Gene Therapy for Hearing and Balance defects: How close are we?
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Since the initial report on hearing restoration by cochlear gene transfer in a mouse mutant defective for vesicular glutamate transporter-3 (VGLUT3-/-), a growing number of studies tackle similar objectives in the perspective of developing inner ear gene therapy in humans. This presentation will focus on our main approaches to inner ear gene therapy, including the prevention of noise-induced hearing loss and the restoration of balance in vestibulopathies.

The first issue was addressed upon the finding that the mutations in the gene encoding pejvakin (Pjvk) result in an hypervulnerability to sound in mice and humans caused by a marked oxidative stress; this stress develops as a consequence of the defect in the adaptive peroxisome proliferation in response to noise exposure. The results of a comparative analysis of the prevention of noise-induced hearing loss by anti-oxidant drugs and adeno-associated virus (AAV) gene transfer of the murine Pjvk cDNA in Pjvk-/- mice will be discussed.

The second issue was addressed in a mouse model for Usher syndrome of type 1G (USH 1G), that is characterized by congenital profound deafness, vestibular dysfunction, and retinitis pigmentosa. This gene encodes Sans, a scaffolding protein expressed in the cochlear and the vestibular hair-bundles. The results of the cure of hearing and vestibular disorders of Ush1g-/- mutant mice by an recombinant AAV2/8 carrying the Ush1g cDNA will be presented with a special focus on the vestibulopathy of the syndrome.

NOTE: The presenting author has changed to *Saaid Safieddine.

A Mutation in SLC22A4 Encoding an Organic Cation Transporter Expressed in the Cochlear Strial Endothelium Causes Human Recessive Non-Syndromic Hearing Loss DFNB60
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Introduction
The high prevalence/incidence of hearing loss (HL) in humans makes it the most common sensory defect. The majority of the cases are of genetic origin. Non-syndromic hereditary HL is extremely heterogeneous. Genetic approaches have been instrumental in deciphering genes that are crucial for auditory function.

Goal
Mapping and identification of the gene causing ARNSHL in a large consanguineous Tunisian family (FT13), and characterization of the function of the encoded protein.

Methods
We used NADf chip to screen for common North African HL-causing mutation in FT13. We performed genome-wide linkage analysis to map the causative gene. We performed whole-exome sequencing on patient DNA to identify homozgyous variants in genes within the determined locus that would cause the disease. We screened a cohort of small Tunisian HL families searching for additional patients carrying the same variants. We studied the targeting properties of candidate proteins in cell lines and performed immunofluorescence on rat inner ear preparations to study their tissue and cellular localization. We used morpholino-based gene knockdown, live imaging, immunofluorescence, and electrophysiological recordings to study auditory function of the orthologous genes in zebrafish.

Results
We first excluded the implication of known North-African mutations in deafness in family FT13. We then assigned the deafness gene locus to a 12.8 Mb critical region on chr:5q23.2-31.1, corresponding to DFNB60 locus. Moreover, we uncovered aminoacid substitution p.Cys113Tyr in a novel protein SLC22A4 (OMIM#604190), as the cause of ARNSHL DFNB60. Besides, screening a cohort of 71 Tunisian HL
families led to uncover a deaf proband of consanguineous parents that is homozygous for p.Cys113Tyr. This patient carried a homozygous microsatellite marker haplotype bordering SLC22A4 gene locus that was identical to that transmitted within FT13, indicating that this mutation is ancestral. SLC22A4 transports various compounds including organic cations, such as physiological carnitine, and naturally occurring potent antioxidant ergothioneine. Within tissues, carnitine facilitates fatty-acid transport into mitochondria to enter β-oxidation for energy production. Using immunofluorescence, we demonstrate that Slc22a4 is expressed in the stria vascularis (SV) endothelial cells of adult rat and mouse cochlea and targets their apical plasma membrane. These observations were corroborated by finding Slc22a4 transcripts in our RNA-seq library from purified primary culture of mouse strial endothelial cells. Finally, slc22a4 disruption in zebra fish causes sensorineural HL.

Conclusions
We present SLC22A4 as an organic cation transporter of the SV that is essential for hearing, and its mutation causes DFNB60 form of HL.

