Neurosciences, Developmental Neurobiology Unit, University of Liege, Avenue de l'Hôpital 1, 4000 Liege, Belgium.*

The transcription factors of the SoxE family, including Sox8, Sox9 and Sox10 play important roles in diverse developmental processes and particularly in cochlea development. Among these regulatory genes implicated in the inner ear development, Sox9 has been shown to be important in otic vesicle formation during early embryonic development. Indeed, Sox9 expression is detected as soon as embryonic day E9, in the forming otic placode. As the organ of Corti begins to differentiate, Sox9 is progressively restricted to supporting cells and its expression is absent in hair cells responsible for sound transduction. This expression profile prompted us to evaluate the role of this gene in cochlear cell terminal differentiation. We therefore analysed the role of Sox9 in cell specification using both gain- and loss-of function approaches. We demonstrated, by *ex vivo* electroporation of mouse embryonic cochlea that Sox9 strongly inhibits hair cell fate even when it is forced by ectopic expression of Atoh1, a potent inducer of hair cell differentiation. Accordingly, Sox9 expression needs to be downregulated in cells that are committed to become hair cells as it was observed in the developing inner ear. In parallel, we are investigating the molecular mechanisms underlying Sox9 inhibitory effect on hair cell fate in UB/OC-1 cell line (derived from mouse embryonic organ of Corti) and more specifically its relationship with other important factors for cell specification in the organ of Corti such as Sox2, Notch pathway or Atoh1. We demonstrated that Sox9 is upregulated by Notch activation in mouse embryonic cochlea and partially contributes to Notch inhibition of hair cell fate. We also observed an inhibition of Atoh1 transcriptional activity upon Sox9 overexpression in UB/OC1 cells, concomitant with an induction of Hey1 and HeyL expression, implicated in the Notch cascade pathway and well known to inhibit Atoh1 activity. Taken together, our work is shedding a new light on the role of Sox9 during inner ear development, specification and maintenance of differentiated cells within the cochlea.

EXOGENOUS BDNF AND CHONDROITINASE ABC CONSISTED BIOMIMETIC MICROENVIRONMENT REGULATES SURVIVAL, MIGRATION AND DIFFERENTIATION OF HUMAN NEURAL PROGENITOR CELLS TRANSPLANTED INTO A RAT AUDITORY NERVE

***KALE A.*[1], *ENGLUND U.*[2], *STUPP S.*[3], *NOVOZHILOVA E.*[1], *PALMGREN B.*[1], *OLIVIUS P.*[1,4,5]**

[1] Department of clinical sciences, intervention and technology, Karolinska Institutet, Karolinska University hospital, 171 76 Stockholm, Sweden. [2] Department of Ophthalmology, Institution of Clinical Sciences in Lund, Lund University, 221 84 Lund, Sweden.[3] Department of Chemistry, Materials Science and Engineering, and Medicine and Institute for BioNanotechnology in Medicine, Northwestern University, Chicago, Illinois, USA.[4] Division of Otorhinolaryngology, Linköping University Hospital, Linköping, Sweden. [5] Division of Otorhinolaryngology, Department of Clinical and Experimental Medicine,University of Linköping, Sweden

Current putative regeneration oriented studies express possible role of stem cell based implantation strategy in the restoration of fundamental perception of hearing. The present work utilizes a rat auditory nerve directed transplantation of human neural progenitor cells (HNPCs) as a replacement therapy and for the further investigation of revival of impaired auditory function. Groups of β-bungarotoxin induced auditory function compromised female rats were used to transplant HNPCs in the nerve trunk through internal auditory meatus (IAM). In the treatment groups brain derived neurotrophic factor (BDNF), peptide amphiphile nanofiber gel (PA bioactive gel) and chondroitinase ABC (ChABC), a digestive enzyme that cleaves the core of chondroitin sulphate proteoglycans were added along with HNPCs while the control groups were with PA inert gel and devoid of ChABC. Six weeks post transplantation all the animals were sacrificed by transcardial perfusion. Survival, migration and differentiation of HNPCs were studied and compared. The groups treated with BDNF and PA bioactive gel showed improved survival and differentiation of transplanted HNPCs while the ChABC treated group showed significant migration of HNPCs along the auditory nerve and elongation of neuronal fibers across the nerve towards the cochlear nucleus which was characterized with immunocytochemical markers for human Nuclei (HuNu), human mitochondria (HuM) and neuronal β-tubulin (Tuj1). These findings show that addition of BDNF and ChABC consisted PA bioactive gel environment facilitated HNPCs to better their survival, migration and differentiation along the transplanted rat auditory nerve area towards the cochlear nucleus in vivo. This regenerative transplantation strategy provides unique experimental validation showing futuristic role for cell based biomaterial consisted trophic factor application in clinically transferable treatment of sensorineural hearing loss along with cochlear implants.

Sponsored by a travel award from:
Hearing Health Foundation
Prevention | Research | Cure

RHO GTPASE CDC42 REGULATES STRUCTURAL PATTERNING OF THE DEVELOPING AUDITORY ORGAN

***ANNA KIRJAVAINEN*[1], *TOMMI ANTTONEN*[1], *CORD BRAKEBUSCH*[2], *ULLA PIRVOLA*[1]**

[1]Institute of Biotechnology, University of Helsinki, Finland; [2] Biotech Research and Innovation Centre (BRIC), University of Copenhagen, Denmark

Embryonic and postnatal development of the epithelial cells of the organ of Corti (OC) is characterized by cell polarization, the establishment and modulation of actin and microtubule cytoskeletons and the formation and rearrangement of intercellular junctions. Rho GTPase Cdc42 regulates cytoskeletal dynamics, cell polarity and junctional integrity of epithelial cells. We initially found *Cdc42* expression in the developing organ of Corti and wanted to study its role in developmental processes mentioned above. To inactivate *Cdc42* in the OC, conditional, inducible approach was applied. The *Fgfr3-iCre-ER*^{T2} mice were crossed with the *Cdc4*$^{loxP/loxP}$ mouse line. Recombination characteristics were studied by crossing *Fgfr3-iCre-ER*^{T2} mice with the *Ai14(tdTomato)* reporter mouse line.

First we studied Cdc42's role in structural differentiation of auditory supporting cells. This distinct process takes place during the early postnatal life. Acute *Cdc42* inactivation was induced in pillar and Deiters' cells between P2 and P4. We found that Cdc42 regulates development of the apical actin cytoskeleton. The integrity of circumferential F-actin belts and adherens junctions was lost in *Cdc42*-depleted supporting cells. In addition, misexpression of the apical (atypical PKCλ/ι) and basolateral (CD44) membrane proteins suggested for disturbances in apical polarization. Upon ototoxic insult that kills outer hair cells, supporting cells of mutant animals failed to remodel their apical F-actin cytoskeleton, an event that normally forms a scar at the site of lost hair cells. These results suggest that Cdc42-dependent structural maturation of auditory supporting cells is essential for wound healing in the lesioned OC.

In the second approach, *Cdc42* was inactivated in the embryonic OC, between E13 and E14. At this age both outer hair cells and supporting cells were recombined. *Cdc42* inactivation after the formation of the progenitor cell population but before the onset of the cellular differentiation led to patterning and polarity defects in hair cells. The OC of *Cdc42* mutant animals showed a planar polarity phenotype with misoriented stereociliary bundles accompanied by defects in cell shapes. Together, Cdc42 regulates embryonic and postnatal development of the OC. It plays a role in the establishment of F-actin cytoskeleton and intercellular junctions in postnatal supporting cells as well as the development of correct patterning and orientation of embryonic auditory hair cells.

PLASTIN-1 CONTROLS VESTIBULAR STEREOCILIA LENGTH

***JOCELYN F. KREY*[1], *RACHEL A. DUMONT*[1], *FRANCISCO RIVERO*[2], *PETER G. BARR-GILLESPIE*[1]**

[1] Oregon Hearing Research Center & Vollum Institute, Oregon Health & Science University, Portland, OR 97239, USA; [2] Centre for Biomedical Research, The Hull York Medical School and Department of Biological Sciences, University of Hull, Hull HU6 7RX, United Kingdom

The mechanosensory hair bundle consists of organized rows of actin-filled stereocilia of increasing heights. During development, stereocilia form through the assembly of actin filaments into tight parallel bundles by actin-bundling proteins. Stereocilia then increase in diameter and length, through both the addition of new actin filaments and the elongation of existing filaments within the actin paracrystal. Although plastin-1 (PLS1, I-plastin, fimbrin) is a member of one of the three classes of actin-bundling proteins known to be enriched in hair bundles, very little is known about its function in stereocilia assembly.

We tested the importance of PLS1 in stereocilia development by examining the vestibular hair bundles from *Pls1*-null mice. *Pls1*-null mice do not display any behavioral evidence of overt vestibular dysfunction and auditory and vestibular hair cells appear to develop normally. To test whether loss of PLS1 is compensated by upregulation of another actin-bundling protein, we used mass spectrometry to quantify proteins in purified mouse hair bundles from wild-type and *Pls1*-null mice. In wild-type mice, PLS1 is the second-most abundant protein in the bundle and the most abundant actin-bundling protein of those we detected (PLS1, FSCN2 and ESPN). PLS1 was absent from the bundle in *Pls1*-null mice but no other actin-binding proteins increased in expression or were mislocalized. Using both mass spectrometry and immunoblotting, however, we detected a significant ~30% decrease in the total amount of actin within the bundles of *Pls1*-null mice. When examined by transmission electron microscopy, we did not find any differences in the organization or number of actin filaments in *Pls1*-null mice as compared to wild-type. Morphometric analyses of vestibular bundles, however, indicated that stereocilia of adult (P21-P25) *Pls1*-null mice were significantly shorter in length (~20%) than those of wild-type mice, with no change in kinocilia length or the staircase architecture of the bundle. Utricle stereocilia at early postnatal stages (P4-P6) were also shorter than those of wild-type mice, although the decrease was smaller (~10%). Using immunoblotting and immunocytochemistry, we found that PLS1 undergoes a steep increase in expression between P4 and P23, the same time period during which we found the majority of stereocilia lengthening occurs.

Our results suggest that PLS1 is not essential for the initial assembly of stereocilia, but that it does play a role in regulating the elongation of stereocilia during postnatal development. Together, the timecourse of PLS1 expression and changes in stereocilia length in *Pls1*-null mice suggest that PLS1 contributes to stereocilia lengthening between P4 and P23, and may also contribute to the stability and maintenance of stereocilia throughout adulthood.

OVEREXPRESSION OF TJP2 LEADS TO HEARING LOSS THROUGH APOPTOSIS MEDIATED BY GSK3β ACTIVATION IN A MOUSE MODEL FOR DFNA51

DANIELLE R. LENZ, SHAKED SHIVATZKI, KATHY USHAKOV, YONI BHONKER AND KAREN B. AVRAHAM

Department of Human Molecular Genetics & Biochemistry, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

Age-related hearing loss (ARHL) is a widespread phenomenon, yet the causative factors remain elusive. We have discovered a tandem-inverted genomic duplication of the tight junction protein 2, encoded by the TJP2 gene, leading to adult-onset progressive hearing loss (HL) in an Israeli kindred. In lymphoblasts derived from human patients, TJP2 overexpression results in changes in GSK-3β phosphorylation and apoptosisrelated gene expression (Walsh et al. AJHG 2010). We predict that these changes increase the susceptibility of inner ear cells to apoptosis and could account for the progressive HL observed. Here we describe a transgenic mouse model, carrying approximately two additional copies of the human TJP2 gene, which is similar to the human duplication of TJP2. The mice present high frequency HL already at P30 that begins at high frequencies and progresses as the mice mature, reaching profound HL by 5 months of age. Antibody array analysis revealed alterations in expression levels of several apoptotic-related factors in a manner similar to the changes observed in the human lymphoblast cells. GSK3β is activated in ears from transgenic mice and subsequent induction of Bcl2 pro-apoptotic family members is apparent. As DFNA51 is characterized by adult-onset HL, the Tjp2 transgenic mouse appears to mimic the human phenotype, setting the stage for using this model to elucidate the mechanisms of ARHL.

BONE MORPHOGENETIC PROTEINS ANTAGONIZE HAIR CELL REGENERATION IN POST-HATCH CHICKENS

JESSE KELLER, REBECCA LEWIS, JENNIFER STONE

Department of Speech and Hearing Sciences at the Virginia Merrill Bloedel Hearing Research Center, University of Washington

Most supporting cells (SCs) upregulate *Atoh1* in the post-hatch chicken basilar papilla after hair cell (HC) damage. However, only some cells with elevated *Atoh1* expression transdifferentiate into HCs. Therefore, acquisition of the HC fate after damage is modulated in some SCs that upregulate *Atoh1*. During chick inner ear development, bone morphogenetic proteins (BMPs) stimulate expression of Inhibitors of Differentiation (IDs), which antagonize *Atoh1* expression (Pujades et al., 2006; Kamaid et al., 2010). We are testing the hypothesis that BMPs modulate HC regeneration via IDs during HC regeneration in post-hatch birds. We performed *in situ* hybridization to determine expression patterns of BMPs and IDs in both normal and damaged basilar papillae (BPs) from post-hatch chickens. *C*ontrol BPs expressed high levels of *Id2* and *Id3* in SCs and high levels of *Bmp4* transcripts in HCs. BMP receptor transcripts were expressed in both HCs and SCs. Expression of *Atoh1* was undetected. At 3 days post-Streptomycin, *Atoh1* transcripts were upregulated in the region of damage, whereas *Id2*, *Id3*, and *Bmp4* transcripts were downregulated. Treatment of streptomycin-damaged BPs with BMP2 or BMP4 *in vitro* decreased *Atoh1* transcripts and increased *Id2* and *Id3* transcripts. Treatment of damaged BPs with the BMP inhibitor noggin *in vitro* increased *Atoh1* transcripts but decreased *Id2/3* transcripts. The number of regenerated HCs increased with noggin treatment *in vitro* but decreased with BMP4 treatment. We propose the following model for BMP activity in normal and regenerating BPs. High levels of BMP4 in HCs in undamaged BPs promote *Id2* and *Id3* expression in SCs under normal conditions, which maintains their quiescence. The reduction of BMP4 signaling upon HC loss triggers the downregulation of *Id2* and *Id3*, thereby allowing *Atoh1* to increase in SCs. The renewed activity of BMP4 in regenerating HCs increases *Id2* and *Id3* transcripts and decreases *Atoh1* transcripts in SCs, thereby antagonizing further HC differentiation. We are currently testing the ability of ID2 or ID3 to mediate BMP4's inhibitory effects on HC regeneration.

Sponsored by a travel award from:
Hearing Health Foundation
Prevention | Research | Cure

DEVELOPMENT OF AN *IN VIVO* FUNCTIONAL SCREENING METHOD FOR NEURAL CIRCUIT ASSEMBLY GENES

***CINDY LU*[1], *LISA GOODRICH*[1]**

[1]*Department of Neurobiology, Harvard Medical School, Boston, MA 02115*

Since all sound information detected by hair cells in the cochlea is sent to the brain via bipolar spiral ganglion neurons (SGNs), proper hearing depends on the exquisite pattern of connections between these neurons and both their peripheral and central targets. However, little is known about how this key part of the auditory circuit is established on the molecular level. I have generated a comprehensive gene expression database for SGNs as they progress through circuit assembly stages, from axon guidance through the onset of hearing. However, functionally screening through large numbers of genes by conventional mouse knockouts is prohibitively time-consuming and costly, especially when conditional knockouts are needed or when functional redundancy makes generation of double or even triple mutants necessary. Indeed, although advances in genome-wide techniques have made large molecular datasets widely available, our ability to screen for gene function *in vivo* in mice has not kept pace. This is a huge bottleneck in our ability to advance the field of auditory neuroscience, and neuroscience in general. To address this problem, I am developing a method of rapidly screening for gene function in mice in a single generation. Briefly, a conditional knockdown vector is targeted into embryonic stem cells (ESCs) carrying a tissue-specific Cre allele. These targeted ESCs are then used to generate embryos or pups for phenotypic analysis. Some notable features of the method: 1) Mutant phenotypes can be analyzed immediately, with no additional mouse breeding. 2) Gene function can be conditionally ablated in existing Cre ESC lines. 3) Multiple genes can be knocked down simultaneously. 4) Cloning and ESC targeting are streamlined and highly efficient. 5) Fluorescent labeling of neuronal projections allows fast and easy phenotype screening. Proof-of-principle experiments are currently under way to confirm the validity of this approach.

Sponsored by a travel award from:

NIDCD

CHANGING TRANSCRIPTIONAL ACTIVITY OF THE MURINE COCHLEA IN RESPONSE TO NEUROPATHIC NOISE AND AGING

***ANDREW LYSAGHT*[1,2], *CHRISTINE SEIDMAN*[4], *JONATHAN SEIDMAN*[4], *KONSTANTINA M. STANKOVIC*[1,2,3,5]**

[1]Program in Speech and Hearing Bioscience and Technology, Harvard & MIT, Cambridge, MA, USA; [2]Eaton-Peabody Laboratory, Massachusetts Eye and Ear Infirmary, Boston, MA, USA; [3]Department of Otolaryngology, Massachusetts Eye and Ear Infirmary, Boston, MA, USA;[4]Department of Genetics, Harvard Medical School, Boston, MA, USA; [5]Department of Otology and Laryngology, Harvard Medical School, Boston, MA, USA

Recent studies on the long-term impact of noise exposure have produced startling revelations concerning the health of the auditory nerve. Exposures previously believed to cause only temporary threshold shift (TTS) have been shown, in mouse models, to cause significant long-term declines in spiral ganglion neurons that make up the auditory nerve. This phenomenon of noise-induced death of the auditory nerve without the loss of sensory or supporting tissues is known as noise-induced primary auditory neuropathy (NI-PAN), and has important clinical applications. NI-PAN preferentially affects high-threshold neurons. Although the unaffected low-threshold neurons allow audiograms to appear normal, the reduction in total number of neurons (effective information channels) leads to reduced ability to effectively process complex acoustic stimuli.

To gain insight into the molecular mechanisms underlying the slowly progressive degeneration of spiral ganglion neurons in NI-PAN (>1 year in mice), we used RNA-Seq and defined the transcriptional activity of the micro dissected spiral ganglion and sensory epithelium in mice exposed to neuropathic and non-neuropathic noise at 24 hours, 2 weeks, 2 months and 16 months post exposure. Transcripts differentially expressed as a result of neuropathic exposure were identified using *DESeq* analysis software. GO analysis showed significant functional enrichment for genes involved in endo/ribonuclease activity and carbohydrate binding. Enriched biological processes include chemotaxis and immune/defense responses. Cellular localization shows enrichment for cytoplasmic and secretory vesicles.

This study presents the first description of the genome-wide transcriptional changes that occur within the adult inner ear in response to neuropathic noise that causes NI-PAN. Our results have implications for future development of targeted therapies to prevent or reverse such neuro degeneration.

THE ROLE OF *JAG1* DOWNSTREAM OF *TBX1* IN THE INNER EAR AND CRANIAL GANGLIA

STEPHANIA MACCHIARULO, BERNICE MORROW

Department of Genetics, Albert Einstein College of Medicine, Bronx, New York, USA.

The overall aim of this project is to characterize the genetic relationship between *Tbx1* and *Jag1*, a Notch ligand, in the regulation of organogenesis, particularly in the inner ear and cranial ganglia. We have found that *Tbx1*; *Jag1* double mutants exhibit defects in neurogenic, sensory, and non-sensory structures of the inner ear. With respect to neurogenesis, it has been shown that *NeuroD* expression is expanded in the otic vesicle in *Tbx1* null mice, delineating a duplicated cochleovestibular ganglion (CVG) (Raft et al., 2004). Genes in the Notch pathway are involved in inner ear neurogenesis and CVG formation. Interestingly, *Notch1* overexpression prevents the formation of the CVG, while *Jag1* inactivation results in a smaller CVG (Pan et al., 2010). It has also been suggested that *Tbx1* may act upstream of the *Notch* pathway to regulate neurogenesis in the inner ear, as it has been reported that in *Tbx1* null mice, *Notch1* and *Dll1,* another Notch ligand, expand in expression into the same domain in which *Ngn1* expands (Xu et al., 2007). Similarly, we have found that *Jag1* expression expands into this domain when *Tbx1* is inactivated. We have also observed that *Jag1* co-localizes with *Tbx1* lineage-traced cells in the otic vesicle and distal cranial ganglia. Based on these findings, we decided to inactivate *Jag1* within the *Tbx1* expression domain using *Tbx1*^{Cre} (*Tbx1* heterozygote)(Huynh et al., 2012) and a *Jag1* floxed line (Kiernan et al., 2006) to elucidate whether there was a genetic interaction between the two genes. While we expected to see a rescue of the phenotype, we instead observed an expansion in *NeuroD* expression in the otic vesicle and the distal cranial ganglia at E10.5 compared to controls. This phenotype is significantly enhanced on a *Tbx1* null background (*Tbx1*$^{Cre\ /-}$). Possible explanations for these results are that: 1) inactivation of *Tbx1* causes another gene to compensate for loss of *Jag1*; 2) loss of *Tbx1* alters the function of *Jag1*; 3) the perceived expansion of *NeuroD* is due to loss of surrounding tissue. Future experiments will focus on assessing neurogenic expression changes in *Tbx1; Jag1* mutants at earlier stages in embryonic development (E8.5-E9.5) before major morphological defects occur, as well as inactivating *Jag1* using Pax2 ^{Cre} for comparison. In addition, we observed defects in E15.5 and adult *Tbx1*^{Cre}; Jag $^{flox/flox}$ mutants, including severe truncation of semicircular canals, missing cristae, and a smaller cochlear nerve.

SYNAPTOGENIC ROLE OF THROMPOSPONDINS IN POSTNATAL INNER EAR DEVELOPMENT

***DIANA MENDUS*[1], *SRIVIDYA SUNDARESAN*[1], *NICOLAS GRILLET*[2], *FELIX WANGSAWIHARDJA*[1], *ULRICH MUELLER*[2], *SHERRI JONES*[3], *MIRNA MUSTAPHA*[1]**

[1] Department of Otolaryngology – Head and Neck Surgery, Stanford University, Stanford, CA, USA; [2] Department of Cell Biology, The Scripps Research Institute, La Jolla, CA, USA; [3] Department of Special Education & Communication Disorders, University of Nebraska-Lincoln, Lincoln, NE, USA

Background. Synaptogenesis is typically viewed as a process involving pre- and postsynaptic targets, but recent studies suggest that glial cells are also involved. One of such glial signals involved in synaptogenesis is the release of cell-adhesion molecules thrompospondins (TSPs). Little information is available on the role of molecules released by glia during the late stages of the vestibular and cochlear synaptic formation and maturation. Here we studied 1) whether TSPs are expressed in the inner ear; 2) which cells of the inner ear produce TSPs; 3) what are the functional consequences of disruption of TSP-induced synaptogenesis in both the hearing and balance organs.

Methods. Using quantitative RT-PCR, we measured TSP1-2 expression at different developmental ages and in adulthood. *In situ* hybridization was used to identify cell types that express TSPs. We examine whether TSP1 and TSP2 are involved in synapse formation and maintenance in the inner ear using transgenic mouse models of these genes. Confocal analysis was applied for synapse quantification. Auditory and vestibular functions were assessed by electrophysiological tests.

Results. Our analysis of TSP1 and TSP2 expression levels revealed significant upregulation in the mRNA both of these genes during the critical window of postnatal inner ear development. We demonstrated for the first time that TSPs which were previously shown to be secreted by astrocytes in central nervous system are also expressed in the cochlea and vestibular system by supporting cells. Moreover, using immunohistochemistry and confocal microscopy, we revealed that TSPs have a synaptogenic role in these organs. Using transgenic TSP1, TSP2 and TSP1/2 KO mouse models, we show that absence of TSPs lead to functional disturbances in the latency and amplitude of the hearing organ and amplitude of the vestibular organ as detected by auditory brain stem response and vestibular evoked potentials, respectively. Thresholds of vestibular performance were not affected in single mutants while auditory thresholds were significantly worse in TSP2 mutants at a young age. Notably, while in the central nervous system, TSP1 and TSP2 had similar effects, we found that the absence of TSP2 had a greater effect on cochlear function. Interestingly, one-year old TSP1 mutant mice show an increase in auditory thresholds compared to wild type controls, suggesting a possible role for TSP1 in synaptic maintenance.

Conclusion. These results indicate that cochlear and vestibular supporting cells contribute *in vivo* to inner ear synapse formation by expression of TSPs. TSP1 and TSP2 are involved in different aspects of inner ear functional maturation and maintenance. Based on our study, TSP1 and TSP2 genes can be suggested as candidates for screening in human patients with congenital and age-related hearing impairment.

TRANSCRIPTIONAL REGULATION OF PMCA2 IN INBRED MOUSE STRAINS

***REBECCA R. MINICH*[1,2], *CLAIRE J. WATSON*[1], *BRUCE L TEMPEL*[1,2]**

[1]The Virginia Merril Bloedel Hearing Research Center, Department of Otolaryngology, and [2]Department of Pharmacology, University of Washington, Seattle, WA, USA.

The plasma membrane Ca^{2+} ATPase 2 (PMCA2) protein regulates intracellular Ca^{2+} levels and is encoded by the *Atp2b2* gene. *Atp2b2* mutations have given rise to an allelic series of *deafwaddler* mouse mutants. Studies in heterozygous *deafwaddler* mutants derived from different strain backgrounds (*dfw*^{2J}and *dfw*^{i5}) suggest that *Atp2b2* expression levels are regulated by ancestral *Atp2b2* haplotypes (Watson and Tempel, submitted). In this study, we show that transcription of the *Atp2b2* C57BL/6J (B6) allele is under-expressed relative to CBA/CaJ (CBA) in heterozygous mice (CBA/B6). A study of the parental strains reveals that both neuronal transcripts of *Atp2b2* (α and β) are down regulated in B6 mice. α-*Atp2b2* is the main transcript of the auditory hair cells and is the focus of this study (Silverstein and Tempel 2006).

To detect sequence nucleotide changes that are polymorphic between CB and B6, the 5kb region upstream of the transcriptional start site of α-*Atp2b2* was sequenced. From this analysis, 9 single nucleotide polymorphisms (SNPs) were identified and analyzed using MatInspector, a software engine that predicts transcription factor (TF) binding sites. This software predicts that 7 SNPs in the B6 promoter of PMCA2 will alter the binding of TFs found in the ear. Of these 7 SNPs, 2 are good candidates for altering TFs important for normal auditory development. This suggests that B6 may have a 'weaker' α-*Atp2b2* promoter than CB, leading to decreased transcriptional expression of α-*Atp2b2* transcript.

To empirically confirm the core promoter, we generated a series of CB like luciferase promoter constructs and transiently transfected them into mammalian cells. Preliminary tests suggest that promoter activity for the α-*Atp2b2* transcript is found in a CpG island directly upstream of the transcriptional start site. To study the 2 SNPs that are predicted to be involved in altering α-*Atp2b2* transcript expression in B6 small 'minigene' luciferase constructs were created. These constructs contain ~250 bases surrounding the SNP of interest cloned in front of the Thymidine Kinase (TK) minimal promoter which is upstream of the translational start of the firefly luciferase gene. Preliminary data (N=3) suggests that the SNP located 4517 bases upstream of the translational start site could be involved in transcriptional repression of *α-Atp2b2* in B6 mice.

INTRINSIC ROLE OF THYROID HORMONE IN DEVELOPMENTALLY REGULATED NEURONAL MATURATION: PRUNING SPIRAL GANGLION TYPE II NEURONS

***MIRNA MUSTAPHA*[1], *QING FANG*[2], *JEE-HYUN KONG*[1], *SRIVIDYA SUNDARESAN*[1], *CAROLINE LEE*[1], *SALLY CAMPER*[2]**

[1]Stanford University, Stanford, CA, USA; [2]University of Michigan, Ann Arbor, MI, USA

The maturation of the organ of Corti involves axonal growth and co-ordination of a massive rearrangement of afferent and efferent fibers and synapses. These processes take place during the thyroid hormone (TH) critical period of cochlear development, in the perinatal period for mice and third trimester in humans. Here we report the characterization of a mouse model of severe, secondary hypothyroidism (*Pit1^{dw}*) with profound, congenital deafness. Although the lack of thyroid hormone causes delay in cochlear development, some processes mature eventually. We previously showed that the sensitivity of cochlear development to hypothyroidism is dependent upon the genetics of the background strain and is intrinsic to the fetus (Fang et al. JARO 13:173, 2012). Here we report that the function of inner hair cells (IHCs) and the patterning of afferent fibers are both slightly delayed, but they mature by P24. In contrast to IHCs, afferent synapses of outer hair cells (OHCs) retain their immature innervation pattern into adulthood. We observed abnormal persistence of temporary spiral ganglion (SG) type II synapses and neurons in hypothyroid OHCs in adult mutants (P42). Hypothyroidism is hypothesized to interfere with neuronal loss necessary for normal cochlear development (Uziel et al., 1982; Rueda et al., 2003). Here we report that defects in OHC afferent refinement are related to the lack of large-scale pruning of SG type II. In addition to the altered pruning of the SG II, OHCs abnormally retain the expression of presynaptic markers that are involved in neurotransmitter release, such as otoferlin. These proteins normally vanish from OHCs by the end of the first postnatal week in parallel with the regression of temporary afferent neurons (Roux et al., 2006). Our studies reveal for the first time that the intrinsic requirement for TH in cochlear development is associated with pre and postsynaptic afferent OHC but not IHC refinement. A comparison of the cochlear transcriptome of *Pit1^{dw}* mutant and wild type littermates reveals differential expression of genes that are involved in afferent synapse pruning in the brain and the retina, suggesting that these genes may mediate afferent refinement during cochlear development. Identification of such genes will enhance our understanding of the mechanism of TH action on synapse refinement in the brain and the periphery.

NOVEL PATHOLOGICAL MODEL OF PROXIMAL SYMPHALANGISM AND CONDUCTIVE HEARING LOSS REVEALED BY DOCKING SIMULATION OF NOGGIN AND HEPARIN

***KAZUNORI NAMBA*[1], *HIROKI KANEKO*[2], *SAWAKO MASUDA*[3], *HIDEKI MUTAI*[1], *SATOKO USUI*[3], *TATSUO MATSUNAGA*[1]**

[1]Laboratory of Auditory Disorders, National Institute of Sensory Organs, National Hospital Organization Tokyo Medical Center, Tokyo, Japan [2]Department of Integrated Sciences in Physics and Biology, College of Humanities and Sciences, Nihon University, Tokyo, Japan [3]Department of Otorhinolaryngology, Institute for Clinical Research, National Mie Hospital, Tsu, Mie, Japan

Background: The access of bone morphogenetic protein (BMP) to the BMP receptors on the cell surface is regulated by its antagonist Noggin. Noggin-BMP complex associates with heparan-sulfate proteoglycans (HSPGs) in extracellular matrices, and is regulated by sulfatases to control the local activity of BMPs. Mutations in *NOG* are associated with various autosomal dominant syndromes associated with conductive hearing loss that are characterized by a spectrum of skeletal defects and synostoses, such as multiple synostoses syndrome, Teunissen-Cremers syndrome, and Proximal symphalangism (SYM1). It is not yet understood how different *NOG* mutations can produce this wide range of symptoms.

Methods: Genetic analysis of *NOG* gene of a family with SYM1 and conductive hearing loss was performed. To compare normal and R136C mutant Noggin structure, we generated modeling structures using crystal structure as a template. To analyze the effect of R136C on the function of Noggin, we conducted docking simulation of the Noggin and pentasaccharide analogue of heparin using AutoDock4.2.

Results: A pedigree diagnosed as SYM1 was found to have a novel heterozygous missense mutation (p.R136C) associated with the symptoms. The position 136 was the heparin-binding site of Noggin, raising the possibility that R136C would change the binding affinity. No mutations of the heparin-binding site of Noggin have previously been reported to associate with diseases. We utilized the crystal structure of wild-type Noggin and investigated whether the p.R136C mutation altered its structure that could lead to some pathogenic effect. An *in silico* docking analysis showed that the heparin analogue appeared to associate with the putative heparin-binding site (K133-K144) of Noggin. However, one of the salt bridges between Noggin and heparin pentasaccharide disappeared following the replacement of the arginine at position 136 with a non-charged cysteine.

Conclusions: Based on our analyses, we predicted that the number of mutant Noggin-BMP complexes that are tethered to HSPGs on the cell surface are decreased because of the reduced binding affinity of the Noggin with R136C mutation to HSPGs. Reduced binding ability of the mutant Noggin to HSPGs is likely to the cause of excess BMP signaling that ultimately resulted in SYM1 and conductive hearing loss.

IGF-1 INDUCES REGENERATION OF SYNAPTIC CONTACTS BETWEEN INNER HAIR CELLS AND SPIRAL GANGLION NEURONS

TAKAUKI NAKAGAWA, NAKARIN ASAKA, YUSHI HAYASHI, NORIO YAMAMOTO, TOMOKO KITA, JUICHI ITO

Kyoto University, Kyoto, Japan

Previous studies have revealed involvement of insulin-like growth factor (IGF) signaling in the development and maintenance of mammalian cochleae. Our previous animal experiments have demonstrated that sustained, topical application of IGF-1 attenuates hearing loss due to noise or ischemia. Based on results in animal experiments, we performed a prospective clinical trial to test the safety and efficacy of topical IGF-1 therapy for sudden deafness refractory to systemic steroid treatment, which indicates the efficacy of topical IGF-1 treatment for sudden deafness. Interestingly, hearing recovery in subjects treated with topical IGF-1 therapy was appeared slowly, suggesting involvement of regenerative processes rather than hair cell protection. We then hypothesized that IGF-1 may induce regeneration of synaptic contacts between inner hair cells (IHCs) and spiral ganglion neurons (SGNs). In the present study, we tested the potential of IGF-1 for regeneration of synaptic contacts between inner hair cells and spiral ganglion neurons using explant cultures of mouse cochleae.

