ABSTRACT BOOK

COMMUNICATIONS

Disease modelling (Aging and HL)

Co01

40 - Reversal of Hearing Loss in Spns2 Mutant Mice

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Progressive hearing loss is common but we have no medical treatments to slow down, stop, or reverse it. In this study, we asked if progressive hearing loss in a mouse mutant can be reversed after it has developed as a proof-of-concept.
Spinster homolog 2, Spns2, is a sphingosine-1-phosphate (S1P) transporter, and Spns2tm1a mutant mice were previously described by our group (Chen et al., 2014) showing a rapidly-progressive hearing loss associated with a decline in endocochlear potential (EP). As EP appears to develop normally at first in mutants, we considered ways of restoring it to normal levels after the onset of hearing loss.
We used a genetic approach to initiate expression of the Spns2 gene, using tamoxifen injection to activate Flp recombinase which recognises FRT sites in the Spns2tm1a allele, removing the targeted insertion and leading to restoration of Spns2 gene activity. Tamoxifen was injected at 4 different ages (Postnatal day (P)14, P17, P21 and P28) and ABRs were recorded at intervals before and after injection up to 8 weeks old when the EP was also measured.
By comparing pre and post tamoxifen ABR thresholds in the same mouse, we observed that injection of tamoxifen at P14 led to development of near-normal thresholds at 6, 12, 18 and 24kHz frequencies in the mutants. At P17, mutants already show raised ABR thresholds but tamoxifen injection reversed this hearing loss at 6, 12 and 18 kHz to near-normal thresholds. For frequencies of 18kHz or over, P21 and P28 injections were too late to improve thresholds, but for 12kHz some improvement was found with injection as late as P28.
The reversal of hearing loss was stable up to 8 weeks old. EP levels at 8 weeks old were generally higher in mutants injected at younger ages than in those injected at P21 or P28, and lower ABR thresholds correlated with higher EP levels. Histological analysis of the marginal cells of the stria vascularis showed normal morphology in the mice injected at P14 and P17. Hair cell degeneration was observed in mice injected at P28, but not in mice injected at P14.
Overall, our results show that hearing loss due to the Spns2 mutation can be reversed and the earlier the reactivation of the Spns2 gene, the more extensive is the reversal. This study provides a proof of concept that certain forms of hearing loss can be reversed after the loss has occurred, which is important support for the development of new treatments for humans.

Co02

86 - Human pluripotent stem cells-derived inner ear organoids recapitulate otic development in vitro

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Inner ear hair cells and auditory neurons are essential for sound detection. Their damage or loss is irreversible in humans and is a major cause of hearing deficit. Stem cell-based models open new opportunities to understand the pathophysiology of hearing loss and to develop novel therapeutics. Furthermore, they could provide new means to gain insight into inner ear development. Inner ear morphogenesis can be in part recapitulated using pluripotent stem cell directed differentiation in inner ear organoids (IEOs). This step-wise differentiation protocol consists of 3 main steps: 1) otic placode differentiation, 2) otic vesicles formation and 3) sensory cell maturation.
The goals of this study were to optimize the initial otic induction phase and to provide a first comparison of the generated inner ear cell types to primary tissue. Multiplex immunostaining was used to characterize human iPSC-derived IEOs from different lines, and compared to human embryos at Carnegie Stages (CS) 11, 12 and 13, or fetal samples at gestational week (GW) 8-12.

Simultaneous inhibition of TGFb and activation of BMP signaling specifies non-neural ectoderm at the surface of IPSC aggregates. Subsequent BMP inhibition and activation of FGF signaling results in cranial placode differentiation by day 8-12 of culture (step 1). Testing of discrete BMP4 concentrations (0.5-10ng/ml) revealed optimal BMP4 levels required for specification of otic placode tissue co-expressing ECAD/AP2/SIX1/NCAD and, by day 8, the otic marker PAX8. Similar marker expression was observed in CS11-12 human embryos.
In a subsequent step (2), IEOs are incubated in presence of the Wnt signaling activator CHIR99021. This leads to the development of otic vesicle-like structure expressing SOX2/PAX2/PAX8/ECAD/FBXO2 and SOX10. In vitro derived otic vesicles at day 30-40 of culture show remarkable similarity to CS13 embryos. Finally, starting from day 55, the differentiation of inner ear specific sensory epithelia is observed. Sensory hair cells (MYO7A/POU4F3/ESPIN positive) developed intercalated to SOX2 supporting cells and received innervation from co-differentiated otic-like neurons, expressing TUBB3/SOX2/BRN3A and ISL1/2 matching the marker expression of cochlea samples at GW12.
Ongoing studies are focusing on the molecular and functional characterization of these cell types by transcriptional profiling and drug-sensitivity assays.
The establishment of robust differentiation methods will deliver a unique tool to expand our knowledge of human development and validate novel therapeutics targeting hearing loss.

Co03

22 - Phospholipid PIP_2 mediates slow adaptation in cochlear and vestibular hair cells

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The mechano-electrical transduction (MET) process allows the transduction of mechanical information from sound and head movements into electrical signals, and it is a fundamental step in auditory and vestibular system function. MET takes place at the level of the hair bundle and is triggered by stereocilia deflection. During a sustained displacement, the receptor current peaks then decays, indicating a gradual decrease in MET channel open probability. This particular process is called "adaptation"; it shifts the operating range of the MET process and might be important in preserving the sensitivity of the system and in filtering (Crawford *et al*., 1989; Eatock *et al*., 1987, Ricci *et al*., 2005). The slow adaptation process operates with a time constant on the order of 10 ms or more and requires Ca^{2+} entry through the MET channels and the activity of myosin motors. Although the myosin motor involved is still unknown in the cochlea, it is known that Myosin1c (Myo1c) is a regulator of adaptation in the vestibular system (Holt *et al*., 2002; Yamoah and Gillespie, 1996; Caprara *et al*., 2020). Recently, we demonstrated that the mechanism of slow adaptation does not involve the upper tip-link insertion movement as hypothesized by the motor model (Caprara *et al*., 2020), questioning the molecular mechanism of the adaptation process.
Using electrophysiological recording in mouse vestibular and cochlear hair cells, we tested a new hypothesis that involves the activity of myosin motors and membrane phospholipids like PIP_2 in the regulation of slow adaptation. In particular, we hypothesized that PIP_2 is the major player in the slow adaptation process, and myosins at the tip of the shorter stereocilia are responsible for transporting PIP_2 to the MET channel proximity to mediate adaptation.
First, using a pharmacological approach, we tested if PIP_2 plays a role in slow adaptation in cochlear and vestibular hair cells, and then we tested its interplay with Myo1c in vestibular hair cells. Our results showed that PIP_2 is necessary to regulate slow adaptation in both auditory and vestibular systems. In vestibular hair cells, the addition of exogenous PIP_2 rescues slow adaptation when Myo1c is inhibited, indicating that also, when the activity of Myo1c is inhibited, exogenous PIP_2 is sufficient to preserve slow adaptation. These results support our hypothesis of PIP_2 being a more direct mediator of slow adaptation and functions downstream of the Myo1c role, likely responsible for transporting and concentrating PIP_2 near MET channels. These data are the first step in determining the underlying molecular mechanism of slow adaptation in mammals, the key process that preserves the sensitivity of the system and allows us to detect a wide range of sound intensities with extremely high precision.

Key words: Mechanotransduction, slow adaptation, PIP_2

New imaging technologies for the inner ear

Co04

148 - 3D reconstruction of the inner ear vascularization and temporal bone marrow in the rat

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Bone marrow from several cranial regions has been found to connect to the dura and CSF spaces through vascular bone channels [Herisson et al. 2018], and to release immune cells into the meningeal compartment, where they mature [Cugurra et al. 2021]. The temporal bone contains marrow, potentially capable of exerting a similar function of local immune hub for the inner ear, but data on its organization are mostly lacking. In order to reconstruct the entire 3D vascular/marrow network, we performed iDISCO clearing on rat temporal bones, while immunolabeling endothelia for vWf, vascular smooth muscle for SMA, macrophages for Iba-1, and counterstaining cell nuclei with TOPRO.
Rat temporal bones were also imaged with microCT at 6-micron resolution, after Lugol treatment to visualize soft tissues. Temporal bone clearing evidenced more details than microCT, thanks to immunolabeling. However, given that clearing protocols include passages that anisotropically modify tissue volume, the comparison with micro-CT allowed to address distortion risks.
Our data displayed the constant presence of three separate bone marrow clusters: one on the medial side of the cochlea (CAM), one (the largest) to the vestibular labyrinth (VAM), in association to the semicircular canal arms, and a third in association with the endolymphatic duct (DAM). These three clusters may correspond to bone marrow associated to the petrous apex, mastoid cells, and endolymphatic duct in humans, although for VAM the comparison to mastoid bone marrow is difficult, given the absence of pneumatization and the presence of the cerebellar paraflocculus in rodent temporal bone.
Bone marrow clusters were connected by bone canaliculi to middle ear, endocranic cavity, and inner ear labyrinth. Most canaliculi are vascular in nature, but not arterial, and display several perivascular macrophages. CAM vascular connections only reach the medial side of the lower turns of the cochlea, where they especially target scala tympani. Given that the latter contains the venous part of spiral ligament, it appears possible that cochlear venules directly receive cells or signals from CAM in selected regions of the cochlea.
VAM displayed vascular canaliculi connecting marrow islands to each other, to the middle ear, and to the endocranic cavity. We could not assess whether all VAM channels were fully connected, therefore providing a continuous pathway from the middle ear to liquoral space. However, the large number of canaliculi connecting the bony labyrinth to VAM suggest an additional route for substances injected in the inner ear to reach liquoral space.
DAM does not appear to be connected to either CAM nor VAM, but only to the vessels surrounding the ED and to liquoral space. Therefore, its role may be different from those of CAM and VAM, given the unique immune status of the ED/ES in the inner ear. Cellular studies of the three marrow clusters will be needed to evidence possible heterogeneities and roles.

Ear Physiology

Co05

36 - Biophysical Maturation of SGN is driven by IHC-Activity during early postnatal Development

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Type-I spiral ganglion neurons (SGNs) transmit sensory information generated in the inner hair cells (IHCs) to the nuclei of the auditory brainstem. The response properties of SGN are highly diverse to ensure faithful representation of acoustic signals over a wide dynamic range. To achieve this heterogeneity, SGNs gradually change their biophysical properties during development. This diversification process is likely to be shaped by a combination of activity-dependent (IHC input) and intrinsic factors (gene expression), the mechanisms of which are poorly understood. Previous biophysical studies have identified 3 subpopulations of SGNs based on their spike adaptation behaviour, which also broadly correlate with their synaptic position on the IHCs. During development, the up-regulation of K+- channel expression, which affects input resistance and spike-timing, has been identified as a driver for the maturation of SGNs.
Here, we use the murine otoferlin knockout model of congenital deafness to identify whether the IHC input is required for the normal development of SGNs. We performed whole-cell patch-clamp recordings from SGNs in ex-vivo organ of Corti preparations from postnatal day 2 (P2) to P8. A broad investigation of the biophysical properties of SGNs was combined with the identification of their innervation pattern around the IHC basolateral membrane.
Compared to wild-type mice, SGNs from littermate otoferlin knockout mice showed a reduction in the size of the both the high-voltage-activated potassium currents and hyperpolarisation-activated currents from as early as P2. Additionally, the transient inward currents were reduced from P5 onwards. This suggests that multiple ion-channel genes in SGNs are normally regulated by the input from the IHCs. Collectively, the above biophysical changes also showed that in absence of otoferlin in the IHCs, the SGNs became hyperexcitable and increased their firing latency. Most of the SGNs from otoferlin-knockout mice were unable to sustain precise firing upon stimulation at 100 Hz, which is likely to limit their ability to retain the temporal precision of incoming acoustic stimuli.
Multi-variate analysis of the dataset has also revealed a wide range of biophysical properties in SGNs from wild-type mice, preventing their classification into distinct sub-populations. This agrees with theoretical considerations about emergence of different spike adaptation patterns in neuron populations. However, this is inconsistent with previous data showing the presence of discrete SGN subpopulations based on transcriptomic studies (Sun et al. 2018, Shreshta et al. 2018).

Sun, S. et al. Hair Cell Mechanotransduction Regulates Spontaneous Activity and Spiral Ganglion Subtype Specification in the Auditory System. Cell 174, 1247-1263.e15 (2018).
Shrestha, B. R. et al. Sensory Neuron Diversity in the Inner Ear Is Shaped by Activity. Cell 174, 1229-1246.e17 (2018).

Co06

111 - Probing the molecular recognition between CIB2 and TMC1 in physiological hearing and deafness

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Calcium and Integrin Binding protein 2 (CIB2) is a small EF-hand protein capable of binding Mg2+ and Ca2+ with remarkably different affinities. Recent lines of evidence suggest that CIB2 is a fundamental component of the mechano-transduction channel, the protein machinery responsible for the proper conversion of sound waves into electrical signals in cochlear hair cells. Moreover, CIB2 seems to be involved in the maintenance of the structural organization of the stereocilia. Two different portions of the pore-forming subunit of the channel, identified as the Transmembrane-Like Channel 1 (TMC1) were described by two independent groups to directly interact with CIB2, the first one encompassing residues belonging to the N-terminal domain, the second one involving the transmembrane domains 2 and 3. To date, several missense point mutations in the genes encoding TMC1 and/or CIB2 were found to be causative of non-syndromic deafness, highlighting their crucial role in hearing. We present a combined in vitro and in cyto approach to investigate the molecular recognition of CIB2 and TMC1 focusing on the affinity and kinetics of the interaction and assessing the putative

CIB2- interacting portions of TMC. The results of our study could be extended to deafness-associated variants of CIB2 and/or TMC1 provide a molecular-level understanding of the role of CIB2 in hearing physiology and pathology.

Co07

42 - cAMP and Voltage Regulate Mechanotransduction Gating-Spring Stiffness

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Hair cells of the inner ear are specialized mechanoreceptors that transform mechanical input from sound into an electrical potential through a process termed mechanotransduction (MET). In turtle auditory papilla hair cells, the second messenger cyclic adenosine monophosphate (cAMP) was shown to regulate the sensitivity of the MET channel through an unknown mechanism (Ricci and Fettiplace, 1997). Here, we investigated the mechanism of cAMP regulation in mammalian MET through pharmacology, patch clamp electrophysiology, fluid-jet stimulation, and high-speed imaging. We first confirmed that cAMP also regulates MET in outer hair cells of the rat cochlea, recapitulating data from non-mammals. Secondly, we found that after a long depolarization, MET sensitivity is regulated in a similar way to upregulation of cAMP. We previously observed that prolonged depolarization induced a transient increase in MET channel resting open probability (Popen) that gradually decreased to steady state over a period of seconds (Peng et al., 2013; Peng et al., 2016). Using force stimulation and high-speed imaging, we found that the decrease in resting Popen during the prolonged depolarization is accompanied by a decrease in hair bundle stiffness and a positive deflection of the hair bundle. These changes in the mechanical properties of the hair bundle are consistent with decreasing gating-spring stiffness, which is the mechanical component responsible for opening and closing MET channels. Immediately after repolarization, MET currents exhibit increased adaptation extent, faster slow adaptation time constants, and decreased resting Popen. We term this phenomenon Long Depolarization Modulation (LDM). We found that pharmacological upregulation of cAMP also shifts the hair cell into an LDM-like state, characterized by increased adaptation extent, faster adaptation time constants, decreased resting Popen, and decreased gating spring stiffness. Additionally, upregulation of cAMP inhibited the changes associated with a long depolarization. Together, these results suggest that cAMP or voltage can regulate MET through a mechanism that modulates gating spring stiffness. This is the first description of a physiological mechanism to regulate gating-spring stiffness in hair cells.

Co08

91 - Prestin structure and function

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We and others have recently solved the sub-nanometer structure of prestin (SLC26a5) with cryo-EM technology. Surprisingly, the structure is similar to its SLC26 family member SLC26a9, providing little obvious insight into how prestin works as a piezoelectric-like protein. Of note, however, is the absence of electromechanical behavior in SLC26a9. That is, while prestin displays voltage-driven displacement currents and nonlinear capacitance (NLC), SLC26a9 does not. I will discuss our data on mechanisms of prestin likely underlying its electrical susceptibility as well as its mechanical susceptibility, features which may arise from intra-protein and inter-protein interactions, respectively.

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Co09

98 - Loss of limbic mineralocorticoid or glucocorticoid receptors impacts auditory processing in the cochlea

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Emerging evidence for associations between hearing impairment and cognitive decline implies that hearing is dependent on the formation and storage of auditory memories in the limbic system in a mood- and arousal-related manner.
Considering glucocorticoid release upon stressful and exciting situations that result in altered auditory perception, we were interested in the contribution of mineralocorticoid- (MR) and glucocorticoid receptors (GR) on hearing function, using a tamoxifen-inducible CreERT2/loxP system to generate single or double deletion of MR and GR in limbic brain regions of adult mice.
While threshold sensitivity in MRGR conditional knockout (cKO) double mutants were unchanged, early and late ABR waves, CAP latencies, and ASSR, suggested a direct beneficial effect of limbic MR/GR function on auditory-nerve processing. Analysis of single MR or GR cKO revealed that the phenotype of MRGR cKO mice resulted from opposing influences on auditory fiber responses, i.e. stimulating and inhibiting action: Limbic MR deletion reduced IHC ribbon numbers and ABR wave I responses, leaving later waves, and synchronization to amplitude-modulated tones, unchanged. This indicates that limbic MR activation mayalter auditory nerve fiber discharge rates. In contrast, limbic GR deletion improved early and late ABR waves without reducing IHC ribbon numbers. CAP thresholds, latency, and synchronization to amplitude-modulated tones were improved. This suggests that limbic GR activation affects neural response synchrony, thus influencing temporal auditory processing.
Our findings suggest that MR/GR stress hormone receptors are candidate factors for positive- and negative cochlear pre-cognitive processing during auditory cue perception and auditory cognitive dysfunction.

Keywords: Stress receptors, Top-down mechanism, Hearing deficits

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Co10

64 - Stress affects central compensation of neural responses to cochlear synaptopathy in a cGMP-dependent way

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Increasing evidence supports a link between hearing loss and dementia. We previously demonstrated in a mouse model that an age-related cochlear synaptopathy (decoupling of inner hair cell synapses from auditory nerve fibers) leads to poorer temporal auditory and memory-related processing. We could show that cochlear synaptopathy can, in some individual cases, be centrally compensated through enhanced input/output function of auditory brainstem responses (neural gain), preventing an age-dependent temporal discrimination loss. Therefore, mice can be subdivided by their compensation capacity into a group of low compensators and another group of high compensators. Low compensators also displayed an associated decrease in memory-linked processes and recruitment of activity-dependent brain-derived neurotrophic factor (BDNF) in hippocampal regions in comparison to high compensators. We aimed to identify factors capable of modifying this compensation mechanism. Animals were injected with either a cGMP-stimulating drug — the “memory-enhancing” phosphodiesterase 9A inhibitor — or a placebo. We surprisingly found that the successful central auditory- and memory-dependent adjustment to cochlear synaptopathy is a cGMP- and glucocorticoid-dependent process.

Keywords: Cochlear synaptopathy, blunted stress response, temporal auditory processing, phosphodiesterase 9A inhibitor (PDE9i), cGMP,

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Age-related hearing loss

Co11

77 - Identification of Genes and Variants Associated with Adult-Onset Hearing Loss

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Introduction: Adult-onset hearing loss is a common, heterogeneous disease with a strong genetic component. However, although to date over 150 genes have been identified as contributing to human hearing loss, many more remain to be discovered, as does most of the underlying genetic variation. Many individual variants have been found to underlie adult-onset hearing loss, but they tend to be rare variants with a high impact upon the gene product. It is likely that combinations of more common, lower impact variants also play a role in the prevalence of the disease.
Methods: We have adopted a variety of approaches to identifying genes and variants associated with adult-onset hearing loss. We have extensive phenotypic data from 532 older adult volunteers, including 78 older adults with normal hearing, which is a very important control set. The audiograms of the 454 people with adult-onset hearing loss have been scored according to audiometric phenotype (Dubno et al., 2013), which may indicate differences in underlying mechanisms of adult-onset hearing loss; these include gradually sloping and steeply sloping audiograms, which indicate metabolic (strial) and sensory components of adult-onset hearing loss, respectively. Whole exome sequencing data from these volunteers were processed and filtered stringently, resulting in a list of high-quality variants, a selection of which have been confirmed by Sanger sequencing, with an accuracy rate of 97.5%.
Results: We have adopted multiple different approaches to assessing which genes are contributing to the hearing loss observed in this cohort. We have carried out an outlier analysis to identify genes with a high variant load in older adults with hearing loss compared to those with normal hearing, and used burden analyses to explore the association of genes carrying variants with specific auditory phenotypes. We have also used the auditory threshold data to identify individual variants which appear to contribute to different audiometric thresholds, and we have found variants linked to better hearing (eg TCEANC2) as well as those linked to worse hearing (eg CAPN9). From these data we have chosen genes for further investigation in silico and in vivo, to confirm their link to hearing loss in older adults.
Conclusions: From these analyses we have identified some known deafness genes with a high variant load, but most of our candidate genes have not previously been associated with hearing loss. While our results support the theory that genes responsible for severe deafness may also be involved in milder hearing loss, they also suggest that there are many more genes involved in hearing which remain to be identified.