Research Funding
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NOTE: Additional authors and affiliations have been added.
cells and primary auditory neurons. Our results reveal that the anti-oxidant activity of peroxisomes protects the auditory system against noise-induced damage. \( Prjk \) gene transfer can rescue auditory dysfunction in \( Prjk^- \) mice.

**Funding**
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**NOTE:** The presenting author has changed to *Saaid Safieddine.*

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**SYMP 21**

**One Woman’s View of Deafness and the Impact of Science, Policy and Culture on Achieving her Career Goals**

Claudia Gordon  
U.S. Department of Labor

**Overview**
Claudia Gordon will provide an overview of her journey that has led her to where she is today, not only as the Chief of Staff for the Department of Labor’s Office of Federal Contract Compliance Programs (OFCCP), but also as an independent deaf woman of color on a mission to promote inclusion and access for individuals with disabilities in our society. Ms. Gordon suddenly lost her ability to hear at the age of eight. Placed in isolation and stigmatized, two years would pass before she emigrated to the U.S. and was enrolled at a school for the deaf. A whole new world opened up for her once she learned to communicate in American Sign Language. When Ms. Gordon expressed an interest in furthering her education and becoming a lawyer, she was advised that she might not do as well in a hearing environment. Undeterred, she attended Howard University and subsequently American University Washington College of Law (WCL). During her time at Howard University and WCL, she had access to sign language interpreters for her classes, and this is still her preferred mode of communication.

Ms. Gordon has served as a senior policy advisor with the U.S. Department of Homeland Security’s Office for Civil Rights and Civil Liberties, staff attorney for the National Association of the Deaf Law and Advocacy Center and Vice-President of National Black Deaf Advocates, Inc. Ms. Gordon is also a member of the Gallaudet University Board of Trustees. As a member of the Obama Administration since 2009, she initially served as Special Assistant to the Director of OFCCP and is now OFCCP’s Chief of Staff. OFCCP enforces the civil rights of applicants and employees of companies benefitting from government contracts. Notably, in 2013 – 2014 she conducted a temporary assignment with the White House Office of Public Engagement as an Associate Director where she served as liaison to the disability community, advising on disability policies. She was awarded the Department of Homeland Security Secretary’s Gold Medal, and the Paul G. Hearne Leadership Award, both for her work with the disabled community.

Ms. Gordon will speak about the numerous challenges faced along her journey, her resiliency and deep commitment to advancing equality of opportunity for people with disabilities. “People with disabilities are defined not by our limits but by our potential. With the right tools and support from the scientific community as well as our policymakers, anything is possible.”

**NOTE:** SYMP 21 has been cancelled.
Minimal Basilar Membrane Motion in Lowfrequency Hearing
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Low-frequency hearing is critically important for speech and music perception, but no mechanical measurements are available from inner ears with intact low-frequency parts. These regions of the cochlea may function in ways different from the extensively studied high-frequency regions, where the sensory outer hair cells produce force that greatly increases the sound-evoked vibrations of the basilar membrane. We used laser interferometry in vitro and optical coherence tomography in vivo to study the low-frequency part of the guinea pig cochlea, and found that a minimal portion of the basilar membrane was moving in response to sound stimulation. The motions that were present had smaller am-plitude and different dependence on stimulus frequency than vibrations measured near the mechanosensitive stereocilia. These measurements show a radically different mechanism for detecting low frequencies that help to explain why low-fre-quency hearing is so resistant to potentially damaging sound levels.

NOTE: This poster has been added to the meeting.

Simultaneous Multigene Mutation Detection in a Multi-Ethnic Cohort of Patients with Sensorineural Hearing Loss Through MiamiOtoGenes Panel
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Background
Genetic causes of hearing loss are very heterogeneous. Thus far, more than 66 genes have been characterized for nonsyn-dromic hearing loss. In addition approximately 150 genetic loci have been mapped, and it is estimated that the number of genes could reach 300, equivalent to 1% of all human genes. Extreme genetic heterogeneity of deafness combined with striking variations in the distribution of causative variants in different ethnic groups has made genetic diagnosis expen-sive and time consuming using traditional methods.