Excised cochleae of neonatal mice were cut into slices including the modiolus. The mid turns of cochleae were maintained in 24-well culture plates. To induce degeneration of afferent dendrites attached to IHCs, a mixture of NMDA and kainate was added to the culture media. Then, cochlear explants were transferred to the culture media containing IGF-1. NMDA and kinate caused degeneration of afferent dendrites, but no significant loss of SGNs. Following 48-h culture with IGF-1, regeneration of afferent dendrites and synaptic contacts between IHC s and SGNs was observed in a dose-dependent manner. An IGF-1 receptor antagonist and inhibitors for PI3K/Akt or MEK/ERK pathway significantly diminished regenerative activity of IGF-1. These findings indicate that IGF-1 has potential for regeneration between IHCs and SGNs, which could be involved in the mechanisms of delayed hearing recovery observed in a clinical trial.

THE SPLICE SITE MUTATION GOYA IN MAP3K1 CAUSES DEAFNESS AND EYE DEFECTS IN THE MOUSE

***ANDREW PARKER*[1], *SALLY CROSS*[2], *RACHEL HARDISTY-HUGHES*[1], *SUSAN MORSE*[1], *GEORGE NICHOLSON*[1,3], *EMMA COGHILL*[1], *RONNA HERTZANO*[4], *IAN J. JACKSON*[2], *MICHAEL R BOWL*[1] *AND STEVE D.M. BROWN*[1]**

[1]MRC Mammalian Genetics Unit, MRC Harwell, OX11 0RD, UK, [2]MRC Human Genetics Unit, Edinburgh, Scotland, UK, [3]Department of Statistics, University of Oxford, UK, [4]Laboratory of Inner Ear Developmental Genetics, University of Maryland, School of Medicine, MD, USA.

We have identified a novel ENU mouse mutation, *goya*, with eyes open at birth and microphthalmia phenotypes. In addition *goya* homozygote mice develop a progressive hearing loss. The *goya* mice carry a splice site mutation in the *Map3k1* gene which has been identified to be causative of both the eye and auditory phenotypes. We show that *goya* and knock-out *Map3k1* homozygotes develop supernumerary outer hair cells in the inner ear and subsequently show extensive degeneration of outer hair cells accompanied by a profound hearing loss by 9 weeks of age. Outer hair cell degeneration is observed by 4 weeks of age demonstrating that MAP3K1 mutants have a progressive hearing loss. Heterozygotes show no hearing loss or degeneration of outer hair cells, but as in the homozygotes develop supernumerary outer hair cells. MAP3K1 is expressed in a number of inner ear cell types, sensory hair cells, stria vascularis and the spiral ganglion. The identification of the *goya* mutant reveals a novel signalling molecule involved with hair cell development and survival, and a new candidate gene for sensorineural hearing loss. Work is currently underway to investigate the molecular mechanisms that lead to the auditory phenotypes.

ACTIN CROSSLINKING AND DISASSEMBLY COORDINATELY REGULATE ADULT STEREOCILIA LENGTH

***BENJAMIN PERRIN*[1], *PRAVEENA NARAYANAN*[1] *AND JAMES ERVASTI*[1]**

[1]*Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, USA*

Stereocilia architecture must be precisely maintained throughout adulthood in order to preserve auditory function. Control of actin dynamics is likely a critical factor in maintenance pathways given that stereocilia are made from parallel bundles of actin filaments. While the majority of actin in stereocilia is very stable, protein turnover is comparatively high in a small region located at stereocilia tips. We have found that stereocilia actin stability is influenced by the b-actin isoform, fascin-2 mediated actin crosslinking and the actin disassembly factor WDR1. Mice lacking β-actin, expressing crosslinking-defective fascin-2 or with reduced WDR1 levels have normal auditory function at young ages but develop a similar pattern of age-related hearing loss. In each case, coincident with hearing loss, the shorter two rows of stereocilia in the auditory hair cell bundle shorten and become irregular in length as compared to their neighbors. In addition, tip link stability influences the hearing loss phenotype, further suggesting that β-actin, fascin-2 and WDR1 coordinately regulate actin turnover in response to tension exerted by tip links or mechanotransduction activity. Additionally, mice that both lack β-actin and express crosslinking-defective fascin-2 mutant have markedly accelerated hearing loss compared to either single mutant, supporting a model where these proteins cooperate in a common stereocilia maintenance pathway. Together these results demonstrate that regulation of actin dynamics mediated by crosslinking, disassembly and isoform composition is required for maintenance of adult stereocilia structure and function.

MIDDLE EAR COCHLIN IMMUNOSTAINING AND HISTOPATHOLOGICAL DEPOSITS IN HUMAN DFNA9-AFFECTED INDIVIDUALS AND *COCH* KNOCK-IN (KI) MOUSE MODEL

***NAHID G. ROBERTSON*[1], *JENNIFER O'MALLEY*[2], *CHENG AI ONG*[2], *ANNE B.S. GIERSCH*[1,3], *KONSTANTINA M. STANKOVIC*[2,3], *CYNTHIA C. MORTON*[1,3]**

[1]Brigham & Women's Hospital, [2]Massachusetts Eye & Ear Infirmary, [3]Harvard Medical School, Boston, MA, USA

Mutations in *COCH*, encoding the secreted protein, cochlin, the most abundant protein detected in the inner ear, are causative of the mid-life onset progressive sensorineural hearing loss and vestibular disorder, DFNA9. To date, 14 missense mutations have been found in *COCH*, throughout four continents. A unique DFNA9 histopathological finding is presence of cochlin-staining, eosinophilic, deposits throughout the spiral ligament and limbus, and the stroma underlying vestibular sensory epithelia, with a marked reduction in number of fibrocytes normally expressing cochlin. A recent study (McCall *et al., JARO,* 2011,12:141-9) showed presence of acellular deposits in middle ear structures of DFNA9-affected temporal bones. This finding prompted us to investigate more thoroughly the nature of these deposits and the role of cochlin in the middle ear by: 1) examining middle ear histology in our $Coch^{G88E/G88E}$ knock-in (KI) and $Coch^{-/-}$ knock-out (KO) mouse models, 2) determining cochlin localization in unaffected human and WT mouse middle ears, and 3) characterizing cochlin immunostaining in human DFNA9-affected and mouse *Coch* KI middle ears.

H&E staining of mouse middle ears revealed presence of eosinophilic deposits in the *Coch* KI mouse model, most notably in the inter-ossicular joints. These aggregates were absent in the WT or *Coch* KO mice, consistent with our hypothesis that DFNA9 pathology is likely a result of a gain of function of mutant cochlin rather than haploinsufficiency, or absence of this protein. Immunostaining with anti-cochlin antibody revealed specific and prominent cochlin localization in the inter-ossicular joints and the pars tensa of the tympanic membrane (TM) in both WT and *Coch* KI mice, including the areas of aggregates. Of note, thickening of, and deposit formation in the TM of one-year-old KI mice was not appreciable, in contrast to prominent deposits present in the joints of the same age, suggesting that aggregate formation in the TM occurs later than that in the inter-ossicular joints. Mice at different ages will be evaluated for onset and progression of this pathology. Cochlin immunostaining of DFNA9-affected and age-matched control middle ears demonstrated cochlin localization in the corresponding murine structures, and within areas of aggregates. It is possible that these deposits contain other proteins in addition to cochlin, as both eosinophilic and basophilic staining was observed. Proteomic studies in progress, will explore the contents of the aggregates to elucidate cochlin interactors and other proteins involved in cochlin functional pathways. Our DFNA9 and *Coch* KI findings provide further insight into, and tools for study of cochlin function and its role in pathology and progression of the disease process.

DELETION OF DICER IN THE INNER EAR REVEALS MULTIPLE FUNCTIONS FOR miRNAS

ROSALIE SACHELI, PRISCILLA VANDENACKERVEKEN, AURÉLIA HUYGHE, FLORENCE ROGISTER, MARIE-LAURE VOLVERT, LAURENT NGUYEN AND BRIGITTE MALGRANGE

GIGA-Research, Developmental Neurobiology Unit, University of Liege, Avenue de l'hopital 1, 4000 Liege, Belgium

The involvement of microRNAs in the development of the auditory portion of the inner ear is still poorly understood. Here, we investigated the global function of miRNAs during early stages of mouse inner ear development by examining Dicer conditional knock-out mice (Dicer-cKO). Dicer is not required for invagination of the otic placode and otic vesicle specification. In contrast, we observed a severe hypoplasia of the cochlea, the vestibule and the cochleovestibular ganglion at E12 in these Dicer-cKO mice. Dicer-cKO deficient otocysts display proliferation defects and apoptosis. Accumulating evidence has suggested that miRNAs play pivotal roles in DNA damage repair. Based on these observations, we showed that mutant otocysts exhibit accumulation of pH2AX protein, a specific marker of DNA damage. Ultimately, this leads to an increased stabilization of p53 inducing its nuclear accumulation and upregulation of its target genes involved in apoptosis such as puma and bax. A transcriptomic comparison of wild-type and Dicer-cKO E12 otic vesicles reveals an up-regulation of numerous direct targets of the highly expressed miRNA cluster miR-183. Work is ongoing to pinpoint mir-183 direct target genes.

TROMBONE – A MODEL OF AGE-RELATED HEARING LOSS SUGGESTS A NOVEL DEAFNESS GENE *SLC4A10* IS REQUIRED FOR NORMAL AUDITORY FUNCTION

***PRASHANTHINI SHANTHAKUMAR*[1], *FEMKE STELMA*[1], *CARLOS AGUILAR*[1], *SUE MORSE*[1], *JOANNE DORNING*[1], *ANDREW PARKER*[1], *LAURA WISBY*[1], *MICHELLE SIMON*[1], *SARA WELLS*[2], *SALLY J. DAWSON*[3], *PAUL POTTER*[1], *MICHAEL R BOWL*[1], *STEVE DM BROWN*[1]**

[1]Mammalian Genetics Unit and [2]Mary Lyon Centre, MRC Harwell, Harwell Oxford, Oxfordshire, OX11 0RD, UK, [3]Centre for Auditory Research, Ear Institute, University College London, 332 Gray's Inn Road, London, WC1X 8EE, United Kingdom

Age-related hearing loss (ARHL), or Presbycusis, is the most prevalent sensory impairment observed in the elderly. It is a progressive symmetrical age-related sensorineural hearing loss, which is most pronounced at higher frequencies. ARHL is a multifactorial disease, with contribution from both environmental as well as genetic factors. To date, little progress has been made in determining the genetic loci involved. Our aim is to elaborate the genetics underlying ARHL through the identification and characterization of ENU-induced mouse models of ARHL.

Our approach has identified *trombone*, a recessive model of ARHL arising from the Harwell Ageing Mutant Screen. Recurrent auditory phenotyping at 3, 6, 9 and 12 months of age shows that affected animals display elevated ABR thresholds from 9 months of age, when compared to littermates. The hearing thresholds are most elevated at 8kHz and 32kHz, and these are further increased at 12 months of age. Genome mapping studies identified a 12.5Mb critical region on chromosome 2 and next generation sequencing identified a T>C mutation in the novel deafness gene *Slc4a10*, which causes a Leucine to Proline substitution in the encoded protein. Immunohistochemical staining of cochlear sections shows that Slc4a10 is expressed in the type II and V fibrocytes of the spiral ligament (no labeling observed in $Slc4a10^{trb/trb}$ mice). Ultrastructural analysis of 12 month old $Slc4a10^{trb/trb}$ mice shows significant hair cell loss (inner and outer), which is most pronounced at the apical and basal regions of the cochlea.

Our findings establish the presence of Slc4a10 in the inner ear and suggest an important role for this sodium-coupled bicarbonate transporter within the fibrocytes of the spiral ligament for normal auditory function. Given that Slc4a10 is thought to regulate intracellular pH and it is expressed by the fibrocytes, it is likely that this mutation affects fluid ion homeostasis, and studies are underway to investigate this. In addition, the contribution of variation in *SLC4A10* gene to human hearing is being investigated. Functional characterization of *trombone* promises to increase our understanding of the genetics underlying hearing and its age-related decline.

IDENTIFICATION OF LMW COMPOUNDS TO ACTIVATE ATOH1 FOR INNER EAR HAIR CELL REGENERATION

***JIANYONG SHOU*[1]**

[1]Novartis Institutes for BioMedical Research, Shanghai, 201203, China

Sensorineural hearing loss resulting from destruction of inner ear sensory cells and/or auditory nerves is an unmet medical need. Humans are born with ~30,000 sensory hair cells, which are vulnerable to ototoxic drugs, loud noise, infections or as a function of aging. Mammals do not regenerate auditory hair cells upon damage or cell loss in contrast to avian. The atonal gene encodes for a developmental master transcriptional regulator, which has been shown to be necessary and sufficient for the generation of sensory cells. We are aiming to develop pharmacological agents to treat sensorineural hearing loss and balance disorders by activation of endogenous ATOH1. We engineered a luciferase reporter cassette including the entire ATOH1 regulatory elements into a surrogating human cell line to screen for LMW compounds and siRNAs that can activate ATOH1. Active hits were further confirmed in secondary assays using mouse primary cells isolated from the Atoh1-GFP transgenic mice as well as in neonatal cochlear explant and adult utricle cultures. The screen results and the follow-up mechanistic studies will be discussed.

CDK ACTIVITY FACILITATES AMINOGLYCOSIDE-INDUCED HAIR CELL DEATH INVOLVING AP-1-MEDIATED TRANSCRIPTION

LITAO TAO[1], SUM-YAN NG[2], NEIL SEGIL[1,2,3]

1,Genetic, Molecular and Cellular Biology Program, University of Southern California; 2,Neuroscience Graduate Program, University of Southern California; 3,Cell Biology and Genetics, House Research Institute

Aminoglycoside-induced sensory hair cell death is a major side effect that limits the clinical use of aminoglycoside antibiotics. However, the underlying molecular mechanisms of hair cell death are not understood. Here we report that in perinatal organ of Corti cultures, Cyclin-Dependent Kinase (CDK) activity is involved in mediating hair cell apoptosis in response to gentamicin. Pharmaceutical inhibitors of CDK activity delay hair cell death in response to aminoglycoside-induced damage, and genetic mutation of CDK2 confers a similar protective response. In addition, immunostaining and Q-PCR analysis indicates that CDK activity is required for the transactivation of the transcription factor c-Jun, a downstream target of JNK pathway that has previously been implicated in hair cell death in response to stress. Our study has identified a novel signaling pathway involved in aminoglycoside-induced hair cell death, and also suggests the importance of CDK activity in stress-induced apoptosis in postmitotic cells.

Sponsored by a travel award from:

march of dimes®

MUTATIONS IN *SLITRK6* CAUSE SENSORINEURAL DEAFNESS AND MYOPIA

Mustafa Tekin[1], Barry A Chioza[2], Yoshifumi Matsumoto[3], Oscar Diaz-Horta[1], Harold E Cross[4], Duygu Duman[5], Haris Kokotas[6], Heather L. Moore-Barton[7], Kazuto Sakoori[3], Maya Ota[3], Yuri S Odaka[3], Joseph Foster II[1], F.Basak Cengiz[5], Suna Tokgoz-Yilmaz[5], Oya Tekeli[8], Maria Grigoriadou[6], Michael B. Petersen[9], Ajith Sreekantan-Nair[2], Kay Gurtz[10], Xia-Juan Xia[11], Arti Pandya[11], Michael A Patton[2], Juan I Young[1], Jun Aruga[3], Andrew H Crosby[2]

1.John P. Hussman Institute for Human Genomics and Dr. John T. Macdonald Department of Human Genetics, University of Miami Miller School of Medicine, Miami, FL 33136, USA; 2. Centre for Human Genetics, St. George's University of London, Cranmer Terrace, London SW17 0RE, UK; 3. Laboratory for Behavioral and Developmental Disorders, RIKEN Brain Science Institute, 2-1 Hirosawa, Wako, Saitama, 351-0198 Japan; 4.Department of Ophthalmology, University of Arizona School of Medicine, 655 N. Alvernon Way, Tucson, Arizona, USA; 5.Division of Pediatric Genetics, Ankara University School of Medicine, 06100, Ankara, Turkey; 6.Department of Genetics, Institute of Child Health, 'Aghia Sophia' Children's Hospital, Athens 11527, Greece; 7.South West Thames Regional Genetics Services, St. George's University London, Cranmer Terrace, London SW17 0RE, UK; 8.Department of Ophthalmology, Ankara University School of Medicine, 06100, Ankara, Turkey; 9.Department of Clinical Genetics, Aalborg Hospital, Aalborg, Denmark; 10.Windows of Hope Genetic Study, Geauga County, Ohio, USA; 11.Department of Human and Molecular Genetics, Virginia Commonwealth University, Richmond, Virginia 23298-0033, USA

In this study we describe a new autosomal recessive syndrome characterized by high myopia and sensorineural deafness. Our molecular investigation in three families led to the identification of three homozygous nonsense mutations (p.R181X, p.S297X, and p.Q414X) in *SLITRK6*, a leucine-rich repeat domain transmembrane protein. Transfection of PC12 cells with wild type and mutant molecules revealed a profound effect of the mutation on the ability of SLITRK6 to regulate neurite extension and number, consistent with a loss of SLITRK6 function. *In situ* hybridization studies of wild type and *Slitrk6*-deficient mice revealed axial length increase in the mutant (the endophenotype of myopia) and synaptogenesis delay associated with hearing loss, mirroring the human phenotype. Taken together our results show that SLITRK6 plays a crucial role in the development of normal hearing as well as vision in humans and in mice and its disruption leads to a novel syndrome characterized by severe myopia and deafness.

ncRNA REGULATION IN SENSORY ORGANS – THE INNER EAR AS A MODEL

***KATHY USHAKOV*[1], *ANYA RUDNICKI*[1], *OFER ISAKOV*[2], *NOAM SHOMRON*[2] *AND KAREN B. AVRAHAM*[1]**

[1]Department of Human Molecular Genetics and Biochemistry, [2]Department of Cell and Developmental Biology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

The discovery that a majority of the genome is extensively transcribed into RNA transcripts that do not code for proteins led to an important and new perspective on the significance of RNA in gene regulation. One form of non-coding RNAs (ncRNAs), microRNAs (miRNAs), regulates gene expression through the RNA interference (RNAi) pathway. They bind to sequences at the 3 untranslated region (UTR) of genes and therefore can inhibit target mRNAs by translational repression and mRNA destabilization. miRNAs play a critical role in the development and regulation of the sensory systems, including the inner ear, which is responsible for hearing and balance in mammals. miRNAs are known to be involved in deafness in both humans and mice. To dissect the function of miRNAs in the inner ear, we performed RNA-Seq on RNA isolated from mouse inner ear sensory epithelia. qRT-PCR confirmed the expression of these miRNAs and *in situ* hybridization was used to determine their spatial expression in the mouse inner ear. We used bioinformatics software to predict the targets of these miRNAs and verified them by *in vitro* over-expression and luciferase assays. Small RNAs found in cochlear and vestibular sensory epithelium (SE) included miRNAs, snoRNAs, transfer RNAs and ribosomal RNAs. Reads were aligned to the mature *Mus musculus* miRNA database (http://www.mirbase.org) and miRDeep2 was used for novel miRNA prediction. Overall, 440 and 458 miRNAs were detected, including known and new miRNAs. From the predicted pre-miRNAs that are expressed in both cochlear and vestibular SE, we selected two miRNAs for further study, included in the introns of the genes *Tectb* and *Gap43*. They have seed regions that are conserved between mouse and human, they are highly expressed in the SE, and they are located in introns of genes expressed in the inner ear. Potential targets were predicted by TargetScan Custom and validated. Dissecting the pathways of miRNA-target interactions may shed light on the role miRNAs play in the regulation of gene expression in the auditory and vestibular systems and the mechanisms leading towards deafness and balance dysfunction.

PRESTINUP-REGULATION IN RESIDUAL OUTER HAIR CELLS AFTER NOISE-INDUCED HEARING LOSS

ANPINGXIA[1], ROSALIE WANG[1], YOHAN SONG[1], SIMON S. GAO[1,3], WILL CLIFTON[2], PATRICK RAPHAEL[1],SUNG-IL CHO[1,4], FRED A. PEREIRA[2], ANDREW K. GROVES[5], JOHN S. OGHALAI[1]

[1]Department of Otolaryngology–Head and Neck Surgery, Stanford University, CA, USA 94305-5739, [2]Bobby R. Alford Department of Otolaryngology - Head and Neck Surgery, Baylor College of Medicine, Houston, TX, USA 77030,[3]Department of Bioengineering, Rice University, Houston, TX, USA 77005,[4]Department of Otolaryngology–Head and Neck Surgery, Chosun University, Gwangju, South Korea 501-717, [5]Department.of Neuroscience, Baylor College of Medicine, Houston, TX, USA 77030

The outer hair cell (OHC) motor protein prestin is necessary for electromotility, which drives cochlear amplification and produces the exquisitely sharp tuning curves associated with mammalian hearing. Previously, we found that OHC prestin expression was higher in Tecta transgenic mice with hearing loss. In the present study, we sought to determine the effects of noise-induced hearing loss on prestin expression. After noise-exposure, we performed cytocochleograms and observed OHC loss only in the basal region of the cochlea. Next, we patch clamped OHCs from the apical turn (9-12 kHz region), where no OHCs were lost, in noise-exposed and age-matched control mice. The non-linear capacitance was increased in noise-exposed mice, consistent with higher functional prestin levels. We then measured prestin protein and mRNA levels in whole-cochlea specimens. Both Western blot and qPCR studies demonstrated increased prestin per hair cell 7 days and 1 month after noise exposure. These increases were larger than would be predicted simply by a shift of the average due to the loss of the smaller, basal OHCs. Finally, we examined the effect of the prestin increase *in vivo*. Immediately after noise exposure, CBA mice had ABR and DPOAE thresholds that were elevated by 30-40 dB. While most of the temporary threshold shifts recovered within 3 days, there were additional improvements over the next month. Basilar membrane vibration and CAP tuning curve measurements from the 9-12 kHz cochlear region demonstrated that tuning curve sharpness after noise exposure was no different than in controls. Taken together, we conclude that prestin is up-regulated~30-40% in residual OHCs after noise exposure. The additional prestin is functional in OHCs, but does not result in any detectable enhancement in cochlear function. These data are consistent with a model whereby perturbations in OHC prestin levels are dynamically modulated to maintain stable auditory thresholds and frequency discrimination.

SORTING NEXIN 9 (SNX9) MAY REGULATE F-ACTIN ASSEMBLY IN THE CUTICULAR PLATE OF HAIR CELLS

XIAOLEI YIN[1], YUJIE CAO[1], CHENGCHENG LIU[1], STEFAN HELLER[2], ZHIGANG XU[1]

[1]Shandong University School of Life Sciences, Jinan, Shandong Province, China;
[2]Stanford University School of Medicine, Stanford, California, United States of America

The cuticular palte is a F-actin-based subcellular structure in inner ear hair cells. It occupies the apical cytoplasm of hair cells and serves as an anchor for stereocilia, hence plays an important role in hearing transduction. At present the mechanism of how the F-actin network in this struture is regulated remains unknown. We show here that a BAR protein family member, Sorting Nexin 9 (SNX9), is expressed in the cuticular plate of mouse auditory hair cells. In vitro experiments show that SNX9 could promote F-actin polymerization in a WASP-Arp2/3-dependent way. FCHSD1, a F-BAR protein family member, binds SNX9 via the F-BAR domain of FCHSD1 and the SH3 domain of SNX9. Although FCHSD1 has no effect on F-actin polymerization when WASP and Arp2/3 complex are present, it greatly enhances SNX9's F-actin polymerization activity. Interestingly, FCHSD1 is also detected in the cuticular plate, where it colocalizes with SNX9. Our results suggest that SNX9, together with FCHSD1, might regulate F-actin assembly in the cuticular plate of hair cells. Conditional knock-out mice are being developed to further explore the role of SNX9 in cuticular plate and hearing transduction.

SCREENING FOR DUPLICATIONS, DELETIONS AND A COMMON INTRONIC MUTATION DETECTS 35% OF SECOND MUTATIONS IN PATIENTS WITH *USH2A* MONOALLELIC MUTATIONS ON SANGER SEQUENCING

MARIA BITNER-GLINDZICZ [1,5], ***HEATHER B. STEELE-STALLARD*** [1], ***POLONA LE QUESNE STABEJ*** [1], ***EVA LENASSI*** [2,3], ***LINDA M. LUXON*** [4,5], ***MIREILLE CLAUSTRES*** [6,7], ***ANNE-FRANCOISE ROUX*** [6,7], ***ANDREW R. WEBSTER*** [2,3],

[1] Clinical and Molecular Genetics, UCL Institute of Child Health, London, UK; [2] UCL Institute of Ophthalmology, London, UK; [3] Moorfields Eye Hospital, London, UK; [4] Audiovestibular Medicine, National Hospital for Neurology and Neurosurgery, London, UK; [5] UCL Ear Institute, London, UK; [6] CHU Montpellier, Laboratoire de Génétique Moléculaire, Montpellier, F-34000, France ;[7] Inserm, U827, Montpellier, F-34000, France.

Usher Syndrome is the leading cause of inherited deaf-blindness. It is divided into three subtypes, of which the most common is Usher type 2, and the *USH2A* gene accounts for 75-80% of cases. Despite recent comprehensive sequencing strategies, a significant proportion of individuals with Usher type 2 have just one heterozygous disease-causing mutation in *USH2A*, or no convincing disease-causing mutation in any other Usher gene.

Forty-nine Usher type 2 or atypical Usher families who had missing mutations (mono-allelic or no mutations following Sanger sequencing of nine Usher genes) were screened for duplications/deletions using the *USH2A* SALSA MLPA reagent kit (MRC-Holland) and for the *USH2A*: c.7595-2144A>G intronic mutation by Sanger sequencing. Mutations were confirmed by a combination of reverse transcription PCR using RNA extracted from nasal epithelial cells or fibroblasts, and by array comparative genomic hybridisation with sequencing across the genomic breakpoints.

Eight mutations were identified in 23 Usher type 2 families (35%) with one previously identified heterozygous disease-causing mutation. These consisted of five heterozygous deletions, one duplication, and two heterozygous instances of the pathogenic variant *USH2A*: c.7595-2144A>G. All five deletions and the heterozygous duplication are novel.

Future mutation detection strategies and genetic counselling will need to take into account the prevalence of these types of mutations in order to provide a more comprehensive diagnostic service.

APPLICATION OF NEW GENERATION TECHNOLOGIES (NGS AND ACGH) TO THE INTEGRAL DIAGNOSIS AND INVESTIGATION OF INHERITED HEARING LOSS

LUCIA BORREGUERO[1], MATÍAS MORÍN[1], MARIA PALOMARES[2], ANGELES MENCÍA[1], LUCIANA S SERRAO DE CASTRO[1], FERNANDO MAYO[1], JULIAN NEVADO[2], PABLO LAPUNZINA[2], IGNACIO DEL CASTILLO[1], JAVIER SANTOYO[3], FELIPE MORENO[1] AND MIGUEL ANGEL MORENO- PELAYO[1].

[1]Unidad de Genética Molecular, Hospital Ramón y Cajal, 28034 Madrid, Spain and Centro de Investigacion Biomedica en Red de Enfermedades Raras (CIBERER), ISCIII, Madrid, Spain. [2]Instituto de Genética Médica y Molecular (INGEMM)-Hospital La Paz-IdIPAZ, Madrid, Spain. [3]Andalusian Human Genome Sequencing Centre (CASEGH), 41092 Sevilla, Spain.

The prevalence of the different subtypes of nonsyndromic hereditary hearing loss (NSHL) is still extensively unknown because of its large genetic heterogeneity, and that is the reason why genetic diagnostic based on traditional practices is not cost-effective. In this study, we have developed and validated two methods based on new generation technologies (NGS and aCGH) to be used in combination for the diagnostic and researching of inherited hearing loss. The first method consists on a modular CGH array based on Agilent technology which allows us to evaluate the existence of pathogenic copy number variations (CNVs) in all NSHL loci. With this tool it is possible to analyse the genomic integrity of more than 700Mb of DNA sequence corresponding to more than 100 NSHL mapped loci. This array has been validated using DNA of patients with pathogenic CNVs previously diagnosed by MLPA technique.

The second method consists on the design and validation of a modular liquid-phase target enrichment system using Nimblegen technology focused on the genomic regions, full exonic and intronic sequences, of more than 70 NSHL genes currently known. The validation of this method on a Solid 5500 NGS platform shows a mean coverage of 140-200X, in which 90% of reads maps in the target region, and 90% of the captured sequence has a coverage higher than 10X. This tool has been validated with already diagnosed patients by Sanger method and in all cases the pathogenic mutation has been identified. The combined application of these tools is making possible the comprehensive analysis of highly heterogeneous genetic pathologies such as NSHL in a cost-effective way due to the fact that they result on more than 90% savings compared to traditional techniques; it is also providing a database of strategic interest about the variations of potential pathogenicity in previously unexplored genomic regions such as the intronic sequences of the NSHL genes or the genomic interval of deafness loci with no gene identified. Altogether they conforms a strong and feasible integral tool for the diagnostic of the inherited hearing loss forms.

A BALANCED CHROMOSOME TRANSLOCATION REVEALS INVOLVEMENT OF A PREDICTED LIPASE IN WEIGHT GAIN, TUMOR SUPPRESSION AND SENSORINEURAL HEARING LOSS

***Benjamin B. Currall*[1,2], *Nahid G. Robertson*[1], *Tatiana Hoyos*[2,3], *Ann E. Hickox*[4], *Kristen E. Wong*[1], *M. Charles Liberman*[2,5], *Eric C. Liao*[2,3], *and Cynthia C. Morton*[1,2]**

[1]Brigham and Women's Hospital, Boston, MA, USA; [2]Harvard Medical School, Boston, MA, USA; [3]Massachusetts General Hospital, Boston, MA, USA; [4]Purdue University, IN, USA; and [5]Massachusetts Eye and Ear Infirmary, Boston, MA, USA

The Developmental Genome Anatomy Project (DGAP; www.dgap.harvard.edu) systematically examines subjects with balanced chromosomal rearrangements to identify genes involved in congenital disorders. Of particular interest for DGAP are rearrangements associated with hearing loss. DGAP056 is an archetypal case with congenital profound sensorineural hearing loss, as well as a constellation of other symptoms including mild craniofacial abnormalities (coloboma, exotropia, blepharophimosis, and low-set posteriorly rotated ears), mitral valve prolapse, hypospadias, and early onset prostate cancer. Genomic analyses of DGAP056, using karyotyping, fluorescence *in situ* hybridization (FISH) and genomic sequencing, revealed a balanced translocation involving chromosomes 2 and 13, t(2;13)(p24.1;q22.3)dn, which disrupts a poorly annotated gene designated *C2orf43.* Bioinformatic analyses indicate that *C2orf43* encodes an evolutionarily conserved protein related to the alpha/beta hydrolase clan of proteins and likely functions as a serine-based ester hydrolase involved in lipid metabolism. Zebrafish and mouse models demonstrate that *C2orf43* is expressed in both the developing and adult inner ear. In knockout (KO) models, older *C2orf43* KO mice are heavier and have higher rates of hearing loss and tumor formation when compared to their wild-type littermates. In humans, *C2orf43* expression is down-regulated in prostate tumors and has been associated with type II diabetes, lipodystropy and prostate cancer. These findings suggest that *C2orf43* plays an important role in lipid metabolism, and the disruption or dysregulation of this gene results in higher rates of tumor development and hearing loss. Experiments to elucidate the function of this putative lipase in the inner ear and other organ systems are underway. DGAP056 illustrates the value of the DGAP approach to gene discovery in hearing loss and other developmental anomalies.

GENETIC VARIATION IN SUSCEPTIBILITY TO HYPOTHYROIDISM INDUCED HEARING IMPAIRMENT

***QING FANG*[1], *MICHELLE T. FLEMING*[1], *THOMAS J. JONES*[1], *TZY-WEN GONG*[1], *AMANDA H. MORTENSEN*[1], *DOLAN F. DOLAN*[1], *MARGARET I. LOMAX*[1], *KENNETH R. JOHNSON*[2], *MIRNA MUSTAPHA*[3], *SALLY A. CAMPER*[1]**

[1]University of Michigan, Ann Arbor, MI, USA; [2]The Jackson Laboratory, Bar Harbor, ME, USA; [3]Stanford University, Stanford, CA USA

Hypothyroidism during pregnancy or in newborns can impair hearing and cognition, although individual children vary in their sensitivity. Thyroid hormone deficiency has pleiotropic effects on development of neurosensory and conductive aspects of hearing in susceptible mouse strains. The DW/J-*Pou1f1*$^{dw/dw}$ mutants produce little or no thyroid hormone and have severe dwarfism and profound, congenital deafness. Mutant F2 progeny from a cross of this strain with CAST/EiJ had equal growth insufficiency but exhibited a range of hearing abilities, from normal hearing to profound deafness. Initial microsatellite mapping studies suggested that a locus on chromosome 2 could explain about 20% of the variation in hearing, and we named it *Modifier of dw hearing*, *Mdwh*. Both AKR and C57BL/6 are susceptible, and C3H/HeJ and 129P2/Ola are resistant. The strain distribution pattern analysis of the DW/J stock revealed that it is similar to C57BL/6 in the *Mdwh* region. Here we report the results of genome-wide SNP typing for 1500 loci on (DW/J x CAST/EiJ) F2 and (DW/J x129P2/Ola) F2 *Pou1f1*$^{dw/dw}$ mice, whose hearing were quantified by ABR testing. The data from both crosses implicate the same region of chromosome 2 and refine the *Mdwh* to 20 MB with over 500 genes. In addition, we identified two other loci of significant effect on other chromosomes. We carried out gene expression profiling with cochlear RNAs from DW/J-*Pou1f1*$^{dw/dw}$ and wild type mice and identified over 500 genes that are regulated by thyroid hormone in the cochlea, and 10 mapped to the *Mdwh* region. We are comparing the sequence and expression of positional candidate genes in susceptible and resistant strains to evaluate them as candidates for *Mdwh*. Identification of a gene or genes that can protect against the pleiotropic effects of thyroid hormone deficiency on the development of hearing could lay the foundation for designing therapeutics for children who are not responsive to thyroid hormone supplementation.