Keywords: age-related hearing loss, exome sequencing, candidate genes

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References: Dubno et al, J. Assoc. Res. Otolaryngol. (2013) 14:687-701.

Co12

161 - Deepening the genetics of hearing: Genome-Wide Association Studies (GWAS) on Moli-sani cohort

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Introduction: Identifying the genetic determinants of hearing function (HF) and its related diseases, such as Age-Related Hearing Loss (ARHL), is fundamental to better understand the molecular basis of the hearing

system and the implementation of preventive or therapeutic strategies. Nevertheless, despite the high relevance of this topic, these aspects are still poorly elucidated.
Here we performed a series of GWAS analyses on a recently collected Italian cohort, named Moli-Sani, providing novel genetic insights on HF and ARHL.
Materials & Methods: Genotypes and audiometric data were collected from the Moli-sani cohort, consisting of ~1200 individuals aged over 46 years and coming from Southern Italy. For each individual, the best hearing ear was considered, and subjects with a history of occupational risk or pathologies leading to hearing defects were excluded.
GWAS analysis was performed on the hearing thresholds as well as on the three pure-tone averages (PTAs) at low (PTAL, mean of 0.25, 0.5 and 1kHz), medium (PTAM, mean of 0.5, 1 and 2kHz), and high frequencies (PTAH, mean of 4 and 8kHz). Moreover, a GWAS analysis on ARHL was carried out considering people aged >50 years and having PTAH ≥ 40 as cases, and those aged >50 years with PTAH ≤ 25 as controls. Analyses were carried out employing linear mixed model regression. Genome-wide significance was set to $p<5\times10^{-8}$, while an association was considered suggestive with $p<10^{-5}$.
Results: As regards HF, we found ~3400 SNPs with significant or suggestive p-values in 162 genes. Among them, the most interesting results include *CCDC88C, GRAMD1B* and *KRT13* genes.
In particular*, CDCC88C* (topSNP:rs141867491, *p*=4.32E-09 at 0.5kHz; *p*=2.19E-06 at 1kHz) is a negative regulator of the *WNT* signalling pathway and plays a critical role in modulating hearing ability (PMID:**34642354)**.
GRAMD1B (rs188767349: $p=4.87\text{x}10^{-8}$ at 0.25kHz, $p=9.56\text{x}10^{-6}$ at 0.5kHz) encodes a cholesterol transporter: recent studies provide evidence of an intriguing relationship between cholesterol metabolism and hearing loss (PMID:35125240).
KRT13 (rs7211835:*p*=2.39E-07 at 0.5kHz; rs7225519:*p*=8.58E-06 at 1kHz) encodes a member of the keratin protein family, known to play a fundamental role in regulating cellular mechanical properties. All these three genes were also associated with PTAL and PTAM traits.
Considering ARHL, 72 SNPs in 9 different genes were detected. The most significantly associated genes were *AJAP1* and *PCSK5*.
AJAP1 gene (rs36055235, $p=4.74\text{x}10^{-7}$) encodes an adherens junction-associated protein that is important to ensure proper mechanotransduction in cochlear cells.
PCSKC5 gene (rs1974322, $p=1.42\text{x}10^{-6}$) encodes a pro-protein convertase, and its ortholog in Zebrafish is expressed in the lateral line, a sensory organ widely used to model human hair cell pathologies.
Conclusions: These results shed light on novel promising candidate genes unveiling interesting findings on the genetics of HF and ARHL. Further analyses on independent replication cohorts and *in vitro/in vivo* studies will be needed to confirm their relevance in modulating hearing ability, thus contributing to new therapeutic approaches development.

Keywords: GWAS;Genetics;Moli-sani

Co13

146 - Genetics of sensory impairment in Italian semi-supercentenarians

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ARSD (Age Related Sensory Decline) is the slow and gradual deterioration of function of single or multiple senses simultaneously during the life span. Sensory impairment, especially the olfactory deficiency, has been shown to be associated with mortality [1,2,3]. Centenarians are widely considered an ideal model of healthy aging owing to their abilities to avoid or largely postpone major age-related diseases.
The aim of this research is to investigate if centenarians are protected from ARSD by their genetic background. Here we described sensory impairment in centenarians, centenarian's offspring and a group of controls recruited in Milano, Bologna and Calabria regions at phenotypic level (N=202). The sensory evaluation of our cohort included assessment of general hearing issues. We have observed high prevalence of sensory

impairment in centenarians i.e. 56% of long-living subjects had compromised hearing. We did not find any evidence on phenotypical advantage of centenarian offsprings over age-matched healthy controls.
Given the importance of sensory decline in the elderly, different PRS (Polygenic Risk Scores) were generated to assess the relative genetic risk of sensory decline in the general population. We searched for publicly available PRS of age-related hearing loss and we evaluated them in WGS (whole genome sequencing) data of Italian semi-supercentenarians and controls (N=117) [4].
Finally we identified the most significant genetic signals associated to general sensory impairment (based ob evaluation of hearing, smell and taste) for the Italian populations and we used them to construct a PRS estimating the risk of development of ARSD. Performance of proposed PRS was evaluated in our WGS dataset.

Keywords: Aging, Sensory Decline, Hearing Loss, Polygenic Risk Score

References:
1. Pinto, J. M., Wroblewski, K. E., Kern, D. W., Schumm, L. P., & McClintock, M. K. (2014). Olfactory Dysfunction Predicts 5-Year Mortality in Older Adults. PLoS ONE, 9(10), e107541. https://doi.org/10.1371/journal.pone.0107541
2. Schubert, C. R., Fischer, M. E., Pinto, A. A., Klein, B. E. K., Klein, R., Tweed, T. S., & Cruickshanks, K. J. (2017). Sensory Impairments and Risk of Mortality in Older Adults. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 72(5), 710–715. https://doi.org/10.1093/gerona/glw036
3. Sun, J., Li, L., & Sun, J. (2020). Sensory impairment and all-cause mortality among the elderly adults in China: A population-based cohort study. Aging. https://doi.org/10.18632/aging.202198.
4. Garagnani, P., Marquis, J., Delledonne, M., Pirazzini, C., Marasco, E., Kwiatkowska, K. M., Iannuzzi, V., Bacalini, M. G., Valsesia, A., Carayol, J., Raymond, F., Ferrarini, A., Xumerle, L., Collino, S., Mari, D., Arosio, B., Casati, M., Ferri, E., Monti, D., & Nacmias, B. (2021). Whole-genome sequencing analysis of semi-supercentenarians. ELife, 10, e57849. https://doi.org/10.7554/eLife.57849

Tinnitus and vestibular disorders

Co14

92 - Novel variants in TECTA gene related with the tectorial membrane in familial Meniere's disease cases

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Background Meniere's disease (MD) is a chronic inner ear disease characterized by episodes of vertigo, associated with low to mid-frequency sensorineural hearing loss (SNHL), tinnitus and aural fullness. Familial Meniere's Disease (FMD) represent 9-10% of patients with MD, mostly showing an autosomal dominant pattern of inheritance with incomplete penetrance. FMD is a genetically heterogeneous disorder with few genes associated with this pattern, such as FAM136A, DTNA, PRKCB, SEMA3D and DPT. In fact, other inheritance patterns have been proposed, such as recessive or digenic inheritance, involving rare variants in OTOG and MYO7A genes. In this study, we have identified several families segregating rare missense and frameshift variants in the TECTA gene.
Methods Exome sequencing data from 99 MD patients (from 77 families), diagnosed according to the diagnostic criteria defined by the Barany Society, were analyzed to search for rare variants. The American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) guidelines were used to classify candidate variants, and their functional effects were evaluated by protein modeling of α-tectorin. Audiological and vestibular examinations were performed, and clinical features on each family were compared to evaluate the genotype-phenotype correlations.

Results We found that 6/77 families (7.8%) had rare variants in the TECTA gene, segregating the MD phenotype. Two families presented the same missense heterozygous variant: p.Val1494Ala. Another 2 families showed a missense and frameshift variants p.Cys1402Ser and p.Asn1474LysfsTer91, respectively. Moreover, two additional variants in TECTA were also found in two families with one MD patient and relatives with partial syndromes (p.Pro1790Ser and p.Gly2118ProfsTer22). These variants could change the stability of α-tectorin, according to the predicted structural protein model.
Conclusions Six MD families were identified carrying different rare missense and frameshift variants in the TECTA gene, which encodes one of the main structural non-collagenous proteins of the tectorial membrane (TM). Alterations in the α-tectorin structure and stability, caused by the variants found, could modify the TM stability and the micromechanics involved in the sound-evoked motion of the stereocilia, leading to hearing loss in FMD.

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Keywords: Genomics, Hearing loss, Tectorial membrane, Meniere disease, Vestibular disorders

Co15

121 - Vestibular Complaints Impact on the Long-Term Quality of Life of Vestibular Schwannoma Patients

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Objectives: To analyze the long-term effect of dizziness-related symptoms on the quality of life (QoL) of patients with unilateral vestibular schwannoma.
Materials and Methods: This cross-sectional study was performed in a tertiary referral center for skull base pathology in the Netherlands. Patients diagnosed with a unilateral vestibular schwannoma between 2004-2013 were asked to participate. Study patients underwent either active surveillance, surgery, or radiotherapy. Participants completed a disease-specific quality of life questionnaire (Penn Acoustic Neuroma Quality of Life [PANQOL]) and the Dizziness Handicap Index (DHI). Linear regression was performed to assess the effect of DHI scores on QoL. Potential confounders such as age, sex, tumor size at baseline and treatment modality were included in the model. An additional regression was performed to assess the effect of the different DHI domains (functional, emotional, physical) on QoL.
Results: In total 304 participants completed both the DHI and the PANQOL. After correction for confounders such as age, sex and educational level, the DHI total score was significantly associated with PANQOL total score. For each additional point of the DHI, we found a reduction of QoL of 0.7 points as measured by the PANQOL (CI95% -0.7;-0.6). The DHI emotional domain was the most prominent determinant for poorer QoL. Each point on the emotional subscale was associated with a -1.4 (CI 95% -1.8;-1.1) PANQOL score. An emotional domain score ≥9 thus results in a PANQOL deterioration that exceeds the minimal clinical important difference of 12.5 points. “Treatment strategy (i.e. surveillance, radiotherapy or surgery) did not have a clinically relevant differential effect on dizziness-related QoL.”
Conclusions: Dizziness has a significant and clinically relevant effect on the reported QoL of patients with unilateral vestibular schwannoma, irrespective of the chosen treatment strategy. The emotional subscale of the DHI is the major determinant of deterioration in QoL. These findings suggest that the QoL of vestibular schwannoma patients with vestibular complaints may be improved by counseling targeted at the emotional burden, in addition to conventional vestibular rehabilitation.

References:
Roshal et al. Diagnostics and therapy of vestibular schwannomas- an interdisciplinary challenge. Head and Neck Surgery, Vol 16, 2017.
Rosenberg et al. Natural history of acoustic neuromas. The Laryngoscope, 110, 2000.
Carlson et al. What drives quality of life in patients with sporadic vestibular schwannoma. Laryngoscope, 125, 2015.
Guyat et al. Measurement of health status. Ascertaining the minimal clinically important difference. Control Clin Trials. 1989.

Co16

150 - Frontiers: Neuro-immunology of the Inner Ear

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This introductory talk summarizing the state of the art in inner ear neuroimmunology introduces the call for the Frontiers Special Topic “Neuroimmunology of the ear- part II”.
The inner ear is protected by highly sophisticated defense systems responsible for the preservation of sensory structures (or for its failure). Classic work from decades ago pinpointed the major histological sites of ear immunoprotection (the endolymphatic sac and blood-labyrinth barrier), and subsequent research has identified many cellular and molecular players. Resident and blood-derived cell populations have been characterized, and single-cell mapping of immune cell populations in the inner ear is now available. The leading cellular players in inner ear protection are macrophages. Several populations of macrophages are devoted to separate inner ear compartments, often with contrasting roles. Selective barriers exclude blood-derived immune cells (e.g., neutrophils) from the endolymphatic compartment while allowing perilymphatic access during inflammation. The latter, observed in conjunction with all types of ear damage, is also associated with microvascular alteration. Novel microscopical observation techniques have allowed the development of a mechanistic model for endolymphatic duct fluid management and GWAS studies in Menière’s disease and other inner ear-related conditions (e.g., vertigo) suggest a link between immune-related damage and inner ear cells and acellular structures. These are exciting times for inner ear neuroimmunology.

Cochlear implants

Co17

81 - Application of autologous mononuclear cells via an inner ear catheter: first clinical experience in cochlear implantation

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Introduction: Application of drugs directly into the cochlea can be achieved via a CE certified inner ear catheter that allows the reach of more apical regions. In order to control inflammation and growth of fibrous tissue after cochlear implantation and thus increase the chance for hearing preservation, delivery of autologous mononuclear cells (aMNC) using the inner ear catheter might be an option.
Methods: The inner ear catheter (MED-EL, Innsbruck) consists of a 20 mm long electrode-shaped silicone body with a hollow lumen and an opening at the tip to deliver fluids also into apical regions of the cochlea. Patients without relevant residual hearing were included in the present study and received a cochlear flushing with aMNC derived from the bone marrow (n=5) via the cochlear catheter just prior to cochlear implantation with a Med-El Flex 28 electrode. Impedances and the slope of the eCAP amplitude growth function were measured directly after implantation in the OR, on day 3, at first fitting and up to 2 years postoperatively. Results were compared to recipients of the same electrode array with the use of steroids (steroid catheter group) and to a control group without the use of the catheter or steroids.
Results: Impedances were stable in patients that received a treatment with the cochlear catheter. The mean impedance values of the patients receiving aMNC were within the normal range but overall higher when compared to patients of the control group or to patients of the steroid catheter group. Speech understanding was comparable to the other groups showing a good acceptance of autologous cell transplantation via the cochlear catheter.
Conclusions: Based on our results, aMNC application via the inner ear catheter is a feasible method. Long-term data showed a good speech perception of the patients and no pathological increase of impedances. Thus, our data confirm that aMNC application does not hamper implant function. Complications such as fibrotic obliteration or even ossification of the cochlea as a result of uncontrolled proliferation of the applied cells can be excluded based on our electrophysiological long-term data.

Co18

56 - Evaluating the predictive potential of the polarity effect on auditory nerve health using the electrically evoked compound action potential in guinea pigs

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Introduction: Cochlear implants (CIs) directly stimulate the spiral ganglion cells (SGCs) of the auditory nerve, which in the absence of hair cells degenerate over time. Several computational models predict that anodic currents stimulate the SGC cell body and central axon, whereas cathodic currents stimulate the peripheral processes (PPs) (e.g. Resnick et al., 2018, Hear. Res. 361). The difference in excitation efficacy between the two polarities is known as the polarity effect, which is hypothesized to be an estimate for SGC degeneration – in particular the extent of degeneration of PPs prior to that of the cell bodies. For an intact auditory nerve, a preference for cathodic stimulation is expected, while with substantial PP degeneration anodic stimulation would be more effective. Since this theoretical polarity effect has not been indisputably experimentally demonstrated, we here compare electrically evoked compound action potentials (eCAPs) for separate pulse polarities to SGC and PP histology in order to establish whether the polarity effect is in fact a predictor of auditory nerve health.
Methods: eCAPs were recorded in 42 chronically or acutely implanted guinea pigs using a PULSAR implant (MED-EL, Innsbruck, Austria) with biphasic current pulses with alternating polarity. The polarity effect on various eCAP outcomes, such as amplitude, threshold, and latency was measured and compared between normal-hearing and ototoxically deafened guinea pigs, and related to quantified histology of SGCs and PPs. Artifact-only recordings were performed post-mortem immediately after euthanasia in order to obtain artifact-free eCAPs for the individual pulse polarities (i.e., anodic-first and cathodic-first).
Results: The applied artifact reduction method for individual polarities adequately reduced the stimulus artifact. The cathodic-first stimulus had higher excitation efficacy: in both normal-hearing and deafened guinea pigs the eCAPs evoked by cathodic-first stimulation had higher amplitudes and lower thresholds than those evoked by anodic-first stimulation. For all animals and conditions, the eCAP latency was substantially shorter for cathodic-first stimuli, indicating that the cathodic phase was the excitatory one in both pulse configurations. In all normal-hearing animals eCAPs could be evoked with anodic-first pulses (with roughly half the amplitude of cathodic-first-evoked eCAPs), while in deafened animals with approximately 50% SGC survival anodic-first pulses regularly failed to evoke eCAPs.
Conclusion: The observation of an overall preference for cathodic-leading stimuli and of a shift in relative contribution of both polarities after deafening are in agreement with the existing literature on the polarity effect. Whether or not the polarity is informative of neural health will be based on the ratio of PPs and SGCs in histology.

Keywords: polarity effect, eCAP, cochlear implant, auditory nerve, guinea pigs.

Co19

143 - Piezoelectric nanomaterial biocompatibility on in vitro inner ear sensory-neural models

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To date, cochlear implantation (IC) is the only way to recover deep sensory-neural hearing loss (SNHL). Unfortunately, there are still disadvantages related to the shape and structure of the electrode and the compliance of patients to be solved, such as residual hearing loss, tonotopy, interference with magnetic resonance imaging etc. Researchers are improving CIs by exploiting new technologies, from optogenetics to drug delivery systems, to the development of piezoelectric nanomaterials. Our research aimed to test the biocompatibility of nano-compounds that can be used to produce an innovative new CI.
The BaTiO3 and LiNbO3 nano-compounds have been tested in vitro on Organ of Corti sensory-like cells (OC-k3) and on neuronal-like cells (PC12). Cell viability, cell morphology, apoptosis and oxidative stress were tested on both cell lines for up to 48 hours. The viability tests confirmed the absence of cytotoxic effects; the morphological investigation showed a good shape of the nucleus and cytoskeleton and an improvement of the neuritic-network; oxidative stress tests and apoptotic studies confirmed their biocompatibility.

In conclusion these piezoelectric nanoparticles could be applied in the production of an innovative "self-powered" whole implanted CI, which will allow to stimulate cochlear neurons bypassing damaged inner ear cells preserving residual hearing and cochlear tonotopy.

This study was performed as part of the Italian Health Ministry project “New self-powered devices for cochlear stimulation based on piezoelectric nanomaterials” (RF-2011-02350464), the “4NanoEARDRM” project funded under the frame of EuroNanoMed III, and by the Italian Ministry of Education, University and Research (MIUR), Italy [grant n. PRIN-2010S58B38_004].

Innovative therapies

Co20

104 - AAV-mediated gene therapy improves hearing In a DFNB93 mouse model

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As gene therapy strategies improve and new vectors emerge that offer higher efficiency and higher expression of the target gene, gene therapy of the inner ear is close to becoming a more selective treatment option to target monogenic hearing impairment. Among others, one big advantage is the restoration of close to normal hearing performance. We tested an AAV-based gene therapeutic approach to target non-syndromic autosomal recessive hearing impairment DFNB93. In the field AAV-based gene therapeutics have already proven to be able to efficiently restore hearing in mouse models of genetic hearing disorders. Defects in the CABP2 gene are responsible for DFNB93 as it is coding for Calcium-binding protein 2 (CaBP2) which modifies voltage-gated calcium channels CaV1.3 in inner hair cells (IHCs). Enhanced steady-state inactivation of IHC CaV1.3 channels was proposed as the underlying disease mechanism and therefore, fewer channels are available to trigger synaptic transmission. We employed AAV variant AAV2/1 and synthetic AAV-PHP.eB, which both contained the Cabp2 coding sequence. Both viruses were injected into the inner ear of early postnatal Cabp2–/– mice to promote Cabp2 expression in IHCs. We deployed in vitro and in vivo techniques and observed restoration of CaV1.3 properties close to wild-type levels that resulted in significant hearing improvement of Cabp2–/– mice. This data provides evidence for the possibility of DFNB93 gene therapy.