Methods
We took advantage of the SureSelect target capture system (Agilent; https://earray.chroma.chem.agilent.com/suredesign/) to de-velop a custom capture panel (MiamiOtoGenes) to contain all exons, 5' UTRs and 3' UTRs of 180 known and candidate deafness causing genes. A target size of approximately 1.158 MB comprising 3494 regions was designed to include genes associated with both syndromic and nonsyndromic hereditary hearing loss. We undertook a targeted sequencing of the 180 genes in a multi-ethnic cohort (South American consisting of Brazilian, Gualamalan, Tunisian, Nigerian, indigenous South African, Turkish, Iranian, Indian, South Florida multi-ethnic
patients) of 360 GJB2 mutation–negative probands. Hearing loss (HL) was congenital or prelingual-onset with a severity variant from mild to profound. The Genomes Management Application (GEMapp; https://secureforms.med.miami.edu/hihg/gem-app) was used for data filtering. Single-nucleotide variants (SNVs), insertion/deletions (INDELS) and copy-number variations (CNVs) were determined. Computational functional prediction algorithms [ClinVar; American College of Medical Genetics and Genomics (ACMG) guidelines] and conservation scores (PHASTCONS, GERP) were also applied.

Results
Overall, the mutation detection rates for a likely pathogenic variant are high, at 55% to 75%, for South American, Indian, Tunisian, Turkish, Iranian, Indian, South Florida groups. In contrast, the pickup rates for Nigerian, indigenous South African are currently low, with only approximately 14% to 50% of probands have sequence changes identified as a probable cause of deafness. There are no predominant recurring mutations in the selected genes within ethnic groups. The lower rate of mutations found amongst sub-Saharan African patients may be indicative of the link of infectious disease to deafness.

Conclusion
Our study highlights the utility of next generation sequencing techniques combined with functional studies analysis tools to provide insight into the etiology of a genetically heterogeneous human disorder such as deafness. Furthermore our data indicate that a deafness gene based panel may be a more cost effective way of assessing genetics of hearing loss in a particular population.

Funding
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NOTE: Additional authors and affiliations have been added.

PS 338
Transcription Analyses of Sensory Epithelia in the Inner Ear of Zebra fish
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Background
The transcriptome is the set of all RNA molecules, including mRNA, rRNA, tRNA, and other non-coding RNA transcribed in one cell or a population of cells. (In our study, we primarily focus on the mRNA.) Transcription analysis can provide crucial information that will help in understanding the genetic mechanisms that control differentiation, proliferation, senescence, metabolism, morphology, and function of a cell or tissue under normal and pathological conditions. Recently, zebra fish model is gaining increasing attention for the study of the development and function of the vertebrate inner ear. The aim of this study is to examine the differential gene expression in saccular, utricular, and lagenar maculae of zebra fish, which helps us to understand the molecular basis underlying functional differences among three otolith organs.

Methods
Sensory epithelia were carefully dissected out from the saccule, utricle and lagena of adult transgenic zebra fish (Et(krt4:GFP)sqet4). Total RNA per sample was prepared using the Ovation Pico WTA System V2 and yielded cDNA product that was fragmented and labeled using the Encore Biotin Module according to the manufacturer’s protocol. Affymetrix Zebra fish Gene 1.0 ST Array were scanned using GeneChip Scanner 3000 7G system. Following quality control, the signal estimates were uploaded for differential expression analysis to the Affymetrix Transcriptome Analysis Console (TAC). The results were statistically analyzed using ANOVA and p values <0.05 were considered significant.

Results
We observed that there was differential expression of genes in the saccule, utricle and lagena. We uncovered hundreds of differentially expressed genes in the three otolith organs. Some of these differentially expressed genes are related to the otolith development and balance in zebra fish, or related to the deafness in human. However, some of the genes were conserved among all three otolith organs. Uniquely expressed genes accounted for <10% of all genes in either otolith organ. Gene ontology and pathway enrichment analyses also provided insights into the gene expression signatures of each otolith organ.