CYTOMEGALOVIRUS DNA DIAGNOSIS USING PRESERVED UMBILICAL CORD IN HEARING IMPAIRED CHILDREN

***SATOSHI IWASAKI*[1], *SAKIKO FURUTATE*[2], *SHINYA NISHIO*[2], *TAKUYA YANO*[2], *HIDEAKI MOTEKI*[2], *SHIN-ICHI USAMI*[2]**

[1] Department of Hearing Implant Sciences, Shinshu University School of Medicine, Matsumoto city, Nagano, Japan. [2] Department of Otolaryngology, Shinshu University School of Medicine, Matsumoto city, Nagano, Japan

Introduction: The genetic origins of Sensorineural hearing loss (SNHL) can be identified in half of the prelingual cases; in the others, SNHL is caused by environmental or unidentified genetic factors. The most common environmental cause of SNHL is congenital cytomegalovirus (CMV) infection. The gold standard for diagnosis of congenital CMV infection is the isolation of the virus from urine or saliva in the first 2 weeks of life. However, asymptomatic congenital CMV infection in children who develop late-onset of SNHL after the 2 weeks of birth cannot be diagnosed on basis of viral isolation from urine or saliva. The purpose of the present study is to investigate the prevalence of congenital CMV infection diagnosed retrospectively by detection of CMV DNA extracted from dried umbilical cord specimens in children with unilateral or bilateral SNHL defined at an age of months or even years after birth.

Materials and Methods: This study evaluated 135 patients (70 males and 65 females) with bilateral (46 patients; 34.1%) or unilateral (89 patients; 65.9%) SNHL. The ages of children who were diagnosed with SNHL ranged from 1 month to 138 months (mean age; 37.7 ± 36.2 months). The children with deafness syndrome were excluded by an etiologic work-up of their SNHL in this study. Both deafness gene test and CMV DNA analysis were provided in only children with bilateral SNHL. Audiometric evaluation was performed for each patient by using auditory brainstem response (ABR) as objective audiologic tests and behavioral audiologic tests and/or pure tone audiometry also used. DNA was extracted from the dried umbilical cords and CMV DNA was detected by quantitative PCR. Deafness gene test based on invader assay were performed in children with bilateral SNHL.

Results and Conclusions: Congenital CMV infection was a major cause of bilateral and unilateral SNHL in children. 8.9% of SNHL (bilateral SNHL: 8.7%, unilateral SNHL: 9.0%) are attributed to congenital CMV infection. The frequency of late-onset, hearing fluctuation, and unilateral hearing loss in children with congenital CMV infection was 41.7%, 33.3%, and 66.7%, respectively. Application of congenital CMV infection screening test using a DNA from preserved dried umbilical cords is important for the examination of SNHL in children. Deafness gene mutations (*GJB2* and *SLC26A4*) were detected in 34.6% of children with bilateral severe-moderate SNHL. We could detect the cause in 46.2% of children with bilateral severe-profound SNHL through this test combined with genetic screening test.

A COMPREHENSIVE SCREEN OF THE USH2A GENE IN 185 PATIENTS WITH AUTOSOMAL RECESSIVE RETINAL DISEASE

Eva Lenassi [1, 2], Zubin Saihan[2], Zheng Li[3], Marko Hawlina[4], Anthony T. Moore[1, 2], Linda M. Luxon[5], Karen P. Steel[6], Maria Bitner-Glindzicz[5, 7], Andrew R. Webster[1, 2.]

[1]UCL Institute of Ophthalmology, London, United Kingdom; [2]Moorfields Eye Hospital, London, United Kingdom; [3]Ocular Genetics, Singapore Eye Research Institute, Singapore, Singapore; [4]Eye Hospital, University Medical Centre, Ljubljana, Slovenia; [5]UCL Ear Institute, London, United Kingdom; [6] Kings College London; [7]Clinical and Molecular Genetics, UCL Institute of Child Health, London, United Kingdom.

Mutations in the *USH2A* gene are the commonest cause of both Usher syndrome and autosomal recessive retinitis pigmentosa (RP). The aim of this study is to provide insights into the clinical and genetic characteristics of nonsyndromic USH2A-related retinal disease. Of particular interest was the identification of “retina-specific” alleles as these may point to protein domains essential for photoreceptor function.

USH2A was screened in 185 probands with autosomal recessive retinal dystrophy without documented sensorineural hearing loss.

A total of 68 coding or splice site variants with a minor allele frequency of 0.20% or less in the Exome Sequencing Project dataset were identified (5 nonsense or frameshifting indels, 4 splice site, 47 nonsynonymous); 24/185 (13%) probands were found to harbour at least two such variants. Interestingly, only in one individual were both variants previously associated with syndromic disease. Three alleles that are likely to be specific to those with nonsyndromic disease were found in more than one proband: p.Cys759Phe, p.Cys3358Tyr and c.12295-3T>A. All 24 patients presented with visual symptoms (night blindness and visual field loss; median age 29 years, range 19-43) and none reported early-onset hearing loss. Retinal phenotype was consistent with RP in all cases and a ring of high density on FAF was observed in 19/24 patients. Audiology testing revealed a phenotype consistent with Usher syndrome type 2 in 1/17 patients.

USH2A retinopathy is a common cause of nonsyndromic autosomal recessive retinal disease. Three likely “retina-specific” variants in *USH2A* were identified. The results of this study are expected to increase the sensitivity of molecular testing in this highly heterogeneous condition.

MOLECULAR EPIDEMIOLOGY OF CONGENITAL HEARING IMPAIRMENT.

***MARKOVA TATIANA*[1, 3], *BLIZNEZCH ELENA*[2], *TSYGANKOVA EUGENIA*[1, 3], *BAKHSHINYAN VIGEN*[1,3], *POLYAKOV ALEXANDER*[2], *TAVARTKILADZE GEORGE*[1,3]**

[1]National Research Center for Audiology and Hearing Rehabilitation, Moscow. [2]Medical Genetic Centre, Russian Academy of Medical Sciences, Moscow. [3]Russian Academy of the Postdoctoral Education

Early detection of congenital hearing impairment among infants has become extremely important in preventive medicine to ensure better development of language and higher cognitive functions by means of hearing aids or cochlear implantation and following rehabilitation. The results of approximately 10 years' work provide convincing evidence for the important influence that *GJB2* mutations have on the etiology of congenital hearing loss in Russia.

The results of molecular genetic testing of *GJB2*-mutations revealed mean rate about 50% among patients with bilateral nonsyndromal sensorineural hearing loss (bNSHL) in our population with Eastern Slavic origin. The most common *GJB2* mutation with allelic frequency 81% is c.35delG. Six frequent mutations constitute 95% of pathological *GJB2*-alleles in Russian patients. The prevalence of *GJB2*-linked hearing loss was calculated to be 1/1000. The carrier rate in the general Russian population for the recessive deafness-causing *GJB2* mutation is 1/16. The recent study in 2001 showed mean frequency of 35delG mutation for the whole country as 1/46, but at random between 5 selected regions the survey involved three with rare level of 35delG mutation (Chuvashia, Bashkiria and Yakutia regions). Genetic testing of who the m.1555A>G showed no more then 3% of cases among patients with bNSHL. One child who carried m.1555A>G was homozygote for 35delG.

The results of our investigations showed different rate of mutation in different groups of patients. It depends on the age, severity, presence of the additional signs or pathology, origin of patients and others. When we calculated the frequency of mutation among random sampling of children from special schools and kindergarten we revealed *GJB2*-mutations in 52% of cases. In general population of patients who passed genetic testing for *GJB2* gene we found out *GJB2*-mutations in 46% of cases. When we took into account children before one year old with congenital hearing loss revealed during universal hearing screening who have both normal hearing parents we found out *GJB2*-mutations in about 70% of infants. *GJB2*-mutation rate was different among deaf and hard of hearing children (59%) and children with mild hearing loss (47%). *GJB2*-mutations were the reason for mild hearing loss in 30% of cases in our observing group.

Molecular genetic testing of the *GJB2* gene now is a routine analysis for individuals with bNSHL and might play important role in genetic epidemiology of congenital hearing impairment.

MUTATION IN *MARVELD2* GENE DETECTED IN SLOVAK ROMA PATIENTS WITH NONSYNDROMIC HEARING LOSS

***IVICA MAŠINDOVÁ*[1], *LUKÁŠ VARGA*[1,2], *MILOSLAVA HUČKOVÁ*[1,3], *MILAN PROFANT*[2], *IWAR KLIMEŠ*[1,3], *DANIELA GAŠPERÍKOVÁ*[1,3]**

[1]Diabgene Laboratory, Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia, [2]Otorhinolaryngology Clinic, Faculty of Medicine and University Hospital, Comenius University in Bratislava, Slovakia, [3]Centre for Molecular Medicine, Slovak Academy of Sciences, Bratislava, Slovakia

Introduction: Nonsyndromic hearing loss exhibits high genetic heterogeneity with over forty known recessive genes. The *MARVELD2 (TRIC)* gene, linked to the locus DFNB49, encodes a tight junction protein tricellulin. Mutations in this gene, which manifest by recessive, congenital, moderate to profound deafness, have been found in only few families so far. The only available data from Europe describing mutation of this gene were published in Czech deaf Roma individuals. Therefore, **the aim of our study** was to provide an analysis of the hot spot mutation IVS4+2T>C in *MARVELD2* gene in a cohort of Slovak Roma patients with diagnosis of nonsyndromic bilateral sensorineural hearing loss.

Patients and methods: Among 144 hearing impaired Slovak Roma individuals recruited for DNA analysis to determine the deafness etiology, 93 unrelated subjects have been selected. After excluding the biallelic *GJB2/GJB6* mutations in the DFNB1 locus as the hearing loss cause, we have analysed the *MARVELD2* gene in 68 unrelated patients. To detect the mutation IVS4+2T>C we have used a RFLP method with restriction endonuclease BstUI. In the positive probands the splice site mutation IVS4+2T>C was confirmed also by bidirectional sequencing of the exon 4 with overlapping intron boundaries.

Results: The mutation IVS4+2T>C was detected in 2 of the 68 individuals in *MARVELD2* gene. Homozygous form of IVS4+2T>C was found in one patient, who was also a heterozygous carrier of the *GJB2* mutation (c.71G>A). The second patient was heterozygote for this substitution. Frequency of the IVS4+2T>C mutation was 2.2% of all tested alleles.

Conclusions: This is the first record of *MARVELD2* mutation in our country and only the second one in Europe. Our results suggest that this gene should be considered for testing in deaf Slovak Roma subjects, if the hearing loss could not be attributed to DFNB1 locus.

Sponsored by a travel award from:
march of dimes

COMPREHENSIVE GENETIC TESTING IN A JAPANESE HEARING IMPAIRED POPULATION USING OTOSCOPE®

***HIDEAKI MOTEKI*[1, 2], *HELA AZAIEZ*[1], *KEVIN BOOTH*[1], *A. ELIOT SHEARER*[1], *RICHARD JH SMITH*[1], *SHIN-ICHI USAMI*[2]**

[1]Molecular Otolaryngology and Renal Research Laboratories, Department of Otolaryngology, University of Iowa, Iowa, USA; [2]Department of Otorhinolaryngology, School of Medicine, Shinshu University, Matsumoto, Japan

Non-syndromic hearing loss (NSHL) affects at least 1 in 500 newborns, with genetic causes accounting for 50-70% of all childhood hearing loss. The development of OtoSCOPE® has made comprehensive genetic testing for NSHL possible using targeted genomic capture and massively parallel sequencing technology. In this study, we used OtoSCOPE® to evaluate the spectrum of genetic causes of hereditary hearing loss in Japan. We ascertained 213 probands with NSHL through 33 otolaryngology clinics and hospitals nationwide in Japan, collecting phenotypes, clinical data, hearing thresholds, episodes of progression, tinnitus history and inner ear malformations on each patient. Hearing loss segregated as autosomal dominant in 54 families and autosomal recessive in 37 families; it was sporadic in 122 families. In all probands, mutations in GJB2 were excluded by Sanger sequencing. Probands are now being screened on OtoSCOPE® by pooling libraries from 48 probands, which we sequence on one lane on the Illumina HiSeq. We anticipate total reads averaging 18,745,296 with a 10X target coverage of 97.8%. Data analysis will be performed on a local installation of Galaxy using the Burrows-Wheeler Alignment (BWA) for read mapping, Picard for removal of duplicate reads, GATK for local re-alignment and variant calling, and ANNOVAR and a custom workflow for variant annotation. The larger pedigrees negative for OtoSCOPE® testing will be moved forward on our exome pipeline.

COMPREHENSIVE GENETIC SCREENING OF KCNQ4 IN A LARGE AUTOSOMAL DOMINANT NONSYNDROMIC HEARING LOSS COHORT: GENOTYPE-PHENOTYPE CORRELATIONS AND A FOUNDER MUTATION

***TAKEHIKO NAITO*[1], *SHIN-YA NISHIO*[1], *YOH-ICHIRO IWASA*[1], *TAKUYA YANO*[1], *KOZO KUMAKAWA*[2], *SATOKO ABE*[2], *KOTARO ISHIKAWA*[3], *HIROMI KOJIMA*[4], *ATSUSHI NAMBA*[5], *CHIE OSHIKAWA*[6], *SHIN-ICHI USAMI*[1]**

[1]Department of Otorhinolaryngology, Shinshu University School of Medicine, Matsumoto, Japan;[2]Department of Otolaryngology, Toranomon Hospital, Tokyo, Japan;[3]Department of Otorhinolaryngology, Jichi Medical University, Tochigi, Japan;

[4]Department of Otorhinolaryngology, Jikei University School of Medicine, Tokyo, Japan;[5]Department of Otorhinolaryngology, Hirosaki University School of Medicine, Hirosaki, Japan;[6]Department of Otorhinolaryngology, Kyushu University School of Medicine, Fukuoka, Japan

The present study of KCNQ4 mutations was carried out to 1) determine the prevalence by unbiased population-based genetic screening, 2) clarify the mutation spectrum and genotype/phenotype correlations, and 3) summarize clinical characteristics.

In addition, a review of the reported mutations was performed for better understanding of this deafness gene. The screening using 287 probands from unbiased Japanese autosomal dominant nonsyndromic hearing loss (ADNSHL) families identified 19 families with 7 different disease causing mutations, indicating that the frequency is 6.62% (19/287). While the majority were private mutations, one particular recurrent mutation, c.211delC, was observed in 13 unrelated families. Haplotype analysis in the vicinity of c.211delC suggests existence of a common ancestor. The majority of the patients showed all frequency, but high-frequency predominant, sensorineural hearing loss. The present study adds a new typical audiogram configuration characterized by mid-frequency predominant hearing loss caused by the p.V230E mutation. A variant at the N-terminal site (c.211delC) showed typical ski-slope type audiogram configuration. Concerning clinical features, onset age was from 3 to 40 years old, and mostly in the teens, and hearing loss was gradually progressive. Progressive nature is a common feature of patients with KCNQ4 mutations regardless of the mutation type. In conclusion, KCNQ4 mutations are frequent among ADNSHL patients, and therefore screening of the gene and molecular confirmation of these mutations have become important in the diagnosis of these conditions.

MASSIVELY PARALLEL DNA SEQUENCING SUCCESSFULLY DISCOVERS NEW CAUSATIVE MUTATIONS IN DEAFNESS GENES IN PATIENTS WITH COCHLEAR IMPLANTATION

***MAIKO MIYAGAWA*[1], *SHIN-YA NISHIO*[1], *TAKUO IKEDA*[2], *KUNIHIRO FUKUSHIMA*[3], *SHIN-ICHI USAMI*[1]**

[1]Department of Otorhinolaryngology, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto Japan. [2]Department of Otolaryngology, Tsudumigaura Handicapped Children's Hospital Kume 752-4, Shunan Japan. [3]Department of Otorhinolaryngology, Okayama University School of Medicine, Shikata-cho 2-5-1, Kita-ku, Okayama Japan

Background: Genetic factors, the most common etiology in severe to profound hearing loss, are one of the key determinants of cochlear implantation (CI) outcomes. Satisfactory auditory performance after receiving a CI in patients with certain deafness genes indicates that genetic testing would be helpful in predicting CI outcomes and deciding treatment choices. However, because of the extreme genetic heterogeneity of deafness, clinical application of genetic information still entails difficulties. Exome sequencing using Massively Parallel DNA Sequencing (MPS) is a new powerful strategy to discover rare causative genes in Mendelian disorders such as deafness.

Methods: By the analysis of 8 (4 early-onset, 4 late-onset) Japanese CI patients, who did not have mutations in commonly found genes including *GJB2, SLC26A4*, or mitochondrial 1555A>G or 3243A>G mutations, we attempted to identify genomic variations responsible for deafness by massive sequencing of the exons of 58 target candidate genes.

Results: MPS successfully discovered rare causative genes in CI patients and in electric and acoustic stimulation (EAS) patients, including *MYO15A, TECTA, TMPRRS3,* and *ACTG1*.

Conclusions: MPS successfully identified responsible genes in patients who showed relatively good auditory performance with CI including EAS, suggesting that genetic testing may be able to predict the performance after implantation.

EXPRESSION OF OTOCONIN-90 IN HUMAN TEMPORAL BONES

***OLUWASEYE AYOOLA OGUN*[1], *IVAN A LOPEZ*[2], *YAN ZHANG*[1], *AKIRA ISHIYAMA*[2] *AND YUNXIA WANG LUNDBERG*[1]**

[1] Boys Town National Research Hospital, Omaha, NE 68131, USA; [2] Department of Head and Neck Surgery, David Geffen School of Medicine at UCLA, Los Angeles, California 90095, USA

Background: While there is a general consensus that fallen otoconia in the semicircular canals play an important role in both the origin and treatment of benign paroxysmal positional vertigo (BPPV), not much research has been done in humans to determine the expression of otoconia proteins. One critical reason is the limited availability of temporal bone samples from patients with specific diagnosis such as BPPV.

The objectives of this study are to determine if the human inner ear expresses the same types of otoconial proteins found in the mouse inner ear, and if the expression of these proteins is different between BPPV patients and non-vertiginous controls.

Method: Fluorescent immunostaining for otoconial proteins was performed using celloidin sections of human temporal bone tissues from BPPV patients and controls.

Results: The celloidin removal process was successful, and otoconin-90, the predominant otoconial protein, was detected in the ampulla, utricle and saccule of human temporal bone sections of both BPPV patients and controls.

Conclusion: The predominant otoconial protein is present in older individuals.

Sponsored by a travel award from:

march of dimes

WHOLE-EXOME SEQUENCING IDENTIFIES VARIANTS OF MT-ND1, FAM136A AND LARS GENES IN AUTOSOMAL DOMINANT FAMILIAL MENIERE'S DISEASE

TERESA REQUENA[1], SONIA CABRERA[1], IRENE GAZQUEZ I[1], JOSE ANTONIO LOPEZ-ESCAMEZ[1]

[1]Otology & Neurotology Group CTS495, GENYO, - Centro de Genómica e Investigación Oncológica – Pfizer/Universidad de Granada/ Junta de Andalucía, Granada, Spain

Familial Meniere's disease (MD) is found in 8-10% of cases in European population (1,2). Although genetic heterogeneity is observed, most of the families have an autosomal dominant (AD) pattern of inheritance. We have performed whole-exome sequencing (WES) in a family with three affected women in consecutive generations with anticipation.

DNA was isolated from peripheral blood samples of patients with MD and a healthy brother present in the second generation. The libraries were prepared with the Agilent's All Exon 50MB capture kit (Agilent Tech) and WES was carried out in a SOLiD 5500xl platform (Life Technologies). Bioinformatics analyses were performed by using the Bioscope software and SAM tools, obtaining ~50.000 single nucleotide variants (SNV) per exome. Functional annotation software (ANNOVAR) and minor frequency allele (MAF) <0.05 were used to prioritize SNV according to the effect in protein structure and phylogenetic conservation. Criteria used for priorization were SIFT (Sort Intolerant from Tolerant), PolyPhen2 (Polymorphism Phenotyping v2), Graham's Matrix, GERP+ (Genomic Evolutionary Rate Profiling), Mutation taster, PhastCons y PhyloP.

We have identified and validated by Sanger sequencing SNV in Mt-ND1, FAM136A and LARS genes in all patients in this family. The variant in FAM136A leads to a stop codon not previously described. The other two SNV are rare missense variants located in the Mt-ND1 gene (rs201212638, M31T) and LARS gene, encoding a Leucyl-tRNA Synthetase, (rs151245897, R457W).

However, a single missense mutation probably cannot explain the differences in phenotype among offspring in this family. Thus, different mutations in the genomic DNA and mtDNA may interact, leading to partial or complete phenotypes in autosomal dominant FMD.

We conclude that WES combined with linkage analysis is an excellent tool to define candidate variants associated with FMD. The functional impact of these variants in MD should be studied.

CANDIDATE GENE INTERROGATION USING A CUSTOMIZED TARGETED GENOMIC CAPTURE PLATFORM COUPLED WITH MASSIVELY-PARALLEL SEQUENCING

CHRISTINA M. SLOAN[1, 2], DIANA KOLBE[1], HELA AZAIEZ[1], DAVID J. EISENMAN[3], LORNA SILIPINO[3], RAN ELKON[4], A. ELIOT SHEARER[1,2], RONNA HERTZANO[3], RICHARD JH SMITH[1,2,5]

[1]Molecular Otolaryngology and Renal Research Laboratories Department of Otolaryngology, University of Iowa, Iowa City, Iowa; [2] Department of Molecular Physiology and Biophysics, University of Iowa Carver College of Medicine, Iowa City, Iowa; [3] Department of Otorhinolaryngology-Head and Neck Surgery, University of Maryland School of Medicine, Baltimore, Maryland; [4] Division of Gene Regulation, The Netherlands Cancer Institute, Amsterdam, The Netherlands; [5] Interdepartmental PhD Program in Genetics, University of Iowa, Iowa City, Iowa

The advent of massively parallel sequencing has revolutionized the genetic diagnosis of non-syndromic hearing loss (NSHL). Using OtoSCOPE® we now routinely interrogate all genes implicated in NSHL. This inclusive strategy provides a definitive diagnosis in about 50% of cases of well-documented hereditary hearing loss, implying the existence of both novels genetic causes of NSHL and non-coding deafness-causing mutations. The number of novel genetic causes of NSHL is likely to be high based on HUGO, which reports 66 genes assigned to deafness loci but lists an additional 91 loci for which causative genes remain to be identified. To address this disparity, we have developed a targeted genomic capture platform that uses massively-parallel sequencing to interrogate simultaneously a large number of candidate genes in a large number of probands. We used advanced cell sorting and RNAseq analysis of mouse inner ears to select candidate genes based on selection metrics defined by the analysis of known NSHL genes, 65% of which are highly enriched in cochlear sensory cells and/or cochlear hair cells. We are using our candidate gene panel to interrogate OtoSCOPE®-negative families. As compared to whole exome sequencing (WES), our 'front-end' filtering by candidate gene selection has the obvious limitation of up-front power loss due to omission of all novel deafness genes that are not included on our panel. However, a discovery strategy based on WES as applied to very small nuclear families (2-3 affected) is unlikely to generate an actionable variant list regardless of downstream filtering and statistical analysis. Furthermore, WES applied to hundreds of probands is far more expensive and far less robust. For example, in silico comparison of SureSelect Human All Exon V4 shows that 13.95% of OtoSCOPE® regions are not covered at all, and even with 95.99% 10x WES coverage, in the subset of OtoSCOPE® genes the 10x coverage is only 74.9%. We estimate that a substantial number of the unknown NSHL genes (about 40-50%) satisfy the selection metrics we have chosen and anticipate that our strategy will allow us to identify several novel NSHL genes with the advantages of high throughput, easy data analysis, and low costs.

SLC26A4 MUTATION FREQUENCY AND SPECTRUM IN 109 DANISH PENDRED SYNDROME/DFNB4 PROBANDS AND A REPORT OF NINE NOVEL MUTATIONS

[1,9] LISBETH TRANEBJÆRG, [2] IRIS SCHRIJVER, [1] MARIANNE LODAHL, [3] JUAN RODRIGUEZ-PARIS, [4] TORSTEN JOHNSEN, [5] EVA C. HANSÉN, [1] LISE A NICKELSEN, [6] ZEYNEP TÜMER, [7] TORIL FAGERHEIM, [8] RANDI WETKE, [1] NANNA D. RENDTORFF

[1]Wilhelm Johannsen Centre for Functional Genome Research, Department of Cellular and Molecular Medicine, The Panum Institute, University of Copenhagen, Copenhagen, Denmark; [2]Departments of Pathology and Pediatrics, Stanford University School of Medicine, Stanford, CA, USA; [3]Department of Pathology, Stanford University School of Medicine, Stanford, CA, USA; [4]Gl. Kongevej 25, Copenhagen, Denmark; [5]Audiologiska Klinikken Örebro Universitetssjukhuset, 701 85 Örebro, Sweden; [6]Center for Applied Human Molecular Genetics, Kennedy Center, Glostrup, Denmark; [7]Division of Child and Adolescent Health, Department of Medical Genetics, University Hospital of North Norway, Tromsø, Norway; [8]Øre-Næse-Hals-Sygdomme, Afdeling H, Århus Sygehus, Århus, Denmark; [9]Department of Audiology, Bispebjerg Hospital, Copenhagen, Denmark

We sequenced analysed 109 unrelated Danish probands suspected for recessive Pendred syndrome/DFNB4 for mutation in *SLC26A4*. In parallel, 78 of probands were analysed using a microarray test for 198 hearing loss-associated mutations. We discovered pathogenic mutations in one or both *SLC26A4* alleles in 10% and 67% probands, respectively. Sequencing of *FOXI1, KCNJ10,* and *SIX1* and *SLC26A4* MLPA analysis did not yield additional mutations in *SLC26A4* monoallelic mutated patients. One *SIX1* mutation (p.E125K) was found in a patient with no *SLC26A4* mutations. Twenty-nine different *SLC26A4* mutations were identified and 9 were novel. The most frequent *SLC26A4* mutations were p.T416P, p.V138F, p.L236P, p.E29Q, c.1001+1G>A, and p.E384G in this order. Interestingly, a common ancestral chromosome is responsible for the second most prevalent mutation, as suggested by haplotype analysis. Our study is noteworthy for its high detection rate of *SLC26A4* mutations (77%), and for a solid clinical pre-selection, where 58% of probands had had iodine-perchlorate testing done, a positive result for which provides a strong indicator of Pendred syndrome. Furthermore, the spectrum of *SLC26A4* mutations identified here justifies a diagnostic sequencing of exons 2, 4, 6, 8, 10, and 19, in the first step in Denmark, since they harbour 80% of all detected mutations.

TARGETED EXOME CAPTURE AND PAIRED-END MASSIVELY PARALLEL SEQUENCING REVEALS NEW MUTATIONS FOR HUMAN HEREDITARY DEAFNESS IN THE MIDDLE EAST

***DIEGO VOZZI*[1], *ANNA MORGAN*[1], *MARCELLO MORGUTTI*[1], *STEFANIA LENARDUZZI*[1], *ELISA RUBINATO*[1], *FLAVIO FALETRA*[1], *GIORGIA GIROTTO*[1] *AND PAOLO GASPARINI*[1]**

[1]*Med Genet, IRCCS-Burlo Garofolo Children Hospital, Trieste Univ, Trieste, Italy*

Hearing Loss (HL) patients need improved diagnosis, prevention and treatment. Specific genetic tests might be developed to improve genetic counselling and molecular diagnosis. In this light, efficient strategies for selectively sequencing panel of genes (i.e. “Targeted Re-Sequencing” or “TRS”) have the potential to contribute to reach this goal. Here, we report the results obtained using two TRS protocols, one for HHL and one for Usher syndrome (USH), based on Ion Torrent™ technology (Life-Technologies). The first one analyzes 96 genes. Oligomeric primers amenable for multiplex-PCR have been designed by Ion AmpliSeq™ Designer tool, accounting for 3487 amplicons and ensuring approximately 92% coverage of the target region (a total of 411.420 bp). The USH panel analyzes the coding regions of 10 USH genes for a coverage of about 96% of the target region (94.980 bp). Multiplex PCR is employed to prepare an amplicon library representing the entire coding region of the selected genes and the 5’-UTR and 3’-UTR regions; sequencing is carried out on an Ion PGM™ Sequencer system. All reads are mapped back to the entire genome, hg19 version, and the percentage of reads mapped to target regions are used to estimate the coverage. Burrows-Wheeler Aligner (BWA) software is used to allow reads mapping process. The identification of variants is performed using GATK (Genome Analysis Toolkit). After quality control, data are filtered a) using an internal database to reduce false positives, b) according to the pattern of inheritance, pedigree structure, linkage data, c) using NCBI dbSNP v137 and 1000 Genomes project databases. Filtered bases are annotated as follows: (i) novelty; (ii) effect on the encoded protein based on the prediction defined by different softwares, (iii) evolutionary analysis; and (iv) expression pattern by using databases on genes expressed in the cochlea. After a validation step, aimed to demonstrate that both protocols are accurate, robust and reliable, they have been used to analyze a series of 50 HHL cases (familial and sporadic ones) and 20 USH cases. The analyses led to both the identification of new genes and alleles whose role/function is now under investigations. In conclusion, this strategy allows for improved diagnostics, in an economically and temporally feasible manner, and establishing etiologically-based genetic counseling and hearing loss management.

A NONSENSE MUTATION IN *CLIC5* CAUSES AUTOSOMAL RECESSIVE SENSORINEURAL HEARING IMPAIRMENT WITH VESTIBULAR DYSFUNCTION.

***CELIA ZAZO SECO*[1,2,3], *ANNE OONK*[1], *JAAP OOSTRIK*[1,2,3], *RONALD ADMIRAAL*[1], *HANNIE KREMER*[1,2,3,4], *MARGIT SCHRADERS*[1,2,3]**

[1]Department of Otorhinolaryngology; [2]Donders Institute for Brain, Cognition and Behaviour; [3]Nijmegen Centre for Molecular Life Sciences; [4]Department of Human Genetics, Radboud University Medical Centre, Nijmegen, The Netherlands.

In a Turkish consanguineous family (W05-009) with autosomal recessive non-syndromic hearing impairment (arNSHI), homozygosity mapping revealed a 47 Mb homozygous region in chromosome 6p21.1-q15. This region includes the known arNSHI gene *MYO6*, however, no pathogenic variants were found in this gene neither with sequence analysis of the coding region and splice site boundaries nor with *MYO6* mRNA analysis. The homozygous region contained another excellent candidate gene, *CLIC5*, and the orthologous mouse gene was described to be mutated in the jitterbug (*jbg*) mouse exhibiting congenital progressive hearing loss and vestibular dysfunction. Sequence analysis of the *CLIC5* gene in family W05-009 revealed a homozygous nonsense mutation (c.96C>T; p.Cys32X; NM_016929_3), which segregated with the hearing loss in the family. This nonsense mutation may result either in the degradation of the mRNA by nonsense mediated decay or in a truncated protein. The c.96C>T variant was not present in 222 Turkish control alleles, the Exome Variant Server and the Nijmegen in-house Mutation Database (1302 exomes). Screening of *CLIC5* in 55 Dutch arNSHI index patients with a comparable ski-slope audiogram configuration as seen in the affected subjects of family W05-009, did not reveal any putative causative variants. The hearing loss in the patients of family W05-009 is likely to be congenital and shows progression. Although motor development was normal, vestibular testing revealed vestibular areflexia at the ages of 16 and 11 years in the two affected sibs. Therefore, the phenotype of the patients closely resembles that seen in the *jbg* mice.

The CLIC5 protein is a member of the chloride intracellular channel protein family. It localizes to stereocilia of both the cochlear and vestibular hair cells and also on the surface of Kolliker's organ during cochlea development in mice. The *jbg* mice have dysmorphic stereocilia and progressive hair cell degeneration. The CLIC5 protein colocalizes with radixin in hair cell stereocilia and may help form or stabilize connections between the plasma membrane and the filamentous actin core.

Sponsored by a travel award from:
Hearing Health Foundation
Prevention | Research | Cure

Mechanotransduction Defects in Sensory Hair Cells of USH1C Knock-In Mice

GWENAËLLE S. G. GÉLÉOC[1], BIFENG PAN[1], YUKAKO ASAI[1], JENNIFER J. LENTZ[2] AND CHARLES ASKEW[1]

[1]Dpt of Otolaryngology, Kirby Center for Neurobiology; Boston Children's Hospital, Harvard Medical School, Boston, MA. [2]Dpt of Otorhinolaryngology & Biocommunications and Neuroscience Center, LSU Health Sciences Center, New Orleans, LA.

Ush1c encodes harmonin, a scaffolding protein expressed in the retina and the inner ear. A cryptic splice site mutation in *Ush1c* (*216G>A*) causes deaf-blindness in French-Acadian USH1C patients (Lentz et al. 2005). Lentz et al. (2007) generated an *Ush1c.216G>A* knock-in mouse model that resulted in abnormal or no ABR responses in homozygous mutants at one month of age, indicating profound deafness. Cochlear histology at P30 showed disorganized hair cell rows, abnormal bundles, and hair cell loss along the organ (Lentz et al. 2010 & 2013). To assess the development of hair cells in *Ush1c.216G>A* mutant mice, we analyzed hair bundle morphology, expression of voltage-dependent channels, as well as the presence of functional mechanosensitive ion channels in vestibular hair cells (VHCs) and inner (IHCs) and outer hair cells (OHCs) of wild type *Ush1c.216GG*, heterozygous *Ush1c.216GA* and homozygous *Ush1c.216AA* littermates during the first postnatal week. In the cochlea, disorganized hair bundles were evident in *Ush1c.216AA* mice all along the organ of Corti in both IHCs and OHCs. Normal voltage-dependent currents and resting potentials were recorded for all genotypes. Analysis of FM1-43 uptake revealed normal uptake in wild type and *Ush1c.216GA* mice but weaker and uneven uptake in *Ush1c.216AA* mutants. Direct measurement of transduction currents, evoked by stiff-probe deflections of OHC and IHC bundles, revealed similar properties in *Ush1c.216GA* and wild type littermates. However, OHCs and IHCs from *Ush1c.216AA* mutants had significantly smaller transduction currents. Adaptation was typically slower with decreased extent of adaptation, similar to that described for other harmonin mutants. More complete adaptation was occasionally observed in *Ush1c.216GA* mutants. In the utricle, while VHC's bundle morphology was well preserved in *Ush1c.216AA* mutants, transduction currents were also markedly reduced during the first postnatal week.