Co21

41 - Third-generation lentiviral gene therapy rescues function in a mouse model of Usher 1B

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Usher syndrome 1B is a devastating genetic disorder with congenital deafness, loss of vestibular function and blindness caused by mutations in the myosin-VIIa (MYO7A) gene. The auditory-vestibular deficits of this disorder can be modelled in homozygous Shaker-1 mice, which develop hearing and balance loss after postnatal day 14 due to Myo7a mutation. Heterozygous animals were found to have normal hearing and vestibular function until 6 months of age, when they developed severe hearing loss across all frequencies. We used a novel third-generation, high-capacity lentiviral vector system to deliver the large 6645 bp MYO7A cDNA plus a dTomato reporter gene in one vector. MYO7A and dTomato were successfully expressed upon in-vitro transduction of the cochlea-derived cell line HEI-OC1. Ectopic in-vivo expression of MYO7A upon lentiviral vector injection into normal hearing mice did not show any adverse effects. In Shaker-1 mice, the dTomato reporter indicated successful transduction of inner, outer and vestibular hair cells, the main inner ear cells targeted by Usher syndrome. Delivery of MYO7A at postnatal day 17 achieved a partial recovery of auditory function and strongly reduced balance deficits in homozygous mutant mice. MYO7A delivery prior to the onset of hearing did not improve hearing outcomes but reduced impaired balance-associated circling behavior. In heterozygous mutant mice, lentiviral MYO7A gene therapy completely rescued hearing with hearing thresholds similar to wild-type littermates. This is the first demonstration of a functional effect using lentiviral vector technology in the inner ear to treat a hearing and balance disorder.

Co22

96 - AAV gene therapy and iPS cell-based disease modeling for GJB2 related hearing loss

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Mutation of the Gap Junction Beta 2 gene (GJB2) is the most frequent cause of hereditary deafness worldwide and accounts for up to 50% of non-syndromic sensorineural hearing loss. GJB2 encodes connexin (CX) 26, a component in cochlear gap junction. We have demonstrated that the degradation of gap junction plaque (GJP) macromolecular complex composed of CX26 and CX30 are critical pathogenesis starting at the embryonic days (Kamiya, J Clin Invest, 2014). We also demonstrated that the cochlear gene delivery of Gjb2 using Adeno Associated Virus (AAV) significantly improved the GJPs and auditory responses of Cx26 deficient mice (Iizuka, Hum Mol Genet, 2015). As several AAV serotypes has different cell tropism to cochlear cell types such as hair cells without gap junction and supporting cells with gap junctions, we examined various AAV serotypes to develop the effective gene therapy for GJB2 related hearing loss. For the disease modeling, we developed a novel strategy to differentiate induced pluripotent stem (iPS) cells into functional CX26-GJP-forming cells that exhibit physiological properties typical of the developing cochlea (Fukunaga, Stem Cell Reports, 2016). To establish the disease model cells from the patients, we generated human iPS cells from the patients with Japanese and East Asian typical GJB2 mutations, GJB2 V37I, G45E+Y136X and 235delC and their disease model cells (Fukunaga, Hum Mol Genet, 2021). To utilize disease model cells for the drug development, we established a gap junction-based screening system with high throughput imaging cytometer. This screening system will enable us to develop the drugs and gene therapy vectors for GJB2 related hearing loss.

Noise-induced hearing loss

Co23

153 - Noise exposure accelerates cognitive decline and hippocampal dysfunction in a mouse model of Alzheimer's Disease

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Recent epidemiological evidence suggests a strong association between hearing loss and cognitive decline. Specifically, impairments in peripheral and central auditory structures have been linked to incidence and acceleration of cognitive deficits, and to increased risk for the onset of neurodegenerative disorders, including Alzheimer's disease (AD). Several hypotheses have been proposed to explain the relationship between auditory sensory deprivation and cognitive impairment, but such association remains still controversial. The aim of this study was to evaluate in an animal model of AD (3xTg-AD mice) if and how hearing loss induced by noise exposure at an age of 2 months (when the AD phenotype is not manifest yet) can accelerate and/or worsen AD-associated cognitive impairment, affecting auditory and hippocampal functions. To this purpose, we exposed both 3xTg-AD and B6129SF2/J wild-type mice of 2 months of age (M) to acoustic trauma (pure tone, 10 kHz, 100 dB SPL, 10 consecutive days, 60 min/day). Animals were analyzed 1 and 4 months after noise exposure (corresponding to 3 and 6 M). The detrimental effects of hearing loss in the auditory cortex and hippocampus have been investigated by using morphological (spine density analyses), electrophysiological (ex-vivo recordings of field-excitatory post-synaptic potentials -fEPSPs), behavioral, and molecular biology experiments (focusing on redox imbalance, neuroinflammation, and tau phosphorylation).
Our results show that hearing loss in the 3×Tg-AD mice caused persistent synaptic and morphological alterations in the auditory cortex by altering both fEPSP recording and dendritic spine density at all time points analyzed. This was associated with earlier hippocampal dysfunctions, as indicated by decreased spine density both in CA1 and dentate gyrus, decreased fEPSP amplitude, increased tau-phosphorylation, neuroinflammation (i.e increased expression of IL-1beta and TNF-alpha), and oxidative stress, along with anticipated memory deficits compared to the expected time-course of the neurodegenerative phenotype.

Collectively, our data suggest that a mouse model of Alzheimer's disease is more vulnerable to central damage induced by hearing loss and shows reduced ability to counteract noise-induced detrimental effects, which accelerates the neurodegenerative disease onset.

Co24

33 - Cochlin Deficiency Protects Aged Mice from Noise-Induced Hearing Loss

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Introduction: Exposure to loud noise can lead to decreased hearing function and tinnitus due to damage to inner ear cells. Interestingly, the region that is most sensitive to noise exposure is the inferior region of the spiral ligament where the type IV fibrocytes are located, and COCH expression is most abundant. The COCH gene is located on the long arm of chromosome 14 and encodes for the COCH protein, cochlin. The function of cochlin is not yet fully known, but it is believed that cochlin plays a role in regulating the innate immune system and maintaining the extracellular matrix (ECM) of the inner ear. Furthermore, it has a key role in maintaining the ion homeostasis in the endolymph, the regulation of the cochlear blood flow and immune response in the cochlea. Noise exposure can induce temporary (TTS) and permanent (PTS) threshold shifts resulting in noise-induced hearing loss (NIHL). Damage to sensory cells is irreversible because these cells are incapable of regeneration leading to cochlear dysfunction and permanent hearing loss. The key mechanism in NIHL is the presence of oxidative stress in the cochlea involving the production of reactive oxygen species (ROS) and free radicals in cochlear tissues. In addition, cochlear inflammation is also a major contributor to NIHL. The objective of this study is to assess the long-term hearing and vestibular function of Coch−/− mice and to investigate the role of the COCH protein in inner ear inflammation after noise exposure. Hypothetically, as COCH maintains the ECM of the inner ear due to its affinity for other ECM proteins, we assumed that Coch−/− mice may suffer more from the NIHL due to alternations in their ECM. However, we brought forward an alternative hypothesis related to the role of COCH plays in the innate immune system: a decreased inflammatory response to noise exposure may potentially result in less hearing loss in the Coch−/− mice.
Methods: Animals were randomly allocated to a noise exposure group and a control group. Vestibular and auditory testing were perfomed at different time points after noise trauma. Whole mount staining and cryosectioning of the cochlea was performed to investigate hair cells, spiral ganglion neurons, inner ear inflammation, Coch expression, and fibrocyte degeneration.
Results: Hearing assessment revealed that Coch+/+ mice had significantly larger threshold shifts than Coch−/− mice after noise exposure. We were unable to identify any differences in hair cells, neurons, fibrocytes, and influx of macrophages in the inner ear between both groups. Interestingly, Coch expression was significantly lower in the group exposed to noise.
Conclusions: Our results indicate that the absence of Coch has a protective influence on hearing thresholds after noise exposure, but this is not related to reduced inner ear inflammation in the knockout.

Keywords: inner ear inflammation, cochlin, noise exposure, spiral ligament

Regeneration, stem cells and developmental biology

Co25

151 - GMP-grade cell purification for clinical application – a tale of two sorters

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Moving away from the research bench and into the Clean Room requires a great deal of thought and consideration to the underlying mechanics of cell manufacturing. Products and processes developed for research application need to be adapted to Good Manufacturing Practice (GMP) standards, to meet regulatory requirements and make them suitable for clinical application.
By using human pluripotent stem cells (hPSCs) as a starting material, we have shown that it is possible to reliably produce human otic neural progenitors (hONPs) using a relatively simple differentiation protocol.

However, off-target cells are also produced, and these must be purged from the cultures prior to expansion – the ultimate aim is to produce an Advanced Therapy Medicinal Product (ATMP) of the highest achievable purity. Fluorescence activated cell sorting (FACS) for specific surface markers is used to pull out the desired hONP population and in research-grade iterations of the derivation protocol, this was performed using the BD FACSJazz machine and resulted in a target population with over 95% purity. However, the 'jet-in-air' nature of the sorting technology employed entails that it is not acceptable for use in ATMP manufacture, notwithstanding the low cell yields and high attrition rate. Therefore, an alternative sorting platform has been explored – the Miltenyi Tyto.
The Tyto differs from conventional cell sorters as it uses an entirely closed system. Cells are loaded and retrieved from a sealed cartridge with no exposure to the 'outside world'. Moreover, the sorting technology is far gentler and gives better yields, making it ideally placed to take forward into a manufacturing suite. The Tyto can routinely produce sorted hONPs at >95% purity and these cells perform as expected in terms of expansion, identity and ability to neuralise in vitro.

Keywords: auditory neuropathy, hPSCs, regeneration

Co26

128 - Protease-Activated Receptor is Essential for Mitotic Basilar Papilla Hair Cell Regeneration

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Loss of auditory hair cells due to genetic predisposition, aging, noise, and ototoxic drugs results in permanent hearing loss. However, hair cell loss in the hearing organs of non-mammals is temporary because supporting cells act as facultative stem cells that can proliferate and regenerate hair cells. The mechanism of how the normally mitotically quiescent avian basilar papilla orchestrates a coordinated regenerative program is unknown.
Towards unraveling the mechanisms of avian hair cell regeneration, we have conducted single-cell RNA sequencing after infusing a single dose of sisomicin into the posterior semicircular canal of 7-days-old chickens at specific time points. We computationally reconstructed the temporal trajectory of gene expression changes in supporting cells upon sisomicin-induced hair cell demise. We focused on the earliest detectable changes in responding supporting cells. The major constituents of the identified candidate signaling pathway that potentially initiates hair cell regeneration were functionally assessed in vivo using pharmacological approaches.
Our results show that supporting cells display distinct gene expression profiles changes as early as 12h post sisomicin infusion when the first sign of DNA fragmentation in hair cell nuclei is detected. In situ validation confirmed the upregulation of distinct genes in early responding supporting cells. We identified a specific signaling pathway associated with proteolytic activation of the receptor F2RL1 followed by a matrix metalloprotease (MMP)-mediated cascade involving EGF receptor activity, MAP kinase signaling, and STAT3 phosphorylation that ultimately leads to upregulation of several transcription factors and proliferative hair cell regeneration. We hypothesize that sensing extracellular proteolytic activity is a key trigger of supporting cell proliferation.
We provide functional evidence showing that the key components of the proposed mechanism are essential for mitotic hair cell regeneration in the avian hearing organ. Our findings inform novel therapeutic strategies aimed at reversing hearing loss in mammals.

Co27

39 - Single-cell RNA-sequencing Reveals Novel Populations of Supporting Cells in the Adult Human Utricle and Profiles Transcriptome Changes after Ototoxic Damage

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Introduction: The utricle, an inner ear balance organ, is comprised of a mosaic of cells that can be divided into two major types: sensory and supporting cells. Sensory cells are responsible for detection of movement and supporting cells for maintaining homeostatic functions. Loss of sensory hair cells due to aging, genetic factors, ototoxic drugs, and infections, leads to permanent balance deficits affecting millions of people worldwide. Since supporting cells survive after damage, they represent an excellent target for endogenous regeneration.
Methods: We used single-cell RNA-sequencing (scRNA-seq) to elucidate supporting cell heterogeneity in utricles from patients with acoustic neuroma. We identified, for the first time, 6 putative types of supporting cells including a novel transitional supporting cell type. Characterization of the different types of supporting cells is a first step towards identifying which subtype(s) represent 'stem' cells for hair cell regeneration.
We used a gentamicin hair cell damage paradigm to assess human utricle's genes and transcription factors that are involved in the damage and potential regenerative response. We damaged, in culture, utricles from the same cohort of patients and evaluated the early response to damage as this might represent a critical time window to set the stage for hair cell regeneration. After 24 h, we isolated the RNA from the sensory epithelia and performed bulk and scRNA sequencing in control and gentamicin-treated samples.
Results: We found genes with significant differences in expression between control and damage conditions highlighting an immediate cellular response of the adult sensory epithelium to damage. Among these differentially expressed genes, we identified transcription factors involved in damage and regeneration response like NFIB known to be a regulator of cell proliferation and epithelial differentiation.
Conclusions: We discovered human-specific genes including a novel gene, and identified the earliest response to gentamicin damage, paving the way for developing therapeutics for the treatment of balance dysfunction.

Keywords: scRNA-seq, human utricle, ototoxic damage.

Co28

133 - Reactivating dormant stemness pathways in presenescent mammalian auditory neuroprogenitors in vitro

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Hearing loss affects over 460 million people worldwide and is a major socioeconomic burden. Both genetic and environmental factors (i.e. noise overexposure, ototoxic drug treatment or ageing), promote irreversible degeneration of cochlear hair cells and associated auditory neurons, known as sensorineural hearing loss. In contrast to birds, fish or amphibians, the mammalian inner ear is virtually unable to regenerate due to the limited stemness of auditory progenitors and no causal treatment is able to prevent or reverse hearing loss. As of today, a main limitation for the development of otoprotective or otoregenerative therapies is the lack of robust and easy preclinical model for drug development. As a consequence, research in the field mostly relies on animal experimentation, resulting in high variability, low throughput and high associated cost and ethical concerns. We have previously identified and characterized the phoenix auditory neuroprogenitors (ANPGs) as highly proliferative progenitor cells isolated from the A/J mouse cochlea. In the present study, we aimed at identifying signalling pathways responsible for the intrinsic high stemness of phoenix ANPGs. We therefore compared transcriptome of phoenix cells to traditionally low stemness ANPGs, isolated from C57Bl/6. Based on the differentially expressed pathways, we developed a reprogramming protocol with the aim to reactivate dormant stemness pathways in presenescent ANPGs (i.e. from C57Bl/6 mouse). Pharmacological treatment

with WNT agonist and dual smad inhibitors resulted in a dramatic increase in neurospheres growth and virtually unlimited passage number in stemness-induced ANPGs. As with the phoenix ANPGs, stemness-induced ANPGs could be frozen and thawed, allowing distribution to other laboratories. Importantly, even after 20 passages at an exponential expansion rate, stemness-induced ANPGs retained their ability to generate new mature and electrophysiologically active type I and type II auditory neurons. Our findings are not only of relevance to the 3R principles, promoting the development of novel alternatives to animal experimentation, but may also open a novel path toward therapeutic strategy aiming at auditory neurons regeneration.

Co29

43 - Cellular diversity of human pluripotent stem cell-derived inner ear organoids

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Genetic inner ear pathologies are one of the most common congenital disorders. Over 400 genes have been linked to non-syndromic and/or syndromic sensorineural hearing loss (SNHL) or balance disorders. Recently, in vitro inner ear models derived from human pluripotent stem cells have been described that could be used as a model to evaluate the impact of these gene mutations in human inner ear function. To date, a major focus within these models is on inner ear neurosensory cell types, such as the hair cells. However, normal function of the inner ear is dependent on other, non-sensory, cell types. Since genetic inner ear pathologies are not limited to the sensory epithelia, in vitro models should, ideally, contain the integrated functional unit of these epithelia to truly recapitulate genetic inner ear disorders. We have evaluated the clinically relevant neurosensory and non-sensory gene and protein expressions in human inner ear organoids present in a multilineage aggregate.
To this end, we generated inner ear organoids in a three-dimensional culture by modulating the FGF, TGF, BMP and WNT pathways in seven different human pluripotent stem cell lines. Clinically relevant protein expression was assessed by immunohistochemistry and gene expression by single-cell or single-nucleus RNA sequencing.
Our results show that based on ultrastructure and gene expression, aggregates at differentiation day 65 and later contained a diversity of inner ear cell types, including sensory and non-sensory inner ear epithelium, periotic mesenchyme, and neurons. Additional cell types were identified in our aggregate, including myocytes, endothelial cells and choroid plexus epithelium. The inner ear organoids within these aggregates expressed genes and proteins known to be mutated in the most common types of syndromic SNHL, including Usher (USH1C, CDH23), Waardenburg (SOX10), Pendred (SLC26A4), and Bartter (BSND) syndromes. Similarly, non-syndromic genes and proteins are expressed, including GJB2 which accounts for 50% of cases of non-syndromic SNHL.
Our study shows the cellular diversity and clinical relevance of inner ear organoids. For the first time, the expression of neurosensory and non-sensory genes and proteins in a single in vitro inner ear model have been established. This opens the way for in vitro genetic SNHL modelling, as well as the discovery and evaluation of (gene) therapies using this inner ear organoid model.

Co30

90 - Cytomegalovirus Entry Protein Expression in the Fetal Human Inner Ear

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Congenital cytomegalovirus (CMV) infection is the most common non-genetic cause of sensorineural hearing loss (SNHL). In addition, vestibular impairment is frequently observed in children infected with CMV. However, the pathogenesis of CMV-induced hearing loss and vestibular impairment remains unclear. In order to gain understanding in the pathogenic mechanism, it is important to know which inner ear cell types are susceptible to CMV infection.

CMV is known to have a wide tropism. The virus enters the human cell using several glycoproteins among which are gB, the pentameric complex and the trimeric complex. The platelet-derived growth factor alpha (PDGFRα), neuropilin-2 (NRP2) and endothelial growth factor receptor (EGFR) have been identified as receptors ('entry proteins') for these viral glycoproteins. However, it is unknown if and at what fetal stage these entry proteins are expressed in the developing human inner ear.
Methods
Fetal human inner ears from both the first (8-11 weeks fetal age (W), N=6) and second trimester (W12-15, N=4) were obtained by means of elective abortion and fixed directly upon collection. Paraffin sections were immunostained for NRP2, PDGFRα and EGFR. Furthermore, inner ear organoids derived from induced pluripotent stem cells were used in order to verify the staining data. RNA-sequencing data from both single nuclei and single cells were acquired of these organoids.
Results
Immunofluorescent staining showed that NRP2 was expressed in the spiral ganglion cells and, in a lesser degree, in the mesenchyme. EGFR was expressed in the mesenchyme, epithelial cells, spiral ganglion cells and stereocilia in the vestibular organs. PDGFRα was also expressed in the mesenchyme, but not always co-expressed with EGFR. All CMV entry proteins were detected in tissue from the first and second trimester. RNA-sequencing data confirm these results.
Conclusion
CMV entry proteins NRP2, PDGFRα and EGFR are already expressed during the first trimester, both in the cochlea and vestibular organs. The proteins are co-expressed in the mesenchymal cells and spiral ganglion cells, which makes these cells potential targets for CMV infection. In future experiments, this hypothesis will be tested in an ex vivo fetal human inner ear model that will be co-cultured with several CMV strains.

Keywords: cytomegalovirus, entry proteins, pathogenesis

Genetics and diseases

Co31

131 - Dual molecular diagnosis in complex hearing loss patients: when a single gene is not enough

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Background: Hearing loss (HL) is the most frequent sensory disorder, affecting approximately 1:1000 newborns, and is characterised by a large clinical and genetic heterogeneity. Indeed, more than 120 genes have been already associated with non-syndromic HL (NSHL) and about 400 syndromes that include HL have been described (SHL). In this tangled clinical and genetic picture, the application of Next Generation Sequencing approaches in the diagnostic work-up has greatly implemented our ability to reach a molecular diagnosis. In particular, we recently highlighted the value of Whole Exome Sequencing (WES) to detect dual molecular diagnosis in patients with signs and symptoms that do not precisely fit a known syndrome (PMID: 35052694). Here we report on further patients concurrently affected by HL and other Mendelian disorders caused by variants in two independently segregating loci.
Methods: In the last 18 months we have molecularly characterised 110 patients affected by HL. All patients underwent a deep clinical evaluation and first-tier genetic testing (evaluation of both *GJB2* and *STRC*). Finally, in subjects that resulted negative, WES analysis was performed.
Results: In our cohort, we detected four additional patients presenting a dual molecular diagnosis. Patients may be divided in two groups: a) subjects with distinct phenotypes, i.e. HL and other clinical features due to a second condition; b) subjects carrying variants in different genes, both associated with HL. In group a) **Patient-1** was affected by HL, due to a pathogenic homozygous variant in *GJB2*, and Retinitis pigmentosa, caused by

a heterozygous *de novo* variant in *RPGR*. **Patient-2** presented Pseudoxanthoma elasticum and HL. Her first clinical condition may be explained by a homozygous variant in *ABCC6*; as regards HL, we detected a heterozygous variant in *WFS1*, associated with Wolfram and Wolfram-like syndromes. In group b) **Patient-3** presented NSHL and resulted to be a carrier of both a heterozygous variant in *EDN3* and a heterozygous variant in *ATP2B2*. The former gene is causative of Waardenburg syndrome type 4B and the latter causes autosomal dominant HL and digenic autosomal recessive HL, acting as a modifier of *CDH23*. Finally, an interesting case is represented by **Patient-4**, who does not fit into any of the two above mentioned groups. The subject presented with HL due to necrotising otitis: WES analysis detected two compound heterozygous variants in *C3* (responsible of C3 deficiency), which may explain his clinical presentation, and a heterozygous variant in *GATA1*, associated with X-linked Thrombocytopenia, whose presence still needs to be assessed.
Conclusion: The reported cases support our previously demonstrated hypothesis that complex HL patients may be simultaneously affected by more than one genetic condition. This occurrence needs to be considered, especially in subjects presenting HL and other clinical features that do not fit into a known syndrome. Our results highlight once again the importance of WES analysis together with a multidisciplinary approach to correctly evaluate these patients and achieve a precise genetic diagnosis.