Conclusions
The present study provides a dataset that will help in identifying and exploring the role of deafness and balance related genes. It will help in validating the utilization of zebra fish as a model to study human auditory and vestibular disorders. Morphants and mutants would help in deciphering the physiological effects of these genes in hearing and balance functions.
Funding
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PS 658
Visualizing Population Model Responses of Peripheral, Brainstem and Midbrain Neurons to Complex Sounds
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Complex sounds are represented by the pattern of discharge rates across the population of auditory-nerve (AN) fibers. This representation is transformed as the information ascends the auditory pathway. There are several ways in which the fine-structure and envelope temporal properties are altered. At low stimulus frequencies (< 600-700 Hz), in the anteroventral cochlear nucleus (AVCN), the synchrony of primary-like response type neurons to the fine-structure of stimuli is enhanced, but at high frequencies their synchrony is reduced. Temporal envelope cues, or amplitude fluctuations, in the stimulus are encoded by fluctuations in the instantaneous discharge rates of AN responses. The neural representations of these envelope cues are shaped by narrowband filtering in the auditory periphery. The AN neural fluctuations are further shaped by nonlinearities such as rate saturation and synchrony capture. All types of AVCN neurons across all frequency channels have enhanced synchrony to the neural fluctuations. This enhanced synchrony to the neural fluctuations increases contrasts in the amplitudes of fluctuations across frequency channels. These contrasts set up differences in discharge rates at the level of the inferior colliculus (IC), where neurons are tuned to low-frequency fluctuations of their inputs. Visualization of these envelope-based contrasts for complex stimuli requires population responses of actual or model neurons. We have developed software to display the responses of the Zilany et al. (2014, JASA 135:283-286) AN model, and simplified models for AVCN and IC neurons (Carney et al., 2015, eNeuro 2:e0004-15). Contrasts in the fluctuations across frequency channels provide a basis for coding complex sounds. Visualizing responses at several stages of the ascending pathway to stimuli from classic psychophysical studies provides insight as to the cues that are available to listeners based on neural fluctuations. We will illustrate responses to several stimulus paradigms including tones in bandlimited noise and notched noise maskers, profile analysis stimuli, and stimuli with steep spectral slopes, such as those used in studies of edge pitch and pinna cues.

NOTE: This poster has been added to the meeting.
Simulating Cochlear Pathology in a Distributed-Diameter Auditory Nerve Population Model
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In theory, comparing the neural coding deficits associated with different cochlear pathologies could inform the development of new cochlear implant stimulation strategies optimized for individual listeners. To this end, we demonstrate modifications that simulate pathology in a previously published biophysical computational model of a population of myelinated fibers with normally distributed diameters. This model has been shown to accurately describe temporal response characteristics of single fibers in acutely deafened felines and predict discrimination of Schroeder-phase and sinusoidally amplitude modulated stimuli. Presently, we simulate three distinct cochlear pathologies in the model. First, we evaluate the effects of removing randomly selected fibers from a population of 30,000 auditory nerve fibers on temporal characteristics such as population latency, relative spread, and jitter. The combined population response is analogous to the electrically evoked compound action potential. The random removal of fibers describes a cochlear trauma that does not preferentially affect any subpopulation. Second, we compute the same temporal response measures in a population where small diameter fibers are preferentially removed, simulating the selective neuronal death characteristic of acoustic overexposure (Furman et al. 2013). Finally, we modify the myelinated internodes of the cable model by increasing the membrane capacitance and decreasing the transversal resistance of these segments. We demonstrate the effects of the coordinated manipulation of these two parameters on axonal conduction velocity in a single fiber and select a parameter set that best describes physiological demyelination. In this new model for a demyelinated auditory nerve fiber, we quantify the chronaxie and absolute and relative refractory periods. Taken as a whole, these manipulations of the computational model provide a toolkit for exploring the effects of cochlear pathology on complex stimulus coding.

NOTE: This poster has been added to the meeting.