DIRECT CALMODULIN BINDING HYPERPOLARIZES PRESTIN'S VOLTAGE OPERATING POINT

***KAZUAKI HOMMA*[1,2], *JACOB KELLER*[3,4], *CHONGWEN DUAN*[1], *JING ZHENG*[1,2], *PETER DALLOS*[2,3] , *AND MARY ANN CHEATHAM*[2,3]**

[1]Northwestern University, Chicago, IL, USA; [2]The Hugh Knowles Center for Clinical and Basic Science in Hearing and Its Disorders, Evanston, IL, USA; [3]Northwestern University, Evanston, IL, USA; [4]Howard Hughes Medical Institute, Ashburn, VA, USA

We have previously demonstrated that the calcium-binding protein, calmodulin (CaM), directly binds to an intrinsically-disordered region (IDR) in the intracellular C-terminal domain (CTD) of SLC26A family members in a Ca^{2+}-dependent manner (Keller and Dallos, 2009). The objective of this study was to define the functional consequences of this direct Ca^{2+}/CaM binding to prestin, SLC26A5. To this end, Ca^{2+}-dependent whole-cell recordings were performed on isolated outer hair cells (OHC) expressing wild-type prestin and on HEK293T cells expressing either wild-type or mutated prestin proteins that lack the ability to bind CaM. Results indicate that intracellularly applied Ca^{2+} induces a significant hyperpolarizing shift in the voltage-operating point (V_{pk}), and that this hyperpolarizing V_{pk} shift is blocked by the CaM inhibitor, trifluoperazine. A similar effect was obtained when the CaM binding site was either deleted or mutated to abolish direct CaM binding.

Our results indicate that prestin's motor acitivty can be directly modulated by Ca^{2+}/CaM binding to the IDR region in the C-terminal domain. We speculate that the well-known acetylcholine (ACh)-elicited change in OHC motor properties can be at least partially ascribed to this direct modulation of prestin's motor function by Ca^{2+}/CaM binding. This possibility is based on the knowledge that ACh elicits an increase in intracellular Ca^{2+}, followed by subsequent reduction in OHC axial stiffness (Dallos et al., 1997), which is thought to reflect changes in the cortical lattice as well as changes in the stiffness of the motor protein (He et al., 2003). These changes in physical properties may contribute to protective feedback regulation via the medial olivocochlear (MOC) efferents (Rajan and Johnstone, 1988).

FUNCTIONAL CONTRIBUTIONS OF HYPERPOLARZIATION-ACTIVATED, CYCLIC-NUCLEOTIDE-GATED (HCN) CHANNELS IN MOUSE SPIRAL GANGLION NEURONS

YE-HYUN KIM[1, 2], JEFFREY HOLT[2]

[1]Neuroscience Graduate Program, University of Virginia School of Medicine, Charlottesville, VA, USA, [2] Department of Otolaryngology, F. M. Kirby Neurobiology Center, Boston Children's Hospital and Harvard Medical School, Boston, MA USA

The hyperpolarization-activated current, Ih, is carried by members of the Hcn channel family and contributes to resting potential and firing properties in excitable cells of various systems, including the auditory system. Ih has been identified in spiral ganglion neurons (SGNs), however, its molecular correlates and their functional relevance have not been established. To examine the molecular composition of the channels that carry Ih in SGNs, we examined Hcn mRNA harvested from spiral ganglia of wild-type (WT) neonatal and adult mice using quantitative RT-PCR. We show expression of Hcn1, 2, and 4 subunits in SGNs, with Hcn1 being the most highly expressed at both stages. To determine the physiological expression of HCN subunits in SGN cell bodies, we used the whole-cell, tight-seal technique in voltage-clamp mode to record Ih from WT and those deficient in Hcn1, Hcn2 or both. We found that HCN1 is a major functional subunit contributing to Ih in SGNs. Deletion of Hcn1 resulted in significantly reduced conductance, slower activation kinetics and hyperpolarized half-activation potentials. To investigate the contribution of Ih to SGN function, we recorded membrane responses in current-clamp mode. We demonstrate that Ih contributes to depolarized resting potentials, sag and rebound potentials, accelerates rebound spikes following hyperpolarization, and minimizes spike jitter for small depolarizing stimuli. Auditory brainstem responses of Hcn1-deficient mice showed longer latencies, suggesting that HCN1-mediated Ih is critical for synchronized action potential firing in SGNs. Together, our data indicate Ih contributes to SGN membrane properties and plays a role in temporal aspects of signal transmission between the cochlea and the brain, which are critical for normal auditory function.

THE ZEBRAFISH *PINBALL WIZARD* DEAFNESS GENE ENCODES THE TAIL-ANCHORED-PROTEIN RECEPTOR, WRB, REQUIRED FOR PROPER FUNCTION AND DEVELOPMENT OF THE RIBBON SYNAPSE

***SHUH-YOW LIN*[1], *MELISSA VOLLRATH*[2], *SARA MANGOSING*[1], *JUN SHEN*[3], *ELENA CARDENAS*[1], *AND DAVID P. COREY*[3,4]**

[1]*University of California, San Diego,* [2]*McGill University,* [3]*HHMI,* [4]*Harvard Medical School*

In searching for genes important for sensory systems in a large-scale zebrafish insertional mutagenesis screen, we found a mutant with deafness and blindness phenotypes, which we named *pinball wizard* (*pwi*). The mutant inner-ear hair cells have reduced but functional mechano-transduction, but neural signaling fatigues rapidly. In the *pwi* retina, the a-wave component of the electroretinogram—generated by phototransduction currents—is nearly normal, but the b-wave—a manifestation of transmission at ribbon synapses—is more transient and smaller. These suggest a defect in synaptic transmission, perhaps in releasing or replenishing vesicles. In mutant *pwi* hair cells, the number of ribbons and vesicles near hair cell ribbons was significantly reduced, while in mutant photoreceptors, the axon connecting the cell body to the synaptic terminals was drastically shortened or missing.

We identified the mutated *pwi* gene and found it encodes an ortholog of the human WRB and yeast Get1 proteins. The WRB protein is thought to be an endoplasmic reticulum membrane receptor for insertion of tail-anchored (TA) proteins, a special class of membrane proteins with C-terminal transmembrane domains that cannot be handled by the conventional Sec61-based translocon. Thus TA proteins may not be properly processed in *pwi* hair cells and photoreceptors. In the mouse genome, we identified 223 genes encoding TA proteins, by homology to previously predicted human TA proteins. By assessing gene expression in FACS-purified neonatal mouse hair cells (shield.hms.harvard.edu), we determined that 153 of these genes are expressed in hair cells. Hair-cell TA proteins include 17 SNARE proteins such as syntaxins and synaptobrevins; improper processing of such proteins might explain a synaptic deficit.

In wild-type fish, an antibody to WRB showed a restricted location in the hair cell, including the area near the synaptic ribbon; in photoreceptors, it is in the inner segment and the axon of the photoreceptor. Consistent with the putative TA-protein-processing function of WRB, synaptic localization of the SNARE synaptobrevin was severely diminished in *pwi* retina, but not the non-TA synaptic vesicle proteins SV2, Rab3 and Cysteine String Protein. Taken together, the data suggest that the *pinball wizard* protein WRB is critical for synaptic TA-protein delivery and for developing the large secretory capability characteristic of ribbon synapses in these receptors.

THE TRYPTOPHAN-RICH BASIC PROTEIN (WRB) IS REQUIRED FOR HAIR CELL EXOCYTOSIS AND HEARING

***ILIANA PANOU*[1,2], *CAROLIN WICHMANN*[2,3], *ARTUR INDZHYKULIAN*[4], *SHUH-YOW LIN*[5], *TINA PANGRŠIČ*[1,2], *DAVID COREY*[4], *TOBIAS MOSER*[1,2]**

[1]InnerEarLab, Department of Otolaryngology, University Medical Center Göttingen, Göttingen, Germany; [2]Collaborative Research Center 889, University of Göttingen, Germany; [3]Molecular Architecture of Synapses group, InnerEarLab, Department of Otolaryngology, University Medical Center Göttingen, Germany; [4]Howard Hughes Medical Institute and Department of Neurobiology, Harvard Medical School, Boston, Massachusetts, USA; [5]UC San Diego, Department of Surgery, School of Medicine, San Diego, California, USA

The tryptophan-rich basic protein (WRB) has recently been shown to interact with another membrane protein of the endoplasmic reticulum (ER), calcium-modulating cyclophilin ligand (CAML), and to form the mammalian ER receptor for the cytosolic ATPase TRC40. During tail-anchored (TA) protein biogenesis, TRC40 loaded with a TA protein docks to WRB/CAML and thus achieves posttranslational membrane insertion of the TA protein. In a forward generic screen, we previously identified a zebrafish mutant called *pinball wizard* (*pwi*) for its visual, hearing, and balance deficits and the mutated gene is a *Wrb* homolog.

Here, we created conditional knockout mice ($Wrb^{flx/flx}$: Vglut3-Cre, CAG-eGFP) to investigate the role of the WRB protein in the inner ear. Our results indicate a progressive hearing loss and vestibular deficits. Furthermore, the knockout animals often do not survive beyond two months after birth potentially due to epileptic seizures. Hair bundles were morphologically normal, and otoacoustic emissions indicated intact hair cell transduction and amplification by outer hair cells at least in young (3-week-old) mice. The cellular mechanism of deafness was further assessed by perforated patch-clamp recordings of Ca^{2+} currents and exocytic membrane capacitance changes in apical inner hair cells (IHCs). We observed normal Ca^{2+} currents and exocytosis of the readily-releasable pool of vesicles, but diminished sustained exocytosis, suggesting a defect in vesicle replenishment. Strikingly, a control lacking Cre ($Wrb^{flx/flx}$: CAG-eGFP), recapitulated the deficit in exocytosis that was observed in the conditional knockout ($Wrb^{flx/flx}$: Vglut3-Cre, CAG-eGFP) mice. The number of ribbon-occupied synapses was initially normal, but later ribbon loss was observed. Ultrastructural analysis of the knockout IHC ribbon synapses revealed clusters of unusually large vesicles and fewer ribbon-associated vesicles. The lack of WRB protein led to a reduction in the protein levels of its transient interacting partner TRC40, further supporting the hypothesis of perturbed posttranslational membrane insertion. Among various synaptic proteins, otoferlin has been predicted to be a TA protein. Therefore, deletion of the *Wrb* gene from IHCs may perturb the membrane insertion of otoferlin and/or affect its downstream trafficking to the synaptic site.

In conclusion, disruption of the *Wrb* gene in IHCs leads to deafness, vestibular dysfunction, and seizures. We propose that efficient membrane insertion of TA synaptic proteins via the WRB/TRC40 pathway is required for normal hair cell presynaptic function.

Sponsored by a travel award from:

NIDCD

PROBING THE CA^{2+}SENSITIVITY OF OTOFERLIN-DEPENDENT EXOCYTOSIS IN INNER EAR HAIR CELLS

***PHILIPPE VINCENT*[1], *YOHAN BOULEAU*[1], *SAAID SAFIEDDINE*[2], *CHRISTINE PETIT*[2] *& DIDIER DULON*[1]**

[1]Neurophysiologie de la Synapse Auditive, INSERM UMRS 1120 & Université Bordeaux Segalen, Hôpital Pellegrin Bat PQR3, 33076 Bordeaux, FRANCE. [2]Unité de Génétique et Physiologie de l'Audition, Institut Pasteur - INSERM UMRS 1120, 25 rue du Docteur Roux 75015 Paris, FRANCE

Otoferlin, a C2 domain Ca^{2+} binding protein is predicted to play a key role as calcium sensor in presynaptic exocytosis of vestibular hair (VHCs) and cochlear inner hair cells (IHCs). Johnson and Chapman (2010) showed, using SNARE-bearing liposomes *in vitro,* that the recombinant otoferlin six-C2 domains accelerate membrane fusion with a rather high Ca^{2+} sensitivity (Kd ~ 2 µM). However, *in situ* in hair cells, the real Ca^{2+}sensitivity of the otoferlin-dependent exocytosis still remains to be determined. By using gradual caged Ca^{2+} photolysis experiments combined with membrane capacitance measurement (ΔCm), we investigated the Ca^{2+} sensitivity of VHCs and IHCs exocytosis in both control and otoferlin knock-out mice. In control mice, upon intracellular Ca^{2+} photo-release, VHCs (type I) and IHCs exocytosis typically displayed a biphasic increase, with a fast and slow component, with both kinetics increasing with the amount of intracellular Ca^{2+} photo-released. In otoferlin-deficient mice, IHCs and VHCs exocytosis only showed a single slow residual component with a very poor Ca^{2+}-dependence. In control VHCs, the otoferlin-dependent fast exocytotic rate could be fitted with a Hill-sigmoidal relationship against Ca^{2+}, with an apparent K_D of 1.98 µM Ca^{2+} and a relatively low cooperative index (n ~1). Surprisingly, in IHCs, the fast exocytotic component showed a much lower Ca^{2+} sensitivity (more than one order of magnitude) than VHCs and did not show saturation below 80 µM Ca^{2+}, the maximum range tested, in agreement with Beutner et al. (2001). Our results confirm that fast vesicular exocytosis in both IHCs and VHCs is otoferlin-dependent but indicate that this process has a higher Ca^{2+}-sensitivity in VHCs.

THE CANDIDATE TUMOR SUPPRESSOR GENE ECRG4 ATTENUATES THE INFLAMMATORY PROLIFERATIVE RESPONSE OF MUCOSAL EPITHELIAL CELLS TO INFECTION

***ARWA KURABI*[1,2], *KWANG PAK*[1,3], *XITONG DANG*, *ANDREW BAIRD*[2], *ALLEN RYAN*[1,3]**

[1]UCSD Surgery/ENT, [2]UCSD Surgery/Trauma, [3]San Diego VAMC

Mucosal membranes play an important role in innate and adaptive immunity acting as physical and biological barriers that prevent and regulate the response to infections. Otitis media (OM) is a common pediatric disease associated with an intense proliferation and differentiation of the epithelial mucosa lining the middle ear (ME) cavity. Using whole-genome gene arrays we identified a small set of regulatory genes that are differentially expressed during OM time course. One such factor, Ecrg4 (Esophageal cancer related gene 4) is unique as it is a tumor suppressor endogenously expressed in quiescent ME. We found that Ecrg4 expression is strongly down-regulated in ME mucosa during OM and recovers as mucosal hyperplasia ceases. When Ecrg4 was over-expressed in vitro, a reduction in the hyper-proliferative response observed by mucosal cells harvested from the ME during OM was seen. When the analogous experiment was performed in vivo, we observed a decrease in the thickness of the mucosal epithelial layer and a reduction in inflammatory infiltrate entering the ME cavity 48 hrs after infection. This suggests that Ecrg4 down-regulation is a major determinant of mucosal hyperplasia during OM. Together these data support that the down-regulation of Ecrg4 gene expression after infection plays a determinant function in mucosal epithelial hyperplasia and are the first that point to the value of a therapeutic strategy targeting Ecrg4 in the inflammation- immunity cascade that precipitates mucosal epithelial proliferation.

HB-EGF STIMULATES HYPERPLASIA OF THE MIDDLE EAR MUCOSA IN BACTERIAL OTITIS MEDIA

***JULIA TOMLIN*[1], *KEIGO SUZUKAWA*[2], *GRACE LUU*[1], *EDUARD CHAVEZ*[1], *KWANG PAK*[1], *STEPHEN WASSERMAN*[1], *ALLEN RYAN*[1]**

[1]*University of California San Diego,* [2]*University of Tokyo*

Background: Otitis media is one of the most common pediatric diseases. While it is usually treated without difficulty, up to 20% of children may progress to long-term complications that include hearing loss, impaired speech and language development, academic underachievement, and irreversible disease. Hyperplasia of middle ear mucosa contributes to the sequelae of acute otitis media and is of important clinical significance. Understanding the role of growth factors in the mediation of mucosal hyperplasia could lead to the development of new therapeutic interventions for this disease and its sequelae.

Poster Abstracts

Monday,
June 24

EARLY TIME COURSE OF GENTAMICIN UPTAKE IN VESTIBULAR CELLS AFTER SYSTEMIC ADMINISTRATION

***HENRY ADLER*[1], *JIANPING LIU*[2,3], *BRIAN CARTER*[2], *CHUNFU DAI*[3], *HONGZHE LI*[2], *PETER STEYGER*[2]**

[1] National Technical Institute for the Deaf, Rochester Institute of Technology, Rochester, NY, USA, [2]Oregon Hearing Research Center, Oregon Health & Science University, Portland, OR, USA, and [3]Eye & Ear Nose and Throat Hospital, Fudan University, Shanghai, China.

Systemic aminoglycoside therapy can induce vestibulotoxicity as well as cochleotoxicity, resulting in imbalance and visual dysfunction. The underlying trafficking routes into vestibular hair cells and subsequent cytotoxicity mechanisms remain unknown. We investigated the early time course of gentamicin trafficking into the peripheral vestibular system after systemic administration using fluorescently-tagged gentamicin (gentamicin-Texas-Red, GTTR).

C57Bl/6 mice received GTTR 2 mg/kg via intra-peritoneal injection; control mice received equivalent doses of Texas Red or PBS. Mice were cardiac-perfused with PBS then fixed with 4% paraformaldehyde at 0.5, 1, 2, 3 or 4 hours, excised vestibular tissues permeabilized and counter-labeled with Alexa-488-conjugated phalloidin prior to examination by sequential scanning laser confocal microscopy. The same laser intensity, iris and gain settings were used for all experimental and control groups. Fluorescence pixel intensities in non-sensory and sensory cells from single optical sections were obtained after removal of extracellular pixels. Pixel intensity-time course plots were constructed.

Vestibular cells of all animals treated with GTTR exhibited changes in GTTR fluorescence over time. Low intensity fluorescence was detected at 0.5 hours, and increased in intensity to peak at 3 hours, before declining 4 hours after injection. The distribution of GTTR fluorescence differed in sensory and non-sensory cells. Sensory cells typically had only diffuse GTTR fluorescence, at all tested time points. In contrast, non-sensory cells displayed an intensely fluorescent granular and a diffuse pattern at weaker fluorescent intensities throughout the cytosol. The area of granular fluorescence in dark cells and transitional cells significantly increased over time. Control vestibular tissues exposed to PBS or hydrolyzed Texas Red had negligible fluorescence.

Systemically-administered GTTR is rapidly taken up by murine peripheral vestibular sensory and non-sensory cells, peaking in fluorescence intensity 3 hours after a single injection. The intensity of GTTR in sensory and non-sensory cells share similar time courses. However, the distribution of GTTR within cells is very different. Diffuse fluorescence suggests entry via GTTR-permeant ion channels, while granular fluorescence suggest either endocytosis and/or compartmentalization of cytosolic GTTR. The identity of these compartments is under investigation. The difference in GTTR distribution within sensory and non-sensory cells suggests differential uptake mechanisms and/or subcellular GTTR targets in these cell types.

DAMAGE REPAIR MECHANISMS IN SENSORY HAIR CELLS

***NICOLA ALLEN*[1], *RICHARD GOODYEAR*[1] *AND GUY RICHARDSON*[1]**

[1]*School of Life Sciences, University of Sussex, Brighton, UK*

Aminoglycoside antibiotics are a class of drug used to treat bacterial infections but have the unfortunate side effect of being both oto- and nephro-toxic. Deafness caused by aminoglycoside ototoxicity results from a loss of sensory hair cells from the inner ear. In vitro, the aminoglycosides have two immediate effects on sensory hair cells. First, membrane blebs form around the perimeter of the hair cell's apex. Second, phosphatidylserine (an aminophospholipid that is normally restricted to the inner leaflet of the plasma membrane) flops to the outer leaflet. This disruption of membrane lipid asymmetry and hair-cell surface structure occurs rapidly and begins within 90-120 seconds of drug exposure. Damage to the hair cells is thought to occur via stimulation of lipid scramblase activity and the addition of new membrane to the apical plasma membrane consequent to aminoglycoside entry through mechanotransducer channels that are located toward the top of the sensory hair bundle (Goodyear et al., 2008). The damage caused by short-term drug exposure is reversible. Following drug washout and incubation in medium at 37°C for 2 hours, phospholipid asymmetry is restored and membrane blebs are no longer observed on the apical surface of hair cells (Goodyear et al., 2008). Further investigations now show that recovery can occur very rapidly and is complete within 15 minutes of drug washout, suggesting hair cells have an efficient mechanism for repairing damage to the apical membrane. Data obtained so far suggest the dynamin-independent, clathrin and caveolar independent carrier (DI-CLIC) pathway (Kirkham et al., 2005) may underlie the retrieval of damaged membrane. Evidence for this pathway includes the presence of surface-connected tubular membrane invaginations within hair cells recovering from aminoglycoside exposure. These tubules lack a clathrin coat and are a typical feature of the DI-CLIC pathway. In addition, there is a lack of co-localisation between endocytosed membrane and components of caveolae. Although the DI-CLIC pathway may be involved, a full characterisation of the damage repair process is required to determine the involvement of different endocytic pathways. Inhibitors of macropinocytosis are currently being tested to see if the repair process can be blocked. Preliminary data suggests membrane repair in inner hair cells but not outer hair cells is blocked by the actin polymerisation inhibitor jasplakinolide. Understanding the mechanisms underlying the damage repair process may allow the design of drugs that will ameliorate recovery from exposure to ototoxins.

Sponsored by a travel award from:

ACTION ON HEARING LOSS

THE ROLE OF IGF-1 FOR RE-INNERVATION AND SYNAPSE FORMATION BY SPIRAL GANGLION NEURONS ON INNER HAIR CELLS AFTER EXCITOTOXIC TRAUMA IN COCHLEAR SLICE CULTURES

NAKARIN ASAKA, TAKAYUKI NAKAGAWA, YUSHI HAYASHI, NORIO YAMAMOTO, TOMOKO KITA, JUICHI ITO

Kyoto University, Kyoto, Japan

For effective clinical treatment of sensory neural hearing loss using cochlear implant, it is essential to maintain the spiral ganglion neuron (SGN) population and functional excitable neurites available for electrical stimulation. In this study, the original auditory nerve preserved dissection technique and culture system where the neuronal responses particularly physiological interaction (synapses) between hair cells and SGNs were examined more precisely. Using this method, the therapeutic effect of Insulin-like growth factor 1(IGF-1) and its mechanism for SGN dendrites and synapses regeneration were investigated.

In the experiment, cochlea and SGNs of postnatal day 2-3 ICR mice were cultured and damaged with 0.5mM NMDA and Kainate for 8 hours exposure. Then the explants were treated with IGF-1. In some culture condition, the IGF-1 addition with a different kind of IGF-1 signal inhibitors, were performed to investigate the mechanism of the IGF-1 pathway. At the end of the culture period, the amount of spiral ganglion cell, afferent dendrite, pre and post synapses in each condition were examined. Immunofluorescence for anti-NF-H was used to label SGN somatas and afferent dendrites. Anti-CTBP2 and anti-PSD-95 were used to label pre and post synapses respectively. Quantitative analysis of the SGN somatas, afferent dendrites, presynaptic ribbon and post synapses were performed using LAS AF Lite software and Image J software. Briefly, the SGN somata at the modiolus for each specimen were counted. All axons that contacted with the IHC were counted for afferent dendrites. CTBP2 ribbons were counted for pre synapses and double labeled CTBP2/PSD-95 punctas were counted for post synapses.

NMDA and Kainate showed specific damage to type-I SGN at the SGN afferent dendrite and the inner hair cell (IHC) area while the spiral ganglion cell in auditory nerve remains preserved. Dose-dependent therapeutic effect of IGF-1 was shown at SGN-IHC. Increase of the dendrites, pre and post synapses number in IGF-1 treatment, compared to the control group were found. The results of IGF-1 treatment and its role for SGN and synaptic regeneration with further studies in patho-physiological mechanism will be discussed.

AUDITORY HAIR CELL STEREOCILIA LENGTH CONTROL DOES NOT RELY ON TWINFILIN2

***LARS BECKER*[1,2], *HAYDN PROSSER*[3], *AGNIESZKA RZADZINSKA*[3], *KAZUO OSHIMA*[1,2], *STEFAN HELLER*[1,2]**

[1]Departments of Otolaryngology-Head & Neck Surgery and [2]Molecular & Cellular Physiology, Stanford University School of Medicine, Stanford, California; [3]Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, United Kingtom

Twinfilin2 is an actin binding protein known to sequester globular actin and to cap filamentous barbed ends and thereby inhibiting filament elongation. It has been hypothesized the distinct staircase-like architecture of mammalian auditory hair cells is regulated by twinfilin2. Therefore the actin binding activity would slow down actin turnover rates in middle and short row stereocilia during development and maintain them at a constant length throughout adulthood.

To further elucidate the role of twinfilin2 in stereocilia length control, both twinfilin2 isoforms have been targeted in a conditional knockout approach and the impact of a loss of function in the inner ear has been studied. To our surprise knockout animals have normal hearing thresholds and also hair cell architecture remains undisturbed.

IDENTIFICATION OF NOVEL ENU-INDUCED ALLELES OF KNOWN DEAFNESS GENES

Lauren Chessum[1], Carlos Aguilar[1], Joanne Dorning[1], Sue Morse[1], Andy Parker[1], Prashanthini Shanthakumar[1], Ruairidh King[1], Laura Wisby[1], Sara Wells[2], Paul Potter[1], Steve DM Brown[1], Michael R Bowl[1].

[1]Mammalian Genetics Unit and [2]Mary Lyon Centre, MRC Harwell, OX11 0RD, UK.

MRC Harwell is undertaking a large-scale *N*-ethyl-*N*-nitrosourea (ENU) mutagenesis screen to identify new mouse models of inherited hearing loss. Auditory phenotyping at 3 months of age, using a clickbox test and Auditory-evoked Brainstem Response (ABR), has identified ten pedigrees with recessive hearing loss.

Of these, three display circling or headbobbing behaviour, suggesting vestibular dysfunction. Currently one of these lines (*MPC-142*) has been mapped and sequenced, identifying a novel mutation in *Myo7a*, causing a N505K substitution within the highly conserved myosin head motor domain. Mice homozygous for this mutation have severely elevated auditory thresholds compared to littermates.

Of the remaining seven early-onset hearing loss models, three map to novel hearing loss loci (*MPC-169*, *MPC-173, MPC-227*), three map to known loci (*MPC-188a*, *MPC-188b, MPC-190*) and one is yet to be mapped (*MPC-234*). *MPC-188a* affected animals have +40dB SPL ABR thresholds. Whole genome sequencing (WGS) of one affected animal identified an ENU-induced lesion within the *Gpr98* gene, known to be mutated in USH2C. This mutates the invariant donor splice site of exon 37 from gt>gc, which is predicted to affect splicing.

MPC-188b affected animals have no detectable auditory responses at stimuli <90dB SPL. WGS identified two ENU-induced lesions within the *Loxhd1* gene, known to be mutated in DFNB77. These two lesions both cause substitutions within PLAT domains - I1457N and T1775A - and thus are likely to affect protein function.

MPC-190 affected animals have no detectable auditory responses at stimuli <80dB SPL. WGS identified an ENU-induced lesion in *Myo15*, known to be mutated in DFNB3. This causes a D1647G substitution in the highly conserved myosin head motor domain.

The three models mapping to novel loci are yet to be sequenced, but promise to provide additional insights to the proteins and molecular pathways required for hearing. Functional characterisation and investigation of these ten newly identified mouse models will help elaborate the mechanisms underlying hearing and the processes involved in hearing loss.

AUDITORY HAIR CELL CENTRIOLES UNDERGO CONFINED BROWNIAN MOTION THROUGHOUT THE DEVELOPMENTAL MIGRATION OF THE KINOCILIUM

JACQUES BOUTET DE MONVEL[1,2], LÉA LEPELLETIER[1,4,5], JOHANNA BUISSON[1,6], CHANTAL DESDOUETS[6,7], AND CHRISTINE PETIT[1-3]

1 Pasteur Institute, Unité de Génétique et Physiologie de l'Audition / Inserm UMRS1120, Paris, France.2 Université Pierre et Marie Curie, Paris, France.3 Collège de France,Paris, France. 4 Université Paris VII, Paris, France.5 Institut de Recherches Cliniques de Montréal, Montreal, Quebec, Canada.6 Pasteur Institute, Unité Biologie Cellulaire des Trypanosomes / CNRS URA2581, Paris, France.7 Institut Cochin & Université Paris Descartes, CNRS UMR8104 / Inserm U567, Paris, France.

Planar polarization of the forming hair bundle, the mechanosensory antenna of auditory hair cells, depends on the poorly characterized center-to-edge displacement of a primary cilium, the kinocilium, at their apical surface. Taking advantage of the gradient of hair cell differentiation along the cochlea, we reconstituted a map of the kinocilia displacements in the mouse embryonic cochlea. We then developed a cochlear organotypic culture and video-microscopy approach to monitor the movements of the kinocilium basal body (mother centriole) and its daughter centriole, which we analyzed using particle tracking and modeling. We found that both hair cell centrioles undergo confined Brownian movements around their equilibrium positions, under the apparent constraint of a radial restoring force of ~ 0.1 pN. This magnitude depended little on centriole position, suggesting nonlinear interactions with constraining, presumably cytoskeletal elements. The only dynamic change observed during the period of kinocilium migration was a doubling of the centrioles' confinement area taking place early in the process. It emerges from these static and dynamic observations that kinocilia migrate gradually in parallel with the organization of hair cells into rows during cochlear neuroepithelium extension. Analysis of the confined motion of hair cell centrioles under normal and pathological conditions should help determine which structures contribute to the restoring force exerting on them.

UNRAVELLING THE ROLES OF LYSINE ACETYLATION BY ELP3 DURING INNER EAR DEVELOPMENT

LAURENCE DELACROIX, SUSANA MATEO SANCHEZ, SOPHIE LAGUESSE, SANDRA HUYSSEUNE, ALAIN CHARIOT, LAURENT NGUYEN AND BRIGITTE MALGRANGE.

GIGA-Research, Developmental Neurobiology Unit, University of Liege, Avenue de l'hopital 1, 4000 Liege, Belgium

Lysine acetylation is a post-translational modification that affects gene transcription when it targets histones and that modulates the localization, stability or activity of many cellular proteins. Given the importance of acetylation homeostasis in controlling developmental processes, we planned to investigate its role in inner ear formation and focused our attention on Elp3 acetyl-transferase (Elongator protein 3) that was recently implicated in neurogenesis.

In the inner ear, we detected Elp3 transcripts in the sensory epithelia of the entire otic vesicle at embryonic day E11.5. At later stages, Elp3 mRNA is present in vestibule and cochlea where it is strongly expressed in the spiral ganglion neurons.

To investigate the role of Elp3 *in vivo*, we used conditional knock-out mice in which the expression of the acetyl-transferase is lost in early otocyst. These mice show obvious vestibular defects as indicated by a stereotyped circling ambulation, head bobbing, retropulsion and absence of reaching response in a tail-hanging test. Furthermore, Elp3cKO do not seem to respond to sound stimuli in Preyer's test and we are currently doing auditory brainstem responses to evaluate their hearing threshold. Although we could not detect any gross structural defects in Elp3-depleted vestibule and cochlea, we found significant abnormalities in spiral ganglion neurons. Indeed, Elp3 seems to be necessary for neuronal survival. In addition, spiral ganglion neuronal processes were less numerous and do not turn towards the base. Taken together, these data support a role for Elp3 in hearing and balance and point out an important role for acetylation homeostasis during inner ear formation. We are currently investigating the molecular mechanisms underlying Elp3 effect on neuronal survival and pathfinding.

EFFECT OF OXIDATIVE STRESS ON GENE EXPRESSION IN HAIR CELLS OF THE MOUSE UTRICLE

GILLIAN L. DRURY[1] AND MELISSA A. VOLLRATH[1,2]

1. Department of Physiology 2. Department of Neurology and Neurosurgery, McGill University, Montreal, Quebec, Canada.

Oxidative stress plays an important role in ototoxicity. The sensory epithelia of the inner ear are composed of a mixed population of supporting cells and mechanotransducing hair cells. Studying oxidative stress in intact sensory epithelia leads to potentially ambiguous results since any changes in gene expression can be attributed to supporting cells, hair cells or both cell types.

Our goal was to dissect changes in gene expression in hair cells during oxidative stress. Therefore stringent criteria were used: Only genes that were overexpressed in hair cells relative to supporting cells according to the Shared Harvard Inner-Ear Laboratory Database (SHIELD) were evaluated. In addition, the studied genes were involved in the response to oxidative stress, apoptosis or cell survival according to the gene ontology database (GO). We assayed an array of 84 genes that met the above criteria in untreated and hydrogen peroxide-treated mouse utricles.

We report that, of these 84 genes, 16 had a greater than two fold change in expression between untreated and peroxide treated utricles. In addition, other genes changed significantly ($p \leq 0.05$) with hydrogen peroxide treatment but by less than two fold. These results may allow for the identification of pathways and mechanisms that hair cells employ to cope with oxidative insults. Furthermore, this approach may prove useful for dissecting hair cell specific responses to other stimuli.

MECHANISMS OF GENERATING ENDOLYMPH-LIKE COMPARTMENTS

DANIEL F. EBERL, BETUL ZORA, ELENA SIVAN-LOUKIANOVA, MADHUPARNA ROY, JULIE S. JACOBS

The University of Iowa, Iowa City, Iowa USA

Excitable cell activities rely heavily on ion flow through transduction channels. This is perhaps most important in sensory cells such as in hearing where the demands of high frequency firing are pushed to the extreme, into the 10's of kHz or higher in some animals. To drive ions reliably through mechanotransduction channels with such high temporal precision, animals have evolved specialized compartments containing lymph with high concentrations of cations such as K+. In the mammalian cochlea, this compartment is the scala media containing endolymph, into which the sensory hair cell stereocilia project. In insect chordotonal organs such as the Johnston's organ (JO) which mediates hearing in *Drosophila*, the scolopale cell surrounds sensory dendrites in each sensory unit to form an individual compartment, called the scolopale space, containing the receptor lymph.