Keywords: Hearing Loss, WES, Dual molecular diagnosis

Co32

49 - Exploring the Pathological Mechanisms of miR-96 Mutations in the Inner Ear

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The microRNA miR-96 is important for hearing, as it acts as a transcriptional regulator in the inner ear and coordinates hair cell maturation. Point mutations in the seed region of miR-96 cause dominant progressive hearing loss in humans and mice. Here, we present two mouse mutants carrying two point mutations identified as underlying progressive hearing loss in humans (Mir96s403 and Mir96s1334). This study aims to determine the underlying pathological mechanisms in the inner ear.
Auditory brainstem response (ABR) measurements, scanning electron microscopy (SEM) and immunolabelling of pre- and post-synaptic components were used to determine the onset of hearing impairment, the hair bundles' morphology, and look for synaptic defects, respectively. We performed RNAseq of the organ of Corti and RT-qPCR to determine how the different mutations affect the gene expression profile. We are currently using several approaches such as gene set enrichment analysis (GSEA) to construct the miR-96 regulatory network.
Our results indicate that the two mutations lead to different physiological, structural and transcriptional phenotypes. Homozygotes of both mouse lines exhibit profound hearing loss, but only Mir96s1334 heterozygous mice have a mild progressive hearing loss. Structural analyses showed hair cell degeneration and misshapen hair bundles in both mutants, with Mir96s1334 mice being more severely affected. Moreover, Mir96s403 homozygotes show a reduction in the number of inner hair cell synapses. The structural phenotype of Mir96s1334 mice is more severe than that of Mir96s403 mice, consistent with the audiological features displayed by humans carrying those mutations. However, the lack of hearing impairment in Mir96s403 heterozygotes in contrast to the human findings might indicate that mutant miR-96 is acquiring different targets in mice and humans. Identifying the critical pathways underlying hearing impairment will ultimately allow the pharmacological modulation of the miR-96 regulatory network for the prevention or delay of hearing loss.
Keywords: miR-96, gene regulatory networks, progressive hearing loss.

Co33

157 - TRIOBP5 Recruits ANKRD24 to Reinforce Stereocilia Insertion Points and Enhance Rootlet Resilience

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Background: Stereocilia are composed of hundreds of unidirectionally oriented and tightly packed actin filaments. However, at their insertion into the apical surface of the cell, each stereocilium has only a few dozen actin filaments (F-actin), allowing it to pivot easily. Stereocilia rootlets traverse this pivot point, anchoring stereocilia firmly into the cell's cuticular plate and protecting them from overstimulation and damage. Rootlets are made of actin filaments that are even more tightly compressed together than those of the stereocilium core due to snug bundling of F-actin by TRIOBP4 and TRIOBP5. The upper part of the rootlet extends into a stereocilium and is bundled mostly by TRIOBP4, while the lower rootlet extends into the cuticular plate and are belted by both TRIOBP4 and TRIOBP5. We show that a novel protein of rootlets, ANKRD24, a member of the N-Ank protein family, interacts with TRIOBP5. ANKRD24 is recruited to stereocilia rootlets by TRIOBP5.
Methods: ANKRD24 was identified by mass spectrometry while characterizing proteins isolated from chicken and mouse vestibular stereocilia (Shin et al, 2013; Krey et al, 2015). An ANKRD24 knockout (KO) mouse was generated using CRISPR/CAS9 technology and characterized using ABR, DPOAE and noise overstimulation. Triobp5 KO mice (Katsuno et al, 2019) were used to investigate localization of ANKRD24 in the absence of TRIOBP5. Helios gene gun transfection experiments reveal the role of TRIOBP5 in recruitment of ANKRD24 to stereocilia rootlets. Super-resolution light microscopy, scanning and transmission electron microscopy and co-immunoprecipitation methods were used to show the localization and interaction of ANKRD24 and TRIOBP5.
Results: Using super-resolution microscopy, we show that ANKRD24 concentrates at the stereocilium insertion point, forming a ring at the junction between the lower and upper rootlet. This annular pattern of ANKRD24 continues into the lower rootlet where it surrounds and binds TRIOBP5, which together with TRIOBP4, bundles rootlet F-actin. In mice lacking TRIOBP5, ANKRD24 no longer localizes to rootlets. Gene gun transfection using Triobp5 KO inner ear explants shows that transfected DsRed-TRIOBP5 recruits endogenous ANKRD24 to rootlets and restores its normal localization in these mice. However, in Ankrd24 KO hair cells TRIOBP5 is present but unevenly distributed in lower rootlets. Ankrd24 KO shows stereocilia bundle abnormalities similar to Triobp5 KO at light and electron microscopy levels and also has a similar but slower progressive hearing loss, as well as an increased susceptibility to overstimulation of the hair bundle. Using deletion constructs of ANKRD24 and co-immunoprecipitation experiments, we show that the interaction of ANKRD24 and TRIOBP5 occurs through the coiled coil domains of both proteins.
Conclusions: TRIOBP5 recruits ANKRD24 to the stereocilia insertion points and lower rootlets. ANKRD24, in turn, bridges the apical plasma membrane with the lower rootlet and maintains the normal distribution of TRIOBP5. Together with TRIOBP5, ANKRD24 organizes rootlets to enable long-term resilient hearing.

Keywords: Stereocilia, rootlets, TRIOBP, ANKRD24

Co34

126 - Age-related hearing loss and *SLC7A8*: Identification of molecules able to restore the gene lost activity

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Age-related hearing loss (ARHL) is a common chronic condition affecting more than 30% of individuals aged over 65 years. Generally, the patients experience bilateral sensorineural hearing loss (HL), affecting mainly the high frequencies. ARHL is characterized by a complex multifactorial etiology; indeed, as regards the genetic contributions, few causative genes have been highlighted. Nevertheless, a new candidate gene named *SLC7A8* has been recently described. *SLC7A8* is the catalytic subunit of the heterodimer SLC7A8/SLC3A2 that mediates the exchange of all neutral amino acids. Interestingly, our previous study (Espino Guarch M. et al., 2018) proved that *Slc7a8* expression in mice's cochlear structures increases with ageing and knock-out mice display ARHL. Further, the tertiary analysis of WGS data, available for a cohort of 147 individuals from isolated Italian villages, revealed four heterozygous missense variants (p.Val460Glu, p.Thr402Met, p.Val302Ile and p.Arg418His) in subjects affected by ARHL. The variants were functionally characterized and proved to have a pathogenic effect probably reducing the protein's activity. Considering the relatively high prevalence (around 3% in the analyzed cohort) of these gene variants, we aim to perform a high-throughput screening (HTS) of more than 20000 molecules searching for a compound able to increase the expression of *SLC7A8*. Considering that all the subjects carry a functioning allele, it might be possible to restore the gene function by increasing its expression. To achieve these results, we took advantage of the CRISPR/Cas9 technology to generate three different knock-in (KI) cell lines. The first one is a luciferase KI reporter cell line in which the luciferase gene was inserted in the genomic area upstream of *SLC7A8,* under the control of endogenous *SLC7A8* promoter. The other two KI lines carry at the *SLC7A8* locus two missense variants (p.Thr402Met and p.Arg418His) previously identified in the Italian cohort. The HTS will be performed on the reporter cell line and the luminescence -the readout of the luciferase assay- will be measured to study *SLC7A8* transcriptional regulation and to screen and select the most effective drugs/compounds ('hits') for further evaluation. The hit compounds that meet luciferase threshold criteria will be rescreened in a dose-response experiment. The final validation of the most promising hits will be carried out in the cell lines carrying the point mutations in order to evaluate if these compounds are able to increase *SLC7A8* expression and restore its function. In conclusion, we aim to better characterize the ARHL induced by *SLC7A8* variants and to identify molecules that could be potentially further developed into drugs. Indeed, the restoration of *SLC7A8* activity might be beneficial for a subset of genetically selected patients predisposed to the development of ARHL.

Keywords: Age-related hearing loss, *SLC7A8,* high-throughput screening, luciferase assay

Auditory neuropathy: where are we now?

Co35

167 - Genotype phenotype correlations of 37 DFNB9 patients with auditory neuropathy and 17 new OTOF pathogenic variants

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Auditory neuropathy represents 5-10% of child's hearing loss. Pathogenic bi-allelic variations of OTOF result in autosomal recessive deafness DFNB9. We retrospectively studied the genotype-phenotype correlations of 37 cases from 30 families with pathogenic bi-allelic OTOF variations. Seventeen new pathogenic variants were identified. All patients had isolated auditory neuropathy. Hearing loss was pre-lingual in 78% of cases and profound in 70%. Hearing loss was progressive in 30%, fluctuating in 30% and temperature-sensitive in 22%. The diagnosis of auditory neuropathy was mainly based on the discordance of electrophysiological tests with acoustic otoemissions present (78%) and brainstem auditory evoked responses absent or desynchronized (81%). All patients with homozygous or compound heterozygous "loss of function" variants had congenital bilateral profound hearing loss, patients compound heterozygous for a “loss of function” variant and a missense variant had variable presentations. Those with two missense variants had a mild to severe hearing loss, which could be of secondary onset. 54% received cochlear implant rehabilitation, 16% of which were bilateral. Our study confirms a successful hearing rehabilitation with cochlear implants, with open word perception increasing from 0% before surgery to 80% at 8 years after implantation. However, cochlear implantation cannot be considered as a treatment (disturbance in noise, social difficulties and professional integration...). Gene therapy trials in mutant OTOF -/- adult mice have shown prolonged hearing rehabilitation, making it possible to consider a short-term therapeutic trial for this isolated congenital form of deafness (RHU AUDINNOVE 2019). This phenotype-genotype study is an essential prerequisite for the future therapeutic trial.

Otoprotection

Co36

101 - Insulin-like growth factor 1 otoprotection in basal and stress conditions is mediated through PI3K/AKT activation in HEI-OC1 cells

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Hearing loss is one of the most common sensory impairment in the human population[1]. Mutations in the gene coding for insulin-like growth factor 1 (IGF-1) are associated with hearing loss in man and mice[2]. IGF-1 biological actions are mediated by binding to its high-affinity transmembrane receptor, IGF1R, leading to the activation of the PI3K-AKT and MAPK-ERK pathways[3]. IGF-1 is essential for inner ear development and hearing, since its deficiency leads to abnormal cochlear morphology and impaired innervation of the hearing receptor[4]. The latter is not easily accessible and contains a limited number of the sensory hair cells, which are fragile, hard to isolate, and the ones that can be collected hardly survive *in vitro*. All this has led to develop the House Ear Institute-organ of Corti 1 (HEI-OC1) auditory cell line[5], derived from the auditory organ of the

transgenic Immortomouse™, which emerges as an alternative model to unravel the mechanisms involved in hearing loss associated with IGF-1 deficiency.
Here, we found that IGF system genes (factors and receptors) are expressed in HEI-OC1 cells and their levels are modulated during cell differentiation. We also observed that IGF-1/IGF1R engagement activates PI3K/AKT/mTOR signaling in HEI-OC1 cells to promote survival, constructive metabolism, and to reduce autophagic flux and apoptosis. We also showed that IGF-1 protects HEI-OC1 cells from an ototoxic insult (cisplatin) through increasing cell survival and antioxidant response, and reducing caspase-3 activation.
To conclude, our results indicate that HEI-OC1 cells reproduce the reported *in vivo* actions of IGF-1 in the cochlea, arising as a suitable model to understand the role of IGF-1 in the inner ear. Our data also demonstrate the otoprotective role of IGF-1 against cochlear insults, strengthening the essential role of IGF-1 in normal hearing.

Keywords**:** IGF-1; HEI-OC1; energy homeostasis; cisplatin.

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References:
1. Deafness and Hearing Loss. https://www.who.int/news-room/fact-sheets/detail/deafness-and-hearing-loss
2. García-Mato Á, Cervantes B, Murillo-Cuesta S, Rodríguez-de la Rosa L, Varela-Nieto I. Insulin-like Growth Factor 1 Signaling in Mammalian Hearing. *Genes (Basel)*. **2021**, 12(10):1553.
3. Hakuno F and Takahashi SI. IGF1 Receptor Signaling Pathways. *Mol. Endocrinol*. **2018**, 61, T69–T86.
4. Camarero G, Avendano C, Fernandez-Moreno C, Villar A, Contreras J, de Pablo F, Pichel J.G, Varela-Nieto I. Delayed Inner Ear Maturation and Neuronal Loss in Postnatal Igf-1-Deficient Mice. *Neurosci*. **2001**, 21, 7630–7641.
5. Kalinec GM, Webster P, Lim DJ, Kalinec F. A cochlear cell line as an in vitro system for drug ototoxicity screening. *Audiol Neurootol*. **2003**; 8(4):177-189.

Co37

162 - Superparamagnetic Iron Oxide Nanoparticle-Based Delivery of Dexamethasone using Magnetic Attraction for Otoprotection in Ototoxicity-induced Hearing Loss

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Nanoparticles can deliver a wide variety of drugs, target them directly to injured organs, and stabilize their release, preventing their degradation and inactivation by the body. The aim of this study was to develop a drug carrier of superparamagnetic iron oxide nanoparticles (SPIONs) loaded with dexamethasone and controlled by magnetic attraction for localized treatment of ototoxicity-induced hearing loss. PLGA [poly (lactic-co-glycolic acid)]-coated polymeric SPIONs with dexamethasone (PSD) and without dexamethasone were fabricated using an oil-in-water (O/W) emulsion method and was applied for therapeutic development in hearing loss. PSD delivered via intra-bullar injection under magnetic field penetrated to the inner ear and prevented the progression of hearing loss to a greater degree than equivalent doses of dexamethasone alone and PSD alone (at 28 th day: ototoxic, 80±0 dB; Dexa 100, 60±15.5 dB; PSD 100, 50±8.2 dB; PSD 100 with magnet, 22.5±5 dB, p<0.05). The otoprotective effects of this drug were confirmed in a series of ototoxicity preclinical models from ex vivo explanted sensory epithelium to the phantom of inner ear and in vivo in this study. Taken together, these findings suggest that the encapsulation of SPIONs with dexamethasone in addition to magnetic field application in the inner ear prevents the progression of ototoxicity-induced hearing loss through anti-apoptotic mechanisms.

CLOSING SESSION

164 - Cotugno & Corti: the bicentenary of a passing of the baton

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Domenico Cotugno died of a cerebral stroke on the 5th of November 1822 at the age of 86 in Naples. A few months before, on 15th June 1822 in Gambarana, a small town not far from Pavia, was born Alfonso Corti.

Cotugno was born in Ruvo di Puglia on January 29 of 1736 into a modest family. Corti was the son of Marquis Gaspare Giuseppe Corti of Santo Stefano Belbo and Marquise Beatrice Malaspina of Carbonara.

Cotugno was only 24 years old when his first great work, "De aquaeductibus auris humanae internae anatomica dissertatio", was published in 1760. Cotugno working on fresh ear specimens collected from newborns or foetuses, the existence of two aqueducts (cochleae et vestibuli). Thanks to his revolutionary dissection technique, he also detected the constant presence of labyrinthic fluid. He wrote that the aqueducts were filled with this liquor, and not with air, as was generally held according to the Aristotelian dogma of “aer ingenitus”, uncontested by scholars such as Falloppio, Eustachius and Willis. Cotugno also stated that the perilymphatic and subarachnoid space communicated at the base of the brain.

Corti published his main paper at the age of 30 "Recherches sur l'organe de l'ouie des mammifères” in the journal Zeitschrift für wissenchaftliche Zoologie (3, 109-169, 1851), published in Leipzig by his colleague and friend Albert Kolliker. This was the first histological description of the fine structure of cochlear receptor epithelium, which later became known as the organ of Corti when Professor von Kölliker referred to the inner and outer pillar cells as the rods of Corti and the intervening tunnel as the tunnel of Corti (von Kölliker, 1852) with the entire structure of the organ of hearing eventually being referred to as Corti’s organ.

POSTERS

POSTER SESSION 1

01. Aging

P1.01.01

95 - Attenuation of auditory cell senescence by Urolithin A

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Introduction: Aging of sensory organs is associated with a decline in mitochondrial function and the accumulation of dysfunctional mitochondria. Impaired mitophagy blocks the turnover of dysfunctional mitochondria and leads to their accumulation. Urolithin A (UA) induces mitophagy in various mammalian cells. This study was aimed at investigating the effect of the mitophagy activator, UA, on premature senescent auditory cells.
Methods: Low-dose H2O2 was used to induce premature senescence in House Ear Institute-Organ of Corti 1 (HEI-OC1) cells and cochlear explants. Senescence-associated p53 and p21, and mitophagy-related proteins were examined in HEI-OC1 cells and cochlear explants. Cellular senescence was examined using β-galactosidase activity. The effect of UA on mitophagy and cellular senescence was examined in H2O2-induced senescent HEI-OC1 cells and cochlear explants. And, knockdown of mitophagy genes was conducted in HEI-OC1 cells to investigate whether anti-senescent activity of UA is dependent on mitophagy or by other effects.
Results: UA significantly decreased the expression of senescence-associated p53 and p21, and increased the expression of mitophagy-related proteins, in H2O2-induced senescent HEI-OC1 cells and cochlear explants. The percentage of β-galactosidase-stained senescent cells also reduced in H2O2-treated cells and cochlear explants upon UA pre-treatment. The formation of mitophagosomes and mitophagolysosomes was restored upon UA pre-treatment of H2O2-induced senescent cells. The knockdown of mitophagy-related genes (Parkin and Bnip3) resulted in annulment of UA-induced anti-senescent activity. UA significantly increased the ATP content, mitochondrial DNA (mtDNA) integrity, and mitochondrial membrane potential in senescent HEI-OC1 cells.
Conclusions: These findings indicate that UA counteracted mitophagy decline and prevented premature senescence in auditory cells. Hence, UA administration might be a promising strategy for preventing mitochondrial dysfunction in patients with age-related hearing loss.

Keywords: urolithin A, age-related hearing loss, mitochondria, mitophagy

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P1.01.02

15 - Age dynamics of the upper frequency boundary of the sound range by G. von Bekesy (analytical solution)

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During life, the perceived human sound range undergoes changes, while its upper limit decreases, and the lower one increases. This is evidenced by many scientific works of both scientists of previous years and our contemporaries, but they have not yet found theoretical confirmation.
According to the Nobel Prize winner G. von Békésy (1947), the decrease in the threshold of high –frequency susceptibility after 40 years is 80 Hz for every six months. Mathematically, this ratio is written in the form – Δf=RBΔt, where RB is the proportionality coefficient between the determined values.
The analytical approach to the problem of determining the upper limit of the perceived range will consist in the transition from finite differences to infinitesimal differences (differentials) of these quantities. Thus, we get the differential equation –df=RB.dt. Integrating the resulting equation ∫df=–RB ∫dt with the initial conditions at the

time of birth (t = 0 years), at which f(0 years) = fm0=20 kHz, we get the desired analytical solution f(t)=–RB.t+fm0. It can be noted that the research and judgments of G. von Bekesy are valid only in a small period of time. It requires a different approach, the choice and development of a new model in solving the specific problem of changing the sound perception.
Key words: sound range, upper frequency threshold limit, linear function, experiment of G. fon Bekesy, analytical solution.