Age-regulated Function of Autophagy in the Mouse Inner Ear
Sara Pulido1; Esperanza Bas6; Rocío de Iriarte Rodríguez2; Isabel Checa2; Marta Magariños2,3,4; Isabel Varela-Nieto2,3,5
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Background
Autophagy is a highly conserved catabolic process essential for vertebrate embryonic development and adult homeostasis. Autophagy induction has been reported to resolve inflammation, to ameliorate ageing and to protect from neurodegeneration. In the inner ear, autophagy has been reported to play roles in chicken otic neurogenesis and in the response to otic injury in the adult mouse (1,2). Here, we will discuss the role of autophagy in late cochlear development and functional maturation.

Methods
Animals. For this study two mouse strains have been used (HsdOla:MF1*129/Sv and HsdOla:MF1).
RT-qPCR. Autophagy genes TaqMan® probes were used and referred to Rplp0 and 18S rRNA as the endogenous housekeeping genes. The estimated gene expression was calculated as 2−ΔΔCt.
Western blotting. Autophagic flux was assessed by measuring the levels of microtubule associated protein light chain 3-II (LC3-II) and sequestosome 1 (SQSTM1/p62).
Statistical analysis. ANOVA or Student t-test were carried out with SPSS v19.0 to compare gene expression in the organs, time points and genotypes of each strain. Post hoc analyses included the Bonferroni test. Results were considered significant at p < 0.05.

Results
Autophagy machinery genes (Becn1, Atg4b, Atg5 and Atg9) were expressed in the mouse cochlea, vestibular system and brainstem cochlear nuclei, although with different expression levels and temporal patterns. Gene expression was up-regulated from perinatal to adult ages in the cochlea of two mouse strains. Protein levels of LC3-II and p62 confirmed that autophagic flux was increased with age. Immunohistochemistry revealed that LC3B was mainly localized in the neurons of the spiral ganglion. Cox2, an inflammation marker, showed the same temporal expression profiles as those obtained from autophagy transcripts. In contrast, no evident association with IGF-1 deficiency was observed.

Conclusion
Our data suggests that autophagy is regulated with age in the cochlea and that it plays a role in the functional maturation of the mouse inner ear.
Echo-acoustic Flow Guides Flight in Bats
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Common to all airborne animals is the need to react fast and correct to rapid changes in their environment during flight. Visually guided animals tackle this challenge by evaluating optic flow, generated by their movement through structured environments.

Echolocating bats flying in complete darkness cannot make use of optic flow; they can navigate solely by echolocation, i.e. the auditory analysis of self-generated sounds. In contrast to vision, echolocation provides explicit distance information through the analysis of echo delay.

Here we show that bats exploit echo-acoustic flow to navigate rapidly through narrow passages. Specifically, we find that bats’ navigation between lateral structures is significantly affected by the echo-acoustic salience of those structures, independent of their physical distance. This is true despite the stroboscopic nature of echolocation which interferes with a motion percept and although echolocation, unlike vision, provides explicit distance cues.

The results demonstrate that sensory flow elicited by self motion is a ubiquitous principle for guidance of flight in the animal kingdom, independent of the fundamentally different peripheral representation of flow information across the senses of vision and echolocation.

Funding
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NOTE: Additional authors and affiliations have been added.
Both stimulus strength and rate alter the responding patterns of non-determinant learning procedures in vestibular nucleus.

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Background
The vestibular system is known as one of the most dynamic areas for the non-determinant learning procedures, habituation and sensitization, which the responding intensity decreases or increases, respectively. In general, the neuronal responding intensity changes depending on the stimulus characteristics (strength, type, rate, etc.). However, it is elusive if there is any dominant stimulus factor over others to change the neuronal responding intensity. Here, we investigated the effects of stimulus characteristics, comparing the slopes of habituated or sensitized responses.

Method
We used the galvanic vestibular stimulation (GVS) as a main stimulus type, and all the neuronal activities were recorded in the vestibular nucleus, originated from the lateral vestibular afferents. The obtained data were filtered (bandpass, 0.3-5 kHz) and stored at a sampling rate of 40 kHz (Plexon, US). For a control response, a multiple set of GVS (100μA DC) with a 3-second stimulation and a 60-second resting period (type I stimulation) was applied on the animal’s temporal bone and around its muscle. The responding effect by strength or rate was induced by increasing the interval of resting period (120-second) (type II stimulation) or the DC amplitude (200μA DC) (type III stimulation), respectively.