The Na+/K+ ATPase (also referred to as the Na pump), composed of a catalytic α subunit and a β subunit required for its transport to the plasma membrane and for regulating its activity, is essential for ion homeostasis. We show that α and β subunits are expressed in JO. We knocked down expression of the α subunit *ATPα* and β subunits (*nrv1*, *nrv2* and *nrv3*) individually in JO with UAS/Gal4-mediated RNA-interference. ATPα shows elevated expression in the ablumenal membrane of scolopale cells, which enwrap JO neuronal dendrites in endolymph-like compartments. Knocking down *ATPα* in the entire JO or only in scolopale cells using specific drivers, resulted in complete deafness. Among β subunits, *nrv2* is expressed in scolopale cells and *nrv3* in JO neurons. Knocking down *nrv2* in scolopale cells blocked Nrv2 expression, reduced ATPα expression in the scolopale cells and caused almost complete deafness. Furthermore, knockdown of either *nrv2* or *ATPα* specifically in scolopale cells causes abnormal electron-dense material accumulation in the scolopale space. Similarly, *nrv3* functions in JO but not in scolopale cells, suggesting neuron-specificity that parallels *nrv2* scolopale cell-specific support of the catalytic ATPα.

We are now investigating the role of additional ion pumps in the generation of endolymph-like extracellular compartments.

TOWARDS A STEM CELL-BASED "OTOTOXIC HEARING LOSS-IN-A-DISH" MODEL – COMPARISON OF THREE APPROACHES USING FACS, MACS AND NOTCH SIGNALING-INHIBITION FOR THE ENRICHMENT OF OTIC DIFFERENTIATED CELLS

***ANDREAS ECKHARD*[1], *NGOC-NHI LUU*[1], *AURÉLIE DOSSANTOS*[1], *JÖRG WALDHAUS*[2], *MARCUS MÜLLER*[1], *HUBERT LÖWENHEIM*[1]**

[1]Department of Otorhinolaryngology, Head and Neck Surgery, Hearing Research Center Tübingen, University of Tübingen Medical Center, Tübingen, Germany; [2]Departments of Otolaryngology, Head and Neck Surgery and Molecular & Cellular Physiology, School of Medicine, Stanford University, Stanford, California, USA

Introduction: Ototoxic insults combined with the lack of hair cell regeneration in the mammalian organ of Corti cause irreversible hearing loss. Towards a rational therapy for the cure of hearing loss, an *in vitro* culture model of ototoxic hair cell loss that can be used to screen for otoprotective or otoregenerative drugs is needed. To establish a "ototoxic hearing loss-in-a-dish" model, we aimed at an increase in the number of *in vitro* differentiated otic hair- and supporting cell like cells and the subsequent neomycin induced hair cell loss.

Materials and Methods: Using FACS and MACS, inner supporting cells that express the glutamate aspartate transporter GLAST (GLAST+ cells) and outer supporting cells that express the p75-neurotrophine receptor (p75-NTR+ cells) were purified from the murine neonatal (P4) organ of Corti. The *in vitro* proliferative capacity and the potential to re-differentiate into otic hair and supporting cell like cells were investigated by immunohistochemical and qRT-PCR analyses. Further, the potential of the gamma-secretase inhibitor L-685458 to induce otic cell differentiation was analysed in *in vitro* cultures of the murine P0 organ of Corti. As a "hearing loss in a dish model" Neomycin (1 mM) was added for 24 h and the number of supporting and hair cell like cells were analysed after 48 h, 72 h, 96 h and 120 h.

Results: FACS and MACS purified GLAST+, p75-NTR+ and GLAST-/p75-NTR- cells proliferated *in vitro* as determined by EdU-incorporation and express stem cell markers. MACS purified GLAST+ and p75-NTR+ cells showed a higher differentiation potential for hair and supporting cell like cells *in vitro* than GLAST-/ p75-NTR- cells; however, due to the sorting process the absolute number of MACS-derived *in vitro* cultured cells was reduced by a factor of 100 compared to unsorted cells. Treatment of *in vitro* cultured cells from the P0 organ of Corti for 24 h with the gamma secretase inhibitor L-685458 increased the absolute number of hair cells and supporting cells *in vitro* by a factor of 6.4 and 19.6, respectively. Addition of neomycin (1 mM, 24 h) *in vitro* 120 h before the end of the culture period resulted in an almost complete loss of hair cell like cells, while the number of supporting cell like cells remained constant.

Conclusions: Treatment of *in vitro* cultured cells from the murine P0 organ of Corti with L-685458 (0.5 µM, 24 h) increases the number of hair and supporting cell like cells and allows quantitative analyses. Neomycin (1 mM, 24 h) selectively kills hair cell like cells *in vitro*, and therefore can be used for an "ototoxic hearing loss-in-a-dish" model.

Sponsored by a travel award from:

NIDCD

GENETIC SIGNATURE OF NEURAL CREST-DERIVED CELLS IN THE OTIC VESICLE

***LAINA FREYER*[1] *AND BERNICE E. MORROW*[1,2]**

[1]Department of Genetics, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461; [2]Departments of Ob/Gyn and Pediatrics, Albert Einstein College of Medicine, 1301 Morris Park Avenue, Bronx, NY 10461, USA

The mammalian inner ear originates from the otic placode, a specialized region of head ectoderm. The otic placode invaginates into the head to form the otic vesicle (OV), the developmental primordium of the inner ear and cocheovestibular ganglion (CVG). We have previously shown that the otic placode ectoderm and neural crest cells (NCCs) contribute directly to the OV (Freyer et al., 2011 PMID: 22110056). In our previous study, we used several NCC-specific Cre drivers to fate map NCCs to the OV and CVG. In particular, *Pax3*$^{Cre/+}$ labeled cells represent a significant subpopulation of the OV. Antibodies to neural and sensory cell markers demonstrated that NCC derivatives constitute approximately 20% of differentiated neurons, hair cells, and supporting cells. In order to gain insight into the function and regulation of NCC contribution to the OV, our current goal is to identify gene expression signatures of cells with different embryonic origins in the inner ear. For this, we have performed comparative gene expression analysis of NCC-derived cells (as labeled by *Pax3*$^{Cre/+}$) versus placode-derived cells (as labeled by *Pax2-Cre*). The OV region was dissected from fate-mapped embryos and subjected to fluorescence-activated cell sorting (FACS) followed by microarray analysis of total RNA from GFP+ and GFP- fate mapped and sorted cell populations. We identified >200 genes that were upregulated in both GFP+ cell populations. In addition, there were 134 genes (FC>1.5, pv<0.01) unique to NCC derivatives that are known to be essential for NCC specification in neuroepithelial cells such as *Wnt1, Pax3, Olig3,* and *Tcfap2b*. Additionally, there was enrichment of 129 genes specific to the *Pax2-Cre* labeled population such as *Slitrk6, NeuroD, Fgf10,* and *Dlx5*. Since we are primarily interested studying NCC derivatives that have incorporated into the otic epithelium, we wanted to eliminate candidate genes that were likely upregulated in neuroepithelial cells from tissue surrounding the OV. To do this, we compared our gene expression data sets with microarray results from highly-purified wildtype OVs that had been treated with dispase in order to remove all surrounding non-otic tissue. By doing so, we narrowed down our results to <20 candidate genes whose upregulation is specific to *Pax3*$^{Cre/+}$ fate mapped cells in the OV. These include some genes associated with sensorineural hearing loss as well as genes likely important for neural progenitor cell maintenance. Additionally, we identified components of known signaling pathways that mediate cell-cell interactions, whose cognate receptor-ligand partners are differentially expressed between placode-derived and NCC-derived cells and may therefore be required for normal inner ear development and gangliogenesis.

INSIGHTS INTO GENETIC REGULATION OF OTIC LINEAGE SPECIFICATION

***BYRON HARTMAN*[1], *ROMAN LASKE*[1], *STEFAN HELLER*[1, 2]**

[1]Department of Otolaryngology – Head & Neck Surgery, Stanford University School of Medicine, Stanford, CA 94305; [2]Department of Molecular & Cellular Physiology, Stanford University School of Medicine, Stanford, CA 94305

Sensorineural hearing loss due to auditory hair cell damage affects millions of Americans. Embryonic stem cells are a potential source for generation of otic progenitors and hair cells that could be highly useful for cell replacement therapies, developmental studies, and other applications. For the stem cell system to reach its full potential to recapitulate otic development and generate pure sensory epithelia *in vitro*, two critical barriers must be addressed. The first barrier is a lack of biomarkers unique to the otic sensory lineage that would enable unambiguous identification of otic cells. The second barrier is poor understanding of the genetic regulation of otic gene expression and lack of tools for live reporting in cells and embryos for purification of specific otic cell types during development. The overall goal of this project is to address these barriers through combined study of mouse genetics, inner ear development and stem cell culture to further our understanding of the molecular genetics of otic specification.

We seek to understand the mechanisms by which otic-specific genes are regulated, including identification of inner ear specific cis-regulatory elements and combinatorial roles of multiple transcription factors in controlling otic genes. To identify genes highly specific to the otic sensory lineage we performed microarray experiments comparing purified otic cell populations to non-otic tissues. Among the most highly ranked otic-specific genes identified was *Fbxo2*, which encodes a ubiquitin ligase (Fbx2) previously shown to be strongly expressed in adult cochlea and required for protein quality control and cochlear homeostasis. We further characterized the expression pattern of *Fbxo2*/Fbx2, which we found is indeed highly specific to the otic sensory lineage and robustly expressed from early in development to adulthood. For lineage tracing and live reporting, we are generating a multifunctional knock-in mouse line for *Fbxo2*. Phylogenetic and transcription factor binding site analyses suggest that *Fbxo2* may be regulated by one or more cis-regulatory modules that integrate activity of multiple transcription factors, including Pax2 and Gata3. We are developing transgenic reporter mouse lines and *in vitro* assays to characterize the minimal promoter/enhancer elements and transcription factor interactions that regulate expression of this gene. In future directions, *Fbxo2* regulatory element-based reporters and knowledge of transcriptional regulation of the otic lineage will be applied to improve the efficacy of guidance protocols for stem cells as a model system for otic development and enable the *in vitro* culture and propagation of highly pure and distinct progenitor cell populations within the otic lineage.

ACOUSTIC STRESS ACTIVATES RAPID INNATE RESPONSES IN THE ORGAN OF CORTI

***Bo Hua Hu*[1], *Qunfeng Cai*[1]**

[1]*Center for Hearing and Deafness, University at Buffalo, Buffalo, NY, USA*

Acoustic overstimulation compromises inner ear structures and damage sensory cells. To remove damaged cells and re-establish cochlear homeostasis, the immune defense system is activated. Previous observations have revealed the involvement of phagocytic cells and cochlear local cells in the cochlear degenerative/recovery process. However, the molecular mechanisms responsible for this process are not clear. The current study was designed to investigate the involvement of innate immune components. We screened the expression of 84 genes related to the innate immune system in the organ of Corti of the C57BL/6J mouse using a PCR array technique. We identified the expression of 39 genes under normal conditions, accounting for 46.4 % of the 84 genes examined. Most of the genes identified in the current investigation have previously been identified in rat cochlear sensory epithelium tissues reported in our previous observation. Using DAVID Bioinformatics Resources, we analyzed the possible functional pathways associated with these expressed genes. Based on the number of the genes involved, we ranked the top five pathways: toll-like receptor signaling pathway, NOD-like receptor signaling pathway, cytosolic DNA-sensing pathway, RIG-I-like receptor signaling pathway and Jak-STAT signaling pathway. To investigate the noise-induced expression changes of these genes, we exposed the animals to a broadband noise at 120 dB SPL for 1 h, and collected the tissues of the organ of Corti at 1 and 4 d post-noise exposure. At 1 d post-noise exposure, we identified 21 genes that showed the fold changes > 1.5, seven of which showed statistically-significant changes with six genes being upregulated (CD14, Ddx58, Irf7, Stat1, Myd88 and Stat3) and one gene being downregualted (Il17a). At 4 day after the noise exposure, 22 genes showed expression changes > 1.5 folds. However, only one gene showed a statistically significant expression change (Ifngr1), suggesting that the innate response is more active in the early phase of cochlear pathogenesis. CD14 antigen has previously been found to participate in the innate response via the Myd88 dependent pathway. We found strong immunoreactivity of CD14 protein in the cytoplasm of inner hair cells as well as in certain Hensen cells in the basal region of the cochlea. Together, these results suggest that the components of the innate immune system are involved in acoustic trauma. Understanding of the functional roles of these innate components may provide novel targets for therapeutic intervention against noise-induced cochlear damage.

COMBINATORIAL REGULATION OF HAIR CELL INDUCTION BY CO-TRANSFECTION OF ATOH1 AND TRANSCRIPTION FACTORS

***RYOUKICHI IKEDA*[1,2], *KWANG PAK*[1,2], *EDUARDO CHAVEZ*[1,2], *ALLEN RYAN*[1,2]**

[1]Department of Surgery, UCSD, [2]VA Medical Center, San Diego

Introduction: Overexpression of the basic helix-loop-helix transcription factor (TF) Atoh1 has been shown to induce the transformation of supporting cells in the organ of Corti into hair cells (HCs). Evaluating potential regulatory region of the pou4f3 gene, a likely target of Atoh1 in HCs, we identified a cluster of binding sites for Atoh1 and a number of other TFs, a feature that was highly conserved across a widely separated mammalian species. We previously tested three of these (TCF3, GATA3 and SP1) and found that two (TCF3 and GATA3) enhanced the ability of Atoh1 to activate the gene and induce Myo7A expression in non-sensory cells.

Methods: We transfected by electroporation neonatal mouse (postnatal day 1.5) organ of Corti with plasmids encoding these TFs for which highly conserved biding sites have been identified in the pou4f3 gene and which are expressed in the developing organ of Corti: CUX1, E2F1, ETS2, ETV4, FOXM1, HES1, HES5, GABPA, GATA1, GATA2, MAX, MAZ, MEIS, NERF, NFE2, NMYC, PATZ1 and USF2.

Results: Co-transfection of hATOH1 with hETV4, hNMYC or hETS2 produced significantly more pou4f3/GFP+ and myosin7A+ cells in the GER than hATOH1 alone ($p < 0.05$). Co-transfection of hATOH1 with hHES1, hHES5 or hNEUROD1 produced significantly less pou4f3/GFP+ and myosin7A+ cells in the GER than hATOH1 alone ($p < 0.05$).

Conclusion: These results suggest that Atoh1 acts in concert with a subset of other TFs that find that find to adjacent DNA element to control the expression of the pou4f3 gene, and more generally to induce a HC phenotype. These TFs could be used to enhance HC regeneration.

THE ROLE OF PI3K SIGNALING PATHWAY IN HAIR CELL REGENERATION

AZADEH JADALI, KELVIN Y. KWAN

Department of Cell Biology & Neuroscience, Rutgers Stem Cell Research Center, Rutgers University, Piscataway, NJ, USA

The inner ear is responsible for our ability to hear and balance. Hair cells within the cochlea are the mechanosensory cells that convert sound to neural signals. A major cause of sensorineural hearing loss is the loss of hair cells. In the mature mammalian cochlea, one approach for regeneration is to re-initiate proliferation in terminally differentiated cells and guide them towards a neurosensory precursor fate to prime them for differentiation. To generate a cellular model of inner ear regeneration, we have developed an induced multipotent otic progenitor (iMOP) cell line using otic neurosensory precursors. These iMOP cells continue to proliferate, maintain the molecular signature of undifferentiated neurosensory precursors and have the ability to differentiate. To identify signaling pathways that can be repurposed to promote auditory regeneration, we compared RNA-Seq data from iMOP cells that are rapidly dividing and post-mitotic differentiating cells. Using pathway and network analysis, we identified high-confidence candidate signaling pathways involved in proliferation. To validate the signaling pathways, we used small molecules to perturb signaling and determined the effects on proliferation. Blocking PI3K signaling using the small molecule LY294002, results in a dose dependent inhibition of proliferation and cell survival in vitro. To extend these findings in vivo, we are activating the PI3K signaling pathway in supporting cells using GFAP-Cre PTEN conditional knockout mice to determine if the PI3K pathway can be repurposed for cochlear regeneration.

MECHANICAL MEASUREMENTS SUGGEST SPECIES-DEPENDENT DIFFERENCES IN THE FUNCTION AND MOLECULAR COMPOSITION OF HORIZONTAL TOP CONNECTORS

K. DOMENICA KARAVITAKI, PAUL D. NIKSCH, DAVID P. COREY

Harvard Medical School, Boston, MA, USA

Horizontal top connectors (HTC) join adjacent stereocilia near their tips and, in bullfrog saccule hair cells, have been shown to confer coherent motion to hair cell stereocilia via sliding adhesion (Karavitaki and Corey, 2010). Such coherent motion puts all transduction channels of a hair cell mechanically in parallel, an arrangement that allows all channels to get essentially the same stimulus and may enhance amplification in the inner ear. Is this true for cochlear hair bundles? In cochlear inner and outer hair cells (IHCs and OHCs), the mature complement of HTC is established by postnatal day 12 (P12); they extend between adjacent stereocilia of both rows and columns (Goodyear et al., 2005). We therefore expect, similar to the bullfrog saccule, that bundle cohesion should be maintained in both directions. We cultured organs of Corti from P4 to P12 gerbils. A stiff probe with a blunt, ~0.5-μm tip was used to push IHC and OHC stereocilia in the excitatory direction. Stimuli were slow sinusoids of 120, 240, and 480 nm, corresponding (depending on bundle height) to 1-14 degrees. Stimuli were delivered at multiple locations along the tallest stereociliary row, and along the stereociliary height. Displacements of individual stereocilia were recorded with a CCD camera and analyzed with cross-correlation methods (Karavitaki and Corey, 2010). Contrary to our expectation, our data show that lateral coupling in both IHC and OHC stereocilia of the tallest raw is weak. Movement in the excitatory direction propagated laterally by only a few stereocilia and the bundle did not move coherently. This was true for all developmental stages, all cochlear turns and all stimulus positions and amplitudes tested. These findings suggest that the function of the HTC in the cochlea is different from those in bullfrog hair cells. Other investigators have shown morphological differences in HTC across species: in the cochlea HTC have been described as a zipper-like lattice (for example see Tsuprun and Santi, 1998; Goodyear et al., 2005); in the frog as amorphous mats (for example see Jacobs and Hudspeth, 1990; Nagel et al., 1991). Together these observations suggest species-dependent differences in the molecular composition of horizontal top connectors. Weak lateral coupling between stereocilia of mammalian bundles also raises concern for current stimulus methods. These involve glass probes that are often small compared to the bundle width. Our data suggest that only stereocilia in contact with the probe will be stimulated, and delivery of the stimulus to the remaining stereocilia will be weak and therefore not homogeneous. To mimic the simultaneous and equal OHC stimulus delivered by the overlying tectorial membrane to stereocilia of the tallest row in vivo, better stimulation methods must be developed.

GENERATION AND REGENERATION OF HAIR CELLS IN THE POSTNATAL MOUSE ORGAN OF CORTI

***PIN-YI KO*[1], *KYUHEE HAN*[1], *RENJIE CHAI*[1], *KAVITA CHALASANI*[1], *ALAN G. CHENG*[1]**

[1]*Department of Otolaryngology-HNS, Stanford University School of Medicine, Stanford, CA, USA*

In the developing mouse cochlea, cell proliferation in the prosensory region terminates on embryonic day 12. The organ of Corti remains mitotically quiescent through late embryonic and early postnatal periods. However, independent studies have shown that postnatal supporting cells are capable of differentiating into hair cells both *in vitro* and *in vivo.* The current study is designed to characterize the time course of hair cell addition and its correlation to the regenerative capacity of the postnatal mouse cochlea.

To characterize hair cell addition, myosin7a-positive cells were manually counted in cochleae harvested from wild type mice at several time points: postnatal days (P) 1, 6, 10, and 21. From P1 to P6, there was a 14% increase in myosin7a-positive hair cells (N=5-7, $p<0.001$), including an increase in outer hair cells, inner hair cells and cochlear length of 15% ($p<0.001$), 11% ($p<0.01$), and 14% ($p<0.01$), respectively. Compared to P6, there was no significant increase in hair cells or cochlear length observed at P10 and P21 (N=3-4, $p=0.14$). We also quantified hair cells using a separate hair cell marker (Atoh1), and found no significant difference between Atoh1- and myosin7a-positive cell counts at P1 (N=6, $p=0.38$).

To determine the developing cochlea's capacity to regenerate hair cells, we selectively ablated hair cells using the Pou4f3-DTR transgenic mice, where human diphtheria toxin receptors (DTR) are expressed in hair cells. After hair cell ablation via systemic diphtheria toxin injection at P1, progressive loss of myosin7a-positive hair cells was observed with maximum loss first noted at P5, and hair cell survival limited to 26.1%, 30.1%, and 37.0% of age-matched controls at the apical, middle, and basal turns, respectively (N=8). In the apical turn only, there was a temporary recovery of hair cells followed by decline, with hair cells quantified as 51.1% at P7, 11.9% at P30, and 7.9% at P60 (N=4-10, $p<0.001$). In contrast, when hair cell ablation was induced at P6, hair cells at P10 were quantified as 24.9%, 24%, and 20.6% at the apical, middle, and basal turns, respectively; and at P15 0.1%, 0%, and 0%, demonstrating that there was progressive hair cell loss without any evidence of temporary recovery (N=4-10).

Together, these results lead us to conclude that a critical window exists in the neonatal when hair cell addition continues and hair cell regeneration is possible. Regeneration was the most robust in the apex, but modest in number, and regenerated hair cells were likely short-lived.

LACK OF CLASSICAL MHC1 GENES CAUSES LOSS IN HIGH FREQUENCY HEARING BUT DOES NOT DISRUPT COCHLEAR SYNAPSE MATURATION IN MICE

***CAROLINE LEE*[1], *MELISSA A CALTON*[1], *DIANA MENDUS*[1], *FELIX WANGSAWIHARDJA*[1], *SRIVIDYA SUNDARESAN*[1], *MIRNA MUSTAPHA*[1]**

[1]*Stanford University, Stanford, CA, USA*

Early cochlear development is marked by exuberant outgrowth of axons that innervate multiple targets. Important for the establishment of mature cochlear neural circuits is the pruning of inappropriate axons and synapse connections. Such synaptic refinement also occurs in the central nervous system and recently, genes ordinarily associated with immune and inflammatory processes have been shown to play non-immune roles in synaptic pruning in the brain. These molecules include the major histocompatibility complex class 1 (MHC1) genes, H2-K^b and H2-D^b. Since the factors involved in synaptic refinement in the cochlea are not well understood, we investigated whether MHCI genes K^b and D^b may be involved in this process. These immune genes are upregulated during the period of cochlear functional development (Lu et al 2011) making them very attractive candidates for our study. We characterized mice lacking both MHC1 genes H2-K^b and H2-D^b (termed K^bD^b-/-) for auditory function and synaptic patterning. Our data were collected from postnatal day 15 (P15) and P29. These ages were chosen to establish morphology after completion of synaptic refinement and synaptogenesis in both inner hair cells (IHCs) and outer hair cells (OHCs). Immunostaining with pre- and postsynaptic markers on whole mount cochlear tissue followed by confocal and Volocity 3DImage analysis suggests that afferent synapse pruning occurs normally beneath both IHCs and OHCs of K^bD^b-/- mice as compared to wild type controls. Patterning of afferent fibers type I and type II that innervate IHCs and OHCs respectively, are normal as well. In addition to afferent fibers, we analyzed efferent synapse and fiber patterns and these appeared unaltered in K^bD^b-/- as compared to control mice. Auditory brain stem recordings of hearing function show that at P29, K^bD^b-/- mice do not have significantly different thresholds at low and middle frequencies (8kHz and 16kHz) but interestingly, K^bD^b-/- mice have elevated thresholds only at high frequency (32kHz) at P29 and 2 months of age compared to their wild type controls. The difference in thresholds at the higher frequency suggests an alteration in OHC function in the basal turn of the cochlea. To confirm this, we then tested the distortion product otoacoustic emission in K^bD^b-/- mice and observed a significant difference compared to controls at 23kHz and 32kHz. Taken together, these results indicate that while MHC1 genes, H2-K^b and H2-D^b are not primary players in cochlear synaptic pruning, lack of these genes results in loss in hearing in the high frequency range. Further studies will be necessary to explain the increased threshold at high frequencies in K^bD^b-/- mice and to identify the other candidate factors that may be involved in the critical process of synaptic refinement in the cochlea.

CONTRIBUTIONS OF PTPRQ AND PITPNM1 TO THE PHENOTYPE OF MICE CARRYING A MUTATION IN MIR-96

***MORAG LEWIS*[1], *JING CHEN*[1], *FRANCESCA CARLISLE*[2], *JENNY HILTON*[2], *SELINA PEARSON*[2], *ANDREEA HUMA*[2] *AND KAREN P. STEEL*[1,2]**

1. Wolfson Centre for Age-Related Diseases, King's College London, London, UK SE1 1UL.2. Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK CB10 1SA.

The diminuendo mouse carries a single base change mutation in the microRNA miR-96. Heterozygotes and homozygotes are deaf, with homozygotes also exhibiting vestibular dysfunction. Homozygote hair cells are abnormal from an early age and have degenerated by P28, and heterozygote hair cells, while still present at P28, have disorganised and malformed stereociliar bundles. The physiological and morphological maturation of mutant hair cells appears to stall at an early stage, before the onset of degeneration (Kuhn *et al*, 2011). Microarrays comparing homozygote and wildtype littermates identifed multiple affected genes, of which five were particularly notable; *Slc26a5* (Prestin), *Ocm*, *Ptprq*, *Gfi1* and *Pitpnm1*. All five are specifically and strongly expressed in hair cells and are markedly downregulated in diminuendo homozygote hair cells. However, the contribution made to the diminuendo phenotype by the downregulation of each gene is unknown.

We have studied two of these genes in detail; *Pitpnm1* and *Ptprq*. Pitpnm1 was not previously known to be present in the inner ear, so we examined its expression at several ages pre- and postnatally. It is specifically expressed in the inner hair cells from late embryonic stages until adulthood, and transiently in the outer hair cells during early postnatal stages. Mice null for the gene, however, display no hearing defects. It is possible that the lack of Pitpnm1 is compensated for by Pitpnm2 and/or Pitpnm3. Despite its specific expression, it is unlikely that the reduced expression of *Pitpnm1* seen in diminuendo homozygotes contributes to the diminuendo phenotype.

Ptprq has been previously shown to result in deafness and hair cell degeneration in mice when mutated (Goodyear *et al*, 2003). We used scanning electron microscopy to compare hair cells from mice lacking Ptprq to diminuendo heterozygote and homozygote hair cells at P4. The developmental retardation and stalling seen in Ptprq null hair cells appears intermediate between that observed in diminuendo heterozygotes and homozygotes. A microarray carried out on Ptprq homozygote mice identified several genes which are also perturbed in diminuendo homozygotes. The reduction in Ptprq observed in diminuendo mice appears to be a major contributor to the phenotype, although it does not account for all of the observations.

DNA DAMAGE RESPONSE OF CELL CYCLE REACTIVATED INNER EAR SUPPORTING CELLS

***MAARJA LAOS*[1], *JEFFREY H. ALBRECHT*[2], *ULLA PIRVOLA*[1]**

[1]*Institute of Biotechnology, University of Helsinki, Helsinki, Finland;* [2]*Division of Gastroenterology, Hennepin County Medical Center, Minneapolis, Minnesota, United States of America*

Supporting cells (SCs) of the mammalian inner ear show a prominent decline in proliferative capacity at early postnatal life, an event that coincides with their morphological maturation. Our earlier data showed that a large part of the cell cycle reactivated adult utricular SCs are arrested in G2 phase and fail to proceed into mitosis. Thus, impaired cell cycle progression may form a significant barrier for the expansion of adult SCs. We found that DNA double-strand breaks (DSBs), a detrimental form of DNA damage, accumulate in SCs with unscheduled DNA replication. We suggested that DSB formation is linked with the observed cell cycle arrest (Loponen et al., 2011). Generally, cells respond to DNA damage by activation of cell cycle checkpoints, resulting in transient cell cycle arrest and DNA repair. In case of severe DNA lesions, the outcome is permanent arrest, linked with genomic instability, or apoptosis. Very little is known about DNA damage signaling and DNA repair in differentiated cells that have been stimulated to re-enter the cell cycle, an event often included in reprogramming approaches. In the inner ear, this understanding may be important if the use of SCs as a platform for hair cell regeneration is realized.

By comparing early postnatal and adult cells, we studied the age-dependency regarding cell cycle progression and the activation of the DNA damage response (DDR) pathway and DNA repair. We used adenovirally-mediated cyclin D1 expression to trigger cell cycle re-entry selectively in SCs in explant cultures prepared from P6 cochleas and utricles, and from adult utricles. This approach was combined with pharmacological and genetic suppression of components of the DDR pathway.

Early postnatal utricular SCs and cochlear Deiters' cells responded by hyperproliferation to ectopic cyclin D1 expression. Clonal expansion was evident in these cultures. In contrast, despite S-phase entry, most adult utricular SCs did not progress through the cell cycle. DSBs, as detected by nuclear foci of phosphorylated H2AX (γH2AX), accumulated in cell cycle reactivated SCs of both ages. However, early postnatal SCs, but not adult SCs, showed resolution of γH2AX foci, suggestive of successful DNA repair. Compared to ectopic cyclin D1-induced proliferation, additional abrogation of the DDR pathway robustly augmented proliferation in young SCs, leading to a malignant cell-like phenotype. In contrast, this manipulation failed to increase proliferative activity in adult SCs. Rather, these cells showed a death-prone phenotype.

Our results suggest that upon cell cycle reactivation, early postnatal and adult SCs can respond to DSBs by activating components of the DDR pathway. DSBs can be repaired early postnatally, whereas adult SCs show decreased DNA repair capacity, consistent with the arrest of these cells upon forced cell cycle re-entry. We speculate that there exist differentiation-associated mechanisms, possibly linked to the chromatin status, that modulate DDR and direct the behavior and fate of cell cycle reactivated SCs.

A ZEBRAFISH MUTAGENESIS SCREEN FOR SENSORY HAIR CELL FORMATION, FUNCTION AND REGENERATION

***MARK E. LUSH*[1], *MEGAN SMITH*[2] *AND TATJANA PIOTROWSKI*[1,2].**

[1]Stowers Institute for Medical Research, Kansas City, MO 64110. [2]University of Utah, Department of Neurobiology and Anatomy, Salt Lake City, UT 84132.

The zebrafish mechanosensory lateral line consists of neuromasts that contain sensory hair cells, support cells and stem cells spaced along the head and trunk. The sensory hair cells of the lateral line are analogous to sensory hair cells in the inner ear of mammals. Importantly zebrafish hair cells are able to regenerate after damage unlike their mammalian counterparts. The genes and signaling pathways required for zebrafish hair cell regeneration are currently unknown. Our laboratory is performing an ENU based forward genetic screen to identify novel genes required for hair cell formation and regeneration. We have identified one mutant, *kt28*, which fails to form hair cells during development. *kt28* also exhibits retina defects and shows degeneration of the lateral line axon. A second mutant, *kt51HC*, forms hair cells with stereocilia but they fail to take up the mechanosensitive dye DASPEI, indicating a defect in mechanotransduction. Both *kt28* and *kt51HC* mutants exhibit a circling swimming behavior indicative of vestibular dysfunction. Additionally we have identified one mutant termed *godot* that initially forms hair cells but fails to regenerate appropriate numbers after neomycin induced hair cell death. *godot* neuromasts express similar levels of *sox2* and *atoh1* when compared to controls but show reduced expression of the more differentiated markers *pou4f3* and *gfi* after neomycin. Neuromast support cells do proliferate in *godot* mutants after neomycin treatment. These results suggest that either differentiation or survival of newly formed hair cells is reduced in *godot* mutants during regeneration. We are currently working to identify the genes affected in all three mutants. This unbiased approach to identify genes required for sensory hair cell regeneration in zebrafish will lead to a better understanding of the signaling pathways involved in regeneration and guide the development of potential therapeutic targets for people.

ANALYSIS OF CLAUDIN GENE EXPRESSION AS A CAUSAL BASIS OF DIFFERENTIAL NOISE RESISTANCE IN 129S6/SVEVTAC AND CBA/CAJ MICE

***AYAKA J. IWATA*[1,2], *JIN LI*[1,2], *BRUCE L TEMPEL*[1,2,3]**

[1]Department of Otolaryngology-Head and Neck Surgery, University of Washington, Seattle, Washington, U.S.A. [2]V.M. Bloedel Hearing Research Center, University of Washington, Seattle, Washington, U.S.A. [3]Department of Pharmacology, University of Washington, Seattle, Washington, U.S.A

Inter-subject variability in noise-induced hearing loss suggests that genetic contributions account for differences in susceptibility to acoustic injury. By comparing the remarkably noise resistant (NR) mouse strain 129S6/SvEvTac (S6) and the normally noise sensitive strain CBA/CaJ (CB), the Tempel Lab identified four genetic loci that contribute to the NR phenotype. At one locus, the gene encoding claudin-6 (*Cldn6*) may contribute to the NR phenotype.

In the organ of Corti (OC), claudin-6 forms intercellular junctions between hair cells and support cells on the reticular lamina, thereby constituting a major diffusion barrier and lending it structural support. Gene microarrays previously showed that *Cldn6* has decreased expression in the cochleae of S6 compared to CB after noise exposure. We hypothesize that the decreased expression levels of *Cldn6* in S6 make the reticular lamina more compliant, thereby protecting it from rupture by intense sound vibrations and rendering S6 more NR. To further validate this differential expression, we isolated cochleae after noise exposure and measured their *Cldn6* mRNA transcript levels using RT-qPCR. As expected, the mRNA levels are lower in S6 compared to CB.

As *Cldn6* has identical protein-coding regions but different 5' non-coding regions between S6 and CB, we hypothesized that the non-coding sequences of *Cldn6* are the underlying cause of its differential expression. To test this, we compared luciferase expression of these promoter regions in OC cell line OC-1. As expected, the S6 expression levels were lower compared to CB expression levels.