P1.01.03

107 - OCT-a Retinal Vascular Density, Age-Related Central hearing loss as predictors for global cognitive function in an Italian Older Population

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Introduction: Hearing, balance, olfaction, and taste represent a complex sensory neural construct biologically linked to aging. Hearing and vision are the most explored senses, providing robust population-based evidence. Several studies have described their role as predictors in age-related neurodegenerative processes, particularly those related to cognitive decline. In particular, their role in the early and preclinical stages of cognitive declines, such as mild cognitive impairment (MCI), is not clear. Furthermore, new retinal imaging, based on OCT with angiography, provides further evidence of its role as an in vivo model of retinal neurodegeneration. This study aims to assess the role of peripheral and central hearing loss and retinal abnormalities as predictors for global cognitive impairment in the older population of Great Age \ Salus in Apulia Study.
Methods: We analyzed data on 1829 older participants (65+) in the cross-sectional population-based Salus in Apulia Study. Optical coherence tomography angiography was used to measure SVD and DVD of the capillary plexus of the macula at the 3-mm circle area centered on the fovea (whole retina), the parafoveal quadrant, and foveal quadrant. Disabling peripheral ARHL was defined as >40 dB hearing level of pure tone average on the frequencies from 0.5, 1, 2, and 4 kHz in the better ear, and age-related CAPD as <50% at the Synthetic Sentence Identification with Ipsilateral Competitive Message test in at least one ear. Global Cognitive functions were measured with Mini-mental State Examination (MMSE).
Results: The median age of the sample was of 75 years (age range of 65–92 years), median education of 5, and 45% of males. For every unitary increase of DVD, there was a 0.07 increase in MMSE on average (95% C.I.: 0.02 to 0.12). When adjusted for both PTA and SSI-ICM in the same model we have noticed an effect modification and we tried an interaction using the new variable PTA multiplied for SSI-ICM. PTA and DVD used in the same model showed an increased effect of association and prediction power (Beta 0.48 95% 95% C.I.:0.31 to 0.51; c-statistic: 0.78).
Conclusions: We identified a consistent association between age-related CAPD and deep retinal vessel density. ARHL, both at the peripheral and central level, and retinal vessel changes have both been associated with neurodegeneration/dementia, exploration of the retina by OCT-A imaging, as an in-vivo model, may help to better understand the complex neurobiological relationship between auditory changes and dementia. Early deep retinal vessel changes in older subjects without hearing disorders may be predictive of the subsequent development of age-related CAPD. Age and peripheral hearing loss and deep retinal vascular features are interlinked in some way. This finding should be investigated in relation to other senses (taste and olfaction).

P1.01.04

174 - Variability in Early Markers of Sensorineural Hearing Loss with Age

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Age-induced cochlear synaptopathy (CS) was demonstrated in rodent and human temporal bone studies, but it remains unclear whether this pathology is the underlying cause of speech intelligibility declines in the aging population. Studying the causality of this relationship in human is not straightforward, since thus far developed EEG-based diagnostic tools of sensorineural hearing loss (SNHL), do not explicitly quantify the CS component and several pathologies can coexist in age-induced SNHL. To better understand the functional role of different SNHL aspects, we studied how several early markers of SNHL (audiogram or auditory evoked potential (AEP) based) relate to the speech recognition threshold. We complemented this analysis with model simulations to estimate the CS-related decline-rate of AEPs and to study its relation to speech intelligibility in the aging cohort.
A total of 92 Flemish subjects participated in this study and were divided into two groups: (i) a young control group with normal audiograms (n=37, 20-36 years) and (ii) adults with normal audiograms, some with tinnitus or self-reported speech intelligibility problems (n=54, 25-64 years). We applied a test battery that comprised audiograms measured at standard and extended high frequencies (EHF), envelope following responses (EFR) to a rectangularly amplitude modulated (RAM) pure-tone and speech intelligibility scores (Flemish Matrix sentence test). We calculated the age-decline rate of each metric and compared them to (1) the age-related auditory nerve (AN) fiber population decrement observed in human temporal bones, and (2) simulated RAM-EFR magnitudes declines for auditory models with different degrees of CS. Moreover, we computed the correlation strength of each metric with speech reception thresholds.
The RAM-EFR magnitude of our aging cohort declined with a rate of 6% per-decade. Simulated RAM-EFR magnitudes for varying degrees of CS, showed a 7.5% reduction when 15% of the AN fiber populations was assumed lost. Hence, our findings predict an approximate 50% loss of the AN fiber population by the age of 40, corroborating AN fiber counts in human temporal bone studies (loss of the 12% of AN fibers per-decade). While the EHF audiogram of our aging cohort declined with a rate of 13 dB per-decade, the standard audiogram only reduced by 2.5 dB per-decade, and remained clinically normal hearing.
The data from our aging cohort, together with the model simulations and human temporal bone studies, support the view that CS plays an important role in the RAM-EFR magnitude reduction and temporal envelope encoding. We show that a timely-diagnosis of speech intelligibility problems can be made based on either EHF thresholds or RAM-EFRs.

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02. Cochlear implant and implantable prosthesis

P1.02.01

58 - A new customized cochlear implant mapping

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Introduction: To verify the utility of an innovative tool to select, preoperatively, the electrode array best fitting the cochlear length and to match the frequency information delivered by the cochlear implant (CI) and cochlear neural fibers with corresponding characteristic frequencies.
A perfect match is difficult to achieve due to the high inter-patient variability in the cochlear shape and to the extent of electrode array insertion.
Objective: The aim of this study is to evaluate speech perception outcomes after a frequency reallocation performed through the creation of an anatomically based map obtained with "Otoplan", a tablet-based software that allows the cochlear duct length to be calculated starting from CT images and permits to match the frequencies of the array with the cochlear frequencies.
Materials and methods: Thirteen postlingually deafened patients who underwent cochlear implantation with MED-EL company devices from 2015 to 2021 in the Tertiary referral center University Hospital of Verona have been included in a retrospective study.
The differences in the PTA, SAT and SRT (sigle) values before and after the reallocation were determined and statistically evaluated.
Results: The mean values of SRT and SAT were significantly better after the reallocation.

Conclusions: Our preliminary results demonstrate better speech discrimination and rapid adaptation in implanted postlingually deaf patients after anatomic mapping and subsequent frequency reallocation. The adequate frequency match between the cochleas and the electrode array improve the auditory outcomes. The new method is particularly promising in younger and older patients poorly collaborative.

P1.02.02

145 - The Clinical Effects of Steroids Therapy in the Preserving Residual Hearing after Cochlear Implantation with the OTICON Neuro Zti EVO

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Background: A prospective clinical study was conducted to investigate whether two different pharmacotherapy strategies of steroid administration impact hearing preservation in adult patients who underwent cochlear implantation with the Oticon Medical Neuro cochlear implant system. **Methods**: Twenty-nine adult participants were included. Pure tone audiometry was performed before implantation, during processor activation and 12 months after activation. There were three treatment groups: (1) intravenous steroid therapy (standard steroid therapy with dexamethasone administrated intravenously at the dose 0.1 mg/kg body mass twice a day); (2) combined oral and intravenous steroid therapy (extended steroid therapy with dexamethasone administrated intravenously at the dose 0.1 mg/kg b.m. twice a day and prednisone (orally) at the dose 1 mg/kg body mass/24 h), and (3) no steroid therapy (a control group). Patients' hearing thresholds before implantation were on average 103 dB HL, 89 dB HL, and 93 dB HL, respectively. **Results**: Deterioration of hearing thresholds was observed in all three patients' groups. Twelve months after surgery the patients with and without steroid therapy had similar hearing thresholds. **Conclusions**: The steroid regimen used in this study did not play a significant role in patients with non-functional residual hearing, who underwent cochlear implantation with the Oticon Medical Neuro cochlear implant system.

Keywords: partial deafness treatment; steroids; cochlear implants

P1.02.03

71 - The effect of chronic electrical stimulation on structure and function of the auditory nerve in deafened guinea pigs

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Background: Spiral ganglion cell (SGC) loss after severe hair cell loss has been characterized extensively in both animals and humans. We have previously shown that this auditory-nerve degeneration is accompanied by functional changes in deafened guinea pigs (e.g., Ramekers et al., 2014, J Assoc Res Otolaryngol). In the present study we examined whether chronic electrical stimulation (CES) of the auditory nerve, as received by cochlear implant users, reduces either of these structural and functional degenerative processes.
Methods: Normal-hearing guinea pigs were implanted with an intracochlear electrode array and were ototoxically deafened four weeks later by co-administration of kanamycin and furosemide. Starting either one or five weeks after deafening, the auditory nerve was chronically electrically stimulated for two weeks. Using a MED-EL PULSAR cochlear implant, awake eCAP recordings were performed at least weekly during the entire 7-11 weeks period following implantation. In each session eCAPs were recorded in response to single biphasic current pulses of which the current level, phase duration and inter-phase gap (IPG) were systematically varied (Ramekers et al., 2014, J Assoc Res Otolaryngol). Following the final eCAP recording session the animals were sacrificed, and their cochleas were processed for histological quantification of SGCs.
Results: SGC survival was similar for the implanted right and the non-implanted left ears in control animals. Surprisingly, the SGCs in the implanted ear were significantly larger than those in the non-implanted ear. Animals receiving CES showed a moderate but statistically significant increase in SGC survival in their implanted/stimulated ear compared to the contralateral ear; cell size across ears was similar in these animals. Most eCAP measures had stabilized during the four weeks prior to deafening, after which a decrease in both

amplitude and threshold, and an increase in dynamic range was observed. None of the analyzed eCAP measures were affect by CES.
Conclusion: CES slowed down, but did not stop SGC degeneration – consistent with findings in human CI users (Seyyedi et al., 2013, Hear Res 302). The eCAP measures reflected SGC survival in a similar fashion with CES than demonstrated previously without CES. We conclude that since changes in eCAPs after deafening are largely unaltered by CES, application of CES in animal studies is not necessary in order to mimic the clinical human situation.

Keywords: eCAP, cochlear implant, electrical stimulation, auditory nerve, guinea pig

03. Developmental biology

P1.03.01

38 - Transcriptome Analysis of Nascent Hair Cells Identifies Ccer2 as a Novel Gene Upregulated during Differentiation

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Introduction: Atoh1, a helix loop helix transcription factor, is necessary and sufficient for sensory hair cell formation and differentiation. These specialized sensory cells detect sounds and movements in the auditory and vestibular systems, respectively. To discover the genes that are downstream of Atoh1 and involved in hair cells formation we profiled the transcriptome of Atoh1-induced ectopic hair cells at the early stage of mammalian cochlear development.
Methods: We electroporated embryonic (E) day 13 mouse cochlear explants with an Atoh1 GFP reporter construct, or with an empty GFP vector as a control. At this stage of development, overexpression of Atoh1 results in a 100% conversion of electroporated cells into hair cells. To identify the earliest genes regulated by Atoh1 overexpression, we used fluorescence-activated cell sorting (FACS) and sorted the cells overexpressing GFP 24 h after electroporation. We extracted RNA from both Atoh1 GFP and control GFP cells and performed bulk RNA-sequencing (RNA-seq).
Results: We found more than 800 differentially expressed genes (~700 upregulated and ~100 downregulated), and our bioinformatic analysis detected several known hair cell genes (e.g., Gfi1, Jag2, Dll1) in the Atoh1 expressing cells. Furthermore, we identified Ccer2 (coiled-coil glutamate-rich protein 2), a novel gene that was significantly upregulated (6-fold change). CCER2 is an uncharacterized protein; there is no published information about its structure, localization, or function. We confirmed the expression of CCER2 in endogenous cochlear and vestibular hair cells and assessed that is one of the earliest markers expressed during hair cells development. We investigated its spatiotemporal expression during mouse cochlear and vestibular development and found that in the cochlea, CCER2 has a developmental base-to-apex gradient and is transiently expressed starting at E13 up to postnatal day 6, following the spatiotemporal expression of Atoh1. In the balance organs (utricle and saccule), the protein is expressed embryonically and throughout adult stages. We analyzed the function of Ccer2, in hearing and balance, by generating Ccer2 mutant mice (FVB/NJ background) using CRISPR/Cas9 technology, and performed ABR and DPOAE, as well as rotarod balance tests.
Conclusions: Our transcriptomic analysis is the first RNA-seq study that profiled up- and down-regulated Atoh1 downstream targets in the early stages of hair cell differentiation, which led to the discovery of CCER2, a novel and specific protein marker for inner ear sensory hair cells. The characterization of CCER2 will provide insights into both Atoh1 and other signalling pathways where it is involved, advancing our understanding of inner ear development.

Keywords: RNA-seq, Atoh1, hair cell.

P1.03.02

35 - Impact of proteostasis on neuronal diversity in the developing inner ear

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Recently, it has been shown that spiral ganglion neurons not only differ at the physiological and morphological level but also at the molecular level (Shrestha et. al, 2018; Sun et al, 2018; Petitpré et al, 2018). Growing evidence suggests a preferential vulnerability of the Ic subtype (Lypd1+) during age and noise trauma, strongly affecting the ability of patients to understand speech in a noisy background and thus highlighting the importance of the normal functioning of each SGN subtype. But how and when the 3 subtypes emerge is still not yet fully elucidated. Protein aggregation, as it is the case during age, in some genetic diseases and after drug administration, could already be linked to sensorineural hearing loss. In our proteotoxic model of Elp3-deficient mice, we could show that there is an ER-stress induced neuronal cell death during development resulting in complete hearing loss. Thus, we plan to unravel the importance of proteostasis during development on the emergence and maintenance of the spiral ganglion neurons.
We used a Foxg1Cre mouse model to induce protein aggregation from E8.5 onwards in the developing sensory epithelia and the spiral ganglion neurons. By performing RNAscope experiments to detect neuronal subtype specific markers on P0 and P7 Elp3cKO mice, we wanted to know how proteostasis disruption impacts the emergence of the 3 neuronal subtypes. The results show that at P0, there is a predominance of Calb2-positive cells at the base and mid turns whereas at the apical turn there are mainly Lypd1-positive cells present. Surprisingly, at P7 this tonotopic pattern is lost, as Calb2-positive neurons are predominant in all three turns. This suggests that we have either a problem of delayed maturation or neuronal differentiation. Further, we plan to investigate if this phenotype is the result of a cell autonomous or a non-autonomous process by using a Bhlhb5Cre mouse line to specifically delete Elp3 only in the neuronal compartment.
At the level of the vestibular sensory organs, we could demonstrate that Elp3 invalidation increases the presence of aggresome-like structures and apoptosis within the VGNs from E13.5 onwards leading to a significant loss of VGNs at P0. We also observed a loss of Calyx-only afferences at P0 in the crista ampullaris and at P15 in the utricular organs. Interestingly, the number of Calyx-only afferences in the crista ampullaris at P15 is similar in the KOs compared to the WT, suggesting that there is a delayed innervation of vestibular hair cells due to proteotoxic stress. In the future, we will quantify the number of calyx-only afferences in the utricle at P30 to see if, similarly to cristae, a proper innervation pattern is rescued at later time points.
These results suggests that proteostasis during development is at least crucial for neuronal survival and for the normal development of spiral and vestibular ganglion neurons as well as the innervation of their target cells

04. Drug delivery system

P1.04.01

55 - Development of an animal model to test preventive local drug delivery of noise-induced hearing loss

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Noise induced hearing loss (NIHL) is one of the leading causes of sensorineural hearing loss and is caused by excessive overexposure to noise during occupational or recreational activities. Since there is no reliable treatment to restore the noise damaged inner ear, the development of preemptive drugs came into the focus of hearing research. To understand a drug's impact on cochlear cell function the drug delivery needs to enable reliable steady state concentrations. A local delivery into the perilymph is the only method to achieve this. Osmotic pumps are well-established for this purpose, currently mainly utilized in profoundly deaf individuals. In hearing animal models the insertion may cause massive deterioration of hearing thresholds. Such surgery related causes for hearing loss complicate the examination of other factors such as noise. The aim of the presented study was to develop an animal model for the examination of substances potentially preventing NIHL.

Male Hartley guinea pigs (n=6) were unilaterally implanted with a silicon catheter with a hook-shaped microcanula at its tip. The catheter was attached to an artificial perilymph containing osmotic pump. The microcanula was inserted in the scala tympani via the round window. Before and one week after the surgery the hearing status was assessed by acoustically evoked auditory brainstem response (AABR) measurements. Afterwards the anesthetized animal was exposed to a musical piece. The original audio was modified to generate a flat power spectrum between 200Hz and 40kHz (max. range 30dB between frequencies), presented at a peak SPL of 120dB. To examine the effect of the noise insult AABR were measured 30 min, 1 day and 7 days following noise exposure.
The implantation of the hook-catheter-pump device let to moderately increased click evoked hearing thresholds (7.5 ± 6.89 dB SPL peak). The threshold shifts from before to directly after noise did not differ significantly between implanted and contralateral ears. One week after noise insult, the hearing threshold partially recovered. The noise induced threshold shifts of both ears differed significantly ($p = 0.05$ for click condition) with implantation resulting in significantly lower threshold shifts compared to contralateral ears. The regeneration of the hearing function indicates that the induced threshold shift was temporary (TTS). Nevertheless, the combination of implantation and noise insult let to significantly increased thresholds, which were still detectable 7 days after noise.
The implantation of the hook-delivery device attached to an osmotic pump caused a moderate threshold shift that enables to further induce and detect a TTS. This facilitates the investigation of a drug's effects delivered prior to the noise insult via osmotic pumps to establish a preventive therapy against noise induced TTS, a prevalent disease.

Keywords: temporary threshold shift, osmotic pump, preventive treatment

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P1.04.02

132 - Preparation and Evaluation of Poloxamer 407 Hydrogel for Efficient Drug Delivery to the Inner Ear

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Introduction: Thermo-reversible hydrogels which are able to undergo sol-to-gel transition have been extensively utilized for drug delivery systems.
Methods: In this study, Poloxamer 407 (P407), a representative thermo-sensitive hydrogel, was investigated as an injectable hydrogel formulation for delivery of dexamethasone (DEX), an anti-inflammatory drug, to the inner ear. Hydrophobic DEX was introduced to P407 hydrogels by different loading methods, physical mixing (P407-P) and dialysis method (P407-D). Physico-chemical properties of DEX-loaded hydrogels were characterized in terms of thermogelation, drug loading content and efficiency, particle size, and drug release.
Results: The P407 hydrogel could effectively solubilized the hydrophobic DEX without crystallization and demonstrated a sustained release compared to the free DEX. The sol-gel transition temperature of the P407 hydrogel was not changed by presence of DEX. The in vivo study showed that our formulation delivered a considerably higher drug concentrations to the inner ear showing favorable safety profile without any apparent cytotoxicity or inflammation in the middle and inner ear.
Conclusion: Taken together, P407 could be useful as an injectable hydrogel formulation for inner ear delivery due to their high drug solubilization effect, loading capacity and thermosensitivity.

Keyword: Poloxamer 407, dexamethasone, sol-gel transition, thermo-sensitive hydrogel

P1.04.03

19 - In vitro transfection of primary fibroblasts from the inner ear of postnatal rats with recombinant plasmids

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Optimization of the drug-delivery systems to improve the nerve-electrode-interface following cochlear implantation is still in research focus. To ensure long termed supplementation of spiral ganglion neurons (SGN) with neurotrophic factors, innovative delivery systems on the basis of recombinant plasmids containing td-

tomato red and BDNF sequences are under investigation. Hereby, transfection of the cochlear fibroblasts adhering to the electrode surface after implantation with the recombinant vector is aimed to induce intracellular BDNF expression, followed by its release to support neuronal survival and outgrowth of neurites towards the CI-electrode. In this study an in vitro transfection model via lipofection using Lipofectamine™3000 was established for the first time in primary cochlear fibroblasts derived from postnatal rats. Transfection efficiency was characterized at varying cell seeding numbers and concentrations of the recombinant plasmid by counting fluorescent active versus DAPI stained cells. As well, BDNF concentration released by genetically modified cells was determined by using ELISA. Our results revealed transfection efficiencies between 15 and 20 % in comparison to untreated cells used as reference. The most effective transfection rates were achieved at a cell seed between 2000 and 4000 cells as well as at 300-500 ng of the recombinant plasmid. In accordance with the number of transfected cells an increasing BDNF concentration up to 22 ng/ml was found in the supernatants. Our results show that the transfection system as established for cochlear fibroblasts may be used as reference for any transfection on the surface of cochlear implant electrode arrays.