Result
Fifteen neuronal responses from seven healthy guinea pigs (509-604g, males) were recorded by a single tungsten electrode (5-12 MΩ). In each neuron, three slopes by type I, II, and III stimulations were calculated, and each slope was computed by using a linear regression on the multiple averaged firing rates during GVS. The comparison between the slopes during type I and II showed more than half of neurons (9/15, 60%) changed their responding slopes after type II, and the same amount of neurons (9/15, 60%) changed their responding slopes after type III. Seven neurons (47%) changed their slopes in both type II and III, and two neurons (13%) showed no changes in both stimulations.

Conclusion
Based on the data population, we concluded that both stimulus strength and rate of GVS affected the non-determinant learning processes of a neuron. Due to the low modification of neural information in the vestibular nucleus, the changes in neuronal responding intensity were relatively small. However, the conclusion was convinced even after excluding the data with a small slope (absolute value of slope<1). [This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2010-0020163) and the Ministry of Science, ICT & Future Planning (NRF-2013R1A2A2A04014796).]
Transient Block of Ca²⁺ Channels by Exocytosed Protons at Mammalian Auditory Hair Cell Ribbon Synapses

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Background
A synaptic cleft pH regulation of presynaptic Ca²⁺ currents has been described at the ribbon synapses of the retina (DeVries et al., 2001; Palmer et al., 2003) and recently in frog auditory hair cells (Cho and von Gersdorff, 2014). However, this proton regulation has never been reported in mammalian auditory inner hair cells (IHCs). To unmask this process in mouse IHCs, we used a physiological pH buffer solution based on bicarbonate. The transient block of Ca²⁺ currents by exocytosed protons will be used as a proxy for exocytosis that mimics the EPSCs to investigate the mechanisms of vesicular release in mouse IHCs.

Methods
Freshly dissected organs of Corti from pre-hearing (P7) and post-hearing (P14-P18) IHCs from controls (WT and Otof +/−) or otoferlin deficient mice (Otof −/−) were continuously bathed and perfused with a 95% O₂, 5% CO₂ (carbogen) bubbled extracellular solution in the presence of the physiological pH buffer bicarbonate. Ca²⁺ currents and time-resolved changes in membrane capacitance (exocytosis) were recorded in the whole-cell voltage-clamp configuration from IHCs (Vincent et al., 2014).

Results
Using external bicarbonate solutions, Ca²⁺ currents of post-hearing IHCs showed a notch or fast transient block (ICaTB) right after their peak onset. This ICaTB was abolished when adding 10 mM HEPES to the external solution, indicating that it was produced by H⁺ release. Remarkably, pre-hearing IHCs did not display ICaTB, suggesting that this regulation requires a tight coupling organization between Ca²⁺ channels and H⁺ release sites. When varying external Ca²⁺, ICaTB and exocytosis in post-hearing IHCs was best fit with a nonlinear power function with index 3, likely reflecting the cooperativity of the putative Ca²⁺ sensor otoferlin. Indeed, Otof −/− IHCs displayed greatly reduced exocytosis and normal Ca²⁺ currents, but no ICaTB. Remarkably, at a comparably low Otof −/− exocytotic response, WT-IHCs displayed significant ICaTB, suggesting that the lack of otoferlin desynchronized vesicular fusion. Interestingly, ICaTB in WT-IHCs was also prevented with 5 mM intracellular BAPTA, a fast Ca²⁺ buffer known to desynchronize vesicular release.

Conclusion
Fast proton regulation of Ca²⁺ channels in the sub-ms range occurs at mammalian auditory ribbon synapses. This process likely contributes to the initial extraordinarily fast spike firing adaptation component of the auditory nerve fibers. Furthermore, analysis of the proton regulation greatly favors an otoferlin-mediated multivesicular release and a nanodomain coupling of Ca²⁺ channels to docked vesicles at the synaptic ribbons of mammalian hair cells.

Funding
Fondation Agir pour l’Audition

NOTE: The presenting author has changed to *Philippe Vincent
Wbp2 is required for normal glutamatergic synapses in the cochlea and is crucial for hearing in Mice and Humans.