By analyzing the differences in transcriptional regulation of *Cldn6* in NR and normal hearing mice, we aim to better understand the molecular basis for the NR phenotype in S6.

EXPRESSION OF NEBULIN, AN ACTIN BUNDLING PROTEIN, IN THE HAIR CELL STRIATED ORGANELLE, STEREOCILIA AND CUTICULAR PLATE

***ANNA LYSAKOWSKI*[1], *ROBSTEIN L. CHIDAVAENZI*[1], *PAULINA STRUK*[1], *MARIA TERESA REQUENA NAVARRO*[2], *JOSE ANTONIO LÓPEZ-ESCÁMEZ*[2]**

[1]Department of Anatomy and Cell Biology, University of Illinois at Chicago, Chicago, IL, USA, [2]Otology and Neurotology Group CTS495, Human DNA Variability Department, Centro de Genómica e Investigación Oncológica, Pfizer/Universidad de Granada/Junta de Andalucía (GENYO), Spain.

Nebulin, at 600-900kDa, is the largest known actin-bundling protein. Each molecule has the capacity to bind 200 actin monomers. It exists in different splice variants, hence the precise molecular weight in any given tissue can only be assigned upon identification of the respective splice isoform expressed. Extensively studied in, and shown to span the entire length of, vertebrate skeletal muscle thin filaments, nebulin has been investigated for its role in determining the length of actin filaments and in regulating actin-myosin interactions in a calcium-calmodulin dependent manner, hence an integral role in various cytoskeletal assemblages is implied. On that basis, we sought to determine its presence and distribution in mechanically sensitive inner ear auditory and vestibular hair cells. Using immunohistochemistry and western blotting, we report the presence of nebulin in the vertebrate inner ear. Western blots show a single band of high molecular weight in vestibular and cochlear tissue. In the vestibular periphery, confocal microscopy shows nebulin in central calyx afferents and in the cuticular plate and striated organelle of adult type I and type II hair cells. In the early postnatal rat (P0-P6), nebulin also appears to be expressed in the stereocilia (but not kinocilia), and cytoplasm of central, but not peripheral, hair cells of both types. In the cochlea, nebulin is more intense in the cuticular plate of inner, compared to outer, hair cells. In outer hair cells, it is also present in the basolateral cell membranes. We intend to further characterize the observed labeling pattern and discuss the probable role that nebulin that may be playing in inner ear sensory epithelia.

GELSOLIN PLAYS A ROLE IN THE ACTIN POLYMERISATION COMPLEX OF HAIR CELL STEREOCILIA AND IS CRITICAL FOR MAINTAINING THEIR NORMAL FUNCTIONALITY

***PHILOMENA MBURU*[1], *ROSARIO ROMERO*[1], *HELEN HILTON*[1], *ANDREW PARKER*[1], *STUART TOWNSEND*[1], *YOSHIAKI KIKKAWA*[2], *STEPHANIE KUHN*[3], *JENNIFER OLT*[3], *STUART JOHNSON*[3], *WALTER MARCOTTI*[3] *AND STEVE BROWN*[1]**

1. Medical Research Council Mammalian Genetics Unit, Harwell Science and Innovation Campus, Oxfordshire, OX110RD, U.K. 2. Mammalian Genetics Project Tokyo Metropolitan Institute of Medical Science. 2-1-6 Kami-kitazawa, Setagaya-ku, Tokyo 156-8506, Japan. 3. Department of Biomedical Science, University of Sheffield, Sheffield, S10 2TN United Kingdom

A complex of proteins scaffolded by the PDZ protein, whirlin, reside at the stereocilia tip and are critical for stereocilia development and elongation. We have shown that in outer hair cells (OHCs) whirlin is part of a larger complex involving the MAGUK protein, p55, and protein 4.1R. Whirlin interacts with p55 which is expressed exclusively in outer hair cells (OHC) in both the long stereocilia that make up the stereocilia bundle proper as well as surrounding shorter microvilli that will eventually regress. In erythrocytes, p55 forms a tripartite complex with protein 4.1R and glycophorin C promoting the assembly of actin filaments and the interaction of whirlin with p55 indicates that it plays a similar role in OHC stereocilia. We show that the actin capping and severing protein, gelsolin, is a part of the whirlin complex. Gelsolin is detected in OHC where it localizes to the the tips of the shorter rows but not to the longest row of stereocilia and the pattern of localisation at the apical hair cell surface is strikingly similar to p55. Like p55, gelsolin is ablated in the *whirler* and *shaker2* mutants. Moreover, in a gelsolin mutant, stereocilia in the apex of the cochlea become long and straggly indicating defects in the regulation of stereocilia elongation. The identification of gelsolin provides an important link between the whirlin scaffolding protein complex involved in stereocilia elongation and a known actin regulatory molecule. We used single-cell electrophysiological recording to investigate the role of gelsolin in immature cochlear outer hair cells. We found that in the absence of gelsolin mechanoelectrical transduction was normal in pre-hearing outer hair cells, indicating that this actin binding protein is not required for initial growth of hair-cell stereocilia but critical in maintaining the normal functionality in adult cells.

SPECTRIN BV, A SPECTRIN INTERACTING WITH USHER 1 PROTEINS, DISPLAYS DIFFERENT DISTRIBUTION PATTERNS IN THE VISUAL AND AUDITORY SENSORY CELLS

***VINCENT MICHEL*[1], *SAMANTHA PAPAL*[1], *MATTEO CORTESE*[1], *CHRISTINE PETIT*[1,2], *AND AZIZ EL-AMRAOUI*[1]**

[1]Institut Pasteur, Génétique et Physiologie de l'Audition, INSERM UMRS587; UPMC Paris 06; 25 rue du Dr Roux, 75015 Paris, France; [2] Collège de France, 75005 Paris, France

Usher syndrome (USH) is the first cause of inherited dual hearing and visual sensory impairment. The Usher syndrome type I proteins have a key role in the spatial organization and functioning of the hair bundle, the sound-receptive structure. We identified spectrin βV, the mammalian non-classical β spectrin, as a myosin VIIa-interacting partner in photoreceptor cells, and showed that spectrin βV also associates with sans and harmonin.

In the retina, spectrin βV displays a polarized distribution from the Golgi apparatus to the base of the outer segment, the light sensitive compartment. Also, consistent with its distribution along the connecting cilium, spectrin βV binds to rhodopsin and to the microtubule-based motor proteins, kinesin II and dynein. We therefore suggest that spectrin βV couples some USH1 proteins, opsin and other phototransduction proteins to both actin- and microtubule-based motors, thereby contributing to their transport towards the photoreceptor outer disks. A failure of the spectrin βV-mediated coupling between myosin VIIa and opsin molecules probably accounts for the opsin transport delay in myosin VIIa-deficient mice.

In the inner ear, spectrin βV, which is absent from the hair bundle, displays a distinct distribution pattern according to the type of hair cells. In the auditory hair cells, this spectrin, which forms heteromers of αII and βV subunits, was abundant along the plasma membrane of the outer hair cells, contributing to the spectrin-based cytoskeleton that provides these cells with the flexibility required for electromotility. A limited targeting to the plasma membrane was observed for αII and βV subunits in the inner hair cells. By contrast, analysis in the vestibular system revealed that, unlike in outer hair cells, no co-localization was observed with αII spectrin in vestibular hair cells, where spectrin βV displayed a punctate cytoplasmic distribution pattern that overlaps with Golgi-labeled structures. Interestingly, in the xenopus inner ear, the protein was detected also in the hair cells, but essentially in the apical region of the cell, just above the cuticular plate. This spectrin βV labeling in frog is reminiscent of the apical membrane distribution of the drosophila β-Heavy spectrin in epithelial cells. Together, these data illustrate how during evolution, the function of this protein has changed from a protein dedicated to protein/membrane trafficking, to a protein targeted to the plasma membrane of outer hair cells, supporting membrane potential-driven cell length changes.

CHARACTERISATION OF CONTRASTIVE PHENOTYPES OF HEARING IN JAPANESE WILD MICE-DERIVED INBRED STRAINS MSM/MS AND JF1/MS

***YUKI MIYASAKA*[1, 2], *KAZUHIRO OKUMURA*[1, 3], *YASUHIRO OHSHIBA*[1, 2], *SARI SUZUKI*[1, 4], *HIROMICHI YONEKAWA*[1], *KAZUO MORIWAKI*[5], *TOSHIHIKO SHIROISHI*[6], *TSUYOSHI KOIDE*[6], *YOSHIAKI KIKKAWA*[1]**

[1]Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan, [2]Niigata University, Niigata, Japan, [3]Chiba Cancer Center Research Institute, Chiba, Japan, [4]Tokyo University of Agriculture, Abashiri, Japan, [5]RIKEN BioResource Center, Tsukuba, Japan, [6]National Institute of Genetics, Mishima, Japan

MSM/Ms and JF1/Ms are inbred mouse strains established from Japanese wild mice, Mus musculus molossinus. Both strains show unique pathological phenotypes because their genetic backgrounds differ from those of classical laboratory strains. To characterize the phenotypes for hearing in these strains, we investigated hearing acuity and cochlear pathology. Measurement of auditory brainstem response (ABR) thresholds showed that MSM/Ms mice remain resistant to age-related hearing loss until 20 months of age. In addition, the stereocilia of MSM/Ms mice at least 15 months of age display V- and staircase-shaped configurations on the outer hair cells and normal spiral ganglion cell density. These results indicate that the MSM/Ms mouse is a good model for pathological study and useful as partner for genetic studies aiming to identify loci associated with hearing loss in inbred mice. By contrast, most of the JF1/Ms mice, characterized by an old piebald mutant with white spots on a black coat and black eyes caused by an endothelin receptor B (*Ednrb*) mutation, expressed profound congenital hearing loss. However, the ABR thresholds of JF1 mice varied among individuals. Approximately 70% of JF1 mice showed no response to auditory stimuli up to high acoustic pressure (<70 dB). The remaining mice showed evident response to auditory stimuli (31-69 dB). In particular, the ABR thresholds of approximately 2% of the mice indicated normal hearing. Histological analysis in JF1 mice with hearing loss showed that morphological abnormality of the melanocytes coincides with defects of the stria vascularis (SV). Notably, the SV of mice with hearing loss was extremely thin and lacked intermediate cells/melanocytes, whereas mice with normal hearing displayed intermediate cells similar to those of control mice. These results suggest that individual variation in hearing among JF1 mice depends primarily on dysgenesis or the survival of melanocytes in the SV.

NOISE-INDUCED HEARING LOSS IS PREVENTED BY METHYLENE BLUE

***ALFRED NUTTALL*[1], *IRINA OMELCHENKO*[1], *SARAH FOSTER*[1] *AND TERESA WILSON*[1]**

[1]Oregon Hearing Research Center (NRC04), Department of Otolaryngology, Oregon Health & Science University, 3181 SW Sam Jackson Park Road, NRC04, Portland, OR 97239-3098, USA

Rationale: Noise-induced hearing loss (NIHL) is the second most common form of sensorineural hearing deficit and is characterized by loss of high-frequency perception usually brought about by occupational or recreational exposure to loud sounds. The direct mechanical disruption of auditory structures, generation of reactive oxygen and nitrogen species, and glutamate excitotoxicity are several of the underlying mechanisms of noise-induced damage to tissues in the inner ear that leads to NIHL. The present study was performed to determine whether methylene blue (MB), a powerful synthetic antioxidant compound, can provide protection against noise-induced hearing loss. Originally synthesized as a textile dye, MB is used treatment for many human ailments including methemoglobinemia, malaria and septic shock. MB is highly cell permeable and readily crosses the blood-brain barrier. In low doses, MB acts as an electron cycler in the mitochondrial electron transport chain facilitating oxidative respiration and greatly reducing superoxide production. Additionally, MB has been shown to directly inhibit the activity of both inducible and constitutive forms of nitric oxide synthase.

Methods: Male CBA/CaJ (8-10 weeks old) mice (n=5/group) were injected with methylene blue (0.5 mg/kg IP) or water at 24 and 1 hr prior to exposure to the moderately damaging noise levels of 110 dB SPL, 4-48 kHz for 3 hrs. At 1 hour, 1 week and 2 weeks, Auditory Brain Stem Response (ABR) and Distortion Product Otoacoustic Emission (DPOAE) levels were measured. The percent of auditory outer hair cell loss was determined and inner hair cell synaptic ribbons were counted.

Results: The results revealed that prior treatment with MB provided significant protection against noise induced-hearing loss and damage to tissues of the cochlea.

Conclusion: Here, we provide evidence that pre-treatment with low dose MB can prevent hearing loss due to loud sound exposure. The mechanism behind this protection is likely the combination of the antioxidant properties and reduced glutamate excitotoxicity provided by MB that reduced loud sound induced damage to the tissues of the inner ear.

DNA DAMAGE REPAIR IN THE MAMMALIAN ORGAN OF CORTI

SUM-YAN NG[1], ROBERT RAINEY[2], NEIL SEGIL[1,2]

1. University of Southern California, Los Angeles, CA, USA
2. House Research Institute, Los Angeles, CA

Human sensory hair cells are hypersensitive to chemotherapy treatment with cisplatin. Postmortem human studies, as well as animal studies, have linked cisplatin treatment to sensory hair cell death. The ototoxic side effects of cisplatin reduce quality of life for many cancer survivors, especially children.

The cytotoxicity of cisplatin is primarily ascribed to its interaction with DNA, forming DNA adducts or cross-linking DNA. Cells eliminate cisplatin-DNA adducts mainly through the nucleotide excision repair (NER) pathway. NER can be divided into at least 2 sub-pathways: transcription-coupled (TC-NER) and global genome (GG-NER) repair. These pathways rely on common, as well as distinct molecular elements, to bring about repair.

Mutations in certain genes involved in the NER pathway lead to progressive hearing loss in humans. Cockayne syndrome (CS) patients with mutations in the Csa or Csb gene, which affects TC-NER, often experience progressive hearing loss. Interestingly, hearing loss is rarely reported in Xeroderma pigmentosum-complementary group C (XPC) patients, whose mutation only appears to affect the GG-NER pathway, but leaves TC-NER intact. The differential susceptibility to hearing loss in TC-NER versus GG-NER mutations suggests that sensory hair cells depend on TC-NER for nuclear homeostasis and survival.

In spite of the human mutation evidence, the relationship between DNA damage repair and hearing loss is still largely uncharacterized. Our preliminary data show that in cochlear organ cultures from perinatal mice, hair cells carrying TC-NER mutations are differentially hypersensitive to cisplatin, relative to those with mutation in GG-NER. At a sub-lethal cisplatin dosage, TC-NER deficient hair cells are unable to remove cisplatin-DNA adducts effectively, compared to either wild-type hair cells or hair cells deficient in GG-NER. Our results suggest that sensory hair cells in the mammalian inner ear depend differentially on TC-NER for survival following DNA damage caused by the chemotherapeutic agent cisplatin.

Sponsored by a travel award from:
march of dimes

IDENTIFICATION OF NOVEL SUSCEPTIBILITY LOCI LEADING TO EARLY-ONSET HIGH-FREQUENCY HEARING LOSS IN BALB/CA MICE

YASUHIRO OHSHIBA[1, 2], SARI SUZUKI[1, 3], YO OBARA[1, 4], YUKI MIYASAKA[1, 2], SYUMPEI P YASUDA[1], YUTA SEKI[1], KUNIE MATSUOKA[1], YOSHIAKI KIKKAWA[1]

[1]Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan, [2]Niigata University, Niigata, Japan, [3]Tokyo University of Agriculture, Abashiri, Japan, [4]University of Tsukuba, Tsukuba, Japan

Laboratory mouse strains offer important advantages as bioresources for hearing research. Many inbred mouse strains exhibit variable hearing capability and onset of age-related hearing loss (AHL). We found that common inbred BALB/cA mice express high-frequency specific hearing loss (HFHL) in tone-pip stimuli at 20 kHz and higher at 4 weeks of age compared with C57BL/6J mice, which are well-known models of AHL. In particular, the ABR thresholds at 32 and 36 kHz in the BALB/cA are about 20 dB SPL higher than those in C57BL/6J (B6) mice. In addition, the HFHL of BALB mice progressed to severe hearing loss by 12 weeks. We assessed in the F_1 crossed C57BL/6J and MSM/Ms with BALB/cA by analyzing ABR thresholds to 32 and 36 kHz stimuli. The ABR thresholds of (C57BL/6J x BALB/cA) F_1 and (MSM/Ms x BALB/cA) F_1 mice tested at 8 and 12 weeks of age were significantly different from those of BALB/cA mice. The ABR thresholds among the (BALB/cA x C57BL/6J) F_1 x BALB/cA backcrossed mice displayed a roughly bimodal rather than a bell-shaped normal distribution, suggesting a large contribution from 1 or a small number of loci. We identified quantitative trait loci on chromosomes 8 and 19 that significantly affected hearing. Moreover, analysis of (BALB/cA x MSM/Ms) F_1 x BALB/cA backcrossed mice demonstrated that early-onset HFHL in BALB/cA is associated with *Cdh23*^{ahl} mutation. We conclude that quantitative trait loci on chromosomes 8 and 19 are contributors to the HFHL of BALB/cA mice and that their effects depend on the *Cdh23*^{ahl} genotype in this strain.

SINGLE MOLECULE TECHNIQUE PROBES PATTERNING AND CELL FATE DECISIONS IN THE DEVELOPING MAMMALIAN INNER EAR

ANNALISA PAWLOSKY [1] ***AND ALEXANDER VAN OUDENAARDEN*** [1,2]

[1]*Massachusetts Institute of Technology, Cambridge, MA, United States of America;* [2] *Hubrecht Institute, Utrecht, The Netherlands*

We investigate how mammalian cells decide their fate during embryonic development, which can lead to complex cell-type patterns in organs. As a model we study the cochlea, a rigorously patterned structure that houses auditory hair cells. The ordered distribution and specialization of hair cells is vital for proper sensory transduction. Using single molecule fluorescence in situ hybridization of mRNA at different developmental time points, we have investigated expression of the master transcription factor *Atoh1*. Additionally we have studied the expression patterns of components of both the Notch and Wnt pathways, as they have putative roles in regulating the expression of *Atoh1*. Our results reveal novel potential functions for these candidate pathways and shed light on potential models for hair cell specification.

CHARACTERIZATION OF A NOVEL REISSNER'S MEMBRANE PROTEIN; VMO1

LINDA PETERS, BLAISE FORRESTER-GAUNTLETT, HANNAH CROSSAN, OLIVIA PATTY.

The University of Waikato, Hamilton, New Zealand

Vitelline membrane outer layer protein 1 (*vmo-1*) was shown to be exclusively expressed in the Reissner's membrane of the mouse inner ear at postnatal day 5. This gene was named based on its shared homology at the DNA level with a chicken vitelline membrane protein. In a similar functional role to Reissner's membrane, the vitelline membrane separates the yolk from the egg white and consists of two distinct layers. These two layers are discrete in terms of their morphology and biochemical composition, and appear to have separate biological functions.

Vitelline membrane outer layer protein 1 is of interest as a candidate gene involved in human hearing loss and balance disorders such as Ménières disease. This disease is thought to be caused by a build up of excess fluid in the inner ear leading to the distension or disruption of the Reissner's Membrane. Movement of fluid and electrolytes is tightly controlled by proteins in the Reissner's membrane and any alteration to these proteins could influence both hearing and balance.

The key objective of this research was to investigate the expression of vmo-1 at different developmental time points in the mouse auditory system. Protein expression of vmo-1 in the developing auditory system has not been defined. To progress our understanding of vmo-1, knowledge of the spatial and temporal pattern of gene expression, and the functions of those gene products are required. This study required bioformatics analysis, the validation of a vmo-1 antibody using western blotting and performing immunohistochemistry on mouse ear tissue. The data generated has begun unravelling the role vmo-1 has within the auditory system.

MICRO RNAS ARE INVOLVED IN THE INFLAMMATORY RESPONSE IN THE INNER EAR

ANYA RUDNICKI[1], SHAKED SHIVATZKI[1], LISA A. BEYER[2], YOHEI TAKADA[2], YEHOASH RAPHAEL[2] AND KAREN B. AVRAHAM[1]

[1]Department of Human Molecular Genetics and Biochemistry, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel; [2]Kresge Hearing Research Institute, Department of Otolaryngology – Head and Neck Surgery, University of Michigan, Ann Arbor, MI, USA

MicroRNAs (miRNA) are small non-coding RNAs that regulate gene expression through the RNA interference (RNAi) pathway. By binding to sequences in the 3 untranslated region (UTR) of genes, a miRNA can inhibit target mRNAs by translational suppression and mRNA destabilization. miRNAs play an important role in the development and regulation in the inner ear, and mutations in miRNAs lead to deafness in humans and mice. miRNAs have been shown to be involved in the inflammatory response. In this study, we propose that miR-224 and its target Ptx3 play an essential role in the innate inflammatory response in the inner ear. We show that miR-224 is expressed in mouse inner ear sensory epithelia. Ptx3, previously unknown to function in the inner ear, but a well-known regulator of innate immune response in other systems, is expressed in the mouse inner ear. Luciferase and over-expression assays demonstrate that miR-224 targets Ptx3. In an inner ear inflammation model, LPS (lipopolysaccharide) was injected into mouse inner ears, leading to an immune response. miR-224 and Ptx3 expression was increased in LSP-induced inflammation. Both miR-224 and Ptx3 promoters contain an NF-κB binding site, suggesting they are co-expressed under similar conditions. In this work, we propose that miR-224 and Ptx3 are both acting in a regulatory loop in the NF-κB pathway. In this model, the upstream transcription factor NF-κB is inducing both Ptx3 and miR-224, and miR-224 is negatively regulating Ptx3, allowing for fine-tuning regulation. We hypothesize that Ptx3 in a powerful protein in the immune cascade and needs to be subjected to tight regulation. In conclusion, we found that miR-224 directly targets Ptx3 in the NF-κB inflammatory pathway in inner ear LSP-induced inflammation. This finding sheds light on the role of miRNAs in the complex regulation of the inflammatory pathway in the inner ear.

Sponsored by a travel award from:
march of dimes

A NEW SPONTANEOUS MUTATION IN THE MOUSE MYOSIN VI GENE

YUTA SEKI[1], KUNIE MATSUOKA[1], SARI SUZUKI[1], YUKI MIYASAKA[1], YASUHIRO OSHIBA[1], KENTA WADA[2], HIROHIDE TAKEBAYASHI[4], YOSHIAKI KIKKAWA[1]

[1]Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan, [2]Tokyo University of Agriculture, Abashiri Japan, [3]Niigata University, Niigata, Japan.

We have identified a new allele of the myosin VI gene (*Myo6*), designated kumamoto shaker/waltzer (*Myo6^{ksv}*) that arose as a spontaneous, recessive mutation. Homozygous *Myo6^{ksv}* mice exhibit abnormal behavior (circling, head-tossing and hyperactivity) and congenital profound hearing loss and stereocilia morphogenesis effects caused by the fusion and huge of stereocilia on the hair cells in the inner ear. We found that the responsible mutation is a 1381G →A transition that results in lysine instead of glutamic acid, which is highly conserved of vertebrates and *C. elegans* at the motor domain of MYO6. By RT-PCR, we defined that this mutation is located at the splice donor site in exon 13 and is predicted to inactivate the MYO6 protein, as it produces a frameshifts and truncated proteins that lacks large number of amino acids in the C-terminal region by partial and incomplete splicing errors. Moreover, immunoblot analysis with an antibody that recognizes the C-terminus of MYO6 identified a reduction in MYO6 protein in the *Myo6$^{ksv/ksv}$* mutants. The results showed that the relative abundance of MYO6 is approximately 30% of the level in the inner ear of wild-type mice. We performed immunohistochemistry analysis to confirm this reduction of the MYO6 in the hair cells of *Myo6$^{ksv/ksv}$* mice. In the hair cells, MYO6 is normally localized in the cytoplasm of cochler and hair cells within the cell body, cuticular plate and pericuticular necklace, and the taper between the actin core and the plasma membrane of the stereocilia. Although we found the immunofluorescence for MYO6 in the cell body and pericuticular necklace of the hair cells in the *Myo6$^{ksv/ksv}$* mice, the immunofluorescence of MYO6 was absent from the cuticular plates and was mislocalised in the fused stereocilia bundles. Moreover, similar expression and localization of protein tyrosine phosphatase receptor type Q (PTPRQ), a predicted interaction partner with MYO6 for maintenance and construction at the base and taper of stereocilia, was observed in the hair cells of *Myo6$^{ksv/ksv}$* mice. These results suggest that the degeneration of the stereocilia in the *Myo6$^{ksv/ksv}$* mice may cause not only truncation of MYO6 protein via abnormal splicing but also loss-of function through missense mutation and/or inframe deletion of MYO6.

PERIVASCULAR MACROPHAGE-LIKE MELANOCYTE RESPONSIVENESS TO ACOUSTIC TRAUMA— A SALIENT FEATURE OF STRIAL BARRIER ASSOCIATED HEARING LOSS

***XIAORUI SHI*[1], *FEI ZHANG*[1], *MIN DAI*[2], *LINGLING NENG*[1], *JIN HUI, ZHANG*[1], *ZHONGWEI ZHI*[2], *ANDERS FRIDBERGER*[3]**

[1]Oregon Hearing Research Center, Department of Otolaryngology/Head and Neck Surgery, Oregon Health & Science University, Portland, OR USA, [2]Biophotonics and Imaging Laboratory (BAIL), University of Washington, Seattle, WA USA, [3]Karolinska Institute, Department of Clinical Science, Intervention and Technology, Stockholm, Sweden

Tissue perivascular resident macrophages (PVM/Ms), a hybrid cell type with characteristics of both macrophages and melanocytes, are critical for establishing and maintaining the endocochlear potential (EP) required for hearing. The PVM/Ms modulate expression of tight- and adherens-junction proteins in the endothelial barrier of the stria vascularis (intra-strial fluid-blood barrier) through secretion of a signaling molecule, pigment epithelium growth factor (PEDF). Here, we identify a significant link between abnormalities in PVM/Ms and endothelial barrier breakdown during acoustic trauma. We find that acoustic trauma causes activation of PVM/Ms and physical detachment from capillary walls. Concurrent with the detachment we find loosened tight junctions between endothelial cells and decreased production of tight- and adherens-junction protein, resulting in leakage of serum proteins from the damaged barrier. A key factor in the intra-strial fluid-blood barrier hyper-permeability exhibited in mice is down-regulation of PVM/M modulated PEDF production. We demonstrate that delivery of PEDF to the damaged ear ameliorates hearing loss by restoring intra-strial fluid-blood barrier integrity. PEDF up-regulates expression of tight junction-associated proteins (ZO-1 and VE-cadherin) and PVM/M stabilizing neural cell adhesion molecule (NCAM-120). These studies point to the critical role PVM/M plays in regulating intra-strial fluid-blood barrier integrity in healthy and noise-damaged ears.

ENDOTOXEMIA ENHANCES COCHLEAR UPTAKE OF AMINOGLYCOSIDES AND SUBSEQUENT COCHLEOTOXICITY

***PETER STEYGER*[1], *JAWON KOO*[1,2,3], *MEIYAN JIANG*[1], *JIANPING LIU*[1], *ZACHARY URDANG*[1], *HONGZHE LI*[1]**

[1]Oregon Hearing Research Center, Oregon Health & Science University, Portland, OR, USA; and [1,2,3]Seoul National University Bundang Hospital, [3]Seoul National University College of Medicine, Seongnam, Kyunggi-Do 463-707, Korea.

Aminoglycoside antibiotics are clinically essential to treat life-threatening bacterial sepsis. Systemic aminoglycosides rapidly enter the cochlea by crossing the strial blood-labyrinth barrier (BLB) and predominantly enter hair cells from endolymph. We used bacterial lipopolysaccharides (LPS) to induce endotoxemia - a classic experimental model of sepsis - in mice to test the hypothesis that LPS-induced endotoxemia can modulate cochlear uptake of aminoglycosides and subsequent ototoxicity.

Mice (C57Bl/6) received an intravenous injection of up to 10 mg LPS (in PBS), and 24 hours later an intra-peritoneal injection of gentamicin or fluorescent gentamicin (GTTR). One or three hours later, sera and cochleae were collected and processed for serum gentamicin levels, confocal microscopy or ELISA analysis of cochlear gentamicin levels. Mice were also treated with saline, LPS (1 mg/kg every 5 days during kanamycin treatment period), kanamycin (700 mg/kg twice daily) or both kanamycin and LPS over a period of 15 days, to test whether chronic exposure to LPS-induced endotoxemia induction modulates kanamycin-induced ototoxicity as assessed by auditory brainstem responses (ABRs).

For acute treatments with LPS, doses <1 mg/kg did not increase serum levels of gentamicin or GTTR; yet 1 mg/kg LPS increased cochlear levels of both gentamicin or GTTR as assessed by either confocal microscopy or ELISA analysis. Furthermore, 1 mg/kg LPS did not acutely affect the ABR thresholds, a functional measure of BLB integrity. Chronic LPS-induced endotoxemia (or PBS alone) did not induce threshold shifts in ABRs of mice three weeks after treatment. Mice treated with kanamycin alone had significant threshold shifts only at 32 kHz, indicating partial induction of kanamycin-induced ototoxicity. Mice that received both LPS and kanamycin had significant threshold shifts at 16, 24 and 32 kHz compared to age-matched mice treated with kanamycin only, as well as LPS only or saline.

The ABR data indicate that the cochlear BLB remained grossly intact during acute endotoxemia. This suggests that cochlear uptake of aminoglycosides occurs via transcellular trafficking across the BLB, and that this trafficking is enhanced during endotoxemia. Finally, chronic endotoxemia, and likely sepsis, enhances aminoglycoside-induced ototoxicity over that observed in control mice, and likely represents a newly-demonstrated form of ototoxic synergy.

A HIGH-THROUGHPUT DRUG SCREEN FOR PROTECTION AGAINST CISPLATIN OTOTOXICITY USING THE HEI-OC1 IMMORTOMOUSE INNER EAR CELL LINE

***TAL TEITZ*[1], *ASLI GOKTUG*[2], *JIE FANG*[1], *TAOSHENG CHEN*[2], *JIAN ZUO*[1]**

Departments of Developmental Neurobiology[1], Chemical Biology and Therapeutics[2], St. Jude Children's Research Hospital Memphis, TN 38105, USA

Cisplatin is a common chemotherapeutic drug used to treat various childhood malignancies including neuroblastoma, retinoblastoma, bone tumors, medulloblastoma and sarcoma. More than 50% of pediatric cancer patients suffer from mild to severe hearing loss due to cisplatin treatment. At the concentrations given to patients, cisplatin has been shown to cause apoptosis in cochlear hair cells, spiral ganglion neurons, lateral wall cells in the spiral ligament and stria vascularis. Here we have designed a high-throughput drug screen employing the immortomouse inner ear cell line HEI-OC1 derived from the organ of Corti to screen libraries of >4,000 unique FDA approved and biologically-active compounds that could confer protection from cisplatin-induced cell death. The screening assay, done in 384-well plates by robots, was optimized for cell number and viability to achieve a linear response to cisplatin and a wide-range of drug doses to measure protection from cisplatin-induced cell death. The viability assay for cisplatin was corrected for the presence of DMSO in the library compounds. Compounds known in the literature to confer some level of protection against cisplatin in the inner ear cells such as Resveratrol, Sodium butyrate and L-carnitine hydrochloride were tested in this assay as positive controls and were found to show similar levels of protection as previously reported. Compound hits of this primary screen will be validated in the HEI-OC1 cell line and used for secondary screens in additional mouse inner ear cell lines as the UB/OC-1, UB/OC-2, SV-k1 and in neuroblastoma cell lines. Cochlear explants will be employed in the secondary screens to validate protection from cisplatin. Promising compounds will be tested *in vivo*. Our high-throughput screen can be expanded to the complete library of >500,000 compounds at St. Jude. In addition we plan to perform a high-throughput screen for siRNAs that can protect HEI-OC1 cells from cisplatin. These high-throughput screens will identify novel molecular targets and pathways involved in cisplatin-ototoxicity in mammalian cochlea and potential drugs for protection from cisplatin ototoxicity in the clinic.

A COMPARATIVE RIP-SEQ APPROACH REVEALS DISTINCT ROLES FOR CAPRIN-1 AND TIA-1 IN REGULATING PROTEIN TRANSLATION DURING COCHLEAR STRESS

EMILY TOWERS[1], NAILA HAQ[1], JONATHAN GALE*[1], SALLY DAWSON*[1]

*[1]UCL Ear Institute, 332 Gray's Inn Road, London, UK (*These authors contributed equally)*

The cochlea is exposed to a wide variety of stresses throughout life including noise damage, ototoxic drugs and the process of ageing. These types of stress are known to contribute to the loss of cochlear hair cells, a major cause of acquired hearing loss, but despite this we still know relatively little about the mechanisms by which hair cells respond to damage. Our previous work demonstrated that stress granules form following oxidative stress and ototoxic damage in an inner ear cell line (OC-2 cells) and in hair cells in ex vivo cochlear cultures respectively (Towers et al, J Cell Sci. 2011). More recently, we have found stress granule formation also occurs in hair cells in vivo during aminoglycoside damage. Stress granules are aggregates of proteins that bind specific mRNAs preventing their translation and thereby regulating protein translation to promote cell survival during cellular stress. In OC-2 cells and hair cells our data show that stress granule formation involves the proteins TIA-1 and Caprin-1, the latter being a direct transcriptional target of the hair cell-specific transcription factor Pou4f3 (Towers et al, J Cell Sci. 2011). Therefore to understand the role of these two stress granule components in regulating the translation of specific RNAs we used both immunofluorescence, and a combined immuno-precipitation and next generation sequencing approach (RIP-seq) in OC-2 cells exposed to different types of damage.