05. Ear physiology

P1.05.01

82 - How cochlear outer hair cells contribute to high frequency tuning

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Introduction: There are numerous lines of evidence that outer hair cells (OHCs) of the mammalian cochlea are part of an amplification system that enhances incoming sound. Pharmacological manipulations, a wide range of genetic mutations, the anatomical positioning of the cells and a fast voltage driven motility all provide support for OHC involvement in cochlear tuning. However, any low pass electrical filtering by the OHC membrane appears to limit the frequency at which potential driven 'electromotility' can contribute to cochlear mechanics. Recent experimental evidence showing limiting OHC electromotile bandwidths have also been reported from OCT measurements in vivo and from patch clamp recording of isolated cells. Nevertheless, considerations based on descriptions of the OHCs as piezo-electric devices [1] [2] suggest that the OHC membrane capacitance system can be a source of mechanical power up to high acoustic frequencies.
Results:.A biophysical synthesis may clarify such proposals. Experimental results demonstrate that inward currents arise from the deformation of the OHC motor protein prestin/SLC26A5, in part originating from movement of anions in the molecular vestibule [3] but possibly also due to charge rearrangements in the molecule itself. Although conclusions do not depend exclusively on a prestin dependent mechanism, useful parameters can be derived from such OHC data. The results can be included in conventional cochlear mechanical models where OHCs provide 'undamping' of the basilar membrane.
Conclusions: The resulting models show that this source of OHC current balance extends the OHC operating bandwidth up to high acoustic frequencies, as is the case for many mammals. Such modelling is able to explain semi-quantitatively the two distinct types of reported auditory nerve frequency tuning curves: at high best frequencies tuning is sharp and shows a distinct low frequency 'tail' whereas at low best frequencies tuning curves are less 'sharp' and more symmetrical.

Keywords: Outer hair cells, cochlear mechanics

References

1. Iwasa KH: **Negative membrane capacitance of outer hair cells: electromechanical coupling near resonance**. *Sci Rep* 2017, **7**:12118.
2. Rabbitt RD: **The cochlear outer hair cell speed paradox**. *PNAS* 2020, **117**:21880–21888.
3. Gale JE, Ashmore JF: **Charge displacement induced by rapid stretch in the basolateral membrane of the guinea-pig outer hair cell**. *Proc Biol Sci* 1994, **255**:243–249.

P1.05.02

62 - Hearing Impairment in a Mouse Model of Diabetes Is Associated with Mitochondrial Dysfunction, Synaptopathy, and Activation of the Intrinsic Apoptosis Pathway

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Introduction: Although previous studies continuously report an increased risk of hearing loss in diabetes patients, the impact of the disease on the inner ear remains unexplored.
Methods: We examine the pathophysiology of diabetes-associated hearing impairment and cochlear synaptopathy in a mouse model of diabetes. Male B6.BKS(D)-Lepr db /J (db/db, diabetes) and heterozygote (db/+, control) mice were assigned into each experimental group (control vs. diabetes) based on the genotype and tested for hearing sensitivity every week from 6 weeks of age. Each cochlea was collected for histological and biological assays at 14 weeks of age.
Results: The diabetic mice exerted impaired hearing and a reduction in cochlear blood flow and C-terminal-binding protein 2 (CtBP2, a presynaptic ribbon marker) expression. Ultrastructural images revealed severely damaged mitochondria from diabetic cochlea accompanied by a reduction in Cytochrome c oxidase subunit 4 (COX4) and CR6-interacting factor 1 (CRIF1). The diabetic mice presented significantly decreased levels of platelet endothelial cell adhesion molecule (PECAM-1), B-cell lymphoma 2 (BCL-2), and procaspase-9, but not procaspase-8. Importantly, significant changes were not found in necroptotic programmed cell death markers (receptor-interacting serine/threonine-protein kinase 1, RIPK1; RIPK3; and mixed lineage kinase domain-like pseudokinase, MLKL) between the groups. Taken together, diabetic hearing loss is accompanied by synaptopathy, microangiopathy, damage to the mitochondrial structure/function, and activation of the intrinsic apoptosis pathway.
Conclusion: Our results imply that mitochondrial dysfunction is deeply involved in diabetic hearing loss, and further suggests the potential benefits of therapeutic strategies targeting mitochondria.

Keywords: Diabetes, Mitochondria, Synaptopathy, Microangiopathy

06. Genetics of hearing loss and Gene therapy

P1.06.01

127 - Mutational spectrum and clinical features of branchiootorenal syndrome patients in Slovakia

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Background: Branchiootorenal (BOR) syndrome is an autosomal dominant disorder characterized by the association of sensorineural, conductive, or mixed hearing loss, structural defects of the outer, middle, and inner ear, branchiogenic fistulas or cysts, and renal abnormalities. BOR syndrome is caused by mutations in the EYA1, SIX1, and SIX5 genes. However, only about half of patients with clinically relevant symptoms have a detectable genetic abnormality, mostly in the EYA1 gene.
Aim: The aim of our study was to identify the genetic etiology in patients with suspicion of BOR syndrome.
Patients and methods: Six probands with clinical symptoms of BOR syndrome were reported to the Diabgene Laboratory. For determination of the genetic etiology the next-generation sequencing (NGS) approaches, MLPA and Sanger sequencing were used. Two probands without clinical symptom of BOR, were added to the set of patients based on identification of the SIX1 gene variants during a nationwide screening of sensorineural hearing loss patients performed in the Diabgene Laboratory previously.
Results: The genetic etiology was determined in 5 out 6 probands with clinical symptoms of BOR syndrome. Two pathogenic heterozygous dominant nonsense variants in the EYA1 gene were identified in 2 probands. In two probands the heterozygous whole gene deletion was detected. In one proband an 82bp deletion

affecting the splicing of the EYA1 mRNA was identified. In one proband one missense variant of uncertain significance (VUS) in the SIX1 gene was identified. Further, two pathogenic missense variants in the SIX1 gene were identified in other two probands without the clinical symptoms of BOR syndrome.
Among 18 individuals (7 probands plus their 11 family members) with genetically confirmed etiology, the most frequent symptom was hearing loss (16/18, 89%). However, the occurrence of other symptoms in individual patients varied, from an isolated hearing impairment (due to pathogenic variants in the SIX1 gene) to a complex BOR syndrome phenotype.
Subsequently, we focused on characterization of the phenotype in a large multiplex family with 11 affected members and with heterozygous deletion of the entire EYA1 gene without overlapping in adjacent genes. We found a large phenotypic variability, including hearing impairment, microtia, preauricular and cervical fistulas, palatal cleft, facial nerve paralysis and different degree of kidney abnormalities. The age of hearing loss onset in this family varied from 2 to 30 years. Preauricular and cervical fistulas were not observed in two family members.
Conclusion: Our results confirmed the predominance of EYA1 gene variants detected in patients with BOR syndrome and showed high variability of symptoms not only between different probands but also within one family.

Key words: Branchiootorenal (BOR) syndrome, EYA1, clinical phenotype

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P1.06.02

165 - ACTG1: a spectrum ranging from non-syndromic hearing impairment to polymalformative fetal presentations

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Background/Objectives: Pathogenic variants of ACTG1 have been reported for 2 distinct phenotypes: Autosomal dominant deafness DFNA20/26 and Baraitser-Winter syndrome 2, that associates intellectual deficiency, ocular malformations, dysmorphism, epilepsy and cerebral malformations. Surprisingly, hearing impairment is seldom associated to Baraitser-Winter syndrome 2. There is a high prevalence of DFNA20/26 patients identified through gene panel sequencing presenting with isolated sensorineural hearing impairment of dominant transmission. DFNA20/26 usually presents as non-syndromic, progressive, postlingual, hearing impairment with an onset between the first and third decade. The objective is to better characterize the phenotypes associated with ACTG1 variants.
Methods: this is a retrospective study on a French cohort of 35 patients and 2 fetuses.
Results: Most of the patients have a typical presentation of DFNA20/26. 3 patients present with developmental delay and a recognizable dysmorphism with flat face and arched eyebrows. 4 patients present with auditory neuropathy. In the 2 fetal cases we found corpus callosum and cerebral anomalies, associated to cardiac and skeletal malformations for 1 of them.
Conclusion: ACTG1-associated phenotype is broader than currently described. We have identified extra-auditory symptoms and a recognizable dysmorphism is a number of patients.

P1.06.03

84 - Genetic determinants of deafness and Enlarged Vestibular Aqueduct in Austria

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Objective: Hearing loss affects at least 1 in 1000 newborns, is a major cause of disability in children and the most common sensorial deficit in humans. In developed countries, at least 60% of cases of hearing loss are of genetic origin and are frequently associated with inner ear malformations, of which the most commonly detected is the Enlarged Vestibular Aqueduct (EVA). Genes that have been linked to EVA are *SLC26A4/*pendrin, *GJB2, FOXI1, KCNJ10* and *POU3F4*. In Caucasian cohorts, a large fraction of patients (50%) remains undiagnosed, thus providing a strong imperative to further explore the etiology of this condition.
Methods: A cohort of 33 subjects referring to the Otorhinolaryngology Department of the Salzburg general hospital for hearing loss were recruited between 2014 and 2022 (17 females and 16 males aged between 5 and 63 years; average age 32 years). For all patients, imaging studies of the inner ear by computer tomography (CT) of the temporal bones were performed. The entire coding region and intron-exons boundaries of *SLC26A4, GJB2, FOXI1, KCNJ10*, and *POU3F4* were amplified by end-point PCR and Sanger sequenced. To discriminate between pathogenic and non-pathogenic *SLC26A4* sequence alterations, the corresponding protein variants were characterized in cell-based assays to determine their expression levels and ion transport activity. The presence of the newly discovered Caucasian EVA (CEVA) haplotype was verified with the rhAmp™ SNP Genotyping assay (Integrated DNA Technologies).
Results: Hearing loss and EVA in this cohort are associated with biallelic pathogenic variants in the *SLC26A4* (n=4/33, 12% of patients) and *FOXI1* (n=2/33, 6% of patients) genes and monoallelic X-linked pathogenic variants in the *POU3F4* gene (n=2/33, 6% of patients). Non-pathogenic variants in the *SLC26A4* gene were found in 2/33 patients (6%). Three/33 patients (9%) showed monoallelic pathogenic variants in the *SLC26A4* gene, which is a non-diagnostic phenotype. Probably coincidental biallelic pathogenic variants in the *GJB2* gene were also detected in 2/33 patients (6%). The 12 SNPs of the Caucasian EVA haplotype were found in 5/33 patients (15%); of these, 2/33 patients (6%) harbor monoallelic pathogenic variants in the *SLC26A4* gene and one (3%) harbors a monoallelic non-pathogenic variant in the *SLC26A4* gene.
Conclusions: Biallelic pathogenic variants in the *SLC26A4* and *FOXI1* genes and monoallelic X-linked pathogenic variants in the *POU3F4* gene explain EVA and hearing loss in 24% of patients in this cohort. The Caucasian EVA haplotype with or without pathogenic variants in the *SLC26A4* gene may account for EVA and hearing loss in 15% of patients. The remaining patients (n=18/33, 55%) miss the identification of the genetic cause of their condition. Further studies are needed to establish the impact of the CEVA haplotype on a molecular and functional level; next-generation sequencing technologies will be essential to reach a conclusive genetic diagnosis of deafness in patients who are negative for the known causative genes.

P1.06.04

138 - Confirmating the causative role of *SF3B2* in craniofacial microsomia: the first Italian family

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Craniofacial microsomia (CFM, MIM#164210), hemifacial microsomia, oculo-auricular-vertebral spectrum (OAVS) or Goldenhar syndrome, include a broad spectrum of features with inter- and intrafamilial variability. It is mainly characterized by mandibular hypoplasia, microtia, facial and preauricular skin tags. Previous studies have demonstrated that this pattern of malformations have a heterogeneous etiology, with genetic and non-genetic risk factors involved. Most reported cases are sporadic, but there are rare familial cases with autosomal dominant inheritance (PMID: 25118188). Until recently, only one gene, *MYT1* (PMID: 27358179, PMID: 32871052), have been described to be involved in CFM. In 2021 Timberlake *et al* (PMID: 34344887), discovered that haploinsufficient variants in *SF3B2* are the most prevalent genetic cause of CFM, explaining ~3% of sporadic and ~25% of familial cases. In their cohort, individuals with *SF3B2* variants showed phenotypic homogeneity, characterized by external ear malformations involving the tragus and mandibular hypoplasia.
Here we report an Italian family with a CFM and intrafamilial variability. The proband is a 2 years old girl with face asymmetry, mandibular hypoplasia, asymmetric ear anomalies resembling a question mark ear, bilateral preauricular tags and a pit long the brachial arch. She presents bilateral conductive hearing loss (HL). Her mother presented a unilateral ear anomaly, with multiple preauricular and face tags, face asymmetry with mandibular hypoplasia and micrognathia. She has a normal hearing. Proband's grandfather has a fibroma in

the posterior cervical region of the neck and presents a mild mandibular hypoplasia, without ear malformation. He developed a unilateral, sensorineural HL at the age of 40.
All subjects have been evaluated in the Genetic Department of the IRCCS Burlo Garofolo in Trieste, Italy. Whole exome sequencing (WES) analysis was performed using DNA samples from proband and her parents obtained from peripheral blood using standard protocols. Variants' validation and segregation analyses were carried out by Sanger sequencing.
WES analysis allowed us to find a novel heterozygous nonsense variant, c.1660C>T, p.(Arg554*) in *SF3B2* gene. The variant was maternally inherited and it was present also in the grandfather's DNA.
In conclusion, we identified the first Italian kindred showing a CFM, due to a pathogenic variant in *SF3B2*. This result allows to enlarge the cohort of patients with *SF3B2* variants, to emphasize his role in CFM and to confirm dominant inheritance with inter- and intrafamilial phenotypic variability. We highly recommend looking at *SF3B2* variants when screening patients with features of mandibulofacial dysostosis and OAV spectrum.

Keywords: *SF3B2*, microtia, question mark ear, OAV spectrum

P1.06.05

57 - Deafness genes: how can phenotype, mutation spectrum and pathophysiology impact gene therapy potential?

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Introduction: Research towards gene therapy for hearing loss is rapidly emerging and certain genes already proved successful in animal experiments. Its potential might be considerable given the high prevalence of genetic hearing loss and its presumable functional superiority over current hearing rehabilitation options, enabling the restoration of natural hearing. However, not all experiments on gene-specific gene therapy demonstrated positive results and some determinants need to be taken into account to predict the potential for each gene.
Methods: The most common deafness genes were selected based on their prevalence in large retrospective cohorts, supplemented with some genes with high gene therapy potential. A literature review for each of these genes was executed in order to provide an overview of the phenotypical and mutation spectrum, and the inner ear expression pattern. Subsequently, gene therapy trial results were combined with our experience to estimate the gene therapy potential for each gene.
Results: Sixteen genes were selected for analysis. The first appartent parameters are the mode of inheritance and mutational spectrum and effect, which affects the technique of gene therapy. In general, autosomal recessive inheritance requires gene replacement, for which the length of the gene should be taken into account in view of viral empacking. A recurrent autosomal dominant mutation might enable gene editing, and mutations with a dominant-negative effect could be eligible for gene silencing. A second parameter is the onset of hearing loss, which is related to its pathophysiology. If the mutation impairs structural development of the inner ear, postnatal administration of gene therapy might be less successful, e.g. *GJB2*. In this way, mutations resulting in delayed-onset hearing loss, such as autosomal dominant *TMC1*, and mutations leading to a functional defect without early impaired structure, such as *OTOF*, might be most eligible. Of course, a decent animal model for the gene of interest should be available and the gene therapeutic needs to reach the involved cells, which results in research towards inner ear cell transfection and alternative delivery strategies.
Conclusions: Gene therapy for hearing loss is a promising research domain with several genes already in a more advanced stage of development. However, not all deafness genes seem eligible. The most important parameters are the mutational spectrum and its effect, and the inner ear phenotype and pathophysiology, in addition to the prevalence. Genes resulting in delayed-onset hearing loss and genes associated with functional rather than structural defects are most auspicious.

Keywords: gene therapy, genetic deafness, inner ear therapeutics

P1.06.06

46 - *In vivo* AAV-driven *Eps8* gene editing restores hair cell development and function in a mouse model of recessive deafness

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Introduction: Mutations in *EPS8* cause autosomal recessive deafness in humans (DFNB102). EPS8 is an actin-regulatory protein required for the development of actin-based microvilli-like structures of the sensory hair cells in the cochlea. Those structures, also known as stereocilia, are key to transduce acoustic information into receptor potentials, which subsequently drive activities in the auditory afferent fibres. *Eps8* knockout (*Eps8*$^{-/-}$) mice are deaf and their hair cells show immature stereociliary bundles and fail to become sensory receptors. Currently, there is no available therapeutic intervention aimed at delaying or curing *Eps8*-related deafness.
Methods: To test whether gene therapy can be used as a potential treatment for DFNB102, we used Anc80L65 as an AAV vector to deliver exogenous *Eps8* in *Eps8*$^{-/-}$ mice *in vivo*. The AAV was injected through the round window membrane at postnatal-day (P) 1–2 or after P18. Hearing function in mice was measured using auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs). Stereociliary structure was investigated using immunofluorescence microscopy and scanning electron microscopy (SEM). In addition, patch-clamp electrophysiology was used to investigate basolateral and mechanoelectrical transducer (MET) currents from the hair cells.
Results: We show that the delivery of *Eps8 in vivo* at P1–P2 rescued the staircase structure of the stereociliary bundles and the physiological functions of apical-coil hair cells. Rescued hair cells showed correct localization of EPS8 and EPS8-associated proteins. In addition, inner hair cells with rescued stereociliary bundles expressed normal MET current and adult-like basolateral biophysical properties. However, the number of hair cells that underwent full morphological and functional recovery was not sufficient to rescue auditory function. Despite the high transduction efficiency, expressing exogenous EPS8 in basal-coil hair cells or in adult mice did not rescue the bundle structure.
Conclusions: Our results suggest that AAV-mediated gene therapy can be a strategy to recover the complex morphological and functional defects found in *Eps8*$^{-/-}$ mice. However, to recover hearing, we propose that this therapeutic approach should be performed in utero since hair cells at postnatal ages from *Eps8*$^{-/-}$ mice appear to accumulated damage beyond the point of repair.

Keywords: EPS8, hair cell, gene therapy

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07. Imaging and Anatomy

P1.07.01

149 - Automating vascular segmentation in the cleared auditory system

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In recent years, several automated methods for vascular reconstruction have emerged, most of which focused on the brain, because of the availability of clearing and staining protocols for this organ, and because of the essential importance of vascular coupling in nervous tissue. Within these studies, the auditory system (especially subcortical areas such as cochlear nuclei or inferior colliculus) stood out as more densely vascularized than the remaining brain structures (Kirst et al. 2020), but despite higher density, the structure of vascular networks in auditory CNS nuclei was similar as in other parts of the brain. Available pipelines for vascular segmentation and tracing rely on machine learning methods, and in particular the use of convolutional

neural networks. However, this approach needs training on manually segmented or annotated datasets (ground truth), and biases in the ground truth will reflect in the predictive power of the neural network model. In general, a deep learning model developed for a particular problem will display very limited ability to generalize (Winfree 2022).
This posits two problems in the study of the auditory system: 1) methods able to allow vascular labeling in both bone and soft tissue, and 2) models able to automatically recognize and reconstruct vessels both in brain parenchyma and in other regions with different vascular geometry. The first problem is solved by clearing methods able to make bone transparent while preserving soft tissues in place, where the whole auditory system is visible (e.g. Perin et al. 2019). To solve the second problem, we have started building a pipeline for vascular segmentation including FIJI, Python and MATLAB scripts for image preprocessing, and training of a U-Net-based model (Ronneberger et al. 2015) for blood vessel recognition in rat cochlear nuclei, romboencefalic choroid plexus, and inner ear.
The choroid plexus was added, although not part of the auditory pathways, since it forms strong contacts with the surface of the dorsal cochlear nucleus, and it is possibly involved in the neuroimmune modulation of the auditory system (Perin et al. 2019), and since its microvascular network is extremely complex (Perin et al. 2021), and therefore not recognized by most available software tools for vascular reconstruction.
Cochlear nuclei display few large vessels and many homogenous capillaries, whereas vessels in the choroid plexus and cochlea both display a large variability in size and geometry; however, heterogeneities in choroid plexus are found throughout its volume, whereas inner ear vascular patterns are strongly regionalized. Therefore, for cochlea and cochlear nuclei, where vessel location influenced their appearance, the structure of interest was globally segmented (e.g. DCN, modiolus, stria vascularis), and annotations used in network training. For choroid plexus, this was not possible, and only local cues were available.