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Steroid hormones are known to be implicated in normal auditory function, and estrogen signalling protects against noise-induced hearing loss. In order to investigate the functional link between hormonal signalling and hearing impairment and identify new targets for therapies, we used Wbp2-deficient mice as a genetic tool. WBP2 encodes the WW domain-binding protein 2, which is phosphorylated before translocating into the nucleus where it acts as a transcriptional coactivator for the estrogen and progesterone receptors ESR1 and PGR.

Auditory Brainstem Response (ABR) thresholds were raised at high frequencies as early as 4 weeks of age in the Wbp2-deficient mice and progressively increased and extended to lower frequencies by 14, 28 and 44 weeks old, indicating progressive hearing loss. Wbp2-deficient mice also show progressive abnormal emissions at high frequencies after recording of distortion product otoacoustic emissions (DPOEs) at 4 and 20 weeks of age. Interestingly, while the gross and cellular structure of the mouse mutant inner ears showed no obvious damage or degeneration up to 30 weeks old, confocal imaging performed at postnatal day 14 (P14), 4 and 8 weeks showed swollen nerve endings below inner hair cells, which is sign of glutamate excitotoxicity. Ribbon synapses showed abnormal morphology after double labelling with pre- and post-synaptic markers (CtBP2 and Glur2/3) in the mutants at 4 weeks of age, and these results were confirmed by transmission electron microscopy (TEM). We built up a pathway to understand the mechanistic link between the loss of Wbp2 and progressive hearing loss, and our data suggest that the phenotype is associated with reduced expression of Esr1, Esr2 and Pgr in the cochlea, leading to disruption of expression of key post-synaptic proteins such as Shank3 and Psd-95.

Finally we report the cases of two children with severe to profound sensorineural hearing loss, each carrying two different point mutations in heterozygosis in the WBP2 gene. This study describes a new gene involved in the molecular pathway linking hearing impairment to hormonal signalling, and provides new therapeutic targets.

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NOTE: The presenting author has changed to *Karen Steel*

Effects of Round Window Occlusion on Intracochlear Pressures

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Background
Reinforcement of the round window (RW) has gained attention recently as a minimally invasive surgical treatment for superior canal dehiscence (SCD). It is theorized that SCD symptoms are improved by decreasing the compliance of the RW. Clinical outcomes from such procedures are varied and the effects of RW manipulations on inner ear fluid mechanics are not fully understood. Here, we quantify the effects of RW reinforcement on intracochlear fluid pressures in fresh human cadaveric temporal bones in baseline-normal state and in simulated SCD conditions.

Method
To measure intracochlear pressures in scala vestibuli (PSV) and tympani (PST), micro-optical fiber pressure sensors were positioned in the respective spaces at the base of the cochlea. Graded reinforcement of the RW was performed using perichondrium, followed by cartilage and then dental impression material in a step-wise fashion. PSV and PST in response to air-conducted sound stimulus in the frequency range of 25-10,000 Hz were measured at baseline and after each incremental reinforcement of the RW. Next, a small defect was created along the lateral aspect of the superior canal to simulate an SCD. Graded reinforcement of the RW was repeated with corresponding measurements of intracochlear pressures.

Results
In the normal temporal bone, RW reinforcements resulted in a graded increase in PSV, PST, and differential pressure across the cochlear partition (Pdiff) in the lower frequencies (f<500 Hz). Pdiff is a measure of cochlear input pressure drive, which provides an estimate of hearing (Pdiff = PSV - PST).

After the creation of an SCD, RW reinforcement resulted in a small increase in PSV (<5dB) around 500 Hz. A variable increase in PST (5-20 dB) at frequencies between 200-1000 Hz was observed. Together, this resulted in small variable increasing or decreasing (5-10 dB) change in Pdiff between 200-600 Hz.

Conclusion
Reinforcing the RW in a normal temporal bone causes a graded increase in PSV and PST. Contrary to current theories, this resulted in an increase in Pdiff. With an SCD, the effects of RW reinforcement were markedly diminished. The effect on Pdiff is small, frequency band limited and variable across the temporal bones.

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