OC-2 cells were treated with sodium arsenite or heat shocked, fixed and immunostained with antibodies to TIA-1, Caprin-1 and other stress granule markers to compare: (i) the timing of formation and disaggregation of stress granules upon damage and recovery, and (ii) the stress granule components recruited to the granules. Caprin-1 and TIA-1 are recruited to stress granules following both types of damage. To determine which RNAs are bound by Caprin-1 and TIA-1 during heat shock and arsenite treatment immuno-precipitation was performed using Caprin-1, TIA-1 and control IgG antibodies to pull down the interacting RNAs from treated and untreated OC-2 cells. The resulting pools of pulled-down RNAs were identified by Illumina next generation sequencing (Hiseq 2000, PE100). Comparative data analyses identified RNAs with differential binding to Caprin-1 and/or TIA-1 after the damage treatments and pathway analysis (DAVID v6.7, NIAID, NIH) used to highlight the processes prioritized for transcriptional regulation during stress. Elucidating the precise molecular components that are "triaged" by TIA-1 and Caprin-1 during stress could identify novel therapeutic targets to enhance cell survival during cochlear stress.

Sponsored by a travel award from:

ACTION ON HEARING LOSS

CHARACTERIZATION OF PMCA2 LOCALIZATION IN THE MNTB

***JESSICA H. WEATHERSTONE*[1,2], *JENNIFER L. THORNTON*[1], *BRUCE L TEMPEL*[1,2]**

[1]The Virginia Merril Bloedel Hearing Research Center, Department of Otolaryngology, and [2]Department of Pharmacology, University of Washington, Seattle, WA, USA.

The plasma membrane calcium ATPase 2 (PMCA2) pumps calcium from inside the cell to the extracellular space. It is known that PMCA2 is present in neurons involved in sound localization of the chick auditory brainstem (Wang et al., 2009), but PMCA2 has not been well characterized in the mammalian auditory brainstem. We show that PMCA2 is present in the medial nucleus of the trapezoid body (MNTB). Morphologically, MNTB neurons consist of the calyx of Held which wraps around MNTB principal cells forming approximately 600 active sites in the post-synaptic cell body. Understanding if PMCA2 is expressed in calyx, principal cell, or in both is essential to understanding PMCA2's role in the MNTB. Using immunocytochemistry (ICC) we characterize the pre- and post-synaptic expression of PMCA2 in these neurons.

The MNTB is arranged tonotopically with lateral cell responding best to low frequencies and medial cells responding best to high frequencies (Sonntag et al., 2009). There are gradients of ion channels along this tonotopic axis in the MNTB (von Hehn et al., 2004; Leao et al,. 2006; Gazula et al, 2010). We used ICC to determine if PMCA2 is expressed homogenously throughout the mouse MNTB. While total PMCA2 is greater in lateral cells than in medial cells, this is due to a cell size gradient in the MNTB where lateral cells are larger and medial cells are smaller. When controlling for differences in cell size by calculating average optical density, the difference between lateral and medial cells disappears suggesting the density of PMCA2 is homogenous along the tonotopic axis.

Ion channel gradients in the MNTB are disrupted in deaf mice models (Leao et al., 2006). To determine if auditory inputs affect PMCA2 expression we used diphtheria toxin receptor (DTR) mice as a deaf mouse model. DTR mice have been genetically engineered to selectively express the diphtheria toxin receptor in hair cells such that a sub-lethal dose of DT selectively ablates hair cells (Golub et al., 2012). Two weeks after DT injection, DTR mice show no gradient in total or average optical density suggesting that PMCA2 is homogenously expressed in mice lacking auditory activity. There was no significant difference in average optical density between controls and DT injected DTR mice suggesting auditory activity does not affect the density of PMCA2 in the MNTB. Further studies will determine if a longer period of auditory deprivation will decrease PMCA2 density in the MNTB.

MECHANISMS OF COCHLEAR HAIR CELL PROTECTION BY IGF-1

NORIO YAMAMOTO, YUSHI HAYASHI, TAKAYUKI NAKAGAWA, JUICHI ITO

Department of Otolaryngology, Head and Neck Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan

Our previous works showed that insulin like growth factor-1 (IGF-1) protects mammalian cochlear hair cells from noise exposure or from ischemic stress. The clinical trial for the treatment of idiopathic sudden sensorineural hearing loss cases with IGF-1 revealed the positive effect of IGF-1 on the hearing threshold in 56% of patients

To elucidate the mechanisms of cochlear hair cell protection by IGF-1 we adopted the cochlear explant culture system of neonatal mice. We used neomycin as a method of hair cell impairment that was effectively protected by IGF-1 in the explant culture system.

We added various inhibitors at the most effective timing and concentration to clarify the downstream signal pathways of IGF-1 exerting the cochlear hair cell protection. We examined inhibitors of PI3K, Akt, MEK, and PKC that constitute the components of major downstream signal pathways of IGF-1. All tested inhibitors attenuated the effect of IGF-1 although the Akt inhibitor only attenuated the protection of inner hair cells, indicating that IGF-1 treatment could activate several kinds of downstream signal pathways. The specific action of Akt was confirmed by immunohistochemistry of phospho-Akt that was detected around inner hair cells. To clarify the effector of IGF-1 in the protection of hair cells, we performed comprehensive gene expression profiling using microarray. We identified two specific effector genes of IGF-1 signal, Gap-43 and Netrin1, in our experimental system.

To elucidate the cellular mechanisms involved in IGF-1 action, we examined the apoptosis and cell proliferation status after IGF-1 treatment. Decrease of apoptotic hair cell numbers and proliferation of Hensen’s cells and Claudius’ cells were observed when explant culture impaired by neomycin was treated with IGF-1. Contribution of the supporting cell proliferation to the maintenance of outer hair cells was confirmed by treatment with two different proliferation inhibitors, aphidicholin and l-mimosine.

In conclusion, these results indicated that IGF-1 activated several downstream cascades and, as a result, it promoted the proliferation of supporting cells and inhibited the apoptosis of cochlear hair cells in the neonatal mouse cochlea.

GENERATION OF INNER EAR CELLS FROM A GENETICALLY ENGINEERED HUMAN EMBRYONIC STEM CELL LINE

***MOHAMMAD RONAGHI*[1], *MARJAN NASR*[1], *MEGAN EALY*[1], *JOERG WALDHAUS*[1], *KAZUO OSHIMA*[1], *STEFAN HELLER*[1]**

[1]*Stanford University School of Medicine, Department Of Otolaryngology, Head And Neck Surgery, Stanford, CA*

Hearing impairment affects more than 350 million people worldwide. The underlying cause of hearing loss in the vast majority of patients is the degeneration and death of sensory hair cells of the inner ear. It has previously been shown that mouse embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) can be guided *in vitro* to differentiate along the non-neural – otic placodal lineage and subsequently are able to differentiate into hair and supporting cell-like cells when an enabling environment is provided. To investigate the potential of human ESCs to differentiate along the otic lineage and ultimately into hair cell-like cells, we generated a human ESC line with a nuclear EGFP reporter driven by a murine Atoh1 enhancer/basic promoter. We used the same reporter gene that previously has been successfully used to generate the Math1/nGFP line (Lumpkin et a., 2003, Gene Expr. Patterns). ESCs and iPSCs from this mouse line can be guided towards hair cell fate with nGFP-positive hair cell-like cells that can easily be identified for electrophysiological interrogation. Using the hESC-Atoh1/nGFP line as well as H9 hESCs, we have developed a stepwise differentiation protocol to guide human ESCs to inner ear cells. Our *in-vitro* generated cells are firstly directed toward non-neural ectoderm, which was capable of responding to otic-inducing growth factors with upregulation of otic marker genes. The resulting presumptive otic progenitors are capable of differentiation into cells that express marker genes indicative of supporting cells and hair cells. Some morphological specializations are detectable on presumptively nascent hair cell-like cells.

THE *WFS1* HEARING LOSS PHENOTYPE – DO GENETIC MODIFIERS EXIST?

***KEVIN BOOTH*[1], *HELA AZAIEZ*[1], *A ELIOT SHEARER*[1], *RICHARD JH SMITH*[1]**

[1]*Molecular Otolaryngology and Renal Research Laboratories, Department of Otolaryngology - Head and Neck Surgery, University of Iowa, Iowa City, Iowa, USA*

Hearing loss is the most frequent sensory disorder. It affects 1 in 500 newborns and 50% of octogenarians. It is also genetically heterogeneous, with 60 causally-related genes identified to date making molecular diagnosis using traditional Sanger sequencing costly, time intensive and laborious. In this study, we used OtoSCOPE, a next-generation sequencing platform targeting all known deafness-causing genes, to screen one patient from a large family segregating autosomal dominant non-syndromic hearing loss. OtoSCOPE data analysis identified a novel 12-base-pair duplication in exon 8 of the *WFS1* gene that leads to the insertion of four amino acids in the C-terminal domain of the encoded protein, wolframin. Sanger sequencing confirmed the presence of this variant in all affected family members; it was absent in unaffected persons and controls. Although most persons carrying this 12-base-pair duplication in *WFS1* had expected audioprofiles as predicted by AudioGene, a machine-based candidate gene prediction tool for autosomal dominant hearing loss, in one small branch of the family the audioprofiles were not consistent with *WFS1*-related hearing loss. Genetic analysis of this branch of the family is on-going to determine whether genetic modifiers of the *WFS1* phenotype are present.

SHOULD SECONDARY FINDINGS FROM WHOLE EXOME AND WHOLE GENOME SEQUENCING BE RELEASED TO RESEARCH SUBJECTS? OUR ETHICAL RESPONSIBILITY

COLLEEN A. CAMPBELL [1, 2], ***RICHARD J.H. SMITH*** [1, 2, 3]

[1] Molecular Otolaryngology and Renal Research Laboratories, [2] Iowa Institute of Human Genetics, Iowa City, IA, USA, [3] Department of Otolaryngology - Head and Neck Surgery, University of Iowa, Iowa City, IA, USA.

Whole exome (WES) and whole genome sequencing (WGS) are powerful tools that have facilitated the identification of multiple novel genetic causes of non-syndromic hearing loss (NSHL). Genetic variants identified incidental to this primary discovery process are referred to as secondary findings. The American College of Medical Genetics (ACMG) has recently recommended that when WES and/or WGS are requested for clinical diagnostic purposes, the final variant report should include secondary findings in a defined set of 57 disease genes. These 'Recommendations for Reporting of Incidental Findings in Clinical Exome and Genome Sequencing' target genes primarily associated with hereditary cancer and cardiovascular disease since therapeutic intervention and/or screening make disease treatment and/or prevention possible. Analysis of data from the NHLBI Exome Sequencing Project on the Exome Variant Server (EVS) shows that there are 8,483 variants in European-American exomes in these genes. We will present the likelihood of identifying an ACMG-reportable variant in an exome. As researchers, we must address this risk and the ACMG guidelines if we are to enroll patients with appropriate informed consent in genetic studies in which WES and/or WGS is planned. We propose select language in the informed consent document to deal with various reporting options.

Sponsored by a travel award from:

NIDCD

GENETIC SCREENING OF 47 GENES IN 140 SPANISH FAMILIAL CASES OF AUTOSOMAL RECESSIVE NON-SYNDROMIC HEARING IMPAIRMENT: TOWARDS THE ESTABLISHMENT OF ITS GENETIC EPIDEMIOLOGY IN A WESTERN EUROPEAN POPULATION

IGNACIO DEL CASTILLO [1,2], ***MARÍA DOMÍNGUEZ-RUIZ*** [1,2], ***LUCIANA SERRAO DE CASTRO***[1,2], ***MARTA GANDÍA***[1,2], ***LAURA RUIZ-PALMERO***[1,2], ***MONTSERRAT RODRÍGUEZ-BALLESTEROS***[1,2], ***ROMINA CHARAF***[1,2], ***MARGARITA OLARTE***[1,2], ***LUIS A AGUIRRE***[1,2], ***GEMA GARRIDO***[1,2], ***ELENA GÓMEZ-ROSAS***[1,2], ***MIGUEL A MORENO-PELAYO***[1,2], ***FRANCISCO J DEL CASTILLO*** [1,2], ***MANUELA VILLAMAR***[1,2], ***FELIPE MORENO***[1,2].

[1] Hospital Universitario Ramón y Cajal, IRYCIS, Unidad de Genética Molecular, Madrid, Spain.[2] Centro de Investigación Biomédica en Red de Enfermedades Raras, Madrid, Spain.

Up to 80% of cases of hereditary childhood non-syndromic hearing impairment (NSHI) follow an autosomal recessive (AR) pattern of inheritance. To date, forty-seven genes have been reported to contain mutations causing AR-NSHI. Most of these mutations were found in large consanguineous families from Asian or African origin. The spectrum and frequency of mutations in those 47 genes are poorly known in Europe, where the predominant type of familial case contains just two or three affected subjects.

We have investigated this issue in 140 Spanish familial cases of AR-NSHI, each one containing at least two affected siblings. All siblings from each family and their parents were genotyped for microsatellite markers closely linked to each gene. In families showing compatibility with linkage to a specific gene, we sequenced all exons and intron/exon boundaries of one affected family member. The segregation of sequence variants was subsequently followed in other members of the family.

We found causative mutations in 103 of 140 investigated families (73.6%). As expected, the largest contribution corresponds to the DFNB1 locus (GJB2 and GJB6 genes), with 63 cases (45.0% of the investigated families). Eight other genes show significant contributions: OTOF (9 cases, 6.4%), MYO15A (7 cases, 5.0%), SLC26A4 (4 cases, 2.9%), STRC (4 cases, 2.9%), TMC1 (3 cases, 2.1%), MYO7A (2 cases, 1.4%), TMPRSS3 (2 cases, 1.4%), and PCDH15 (2 cases, 1.4%). In addition, CDH23, TECTA, TRIOBP, PJVK, TMHS, LOXHD1, PTPRQ and OTOG contributed just one case (0.7%) each. Most mutations were novel and private.

To our knowledge, this is the first comprehensive study on the genetic epidemiology of AR-NSHI in a Western European population. Our data suggest that diagnostic strategies based on re-sequencing will significantly increase the elucidation rate. The high proportion of private mutations indicates that microarray-based techniques for detecting specific mutations should be much less efficient.

SPECTRUM OF DISCREPANCY IN HEARING LEVELS BETWEEN SIBLINGS WITH IDENTICAL GJB2 MUTATIONS IN JAPANESE

***Masato Fujioka*[1,2], Noriko Morimoto[3], Koichi Sakamoto[4], Masahide Ohtsu[4], Atsuko Nakano[5], Yukiko Arimoto[5], Sawako Masauda[6], Tomoko Sugiuchi[7], Susumu Masuda[8], Kimitaka Kaga[2], Tatsuo Matsunaga[2]**

[1]Department of Otorhinolarygology, Keiyu Hospital. Yokohama, Kanagawa, Japan; [2]Department of Otolaryngology, National Health Organization, Tokyo Medical Center, Tokyo, Japan; [3]Department of Ororhinolaryngology, National Center for Child Health and Development, Tokyo, Japan; [4]Department of Ororhinolaryngology, Hyogo Prefectural Kobe Children's Hospital, Hyogo, Japan; [5]Department of Ororhinolaryngology, Chiba Chilrdren's Hospital, Chiba, Japan; [6]Department of Ororhinolaryngology, National Mie Hospital, Mie, Japan; [3]Department of Ororhinolaryngology, [7]Department of Ororhinolaryngology, Kanto Rosai Hospital, Tokyo, Japan; [8]Department of Pediatric Ororhinolaryngology, Hiroshima Prefectural Hospital, Hiroshima, Japan

Hearing level of the subsequent child is sometimes anticipated to be asked during the genetic counseling with parents when the first child has hereditary hearing loss. There are evidences of genotype-phenotype correlation with protein nontruncating variants producing a milder phenotype than protein truncating variant in GJB2 deafness. However, there is, so far as we searched, no report examining the difference between siblings (brothers and/or sisters), while common genetic background of those might affect the variation.

In this study, we retrospectively examined the hearing levels of 14 pairs of siblings (28 subjects) each of who had the same pathogenic mutation in the GJB2 gene. The result revealed that, as a whole, there was no correlation in between the average hearing level (250, 500Hz, 1, 2 and 4 kHz) of the first and second children (r=0.4886) suggesting that predicting audiometric prognosis in the second child based on the hearing of the first is difficult. However, by looking at individual cases, the differences greater than 40 dB in hearing average in between the siblings were observed only in 4 pairs (29%) and the differences in the rest 10 pairs were within 10 dB.

In summary, the hearing level in the subsequent child can be anticipated in most (70 %) of the siblings in GJB2 hereditary hearing loss and the difference in hearing average is within 10dB. On the other hand, rest pairs have greater differences (larger than 40 dB in average). Possible reasons of the audiometric spectrum may be due to the mutations and genetic background, including common SNPs. Further studies should be continued for finding the parameters affecting on the discrepancy of hearing levels.

NEW MUTATIONS IN *PRPS1* AND *SMPX* GENES IN FRENCH X-LINKED DEAFNESS FAMILIES.

JONARD L [1,2] ***; DÉTRICHÉ C*** [2] ***; LINA-GRANADE G*** [3] ***; DOLLFUS H*** [4] ***; NIASME-GRARE M*** [2] ***; GHERBI S*** [1,5] ***; BONNET C*** [1] ***; LOUNDON N*** [1,6] ***; GARABEDIAN EN*** [1,6] ***; DENOYELLE F*** [1,6] ***; MARLIN S*** [1,5,7] ***.***

[1]. INSERM U1120, Paris, France; [2]. APHP, Trousseau Hospital, biochemistry laboratory, Paris, France; [3]. HCL, Edouard Herriot Hospital, ENT department, Lyon, France; [4]. University Hospital of Strasbourg, Medical Genetics department, Strasbourg, France; [5]. APHP, Trousseau Hospital, Genetic deafness referral centre, Paris, France; [6]. APHP, Necker Hospital, ENT department, Paris, France; [7]. APHP, Trousseau Hospital, Medical Genetics department, Paris, France.

X-linked isolated is a rare condition, probably underestimated. Until now, three genes have been implicated in this disease: *POU3F4, SMPX* and *PRPS1*.

Until 2010, *POU3F4* was the unique gene involved in isolated X-linked deafness. *POU3F4* mutated patients (DFNX2) always present with either mixed or sensorineural progressive deafness associated with perilymphatic gusher at stapes surgery, related to bone abnormalities visible on high-resolution temporal computed tomography (CT) scan.

On the opposite, *SMPX* (DFNX4) and *PRPS1* (DFNX1) mutated patients do not display any abnormality on CT-scan. *SMPX* is a gene encoding a small muscular protein playing an important role in protecting cellular membranes from mechanical stress. *PRPS1* is encoding a phosphoribosylpyrophosphate synthetase involved in purine and pyrimidine biosynthesis.

We have tested a large cohort of French families compatible with an X-linked inheritance mode for both *PRPS1* and *SMPX*, using Sanger sequencing.

We report here four families with mutations in either *PRPS1* gene (one family with a missense new mutation) or *SMPX* gene (three families: one new splice-site mutation and two novel missense mutations). We focus on clinical data of male patients and female carrier.

SMPX and *PRPS1* analysis should be considered when X-linked inheritance mode is not excluded in sensorineural isolated deafness.

A MOLECULAR DIAGNOSTIC SERVICE FOR USHER SYNDROME: A NEXT GENERATION SEQUENCING GENE PANEL APPROACH

***Nick Lench*[1], *Suzanne Drury*[1], *Ann-Marie Differ*[1], *Thomas Cullup*[1], *Lucy Jenkins*[1] *and Maria Bitner-Glindzicz*[1]**

[1]*NE Thames Regional Genetics Service, Great Ormond Street Hospital for Children, London, WC1N 3JH, United Kingdom*

Usher syndrome is a genetic condition that affects both vision and hearing, thought to be responsible for 3-6% of all childhood deafness and approximately 50% deaf-blindness in adults. To date, mutations in 10 genes have been associated with Usher syndrome. Due to the size of the genes (373 exons) a comprehensive molecular diagnostic service by Sanger sequencing has not been feasible in the UK. Next generation sequencing (NGS), where multiple genes and samples are sequenced in parallel is ideal for testing pheno- and geno-typically diverse disorders such as Usher syndrome. We have trialled two NGS approaches, Agilent HaloPlex Custom enrichment and Illumina TruSeq Custom Amplicon, with sequencing on the Illumina MiSeq platform. Using HaloPlex, 11 positive controls and 4 undiagnosed patients were sequenced. Mutations in 1/11 positive controls were not detected as the assay did not contain probes targeting the region. Pathogenic mutations were found in 50% of undiagnosed patients. Using TSCA, 27 patients were sequenced, including 11 positive controls. 2/11 controls were not detected, one because the amplicon covering the mutation failed to amplify and one because of poor read quality. Of 16 patients without a molecular diagnosis, clearly pathogenic mutations were found in 9/16 (56%) including six novel mutations. NGS is therefore a highly effective approach for identifying the molecular basis of Usher syndrome. We are implementing the TSCA protocol due to ease of incorporating the worklflow in the laboratory and improvements in read quality. Large deletions in *USH2A* and *PCDH15* also contribute to a significant proportion of diagnoses and our molecular diagnostic service for Usher syndrome includes this.

OPTIMIZATION OF SIMULTANEOUS SCREENING OF THE MAIN MUTATIONS OF NON-SYNDROMIC DEAFNESS USING TAQMAN OPENARRAY GENOTYPING PLATFORM

***FÁBIO T. A. MARTINS*[1] *AND EDI L. SARTORATO*[1]**

[1] *Center of Molecular Biology and Genetic Engineering (CBMEG), Human Molecular Genetics Laboratory, State University of Campinas (UNICAMP), Campinas, São Paulo, Brazil*

Hearing loss is the most common sensory deficit in humans, affecting approximately 10% of the entire world population. The restriction of communication by the oral expression results in changes in cognitive and psychological development of the affected individual. In developed countries, one in every 500 individuals has severe/profound bilateral sensorineural hearing loss. Among all the causes of hearing loss, more than 60% of congenital hearing losses are genetics. So far, already aware of almost 100 genes and 150 *loci* related with the hearing loss, and most of them have at least 20 changes (point mutations, deletions, insertions, etc.) which may cause the loss. The gene that has the higher number of changes is the *GJB2*, encoding connexin 26, a protein related to ion exchange intercellular maintaining homeostasis potassium auditory system, essential for hearing. Only this gene has over 302 changes confirmed, being the main gene related to cases of hearing loss with genetic origin.

Due to the great clinical and genetic heterogeneity of hearing loss and the importance of correct molecular diagnosis of individuals with hereditary hearing loss, this work proposes standardize a layout to the diagnosis by a genotyping technology using a high-throughput technique based on real-time PCR called *TaqMan OpenArray Genotyping*. With this, we customized a layout to the *OpenArray* genotyping plates, being possible to analyze 32 changes of 96 individuals per plate simultaneously.

Were analyzed 376 individuals, being 94 of them controls listeners, totaling 4 plates in duplicate. All 31 changes analyzed were present in the nuclear genes *GJB2, GJB6, CRYL1, TMC1, SLC26A4, miR-96, OTOF* and in the mitochondrial genes *12S rRNA* and *MT-TR1*. Reactions were subsequently validated by previously established techniques (direct sequencing, Multiplex PCR and RFLP-PCR), tests used for the molecular diagnostic of the hearing loss at Human Genetics Laboratory of the Center for Molecular Biology and Genetic Engineering (CBMEG/UNICAMP). In total, 11.656 reactions of genotyping were performed using this platform. Only 353 reactions failed, representing approximately 3.03% of the reactions. Among the reactions that failed, were samples of nine individuals who did not meet the minimum concentration, purity and integrity of the DNA for the experiments. With this, was calculated the average income of the *OpenArray* genotyping plates, which showed an accuracy of approximately 96.97%. These results and the comparative analysis of the costs among *OpenArray* platform and the others molecular techniques demonstrated the great accuracy, low cost and easy reproducibility of the technique, making this layout customized for the platform *TaqMan OpenArray Genotyping* a good and reliable tool to be used in the molecular diagnostic of hearing loss in the Brazilian population.

Sponsored by a travel award from:

march of dimes

IN VIVO AND IN VITRO EFFECTS OF THE GAMMA- (ACTG1) AND BETA- (ACTB) ACTIN MUTATIONS THAT CAUSE BARAITSER-WINTER SYNDROME

MATIAS MORÍN[1], KUO-KUANG WEN[2], FERNANDO MAYO-MERINO[1], JULIA KORCHAGINA[3], RICHARD GOODYEAR[3], LUCIA BORREGUERO[1], LUCIANA S SERRAO DE CASTRO[1], FELIPE MORENO[1], GUY RICHARDSON[3], PETER A RUBENSTEIN[2], MIGUEL-ANGEL MORENO-PELAYO[1].

[1]Unidad de Genética Molecular, Hospital Ramón y Cajal, 28034 Madrid, Spain and Centro de Investigacion Biomedica en Red de Enfermedades Raras (CIBERER), ISCIII, Madrid, Spain.[2]Department of Biochemistry, Roy A. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, Iowa 52242, USA.[3]Sussex Neuroscience, School of Life Sciences, University of Sussex, Falmer, Brighton, BN1 9QG, UK.

Here we report the functional assessment of six *ACTG1* (T120I, A135V, S155F, T203K, R254W and R256W) and four *ACTB* (N12D, L65V, R196C and R196H) mutations associated with Baraitser-Winter syndrome, a well defined disorder characterized by distinct craniofacial features, ocular coloboma, neural migration defects and hearing loss. We have developed biological analysis in yeast and mammalian cells to explore the functional consequences of these mutations. Our in vivo experiments showed that most of the mutants had a very mild effect on yeast behaviour. The *ACTG1* mutant S155F, however, showed a growth defect and exhibited a significant increased in cell size. The *ACTG* S155F and the *ACTB* R196H mutants had a highly compromised ability to grow on glycerol as a carbon source indicating a mitochondrial detect. In vitro, the R196H and R256W mutants both show an increased critical concentration and an elongated nucleation phase during polymerization compared to wild type gamma actin. Both the 256 and 196 mutations behave abnormally in their interaction with the actin-binding fragment from the yeast formin Bni1. R196H actin shows little if any response to the fragment, whereas with R256W the formin increasingly blocks polymerization as the dose of formin increases. These results therefore support our hypothesis that 256 and 195 are a part of, or affect, an allosteric pathway that allows formin binding to dictate actin filament conformation and dynamics. The *ACTB* mutants, N12D and L65V, showed depositions of thick F-actin bundles randomly in the yeast cells. We have also examined the sensitivity of the mutants to latrunculin A, a drug that sequesters actin monomers and reversibly promotes rapid depolymerisation of actin filaments, and observed that most of the mutants, except T120I, showed elevated sensitivity to the drug. In transiently transfected NIH3T3 cells all the mutant actins were normally incorporated into cytoskeleton structures, although cytoplasmic aggregates were observed in most of the *ACTG1* mutants indicating an element of abnormality caused by the mutations in vivo. The T203K mutation also provoked deposition of thick F-actin bundles in the cell. Interestingly, gene-gun mediated expression of these mutant forms of gamma actin in cochlear hair cells did not result in a gross alteration of cytoskeletal structure or a change in the morphology of stereocilia. Our results provide a more complete picture of the biological consequences of the beta- and gamma-actin mutants associated Baraitser-Winter syndrome and provide interesting clues about the pathogenic mechanism underlying this disorder.

TARGET-CAPTURED NEXT GENERATION SEQUENCING OF REPORTED DEAFNESS GENES REVEALS VARIABILITY OF GENETIC BACKGROUND OF HEREDITARY HEARING LOSS IN JAPAN

***HIDEKI MUTAI*[1], *NAOHIRO SUZUKI*[1], *ATSUSHI SHIMIZU*[2], *CHIHARU TORII*[3], *KAZUNORI NAMBA*[1], *JUN KUDOH*[4], *KENJIRO KOSAKI*[3], *TATSUO MATSUNAGA*[1]**

[1]Laboratory of Auditory Disorders, National Institute of Sensory Organs, National Hospital Organization Tokyo Medical Center, Tokyo, Japan [2]Iwate Tohoku Medical Megabank Organization, Iwate Medical University, Iwate, Japan [3]Center for Medical Genetics, [4]Department of Molecular Biology, Keio University School of Medicine, Tokyo, Japan

Background: Genetic test of patients with hereditary hearing loss is considered useful to determine clinical intervention and the first step toward future development of therapeutics. Genetic test of all the reported deafness genes by Sanger sequencing is extremely time-consuming. Next generation sequencing (NGS) technology has been becoming applied for clinical diagnosis of diseases with heterogenic genetic causes.

Methods: Fifteen unrelated Japanese families with hearing loss were examined. They did not have pathogenic mutations in *GJB2*, mitochondrial m.1555A>G and 3243A>G mutations, nor enlargement of vestibular aqueduct, which excludes out possibility of *SLC26A4* mutations. Genomic DNA from each subject was subjected to custom-designed SureSelect Target Enrichment System to capture coding exons and proximal flanking intronic sequences of 84 genes responsible for nonsyndromic or syndromic hearing loss, and sequenced by Illumina GAIIx (paired-end read). The sequences were mapped and quality-checked by BWA, Novoalign, Picard, and GATK, and analyzed by Avadis NGS.

Results: We detected possible pathogenic mutations or variants with uncertain pathogenicity in 7 of the 15 families. The identified candidate causative genes in each family were *ACTG1, DFNA5, POU4F3*, *SLC26A5*, *MYO7A*, *CDH23*, *PCDH15*, or *USH2A*, suggesting that there is no "common" causative deafness genes in Japanese ethnic groups other than *GJB2* and *SLC26A4*. Mutations in Usher syndrome-related genes were detected in 3 families including one with candidate digenic heterozygous mutations of *CDH23* and *PCDH15*.

Conclusion: Targeted NGS analysis was considered to be time and cost-effective for diagnostic use and would help consideration of prognosis, deciding clinical intervention, and future therapeutic study.

TARGETED EXON SEQUENCING SUCCESSFULLY DISCOVERS RARE CAUSATIVE GENES AND CLARIFIES THE MOLECULAR EPIDEMIOLOGY OF JAPANESE DEAFNESS PATIENTS

***SHIN-YA NISHIO*[1], *MAIKO MIYAGAWA*[1], *TAKEHIKO NAITO*[1], *KAZUYUKI NAKAZONO*[2], *NAOYUKI KAMATANI*[2,3], *SHIN-ICHI USAMI*[1]**

1. Department of Otorhinolaryngology, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto 390-8621, Japan. 2. StaGen Co., Ltd., Tokyo, 111-0051, Japan. 3. Laboratory for Statistical Analysis, RIKEN, Kanagawa, 230-0045, Japan

Although recent advances in discovery of deafness genes have resulted in more accurate molecular diagnosis, clinical application still entails difficulties because of the extreme genetic heterogeneity of deafness. Target exon sequencing using Massively Parallel DNA Sequencing (MPS) is a new powerful strategy to discover causative genes in rare Mendelian disorders such as deafness. We attempted to identify genomic variations responsible for deafness by massive sequencing of the exons of the candidate genes. By the analysis of randomly selected Japanese deafness patients, who had already been evaluated for common genes/mutations by Invader assay, we efficiently identified causative mutations and/or mutation candidates. To determine which genes have the greatest impact on deafness etiology, the number of mutations was counted, showing that GJB2 was exceptionally higher, followed by SLC26A4, MYO15A, and CDH23. In the congenital group, GJB2, SLC26A4, MYO15A, CDH23, and TECTA were frequently found, in contrast to the late-detected group, where GJB3, MYO15A, CDH23, USH2A, EYA1, TMPRSS3, and GPR98 were frequently found.

The present data suggested that targeted exon sequencing of selected genes using the MPS technology will be able to identify rare responsible genes including new candidate genes for individual patients with deafness and improve molecular diagnosis. In addition, using a large number of patients, the present study clarified the molecular epidemiology of deafness in Japanese.

FUNCTIONAL AND CELLULAR RESPONSE TO ARRAY INSERTION IN GUINEA-PIG AND MOUSE MODELS OF COCHLEAR IMPLANTATION

***LISA S. NOLAN*[1], *NINA MISTRY*[1], *KRISTIEN VERHOEVEN*[2], *SHAKEEL SAEED*[1,3], *ANDREW FORGE*[1], *RUTH R. TAYLOR*[1].**

[1]UCL Ear Institute, U.K., [2]Cochlear Technology Centre, Belgium., [3]The Royal National Throat, Nose and Ear Hospital, London, U.K.

Electro-acoustic stimulation (EAS) with CochlearTM Hybrid implants has extended the traditional implantation candidacy criteria to patients that have retained some functional hearing in the low frequencies. Hearing loss in such candidates is targeted with electrical stimulation of the basal cochlear turns, encoding the high frequencies, in concert with acoustic stimulation of the low frequency apical turns. However, the benefits gained through EAS have been hampered by data from clinical trials that have shown some patients eventually lose a considerable amount of their residual low-frequency hearing in the implanted ear. The aetiology of this loss of low-frequency sounds is currently unknown. We have developed animal models of cochlear implantation via the round window in guinea-pig and mice to investigate the molecular mechanisms underlying this loss of low-frequency hearing.

In guinea-pig we have developed a residual hearing model for cochlear implantation by pre-treatment with gentamicin (125mg/kg) for 10 consecutive days to ablate hair cells predominantly restricted to the basal turns. In mice, we have used the C57BL/6J strain at ages prior (4 months) and subsequent (7 months) to the onset of their accelerated form of age-related hearing loss which is typical of this strain. In both animal models, auditory brainstem responses (ABRs) to click and tone pip stimuli were recorded pre-implantation and at specific time-points post-implantation until sacrifice. Auditory bullae were fixed and tissue prepared for histological processing.

In guinea-pig at 2 days post-implantation hearing thresholds at low frequencies were only minimally affected in implanted ears compared to contralateral controls. At one week post-implantation greater threshold shifts of 20dB were identified in implanted ears compared to control, but little further deterioration was evident at one month post-implantation. Initial analysis of mice implanted at 4 months of age showed pronounced shifts in hearing thresholds at 1 week post-implantation. In both animal models, encapsulation of the electrode array with fibrotic-like tissue was observed at 1 week post-implantation.