10. Noise induced hearing loss

P1.10.01

144 - Development of noise-induced hearing loss (NIHL) rat models for preclinical efficacy assessment

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Introduction: Occupational and recreational noise-induced hearing loss is a complex disease with an increasing global prevalence. Exposure to intense or prolonged sounds has a damaging effect on cochlear structures which are irreversible. Acoustic trauma can lead to permanent or transitory threshold shifts (PTS and TTS) with different pathological features, including hair cell loss, degeneration of the auditory nerve, and damage to ribbon synapses, also known as synaptopathy. Preclinical models have been developed in rats to model different patterns of NIHL and give insight into future pharmacological treatments.
Material & Methods: Five noise exposures were performed. Animals received a bilateral noise exposure at a noise band of 8-16 kHz at 105 dB for 2 hours (A), 110 dB for 1 hour (B), 110 dB for 2 hours (C), 115 dB for 2 hours (D), and 120 dB for 2 hours (E). DPOAE amplitudes and ABR thresholds were measured at baseline, T+1DAY, T+7DAYS, T+14DAYS and T+21DAYS for groups A, B, C, and at baseline, T+1DAY, T+7DAYS, T+14DAYS, T+21DAYS and T+35DAYS for groups D and E. Histological analyses were performed at T+21DAYS to count outer and inner hair cells and synaptic ribbons for groups A, B, and C. For groups D and E, histological analyses were performed at T+35DAYS to count outer and inner hair cells, fibers and SGN.
Results:
A- 105 dB 2h: TTS – ABR thresholds and DPOAE amplitudes returned to BL – No hair cell loss – loss of synaptic ribbons at 25 and 32 kHz
B- 110 dB 1h: PTS – permanent increase of ABR thresholds and decrease of DPOAE amplitudes – No hair cell loss – no synaptic ribbon loss
C- 110 dB 2h: PTS - permanent increase of ABR thresholds and decrease of DPOAE amplitudes – No IHC loss, slight loss of OHC, loss of synaptic ribbons at 25 and 32 kHz

D- 115 dB 2h: PTS - permanent increase of ABR thresholds and decrease of DPOAE amplitudes - No IHC loss, significant loss of OHC at 16 and 32 kHz - no synaptic ribbon immunostaining – slight loss of fibers – no SGN loss
E- 120 dB 2h: PTS - permanent increase of ABR thresholds and decrease of DPOAE amplitudes – significant IHC loss from 16 kHz, complete OHC loss from 16 kHz - no synaptic ribbon immunostaining – loss of fibers – loss of SGN
Conclusion: By varying the intensity of the trauma by 5 dB steps and modifying the duration, we obtained different phenotypes of hearing loss, from a simple functional impairment without cell loss to the activation of cell death pathways and thus degeneration of cochlear structures. These models allow to mimic different aspects of NIHL and to test the efficacy of drugs targeting synaptogenesis, cell death (apoptosis or necrosis), oxidative stress, inflammatory responses, or cell regeneration.

11. Otoprotection

P1.11.01

21 - SENS-401 Local Exposure is not altered by severe acoustic trauma in a rat model of sudden sensorineural hearing loss (SSNHL)

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Studies have demonstrated an impact of inner ear lesioning on local drug exposure in various hearing loss animal models, which could be model-specific and affect successful dose-translation from preclinical studies to the clinical sudden sensorineural hearing loss (SSNHL) patient population. SENS-401 (azasetron besylate) is currently in phase 2 clinical trial for the treatment of SSNHL as otoprotective treatment. Preclinical otoprotective efficacy with significant improvement of hearing by SENS-401 treatment vs placebo control has been previously demonstrated in a rat animal model of SSNHL (Petremann et al. Otol Neurotol, 2019). Here, the goal is to determine the impact of acute acoustic trauma performed 24 hours prior to SENS-401 oral administration on systemic (blood plasma) and local (inner ear and perilymph) SENS-401 exposure profiles.
Following baseline audiometry (ABRs and DPOAEs), rats were randomly assigned to two groups of noise exposure: 0 dB SPL (sham trauma group) or 120 dB SPL (acoustic trauma group) octave band noise (8-16 kHz) for 2 hours. 24 hours after sham or acoustic trauma, audiometry recordings were performed on both groups in order to assess hearing loss degree. Four hours after anesthesia induction, fully awake rats received a single oral administration of SENS-401 (13.2 mg/kg), and each group was divided in 4 subgroups for each of the 4 sampling time points: 0.5h to 4 hours post SENS-401 treatment. SENS-401 was quantified in temporal bones and blood plasma by high performance liquid chromatography/tandem mass (LC-MS/MS) spectrometry.
As control, T+24h mean ABR threshold shift across frequencies was significantly higher in the acoustic trauma group in comparison to sham control group (mean difference 53 dB, $p<0.001$), indicative of effective hearing loss induction. Furthermore, no statistically significant differences were observed between rats experiencing acoustic trauma or sham trauma for SENS-401 quantification on inner ear tissue, perilymph and blood plasma ($p=0.921$; $p=0.315$ and $p=0.313$, respectively).
The impact of lesions on enhancement of local exposure is an important consideration which merits being assessed in preclinical models and included in development strategies. Here, we show that acoustic trauma-induced hearing loss did not affect local and systemic exposures of orally administered SENS-401 in an animal hearing loss model where blood-labyrinth barrier (BLB) permeability is supposed to be enhanced. Acoustic trauma did not affect BLB permeability and inner ear local bioavailability as shown with inner ear tissue, perilymph, and plasma SENS-401 concentrations,. The lack of impact of noise trauma on drug local exposure profiles may result from SENS-401 demonstrated good local bioavailability (Petremann et al. Otol Neurotol, 2017).

Keywords: acoustic trauma, local exposure, hearing loss, drug delivery, SENS-401

12. Ototoxicity

P1.12.01

106 - Influence of the platinum nanoparticles on the cell viability of the mouse organ of Corti cell line HEI-OC1 and the rat spiral ganglion neurons

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Introduction: So far, systemic application of cortisone and antibiotics are not able to recover Cochlear Implant (CI) -patients from increased impedances. Recent studies revealed corroded platinum electrode contacts which may cause the reduced speech perception. Aim of this study is the characterisation of the effects of platinum nanoparticles (Pt-NP, 3 nm) on HEI-OC1 cells and rat primary spiral ganglion neurons (SGN).
Methods: The metabolic activity of the HEI-OC1 cells was determined at 50-150 µg/ml Pt-NP by using resazurine. The SGN were dissociated from the cochleae of postnatal rats (P5) and cultivated for 48 h following exposition to 20-100 µg/ml Pt-NP. The SGN survival rates and the neurite outgrowth were quantified by staining of the neurofilament antigen. Also, scanning (SEM) and transmission electron microscopy (TEM) were used to examine morphological and ultrastructural changes.
Results: The determination of the metabolic activity revealed a Pt-NP concentration depending decrease in mitochondrial activities without inducing cell death. TEM imaging demonstrated apoptosis at 100 µg/ml Pt-NP, but also induction of repair processes by means of autophagosomale-lysosomale mechanisms. Neither SGN loss nor reduction of the neurite outgrowth was found at any Pt-NP concentration.
Conclusions: In response to increasing Pt-NP exposure effective repair mechanisms may prevent the HEI-OC1 cells against cell death inducing processes. In contrast to the Organ of Corti cell line, Pt-NP had no effects on the metabolic activities of the SGN. Thus, it has to be elucidated if cellular protection processes are activated to prevent either the entry of Pt-NP to neurons or to induce highly effective repair mechanisms.

Keywords: Cytotoxicity, corrosion of platinum electrode contacts, platinum nanoparticles, HEI-OC1 cell line, spiralganglion neurons.

P1.12.02

156 - Oxidative stress and inflammation caused by the neuro-ototoxic effect of styrene in both cochlea and auditory cortex involve macrophage and microglia activation

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Styrene is an organic solvent used in industry as basic components of different types of plastics resins and fiberglass. Experimental and human investigations have raised the level of concern about styrene neuro-ototoxicity and we previously demonstrated that the combined exposure of noise and styrene insult can have a synergistic impact on cochlear damage, exacerbating apoptosis of the neurons of the spiral ganglion (SGNs), inner and outer hair cells (OHCs and IHCs) and supporting cells (Deiters cells) through and interplay between oxidative and inflammatory processes. It is known that toxic insult can induce activation of the glia and infiltration of macrophages in the cochlea, which can be involved in the onset of oxidative-inflammatory processes, however, the role of macrophages and glial cells (both microglia and astrocytes) in styrene-induced ototoxic damage and in the onset of cochlear inflammation have not been established. Moreover, the neurotoxic effect of styrene in central auditory structures, as a consequence or as a direct toxic effect to the brain, has not been investigated yet.
Thus, in this study we assessed (1) the link between cochlear oxidative stress, inflammation and macrophage and microglial activation induced by styrene exposure, (2) the neurotoxic effects of styrene exposure on the auditory cortex.
For these experiments, adult (2 months of age) male Wistar rats exposed to styrene (5 days a week for 3 weeks, 400 mg/kg) and age-matched not treated controls were used. Auditory brainstem responses were measured to evaluate hearing loss induced by ototoxic insult. At the end of styrene treatment (day 21) both

cochleae and brains were collected to evaluate oxidative stress, macrophages infiltration, the activation of astrocytes and microglia and inflammatory processes by immunoblotting and immunofluorescence analysis.
Our results show an increase of the activation of microglia and macrophages infiltration (increased IBA-1 positive cells) in the cochlea of styrene-treated animals compared to controls. Furthermore, immunoblotting assay shows a remarkable state of cochlear oxidation and inflammation, evaluated by the expression of 3-Nytrotiroasine (3-NT) and cyclooxygenase-2 (COX-2) in styrene-treated rats. Moreover, we found an increase in the activation of GFAP-labeled astrocytes in cochlear samples of styrene-treated animals. Interestingly, we found an increase of the same markers in the auditory cortex of styrene exposed animals, associated with an increase of caspase-3 activation and a decrease in the number of dendritic spines in pyramidal neurons of layer II-III of the auditory cortex, thus suggesting neuronal death and structural damage.
Collectively, our results demonstrate that the activation of the glial cells and the infiltration of macrophages may be the basis of the induction of cochlear inflammatory state and oxidative stress induced by styrene not only in the cochlea but also in the auditory cortex, suggesting a combined neuro and ototoxic insult in the auditory system induced by styrene.

P1.12.03

158 - Evaluation of Corti neural damage in a model of cisplatin-ototoxicity

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Cisplatin is a commonly used chemotherapeutic agent to treat a wide range of solid malignant tumors in adults and children. One of the main dose-limiting side effects of cisplatin chemotherapy is ototoxicity. In the last two decades, several studies assessed Corti structural damage induced by cisplatin and clarified the main mechanism of cisplatin-induced hearing dysfunction, demonstrating that an increased reactive oxygen species (ROS) generation after cisplatin treatment could lead to lipid peroxidation and hair cells death (Fetoni et al., 2014). However, to date, Corti neural impairment after cisplatin treatment is poorly understood.
In this study, we focused on cochlear neural damage induced by cisplatin to evaluate if other alterations other than HCs loss could affect cochlear function.
To this aim, we used an animal model, namely male adult Wistar rats, treated with a cisplatin single dose of 12 mg/kg. Functional and morphological analyses were performed before and 1, 3 and 7 days after cisplatin administration. Oto-functional assessment after cisplatin administration was performed through Auditory Brainstem Responses (ABR). Morphological evaluations were assessed by H&E staining, DAPI, Rh-Ph and NF200 immunofluorescence to clarify Corti neural damage (spiral ganglion neurons and afferent nerve fibers). Western blot analyses for pTrkB, P75 were performed to detect expression of cochlear BDNF receptors before and after cisplatin treatment, as BDNF represent an important factor involved in neural structure maintenance and survival. In addition, we evaluate cochlear biological responses induced by cisplatin, through quantitative analyses of pAKT, pERK and Caspase 3 cleaved expression.
Our results confirmed that cochlear functional impairment after cisplatin administration is related to both OHCs and IHCs cell loss, but also to a selective SNG and neural damage. In fact, in our experimental conditions we observed a significant HCs loss as well as SNG loss mainly located in middle-basal cochlear turn. Differently, neural afferent fibers damage was observed in all cochlear partitions.
These findings could be very interesting in light of oto-funcional analyses which showed a significant threshold worsening over all frequencies tested (6-32 kHz), suggesting that functional impairment could reflect not only HCs loss but also Corti neural damage.

14. Physiopathology of Auditory Pathways

P1.14.01

20 - An auditory neuropathy treatment study - Analysis of the modulatory effect of pegylated IGF1 (pegIGF1) on auditory function of the pmn mouse model.

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The progressive motor neuropathy (pmn) mouse is a model for an inherited motor neuron disease. It is characterized by a progressive degeneration of peripheral motor neurons, which starts at the 2nd postnatal week and leads to death after about 5 weeks due to respiratory depression. A homozygous mutation in the gene of the TBCE protein, which is responsible for the correct dimerization of alpha and beta tubulin, underlies the axonal defect. In addition, the animals develop progressive hearing loss after normal hearing onset, which progresses to deafness in the 4th postnatal week. Previous studies with pegIGF1 in the pmn mouse have shown a benefit in lifespan, weight, muscle strength and motor coordination. This indicates a rescue and survival of the peripheral motor neurons.
The aim of this study was to characterize the effect of pegIGF1 on the cochlea, the type of hearing loss and the underlying defect.
For this, frequency-specific BERA and DPOAEs were performed in animals with and without treatment of pegIGF1 on postnatal day 21 and 28 and the cochlea was histologically processed with auditory neurons. For this purpose, transverse and longitudinal sections of the cochleae were made to examine them by immunofluorescence, immunohistochemistry and electron microscopy. Furthermore, the number and morphology of hair cells, spiral ganglion cells and their axons were determined.
The electrophysiological examinations showed the classic picture of auditory neuropathy, with elevated thresholds in ABR and OAEs compared to control animals. Treatment with pegIGF1 did not significantly worsen the auditory outcome of the animals.
Histological studies show an axonal damage in all animals, which is mainly located in the tubulin skeleton, with conspicuous number and morphology of neuronal fibers outer hair cells in treated pmn mice. Interestingly, pegIGF1 influences the expression of acetyltransferase in cholinergic neuronal fibers, which innervate the hair cells efferent.
In summary, the pmn mouse is an excellent model of auditory neuropathy and offers the opportunity to gain more insights into this disease pattern and possible treatments. The therapeutic effect of pegIGF1 shows stagnation of progressive auditory neuropathy and support for the maintenance of neurons in the cochlear organ of Corti.

Keywords: auditory neuropathy - progressiv motorneuropathy mouse model - outer hair cell/ hearing loss - MOc fibres - cholinergic efferent innervation - pegIGF1

Bommel, H., Xie, G., Rossoll, W., Wiese, S., Jablonka, S., Boehm, T., Sendtner, M. 2002. J Cell Biol 159, 563-9.
Jablonka, S., Holtmann, B., Sendtner, M., Metzger, F. 2011. Exp Neurol 232, 261-9.
Rak, K., Frenz, S., Radeloff, A., Groh, J., Jablonka, S., Martini, R., Hagen, R., Mlynski, R. 2013. Exp Neurol 250, 333-40.
Metzger, F., Sajid, W., Saenger, S., Staudenmaier, C., van der Poel, C., Sobottka, B., Hoeflich, A., De Meyts, P., Schoenfeld, H.J. 2011. J Biol Chem 286, 195

15. Regeneration and Stem cells

P1.15.01

152 - Biosafety and biodistribution of otic neuroprogenitors generated from human pluripotent stem cells

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Although prosthetic devices like cochlear implants can successfully ameliorate the impact of hearing loss, deafness remains an intractable condition. To date, no biological regenerative solution exists with the potential to repair any part of the cochlea.
To address this deficit, our lab has been exploring the use of human pluripotent stem cells to develop a regenerative strategy to target the auditory nerve to treat neuropathic hearing loss.
We have previously shown that human embryonic stem cell-derived otic neuroprogenitors (hONPs) can functionally restore the cochlear nerve in a gerbil model of auditory neuropathy. But for any therapy to successfully translate to the clinic, it needs to demonstrate not only that is efficacious but also that is safe. In this study, we are exploring the distribution of transplanted cells and their behaviour after a long-term follow up. hESC-derived ONPs were purified by FACS using a fluorescent otic reporter, transplanted into ouabain-treated gerbils and monitored for up to a year.
Some cell therapy applications on other systems are based on the systemic delivery of cells, commonly as an intravenous infusion or even via an intravenous bolus. In these modalities, it is expected that cells would target multiple tissues. Furthermore, many cells would be trapped in filter organs, primarily the lungs and the liver. Although this is not to be expected when using a local administration as the one employed here, it is still important to determine their biodistribution on tissues beyond the cochlea.
A qPCR assay was employed to detect human-specific sequences in multiple tissues harvested at the end of the procedure. Analysis showed that cells do not spread systemically and are not detected in other organs. Moreover, a whole body MRI scan using contrast was performed at termination to identify potential lesions before animals underwent a whole post-mortem. No tumours that could be attributed to the transplanted cells were detected.
These findings demonstrate that the application of hONPs is safe, and support their translation into the clinic.

P1.15.02

105 - Characterization of immune suppression approaches for intracochlear transplantation of human induced pluripotent stem cell-derived otic progenitor cells in a rodent model for the denervated cochlea

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Introduction: A large number of patients suffering from severe hearing impairment caused by the loss of function of spiral ganglion neurons (SGN) do not profit from an electrical Cochlea implant (CI). Regeneration of lost or dysfunctional SGN using human optogenetically modified otic progenitor cells (OPCs) would open up the possibility of therapy with an optical CI for this group of patients (Wrobel et al., 2021). Therefore, the focus of our research is to treat the denervated cochlea with a combination of optogenetic and regenerative methods using iPSC derived otic bioengineered neuronal organoids (oBENO) (Zafeiriou et al.,2020) . We adapted a preclinical rodent model (Mongolian gerbil) for the denervated cochlea through application of ouabain to the round window niche (Chen et al., 2012). It is known that treatment with ouabain - performed to reduce type I SGN in the modiolus - induces a strong intracochlear immune response that poses a risk for transplantation of OPCs into the cochlear modiolus. Furthermore, the surgical intracochlear approach violates the immune-privileged integrity of the cochlear additionally enhancing immune response. Thus, a cornerstone for successful transplantation of human iPSC- derived OPCs into the gerbil cochlea is an efficient protocol to suppress the local immune response.
Methods: In this study, we characterize four different approaches to peri-interventional immunosuppression, focusing on the comparison between systemic and local administration of immunomodulatory agents. During

and after OPC onset, gerbils were treated with either cyclosporine A 15mg/kg subcutaneously with daily injections or dexamethasone 40mg/kg weekly or a combination of subcutaneous injection of cyclosporine A daily and dexamethasone weekly. In comparison, local immunosuppression was tested by placing a Gelitta sponge containing cyclosporine A (5mg/0.1 ml) and dexamethasone (4mg/0.5ml) in the round window niche after stem cell injection.
Conclusion: Preliminary results indicate that systemic immunosuppression is necessary to ensure successful transplantation of OPC's.

References:
Chen, W., Jongkamonwiwat, N., Abbas, L., Eshtan, S.J., Johnson, S.L., Kuhn, S., Milo, M., Thurlow, J.K., Andrews, P.W., Marcotti, W., Moore, H.D., Rivolta, M.N., 2012. Restoration of auditory evoked responses by human ES-cell-derived otic progenitors. Nature 490, 278–282.
Wrobel, C., Zafeiriou, M.-P., Moser, T., 2021. Understanding and treating paediatric hearing impairment. EBioMedicine 63, 103171
Zafeiriou, M.-P., Bao, G., Hudson, J., Halder, R., Blenkle, A., Schreiber, M.-K., Fischer, A., Schild, D., Zimmermann, W.-H., 2020. Developmental GABA polarity switch and neuronal plasticity in Bioengineered Neuronal Organoids. Nat. Commun. 11, 3791.

P1.15.03

52 - Human cochlear and vestibular organoids from patients undergoing skull-base tumor resections

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Background: After the discovery of supporting cells that express progenitor cell markers like SOX2 or Lgr5, extensive research has focused to promote hair cell regeneration in vivo using transgenic animal models or in vitro using inner ear organoids. Many reports have focused on neonatal supporting cells because of their increased progenitor potential compared to adult. Only a few (including us) have focused on adult mice showing survival of supporting cells with progenitor cell markers after deafening (Smith-Cortinez et al., 2021). Still, in humans, there is little evidence for hair cell regeneration in adulthood. Three-dimensional cultures have allowed the expansion and experimentation of human-derived cochlear organoids. It has been described that fetal-inner-ear-derived post-mitotic EpCAM+ cells were able to proliferate, expand and generate hair cells in vitro (Roccio. et al., 2018). Others have obtained vestibule-derived organoids after isolation from adult patients (McLean et al., 2017: Senn et al., 2019). Here, we aimed to develop cochlear and vestibular organoids from adult patients undergoing surgery for skull-base tumors.
Methods: Adults undergoing surgery for skull base tumors were included. Sensory epithelium of the cochlea and vestibule was collected in basal medium. The tissue digested and single cell suspension was filtered and mixed with matrigel and 3D drops were made, left to gelidify at 37oC and complete growth factor medium was added to the plates. Medium was changed and cells were imaged every 3 days.
Results: Preliminary data shows that vestibule-derived organoids were generated in expansion medium from all three patients so far included. Cochlea-derived organoids were observed only in 2 out of 3 patients. Both vestibular and cochlear organoids grew in culture and increased their growth rate after Wnt signaling pathway activation.
Conclusions: Cochlear and vestibular tissue from adult patients possess progenitor potential and the capacity to generate organoids in vitro.