IDENTIFICATION AND CHARACTERIZATION OF GENOMIC BREAKPOINTS IN FOUR MOHR-TRANEBJÆRG SYNDROME DELETION PATIENTS – THE UTILITY OF MATE-PAIR NEXT-GENERATION SEQUENCING

[1]NANNA D RENDTORFF, [1]MARIANNE LODAHL, [2]VALERIO CARELLI, [3]JOHN TOLMIE, [4]KIRSTY GARDNER-BERRY, [5]SD VAN DER VELDE-VISSER, [5]FRANS MC CREMERS, [6]GRAHAM REYNOLDS, [1,7] LISBETH TRANEBJÆRG

[1]Department of Cellular and Molecular Medicine, The Panum Institute, University of Copenhagen, Denmark; [2]Laboratory of Neurogenetics, Department of Neurological Sciences, University of Bologna, Bologna, Italy; [3]Clinical Genetics Service, Laboratory Medicine Building, Southern General Hospital, Glasgow, Scotland; [4]SCIC, Old Gladesville Hospital, Gladesville NSW, Australia; [5]Department of Human Genetics, Radboud Unviversity, Nijmegen Medical Centre, Nijmegen, the Netherlands; [6]Department of Paediatrics and Child Health, ANU Medical School, The Canberra Hospital, Australia; [7]Department of Audiology, Bispebjerg Hospital, Copenhagen, Denmark

Mohr-Tranebjærg syndrome (MTS) is an X-linked, deaf-blindness syndrome characterized by sensorineural hearing impairment in childhood followed in adult life by progressive neuro-degeneration affecting the brain and optic nerves, leading to deaf-blindness. MTS is caused by mutations in the TIMM8A gene, encoding a 97 amino acid protein that functions as a translocase for the inner mitochondrial membrane. The neighboring gene of TIMM8A is the Bruton's tyrosine kinase (BTK) gene. Mutations in this gene result in the immunodeficiency X-linked agammaglobulinemia (XLA). Gross gene deletions can result in disruption of both genes causing a contiguous deletion syndrome of MTS and XLA. Large genomic alterations in BTK are part of MTS syndrome in about 50% of published MTS patients. We here identify and characterize genomic deletion breakpoints in four MTS patients. The breakpoints were identified by a combination of successive PCRs testing for the presence or absence of PCR products amplified from TIMM8A and surrounding genomic regions. In two patients, BTK was also affected by the deletion. These deletion breakpoints were identified by means of next-generation sequencing analysis of a mate-pair DNA library from the respective patients, which allowed precise placement of primers for breakpoint-spanning PCRs. In two patients both breakpoints were located in Alu repeats. Our study adds four MTS deletion patients to the list of only 14 published cases and demonstrates the utility of mate-pair next-generation sequencing in elucidation of the genomic extent of deletions.

INTEGRATING LINKAGE AND COPY NUMBER VARIATION ANALYSES WITH GENOME SEQUENCING IN THE CLINICAL DIAGNOSIS OF HEARING LOSS

***JUN SHEN*[1,2,6], *MATTHEW S. LEBO*[1,2,3], *SAMI S. AMR*[1,2,3], *HATICE DUZKALE*[1,2], *TREVOR J. PUGH*[1,2,4], *ELIZABETH D. HYNES*[1], *BRYAN HARRISON*[1], *AMY L. HERNANDEZ*[1], *KATHERINE A. LAFFERTY*[1], *JOHN B. MOESCHLER*[5], *SHEILA UPTON*[5], *SIVAKUMAR GOWRISANKAR*[1], *DIANA MANDELKER*[1,3], *DAVID P. COREY*[6], *HEIDI L. REHM*[1,2,3]**

[1]*Laboratory for Molecular Medicine, Partners Healthcare Center for Personalized Genetic Medicine, Cambridge, MA;* [2]*Genetics Training Program, Harvard Medical School, Boston, MA;* [3]*Department of Pathology, Brigham and Women's Hospital, Boston, MA;* [4]*Broad Institute, Cambridge, MA;* [5]*Dartmouth Hitchcock Medical Center, Lebanon, NH;* [6]*Howard Hughes Medical Institute and Department of Neurobiology, Harvard Medical School, Boston, MA, USA*

Nonsyndromic sensorineural hearing loss is a genetically heterogeneous condition with >150 mapped loci, but the causative genes are known for only half. Available clinical genetic tests for known hearing loss genes often do not return a positive result. Genome sequencing (GS) holds the promise of increasing diagnostic yield by interrogating nearly every nucleotide in a genome, with affordability offered by next-generation sequencing (NGS) technology. However, the large amount of GS data brings to the forefront an interpretation challenge. In a pilot study, we investigated the feasibility of clinical GS to diagnose the genetic cause of hearing loss. A family including five siblings with congenital nonsyndromic mid-frequency sensorineural hearing loss was ascertained. The OtoChip Test on the proband had been inconclusive. The proband and two affected siblings underwent GS and eleven family members were also genotyped by high-density SNP arrays. GS revealed 97-98% coverage of the genome with >3 million variants identified in each sibling. Thirty-two out of the 1.2 million shared variants were novel nonsynonymous, but none was in a gene obviously associated with hearing loss. Linkage analysis using the genotype data revealed a 20 Mb region overlapping the DFNB16 locus on chromosome 15q15.3 with a maximum LOD score of 2.2. Copy number variation (CNV) analysis identified a 100 kb deletion encompassing the *STRC* gene within this region. All affected siblings were homozygous for the deletion, which accounted for their hearing loss. Precise copy numbers of the region in all family members were assessed with digital droplet PCR assays, and the results were consistent with autosomal recessive inheritance. In addition, the proband was later analyzed by the newly developed targeted-NGS-based OtoGenome Test. Consistent with the GS results, the *STRC* deletion was detected using VisCap, an in-house developed CNV detection tool, and showed variable copy number signals as influenced by the remaining two *STRC* pseudogene alleles. We have recently developed a long-range PCR assay for future patients who may have a combination of an *STRC* deletion and a point mutation, not discernable from the pseudogene by NGS. In conclusion, linkage and CNV analyses by SNP array and targeted NGS complement the GS approach and will be used in conjunction with our clinical GS service to offer comprehensive genomic analysis to patients.

GENETIC ASSOCIATION STUDY OF CANDIDATE GENES FOR AGE-RELATED HEARING IMPAIRMENT IN THE JAPANESE POPULATION

***NOBUYOSHI SUZUKI*[1], *SARAH BONNEUX*[2], *ERIK FRANSEN*[2,3], *SHIGENARI HASHIMOTO*[4], *SHIN-ICHI USAMI*[1], *GUY VAN CAMP*[2]**

[1] Department of Otorhinolaryngology, Shinshu University School of Medicine, Matsumoto, Japan. [2] Department of Medical Genetics, University of Antwerp, Antwerp, Belgium. [3] StatUa Center for Statistics, University of Antwerp, Antwerp, Belgium. [4] Institute of Pathogenesis and Disease Prevention, Shinshu university School of Medicine, Matsumoto, Japan

Objective: Age-related hearing impairment (ARHI) is the most common sensory disorder and the most important cause of hearing loss in the elderly, with a negative impact on the quality of life. Different genes have been reported as susceptibility genes for ARHI in the European population: GRHL2, NAT2, GRM7 and IQGAP2 and in the Japanese population: EDN1, UCP2, FAB2, MTHFR and MTR. We performed a replication study for the previously reported associated SNPs in a Japanese population of 620 samples, aged 50-75 years.

Study Design: Genetic association study of candidate genes for ARHI.

Methods: Principal components (PCs) were calculated for the audiometric data of 620 selected Japanese individuals. Association between the genotypes from the reported SNPs in the candidate genes and PC-scores was tested using linear regression.

Results: None of the previously found SNPs showed significant association with ARHI. Several possible causes for the non-replication are possible: the differences in phenotyping and data-analysis, the allele frequency differences between the Japanese and European population, the candidate alleles may be in distinct gene regions according to the population, the small sample size of our study design, leading to a power that is too low to replicate and the possibility that the initially reported findings were false positive.

Conclusion: ARHI is a highly complex disorder, involving many polymorphisms with small effect sizes, which requires larger studies with more power.

LINKAGE STUDIES AND WHOLE EXOME SEQUENCING ANALYSIS AIMED AT THE IDENTIFICATION OF NEW HEREDITARY HEARING LOSS GENES/LOCI

***G.GIROTTO*[1], *F.FALETRA*[1], *D.VOZZI*[1], *D. VUCKOVIC*[1], *KHALID ABDULHADI*[2], *M. KHALIFA ALKOWARI*[3], *R. BADII*[3] *AND P.GASPARINI*[1]**

[1]*Med Genet, IRCCS-Burlo Garofolo Children Hospital, Trieste Univ, Trieste, Italy;* [2]*Audiology/Balance Unit, HMC, Doha (Qatar);* [3]*Lab. Med. Path., HMC, Doha, (Qatar)*

HHL loss is a common disorder accounting for at least 60% of prelingual deafness. Despite the most common genes (*GJB2*, *GJB6* and MTRNR1) play a major role worldwide still there is a need to search for new causative mutations/genes. A combined strategy based on linkage analysis and whole exome sequencing (WES) has been developed. High-density SNPs arrays have been used to get linkage data in order to define a given number of candidate loci for each family to be applied in the WES filtering phase. As regards WES, after the enrichment step, library construction, sample sequencing and reads mapping, nucleotide variants were called by Samtools V0.1.18 and filtered comparing with in-house, dbSNP and 1000G databases. "*In silico"* functional prediction analysis of variants was done using a series of tools such as PolyPhen-2, MutationTester, etc. 6 Italian families and 5 Qatari ones (showing respectively a dominant and recessive inheritance) and all negative for the presence of mutations in the most common HHL genes have been analysed. In a family from Qatar, linkage analysis identified 1 locus with a LOD of 3.8 on chromosome 5q13, subsequently confirmed by NGS with the presence of a mutation (p.*2625Gluext*11) segregating within the family in a new HHL gene called *BDP1.* This variation changes the last stop codon TAA with the coding one GAA and introduces a new string of 11 amino acids in the transcript. Moreover, immunohistochemistry in the mouse inner ear at P5 showed expression in multiple cells type in the cochlea. In one of the Italian families, linkage analysis showed a LOD of 3.3 on chromosome 12q24. WES data of the region confirmed the presence of a new missense mutation (c.1057G>C; pG353R) in *P2X2* gene, a member of P2X receptors family. A three-dimensional model of this protein suggests that the substitution of the hydrophobic Glycine, with a charged residue as the Arginine, it is expected to destabilize the protein folding. As regards the other families, data are under final validation steps. These findings definitely increase our knowledge of molecular bases of HHL genes, further confirming the importance of the combination of both strategies for disease gene identification and may suggest new targets for HHL treatment and prevention.

PREVALENCE OF MUTATIONS IN THE *DFNB1* LOCUS AMONG COCHLEAR IMPLANT USERS IN SLOVAKIA AND THEIR IMPACT ON POSTOPERATIVE HEARING OUTCOMES IN PRELINGUALLY DEAF CHILDREN

***LUKÁŠ VARGA*[1,2], *IVICA MAŠINDOVÁ*[2], *ZUZANA KABÁTOVÁ*[1], *DANIELA GAŠPERÍKOVÁ*[2,3], *IWAR KLIMEŠ*[2,3], *MILAN PROFANT*[1]**

[1]*1st Otorhinolaryngology Clinic, Faculty of Medicine and University Hospital, Comenius University, Bratislava, Slovakia;* [2]*Diabgene Laboratory,Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia;* [3]*Centre for Molecular Medicine, Slovak Academy of Sciences, Bratislava, Slovakia*

Objective: Cochlear implantation (CI) is generally considered as an effective but expensive treatment in patients with profound bilateral deafness. Thus factors with possible impact on hearing and speech rehabilitation success after CI are of great concern. The aim of the presented study was 1. to provide actual data on prevalence of hereditary hearing loss among Slovak implantees, and 2. to detect a possible linkage between the hearing loss etiology and the rehabilitation outcomes following CI in prelingually deaf children.

Methods: We performed DNA analysis of *GJB2* and *GJB6* genes using the MLPA analysis and direct sequencing in 131 unrelated Slovak CI users, of whom 92 were prelingually deaf. Patients with assumed matrilineal inheritance were also tested for mitochondrial mutation A3243G. According to the hearing loss etiology we divided the patients into three groups (1. *DFNB1* related, 2. known, and 3. unknown). Eighty-one genotyped children were subject to hearing evaluation conducted one, three and five years after CI.

Results: Eight causal mutations and one probably disease causing missense variant (c.127 G>A) were detected in the *GJB2* gene in 44.28% of patients tested. One *GJB6* deletion (delD13S1830) was identified in single subject and no patient was carrying the A3243G mutation. In all hearing tests performed, CI users whose deafness resulted from GJB2 gene mutations achieved better results. Statistically significant differences ($p<0.05$) for tone audiometry were obtained after the first year from implantation. The differences in speech audiometry, monosyllabic word test and categories of auditory performance achieved the statistical significance only after the 3rd and 5th year post implantation.

Conclusions: The mutation prevalence in cochlear implant users was by 25% higher than established for non-implanted hearing impaired population in Slovakia. The children with *GJB2* mutations have outperformed the other two control groups in all investigated variables, although a clear cut statistical significance has not always been achieved. Our results may suggest that hearing loss etiology should be considered as an important predictive factor for hearing rehabilitation outcomes after cochlear implantation.

Sponsored by a travel award from:
march of dimes

EPIGENETIC CHANGES IN AGE-RELATED HEARING IMPAIRMENT (ARHI)

***LISA E WOLBER*[1], *CLAIRE J STEVES*[1], *TIM D SPECTOR*[1], *JORDANA T BELL*[1], *FRANCES MK WILLIAMS*[1]**

[1] *Department of Twin Research and Genetic Epidemiology, King's College London, UK.*

Background: Epigenetic regulation of gene expression has been shown to change over time and is associated with environmental exposures [1]. Monozygotic twin pairs share their genetic code as well as many environmental exposures. They provide excellent subjects, therefore, to explore epigenetic changes independent of genetic variation - matched as they are for age, sex, and many environmental factors [2]. ARHI is a complex disorder known to be heritable [3]; epigenetic regulation could, therefore, explain the differences in age of onset and magnitude of ARHI seen in identical twin pairs [4,5].

Methods: We collected pure-tone audiograms (PTA) in female twins (n=1303, age range: 40-86 years) from the TwinsUK cohort. PTA results were expressed as principal components (PC1, PC2), where PC1 represented the overall threshold of the PTA and PC2 its slope. Epigenome-wide DNA methylation (Illumina 27K array) data were available in 115 subjects (age range: 47-83 years). Epigenome-wide association with PCs 1 and 2 were examined in a linear mixed effects model adjusting for batch, age and relatedness of subjects. Significant epigenome-wide associations were validated in a separate replication cohort from TwinsUK (n=203, age range: 41-86 years).

In addition, a sample of discordant monozygotic twins (n=42, age range: 50-72 years) was examined to identify differentially methylated regions (DMRs) within twin pairs associated with hearing.

Results: In the epigenome-wide association study, PC1 was associated with DNA methylation in the promoter region of genes TCF25, PGM3, CDO1, FGFR1 and POLE, while PC2 was associated with DNA methylation in the promoter region of genes ACADM and PEX12 ($p < 9 \times 10^{-5}$). Associations of DMRs in TCF25 ($p = 8.6 \times 10^{-5}$) and PGM3 ($p = 3.7 \times 10^{-3}$) with PC1 were replicated in the replication sample.

In the monozygotic twin pair analysis, intra-pair differences in PC1 were associated with differential DNA methylation of the RNF39 gene ($p = 1.2 \times 10^{-4}$), whereas differences in PC2 were associated with methylation differences in the KLK5 gene ($p = 2.5 \times 10^{-5}$).

Conclusion: Using the TwinsUK data on hearing ability we have identified differential methylation in a number of genes, which may shed light on epigenetic mechanisms operating in ARHI.

Sponsored by a travel award from:

ACTION ON HEARING LOSS

ABSENCE OF CC CHEMOKINE LIGAND 3 DELAYS CLEARANCE OF INFECTION IN EXPERIMENTAL OTITIS MEDIA

***DOMINIK DENIFFEL*[1], *KWANG PAK*[1], *STEPHEN I. WASSERMAN*[2], *ALLEN F. RYAN*[1]**

[1]Department of Surgery, Division of Otolaryngology, University of California, San Diego, La Jolla, the Veterans' Administration Medical Center, San Diego; and the [2]Department of Medicine/Rheumatology, Allergy and Immunology, University of California San Diego, La Jolla, California, USA

Acute otits media (OM) is one of the most common pediatric diseases. Although it has become evident that innate immune system plays a key role in host defense against OM, its precise mechanisms remain not completely understood.

It has been demonstrated in murine models of OM that Toll-like-receptor (TLR) signaling is crucial for innate immune responses to OM infections. TLRs activate the NF-κB pathway, which culminates in the induction of inflammatory cytokines such as tumor necrosis factor (TNF), whose absence was shown to enhance the middle ear inflammation in murine models. This effect can be compensated by in vivo application of recombinant CC chemokine ligand 3 (CCL3), a potent downstream effector of TNF mediated-inflammation. In addition, blocking CCL3 activity with anti-CCL3 antibodies reduced recovery from OM.

To further explore the influence of CCL3 on host defense against OM, we induced OM in CCL3 -/- mice by middle ear (ME) inoculation of nontypeable Haemophilus influenzae (NTHi). Clearance of ME infection was evaluated by bacterial cultures after 1 day, 2 days, 5 days and 10 days.

The CCL3 -/- animals demonstrated compromised bacterial clearance compared to wild-type mice, evidenced by increased bacterial colonization on days 1 and 2 after NTHi inoculation.

The results suggest that the absence of CCL3 results in impaired innate immunity in the ME. In accordance with our previously published data, we believe that CCL3 plays a key role in the TLR-mediated inflammatory response to ME infection. Therapies based on CCL3 could prove useful in treating persistent disease.

POSTTRANSLATIONAL MODIFICATION OF OTOFERLIN SUGGESTS BIOCHEMICAL MODULATION OF SYNAPTIC VESICLE REPLENISHMENT AT THE AFFERENT FIBER SYNAPSE

***MEIKE HERGET*[1], *SANDRA MEESE*[2], *ELLEN REISINGER*[2], *CHRIS ADAMS*[3], *ANTHONY RICCI*[1] *AND STEFAN HELLER*[1]**

[1]Department of Otolaryngology and Head & Neck Surgery, Stanford School of Medicine, Stanford, CA; [2]Georg-August-University, Goettingen, Germany; [3]Department of Chemistry, Stanford University, Stanford, CA.

Hair cell afferent fiber synapses are specialized to operate with high fidelity and in an indefatigable manner at high rates of release and replenishment of synaptic vesicles. Understanding the biochemical regulation of these processes will provide insight into normal hair cell physiology and disease processes and could provide direction to therapeutical approaches. The molecular mechanism underlying vesicle replenishment and release is unknown, however it is suggested to be dependent on otoferlin. Otoferlin is defective in a recessive form of human deafness (DFNB9), causing auditory neuropathy. It posesses a unique and identifying feature of 6 tandem C2 domains, implicated in Ca2+ and phospholipid binding, followed by a single transmembrane domain. Conducting a molecular analysis of endogenous otoferlin immunoprecipitated from chicken utricle hair cells, we found otoferlin to be posttranslationally modified, comprising acetylation, ubiquitinattion and phosphorylation at multiple sites. Also, pilot work shows that CaMKII□, a Ca2+/calmodulin dependent serine/threonine kinase, binds and phosphorylates otoferlin in vitro. CaMKII was previously co-immunoprecipitated with synaptic ribbons from retinal photoreceptors and chicken utricle hair cells. Together with implications of CaMKII in modulation of neurotransmitter release in the brain, we have formed a general hypothesis that posttranslational modifications of otoferlin regulate synaptic vesicle release and replenishment at inner hair cell ribbon synapses. By pharmacological inhibition of CaMKII in inner hair cells we will probe functional consequences of activated CaMKII in vesicle trafficking and release and consequently we will determine a molecular relevance of otoferlin phosphorylation in this process.

THE ACTIVE ZONE PROTEIN RIM2α IS REQUIRED FOR NORMAL SYNAPTIC SOUND ENCODING VIA PROMOTING A LARGE NUMBER OF CA^{2+} CHANNELS AND READILY RELEASABLE VESICLES

***SANGYONG JUNG*[1], *TOMOKO OSHIMA-TAKAGO*[1], *SUSANNE SCHOCH*[3], *NICOLA STRENZKE*[2] *AND TOBIAS MOSER*[1]**

[1]InnerEarLab, Department of Otolaryngology and Collaborative Research Center 889, University Medical Center, 37075, Göttingen, Germany. [2]Auditory Systems Physiology Group, InnerEarLab, Department of Otolaryngology and Collaborative Research Center 889 University Medical Center, 37075, Göttingen, Germany. [3]Department of Neuropathology, University of Bonn, 53105, Bonn, Germany.

RIMs are multidomain proteins of the presynaptic active zone (AZ) that can bind to the vesicular Rab3 protein, to Ca2+ channels and to other AZ proteins. Via those interactions RIMs are thought to mediate vesicle docking and/or priming and to enable the clustering of Ca2+ channel to active zones in central nervous system neurons. Whether RIMs are expressed in inner hair cells (IHCs) of hearing mice and have a role in synaptic sound encoding remains unclear.

Here we report the expression of RIM2□, RIM2□ and RIM3□in mouse inner hair cells (IHCs) by immunohistochemistry and single-cell RT- PCR. We then analyzed IHC presynaptic function and hearing in RIM2□ KO mice. Confocal analysis of immunolabeled organs of Corti revealed that the number of ribbon-occupied IHC synapses was unchanged and the Ca2+ channels remained clustered at the AZ. Ca2+ current, RRP size and sustained exocytosis were reduced. Sound evoked discharge rates of single spiral ganglion neurons were modestly reduced, both at sound onset and after adaptation, while sound thresholds, dynamic range and recovery from forward masking were normal. Auditory brainstem response had slightly elevated thresholds. Together, our data suggest that RIM2□ promotes a large Ca2+ channel complement and readily releasable pool at IHC AZs and supports temporally precise auditory coding at high rates.

USING A TRANSGENIC ZEBRAFISH TO MEASURE LOCAL CALCIUM SIGNALS AT SYNAPTIC RIBBONS

***KATIE KINDT*[1], *TERESA NICOLSON*[1]**

[1]*Oregon Health and Science Univeristy, Vollum Institute, Portland, OR, USA*

Hair cells, photoreceptors and bipolar cells have a specialized presynaptic density, also known as the synaptic ribbon. The synaptic ribbon is an electron dense structure that acts as a scaffold to tether vesicles adjacent to the presynaptic membrane and near Ca2+ channels (CaV1.3). At synaptic ribbons exocytosis is coupled to graded changes in membrane potential rather than an action potential. This graded response is critical; it enables synaptic ribbons to encode the frequency, intensity and phase of stimuli. Synaptic ribbons vary in size and shape depending on specifies and type of hair cell. These variations are thought to reflect diverse sound encoding requirements.

To study activity at ribbon synapses I have created transgenic zebrafish that express a genetically-encoded calcium indicator localized exclusively to the synaptic ribbon. Using this transgenic I am able to measure local calcium signals at synaptic ribbons. Here I show that the Ca2+ signals are precise and local: in caV1.3 mutants I observe no Ca2+ response at synaptic ribbons. Additionally, there is a sizeable range of Ca2+ signals within a population of synaptic ribbons. Despite this heterogeneity in Ca2+ signals among synaptic ribbons, within an individual hair cell, Ca2+ signals are homogeneous. I am investigating the structural differences that impart unique functional attributes at synaptic ribbons. Our preliminary data indicate that it is the amount of CaV1.3 at synaptic ribbon rather than the size of the synaptic ribbon that dictates the magnitude of the calcium signal. Overall these studies will provide insight as to how the size and structure of synaptic ribbons impact local Ca2+ activity and vice versa.

OVERLAPPING AND DISTINCT ROLES OF L-TYPE CA^{2+}-CHANNELS IN THE AUDITORY BRAINSTEM

***HANS GERD NOTHWANG*[1], *VENKATA SATHEESH*[1], *LUKAS RÜTTIGER*[2], *LENA EBBERS*[1], *ECKHARD FRIAUF*[3], *MARLIES KNIPPER*[2]**

[1]Department of Neurogenetics, Carl von Ossietzky University Oldenburg, 26111 Oldenburg, Germany; [2]University of Tübingen, Department of Otolaryngology, Hearing Research Centre Tübingen (THRC), Molecular Physiology of Hearing, Elfriede Aulhorn Str. 5, 72076 Tübingen, Germany;[3]Department of Biology, Animal Physiology Group, University of Kaiserslautern, POB 3049,D-67663 Kaiserslautern, Germany

Seminal work by R. Levi-Montalcini demonstrated in 1949 the requirement of synaptic connections for the maintenance of the auditory brainstem. Ablation of the otocyst resulted in hypoplasia of secondary auditory centers in the chicken. However, the signaling cascades promoting survival in intact auditory circuits remained largely unknown. We recently showed that the L-type channel Cav1.3 is required for normal development and function of the auditory brainstem in mice. Its targeted ablation resulted in abnormal structure and ~45% reduction of nuclei in the superior olivary complex (SOC), decreased expression of Kv1.2, and abnormal auditory brainstem responses (ABR) with decreased amplitude of wave I, and increased amplitudes of waves II and III (Satheesh et al., 2012). In addition to Cav1.3, auditory brainstem neurons also express Cav1.2. To study its role, we generated Egr2::Cre;Cav1.2 mice (Cav1.2Egr2). At P25, SOC nuclei appeared normal in shape, but smaller compared to control mice. Nissl-stained sections revealed a significant volume reduction of the lateral superior olive (LSO) by 35% and of the medial nucleus of the trapezoid body (MNTB) by 45%. Cell counts revealed that the smaller volumes in both nuclei were due to a reduced number of neurons. Cell number of the MNTB was already significantly reduced by 40% at P4. ABRs in young-adult mice were nearly normal. The only difference was a significant increase in the inter-individual variation of the latency of the negative peaks III – V, indicating a higher inter-individual variability in conductance or wiring of the central auditory circuitry. This result is in contrast to the strongly altered ABRs in Cav1.3Egr2 mice. Furthermore, Kv1.2 expression was unchanged in Cav1.2Egr2 mice. Histological analysis of Cav1.2Egr2 or Cav1.3Egr2 animals at P0 demonstrated normal volume and cell number of the MNTB. These data demonstrate that early postnatal survival, but not neuronal birth or migration, is compromised through the absence of L-type Ca2+-channels. This is in agreement with Levi-Montalcini's conclusion that hypoplasia in the chicken auditory brainstem after disruption of synaptic connectivity is the result of on-site elimination of partially differentiated cells.

In summary, our data demonstrate the importance of both Cav1.2 and Cav1.3 signaling for perinatal survival of auditory brainstem neurons. However, only loss of Cav1.3 results in altered LSO structure, abnormal ABRs, and lower Kv1.2 expression. Thus, both channels have overlapping, but also distinct, functions in the developing SOC.

ACOUSTIC OVERSTIMULATION AND MITOCHONDRIAL CA^{2+} OVERLOAD IN OUTER HAIR CELLS

RUBEN STEPANYAN,[1] ARTUR INDZHYKULIAN,[1,2] JULIE MCLEAN,[1] GHANSHYAM SINHA,[1] GREGORY FROLENKOV[1]

[1]Department of Physiology, University of Kentucky, Lexington, KY, USA; [2]Present address: Department of Neurobiology, Harvard Medical School, Boston, MA, USA

Sound-evoked deflections of the hair cell stereociliary bundles are detected by mechano-electrical transduction (MET) channels located at the tips of stereocilia. The MET channel is a non-selective cation channel with particularly high calcium permeability, passing Ca2+ ten times better than Na+ or K+. Calcium ions that enter hair cells through MET channels are extruded via stereociliary plasma membrane Ca2+ ATPase isoform 2 (PMCA2) pumps. Chen et al. 2012 reported that there is no difference in PMCA2 density between stereocilia of outer hair cells from basal and apical turns; therefore, the shorter and thinner stereocilia of outer hair cells from the basal, high frequency, region of the cochlea can harbor a lesser amount of PMCA2 pumps. In these stereocilia, PMCA2 pumps can cope only with resting Ca2+ load (Beurg et al. 2010; Chen et al. 2012), originating due to a fraction of MET channels open at rest. Subsequently, mitochondria – which are concentrated in a belt beneath the cuticular plate, a support structure for stereocilia – contribute to Ca2+ clearance in basal outer hair cells during mechanical stimulation (Beurg et al. 2010). Our data demonstrated significant Ca2+ increases in the soma of mechanically overstimulated basal outer hair cells. Elevated Ca2+ was promptly removed from the cytosol, while Ca2+ accumulation in mitochondria was sustained. Calcium overload in mitochondria is a common event in the pathways of cell death, including increase in mitochondrial reactive oxygen species (ROS) production. When ROS production increases, mitochondria can undergo oxidative damage and further elevate the rate of ROS generation, initiating a vicious cycle of further damaging mitochondria. Antioxidants attenuate noise-induced damage to hair cells; however, the majority of antioxidants did not demonstrate considerable protection, possibly because most antioxidants do not effectively reach the sites of ROS production, particularly in mitochondria. We utilize a novel class of small peptide antioxidants that concentrate 1000-fold to the inner mitochondrial membrane. Our preliminary data indicate that these novel antioxidants reduce noise-induced permanent shifts of hearing thresholds in mice.

Sponsored by a travel award from:
Hearing Health Foundation
Prevention | Research | Cure

STIMULUS FREQUENCY OTOACOUSTIC EMISSION TUNING CURVES IN PRESTIN MOUSE MODELS

MA CHEATHAM, T KIM, J SIEGEL, P DALLOS

[1]*Northwestern University, The Knowles Hearing Center, Evanston, IL, 60208 USA*

It has been reported that when recordings are made by focusing the beam of a laser-diode interferometer through the round window onto the basilar membrane (BM), mechanical tuning curves in prestin knockout (KO) mice are broader than in controls and have a characteristic frequency (CF) of ~45 kHz (Mellado-Lagarde et al., 2008). This CF is lower than the ~60 kHz found in wildtype (WT) mice. The KO data also indicate that sensitivity is similar to WT but, significantly, does not change post-mortem. In contrast to that of the BM, neural tuning is broad and insensitive as reported before in mice lacking prestin (Cheatham et al., 2004). Because outer hair cells (OHC) harvested from KO mice are much less stiff than controls, it was postulated that the frequency/place map shifts to lower frequencies due to the change in mechanical impedance. In a subsequent experiment, Weddell et al. (2011) confirmed the KO data and also demonstrated that mice expressing a mutated form of prestin, which also results in a loss of cochlear amplification (499 prestin, Dallos et al., 2008), possessed BM tuning functions that were insensitive and very broad. In other words, these 499 knockin (KI) tuning curves were similar to those recorded at the auditory nerve using compound action potentials (CAP) and simultaneous masking (Cheatham et al., 2004) and similar to BM functions recorded in WT mice post-mortem. In order to provide another perspective, we recorded stimulus frequency otoacoustic emission (SFOAE) tuning functions (Cheatham et al., 2011) in prestin KO and 499 KI mice. Data indicate that the SFOAE tuning functions for both mouse models are similar to one another and to those obtained previously using the CAP and to those recorded from the BM in WT controls after death. This result suggests that the characteristics of SFOAEs appear to be established at the level of the input to inner hair cells, as are those of neural tuning. Even though the use of SFOAEs allows us to assay frequency selectivity much earlier in the signal-processing pathway, the emission results do not confirm those obtained at the BM in mice lacking prestin. Hence, the discrepancy in selectivity between BM and CAP tuning properties, but not between SFOAE and CAP, in prestin KO mice requires further investigation.

THE ROLE OF TENASCIN C FOR THE GENERATION AND DIFFERENTIATION OF NEURAL PRECURSOR CELLS IN THE AUDITORY SYSTEM

***STEFAN VOLKENSTEIN*[1], *NINA KRAFT*[1], *MONIKA KWIATKOWSKA*[1], *JAQUELINE REINHARD*[2], *ANDREAS FAISSNER*[2], *STEFAN DAZERT*[1]**

[1]Department of Otorhinolaryngology, Head & Neck Surgery, St. Elisabeth-Hospital, Ruhr-University Bochum, Bochum, Germany; [2]Department of Cell Morphology and Molecular Neurobiology, Faculty of Biology and Biotechnology, Ruhr-University Bochum, Bochum, Germany

The auditory nerve does not regenerate after it has been damaged for various reasons. Replacement and regeneration strategies for auditory neurons are studied intensively due to the hope for some patients where a satisfying speech understanding can not be restored successfully by cochlear or brainstem implants. The replacement of auditory neurons alone or in combination with implantable hearing devices is crucial for patients with auditory neuronopathy or secondary auditory nerve degeneration.

Tenascin C is a glycoprotein of the extracellular matrix, which is known to play a role in afferent synaptogenesis in the developing mouse cochlea and the modulation of precursor cells in different sensory systems. There has been no studies about its role on the differentiation of precursor cells in the auditory system published yet.

Therefore, we performed an immunohistological analysis in sections of the mouse brainstem and cochlea at different time points after birth. We generated neurospheres of the postnatal cochlear nucleus and the spiral ganglion of wildtype and Tenascin C knockout mutants. We analysed the influence of a coating of cell culture dishes with Tenascin C on the differentiation of these neurospeheres into neurons and glia cells and effects on neurite outgrowth in primary culture of auditory neurons.

We demonstrated the presence of Tenascin C in the postnatal cochlea and in the brainstem as well as in neurospheres generated from early postnatal auditory neurons. Coating of cell culture dishes with Tenascin C has a significantly influence on differentiation of neural precursor cells and stimulates neurite outgrowth of postnatal auditory neurons.

Our data suggest, that Tenascin C plays a role in the differentiation and generation of neural precursor cells and postnatal neurons in the auditory system. A possible use of Tenascin C in the future in combination with cochlear or brainstem implants may help to achieve a better bioelectrical interface and therefore a better speech understanding for these patients.

Sponsored by a travel award from:
Hearing Health Foundation
Prevention | Research | Cure