Key words: Human organoid, hair cell regeneration, adulthood

References
McLean WJ, Yin X, Lu L, Lenz DR, McLean D, Langer R, Karp JM, Edge ASB. Clonal Expansion of Lgr5-Positive Cells from Mammalian Cochlea and High-Purity Generation of Sensory Hair Cells. Cell Rep. 2017 Feb 21;18(8):1917-1929.
Roccio M, Perny M, Ealy M, Widmer HR, Heller S, Senn P. Molecular characterization and prospective isolation of human fetal cochlear hair cell progenitors. Nat Commun. 2018 Oct 2;9(1):4027.
Senn P, Mina A, Volkenstein S, Kranebitter V, Oshima K, Heller S. Progenitor Cells from the Adult Human Inner Ear. Anat Rec (Hoboken). 2020 Mar;303(3):461-470
Smith-Cortinez N, Yadak R, Hendriksen FGJ, Sanders E, Ramekers D, Stokroos RJ, Versnel H, Straatman LV. LGR5-Positive Supporting Cells Survive Ototoxic Trauma in the Adult Mouse Cochlea. Front Mol Neurosci. 2021 Oct 5;14:729625

16. Tinnitus

P1.16.01

175 - Can Functional Biomarkers Differentiate Tinnitus and Tinnitus with Co-occurring Hyperacusis?

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Tinnitus (a constant phantom humming or ringing) and hyperacusis (the noisy or even painful perception of moderate sounds) are major health impairments with a prevalence of 10% to 20% in the population. These impairments in everyday life can worsen or even cause the condition of psychiatric disorders such as depression and anxiety. Currently, conflicting views on the neural correlate of tinnitus [Knipper et al. – Ruttiger (2020). J Neurosci] hinder the development of effective diagnosis and therapy for tinnitus. Although hyperacusis often co-occurs with tinnitus, it is until now considered neither in clinical diagnosis nor for targeted, individualized therapies. Successful individualized therapy of both sub-entities (tinnitus with or without hyperacusis) requires differentiation, identification and classification of hearing disorders by objective tools. Based on previous observations that link reduced and delayed auditory processing and reduced evoked and resting state BOLD fMRI responses to tinnitus [Hofmeier, Wertz et al. – Wolpert (2021), Refat, Wertz et al. – Wolpert (2021)], we currently investigate pre-defined frequency bands of neural oscillations in combined NIRS/EEG recordings.
Thereby, objective differences could enable individualized therapeutic intervention strategies in patient groups suffering from the above-mentioned sub-entities and thus could increase the urgently needed specificity of future tinnitus intervention.

17. Vestibular disorders

P1.17.01

117 - Baseline characteristic feature of hemispheric dominance of the vestibular cortical system of healthy controls

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Objective: It is well known that compensation like structural change of the central vestibular system plays an important role in the recovery of patients with unilateral vestibular loss. However, most of the studies were conducted through comparison of lesions and normal without considering dominant vestibular cortex. This study was designed to confirm baseline characteristic feature of hemispheric dominance of the vestibular cortical system of healthy controls who were expected to compare with unilateral vestibular failure patients. To this end, gray matter (GMV), white matter (WMV), and brain metabolites of left and right central vestibular system in right-handed healthy person who have never experienced dizziness were compared
Method: We included 23 healthy volunteers who visited Kyung-Hee university hospital at Gang-Dong from Mar.2016 to Mar. 2018. We performed magnetic resonance imaging for calculating GMV and WMV of central vestibular network of both side and 1H MR spectroscopy for collect brain metabolite in the parietal operculum.

Result: In the result of structural comparison, GMV of Rt side parietal operculum, caudate, Insula, percutaneous area were significantly higher than those of Lt side ($p<0.001$). WMV of Rt sided caudate, cutaneous, percutaneous area were significantly higher than those of Lt side ($p<0.001$) but WMV of Rt sided thalamus, Insula were significantly lower than those of Lt side.
Following metabolites of MR spectrography concentration of left side was significantly higher than those of right side. (Total Choline ratio, Glutamine/glutamate ratio) In contrast, following metabolites showed apposite result. (N-acetylaspartate(NAA), N-acetylaspartate to creatinin ratio(NAA/Cr)) Among metabolites, left NAA, Rt glutamate, Rt NAA/Cr showed significant correlation that those metabolite values were decreased as one grows older. Furthermore, Lt NAA, Lt glutamate, showed proportional correlation to GMV.
Conclusion: There were significant difference of structural volume and concentration of brain metabolite in central vestibular system according to dominant hemisphere in healthy subject, So the study for vestibular compensation, we should consider this difference of baseline characteristic feature.

P1.17.02

78 - Vestibular migraine in children. A cross-sectional study

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Introduction: The most common cause of episodic vertigo in children is vestibular migraine (VM) and headache can occur before, simultaneously or after vestibular symptoms.
While the mechanisms that account for vestibular symptoms in adults with VM have been extensively investigated, the debate in children is still open. A careful examination of trigger symptoms is a prerequisite for successful non-pharmacological prophylaxis and rehabilitation. A previous case–control study in children with migraine without vestibular symptoms suggested abnormal visually-evoked postural responses (VEPRs). The aim of this study was to analyse VEPRs in children affected by VM
Materials and methods: This cross-sectional study was based on children referred to the Tertiary Centre for Vestibular and Balance Disorders of the University Hospital of Modena from the Centre for the Diagnosis and Treatment of Headaches of the same institution between 2013 and 2019.
The sample was composed of school-age children (6–12 years old) affected by vestibular migraine (group VM) and by migraine without aura (group M).Posturography was used to investigate the VEPRs of children with VM and compare them to data obtained from children with primary headache and controls (C).
Results: Postural strategies of children with migraine are different to those of healthy subjects. These differences are more relevant in children of group VM. The stabilometric results of this study indicate a bimodal processing of visual information in the pain- and vertigo-free intervals: static visual references are effective in reducing body sway. In contrast, moving visual cues, such as those generated by optokinetic stimulation are detrimental to postural control and a remarkable increase of body sway is clearly documented.
Conclusion: This study has shown that children with VM are critically destabilized by moving visual stimuli in the interictal period. This behavior could be interpreted as a marker of the disease.

19. Miscellaneous

P1.19.01

11 - Prognostic factors for outcomes of idiopathic Sudden onset Sensorineural Hearing Loss: The SeaSHeL national prospective cohort study

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Background: Currently there is insufficient evidence about the patient pathway and hearing outcomes of patients presenting with sudden onset sensorineural hearing loss (SSNHL) in the National Healthcare System (NHS).
Based on recent discoveries in the molecular mechanisms that lead to SSNHL, new therapeutics are being developed that are ready for trialling. To allow for these trials to be run effectively and reliably within the NHS, we need information on where and when patients with SSNHL present and how they fare.
Objectives:
1. To map the patient pathway and identify the characteristics of adult patients presenting to NHS Ears, Nose and Throat (ENT) and Audiology services with SSNHL.
2. To develop a prediction model to predict recovery for patients with idiopathic SSNHL.
3. Establish the impact of idiopathic SSNHL on patients' quality of life
Methods: Study design: National multicentre prospective observational cohort study across 97 trusts.
Inclusion/Exclusion criteria: Adult patients with SSNHL presenting to the NHS
Data collection: Patient demographics, geographical distribution, treatment pathways and outcomes will be recorded. A selection of these sites (n=20) will collect patient quality of life data using questionnaires.
Analysis: A multivariable prognostic model will be created to predict recovery for patients with SSNHL.
Results: 413 patients have been recruited so far in 97 NHS trusts in England and Wales, and 117 clinicians, including doctors, audiologists and medical students are actively contributing to the delivery of the study nationwide.
Conclusion: We will provide an overview of the SeaSHeL study, including progress made so far and next steps. We will discuss the impact that COVID19 has had on the study in terms of patient recruitment and data protection following remote-working indications.
We will explore the lessons learnt on coordinating a multicentre study via ENT trainee (INTEGRATE and SFO), Audiology and NIHR CRN networks to optimise data collection and increase trainee involvement in research.

P1.19.02

28 - The human endolymphatic sac endolymph – novel sampling technique and proteome presentation

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Introduction: Collecting samples from the endolymph in the endolymphatic sac (ES) is difficult due to its small volume and hidden anatomic location. Using a tailored solide phase microextraction-probe we demonstrate a novel way of sampling proteins in the luminal fluid of the ES.
Method: Five endolymph samples and six ES tissue biopsies were collected in patients undergoing trans-labyrinthine surgery for sporadic vestibular schwannoma (VS).
After preparation, the samples were analysed with nano-liquid chromatography - tandem mass spectrometry (nLC-MS/MS) and MaxQuant software to identify the total number of proteins followed by pathway identification using PANTHER classification system.
Results: In total 1656 different proteins were found in the eleven samples. 1101 of the proteins overlapped, thus the proteins were found in both the endolymph samples and the ES tissue biopsies. 110 of the proteins were unique for the endolymph samples.

The results including the unique proteins found in the sac endolymph are discussed in the light of current views of human endolymphatic sac function.
Conclusion: Presenting the proteome of the endolymph in the human ES is to our knowledge unique. Hopefully the results can contribute to a platform for further investigations to better understand the function of this intriguing tissue in the human inner ear.

Keywords: endolymph, proteomics, human, vestibular schwannoma

POSTER SESSION 2

01. Ageing

P2.01.01

109 - Amplitude-Modulation Following Responses in Male and Female C57Bl/6N mice change with increasing age

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Increasing age can lead to numerous challenges for the auditory system, such as problems with speech intelligibility. Altered processing of temporal information within sounds is one means by which such issues can arise. To assess temporal coding and processing in mice, we have measured Amplitude-Modulation Following Responses (AMFRs) in male and female animals at relatively young ages (1, 6 and 9 months) to assess sex- and age- differences.
Stimuli were comprised of 12kHz carrier tones presented at 80dB SPL (100 ms duration, with 5 ms rise / fall times), which were sinusoidally amplitude-modulated (100% depth) at rates (modulation frequency, fm) from 100 to 1750 Hz in 30 Hz steps. Single sweep responses from sub-cutaneous scalp electrodes were recorded and averaged. From averaged responses, Fast Fourier Transformation (FFT) analysis yielded the magnitude (μV) and phase (radians) of the response at each fm. Group delay (ms) was calculated from the slope of the function of unwrapped phase (cycles) vs fm (Hz). From single sweep responses, a distribution of phase at fm, determined from FFT analysis, was used to calculate a synchrony coefficient to express the extent of phase-locking of the AMFR at each fm.
Mean AMFR magnitude transfer functions were generally multi-peaked, having maxima at approximately 250 Hz, 600 Hz and 1100 Hz. No obvious sex differences were noted but the 250 Hz and 600 Hz maxima were reduced in 6 month and 9 month old mice compared to 1 month old mice. Group delays were generally higher at lower fm and lower at higher fm, suggesting that the dominating neural component in the response had longer latency at lower fm (2-4 ms) compared to 1-2 ms at higher fm. No obvious sex or age differences were noted in group delays. In 1 month old mice phase locking to modulation rates above approximately 1100 Hz was more variable and on average reduced compared to that of 6 - 9 months old mice. This may reflect maturation in the auditory system with improved phase locking at higher fm in adult mice.
In mice aged up to 9 months old, we see no obvious differences in AMFR properties suggesting there is no decline in temporal processing of the auditory system, and no obvious sex differences at the age groups tested. Over this age range, these mice show little threshold elevation in the 12 kHz region of the cochlea targeted by these AMFR stimuli, suggesting that age-related threshold elevations in more basal regions do not affect temporal processing of sounds encoded more apically in the cochlea.

Keywords. Aging, Temporal Processing, Phase-Locking, Group Delay

P2.01.02

70 - Individual differences in speech induced MEG in older and hearing-impaired listeners

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Age-related deficits in speech-in-noise understanding pose a significant problem for older adults. Aging people often experience difficulties in perceiving speech in a noisy environment, even without elevated audiometric thresholds, a pathology that is assumed to be attributed to progressive cochlear synaptopathy. We here hypothesize that altered brain oscillations are triggered only by those speech sounds that are encoded deficiently. Thus, it could be that auditory fiber subtypes participate in different ways in the coding of sound classes relevant for speech (nasals, fricatives, liquids, or glides). Depending on articulation type, loudness, functional participation of higher or lower frequency spectra, the relevance of the auditory fiber type for the coding of e.g. fricatives or vowels could differ fundamentally and thus influence speech intelligibility in old age.

Here, the cognitive ability to discriminate between new or rapidly changing stimuli could have a direct influence. The ability to recognize rapidly changing stimuli in repetitive signal sequences can be tested by peaks of mismatch negativity (MMN). We here specifically used speech fragments and sentences to test speech in noise comprehension and deviant detection. To improve spatial recording of these speech stimulus-evoked cortical brain responses in the -frequency band and to validate the EEG results, a 275-channel magnetoencephalograph (MEG) will be used, which has a higher spatial resolution. Young, middle-aged and aged subjects will be tested during exposure of subjects to different sound classes.
We hope to contribute to the development of first diagnostic methods for the early detection of speech coding problems in old age, and possibly also for the early detection of correlated cognitive deficits (up to dementia).

Acknowledgment and funding: This work was supported by the Deutsche Forschungsgemeinschaft DFG KN 316/13-1, DFG RU 713/6-1, KL 1093/12-1; ERA-NET NEURON JTC 2020: BMBF 01EW2102 CoSySpeech and FWO G0H6420N

P2.01.03

16 - Distribution of auditory receptors by perceived frequencies according to G. Von Bekesy

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One of the most important psychophysical problems of hearing is the determination of the distribution of sound frequencies according to the coordinates of the auditory receptors of the Cortical organ of the human inner ear. The solution of this problem will be the goal of our research.
As the results obtained, we will give a representation based on the experiments of Bekesy. For the first time, this assumption was made in his work "A new audiometer". This idea was based on experiments and the idea of Bekesy that for every six months a person loses 80 Hz in the upper frequency area of the sound range. This idea was expressed by him in the assumption of a linear dependence of the decrease of the upper frequency limit: $f_B(t) = -rb \cdot t + fm0$, where fm0 = 20 kHz is the upper maximum audible limit of the auditory range.
This linear dependence takes a special form if you imagine the vertical frequency axis on a logarithmic scale. In general, this image represents the distribution of auditory receptors by coordinates, if the horizontal time axis is replaced by the coordinate axis of the auditory receptors by a simple linear ratio $x(t) = (L0 / Tn)\, t$, where L0 = 32 mm is the length of the basilar membrane that coincides with the Corti organ, Tn is the duration of the process, and t is the current time coordinate.

Key words: sound range, upper frequency threshold limit, linear function, logarithmic function, experiment of G. fon Bekesy, distribution of auditory receptors by G. fon Bekesy.

P2.01.04

73 - Age related hearing loss in Dunkin Hartley Guinea Pigs

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Background: Guinea pigs are the most favored animal model in research on cochlear implant (CI) optimization. In general, their auditory system is damaged by ototoxic drug treatment to induce sensorineural hearing loss (SNHL) and to investigate the respective CI optimization strategy. Most CI patients are older than 60 years and suffer from severe sensorineural hearing loss due to accumulation of insults throughout the lifespan. Therefore, an animal model of age related hearing loss (ARHL) may be a beneficial alternative model in contrast to those using ototoxines. We investigated the hearing loss over time in one strain of guinea pigs to elucidate if it may be used in future studies on CI and ARHL.
Methods: Dunkin Hartley guinea pigs, weighting between 347 and 615 g, from one breeder were included. Frequency specific (1, 2, 4, 8, 16, 32, 40 kHz) acoustically evoked auditory brainstem response (AABR) measurements were performed on day 0 (n=24), month 2 (n=4), 3 (n=4), 6 (n=7), 9 (n=8) and month 12 (n=15). Animals were killed on different time points of the observation period and hair cells were analyzed.
Results: At inclusion, animals showed the regular U-shaped frequency specific threshold curve with 16 kHz being the best frequency (mean: 20 dB SPL). Two months after inclusion the hearing thresholds were moderately increased (frequency dependent from -1 to 14 dB SPL) and increased further until month 12 when the thresholds were shifted by 49, 44, 43, 30, 23, 34, 52 dB SPL (mean values at 1, 2, 4, 8, 16, 32, and 40 kHz).

Conclusion and Outlook: The results demonstrate that there is a significant increase in the auditory thresholds. The detailed analysis (latencies, histology) is in progress and the sum of all data will finally characterize the peripheral ARHL in this specify guinea pig strain which may be a suitable animal model for CI studies in aged and hearing affected individuals.
The project was supported by Förderstiftung MHHPlus.

Keywords: CI research; animal model, ARHL

02. Cochlear implant and implantable prosthesis

P2.02.01

59 - Pediatric Auditory Brainstem Implant and contralateral Cochlear Implant: simultaneous or sequential surgeries?

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Objectives: To evaluate bimodal auditory performance in children with bilateral cochlear nerve deficiency (CND) and simultaneously or sequentially fitted with a cochlear implant (CI) and contralateral auditory brainstem implant (ABI).
Methods: This is a retrospective case review performed at a tertiary referral center. From January 2016 to January 2021, three children (age range: 1 to 3 years) with CND have been fitted with simultaneous (1 child) and sequential (2 children) CI and contralateral ABI.
Preoperatively, cochlear nerve canal, internal auditory canal and number of nerve bundles have been determined using CT and MRI imaging and matched with electrophysiological data obtained with electrocochleography (ECoG), auditory brainstem responses (ABR), electrical ABR (EABR), to choose the device vs the side of implantation.
Postoperatively, the Category of Auditory Performance (CAP) and the Ling Six Sound Test have been used to evaluate the auditory outcomes.
Results: All three children performed better on their auditory perception assessments using both of their devices than with either device alone.
Conclusion: The results observed could suggest that ABI and CI have a synergic effect, more evident in terms of time and auditory performances in simultaneous surgery. The imaging and electrophysiological evaluations are important and must be matched when considering how to make decisions about the side for ABI vs CI in bilateral surgeries.

P2.02.02

67 - Cochlear Implantation in Minipigs - A Potential Animal Model with Similar Inner Ear Dimensions to Humans

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Background: Animal models are a mainstay of inner ear research. However, the clinical translation of findings is hampered by striking anatomical and physiological differences between humans and the frequently used rodent models. In an attempt to bridge this gap, we evaluated two porcine models for cochlear implant surgery with commercially available cochlear implant electrodes. Furthermore, we determined the induced trauma by electrophysiological and histological assessments.
Methods: Initially, anatomical landmarks were defined and the surgical approach was established in porcine cadavers. Then, cochlear implantation using either a Flex20 or a Flex24 electrode was performed in seven piglets and six Aachen minipigs. Before and up to two months after implantation, electrophysiological evaluation including auditory brainstem responses (ABRs) and impedance measurements was performed.

Two months after surgery, animals were euthanized and temporal bones were extracted for histological analysis. Cochlear implant electrode insertion trauma and the resulting foreign body reaction was assessed in Masson's trichrome stained mid-modiolar sections. To verify the electrode position in the scala tympani, Giemsa staining after methylmethacrylate embedding was performed in one implanted cochlea.
Results: Surgery was successfully performed in all animals using a single incision retroauricular approach. Correct intracochlear placement of the electrode was confirmed by X-ray scans performed directly after surgery. While animals displayed no signs of hearing loss prior to surgery with a click ABR threshold of 27 ± 2.8 dB SPL (mean ± SEM), all animals were functionally deaf in the first week after surgery. However, partial hearing recovery was observed at later timepoints of the study. After euthanasia, electrode placement within the cochlear basal and middle turn was confirmed by histology with the electrode in situ. Although conventional histological analysis revealed morphological damage and fibrosis within the implanted cochleae, no significant difference regarding the degeneration of spiral ganglion neurons could be detected.
Conclusions: We were able to demonstrate that the pig is a suitable large animal model for cochlear implantation, which exhibits anatomical and physiological similarities to humans. The easy availability and handling of piglets and minipigs make this large animal model an interesting choice for inner ear and especially cochlear implantation research, which should be further evaluated in future studies.

P2.02.03

74 - Acute effects of cochleostomy and electrode-array insertion on compound action potential and cochlear microphonics in normal-hearing guinea pigs

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Introduction: Electrocochleography (ECochG) refers to the recording of the electrical activity of hair cells and the auditory nerve in response to acoustic stimuli. ECochG is increasingly used in cochlear implant (CI) surgery, in order to monitor the effect of insertion of the electrode array. However, the obtained results are often poorly understood. Here we aim to elucidate effect of cochlear implantation on ECochG in normal-hearing guinea pigs at multiple time points during the procedure.
Methods: Thirteen guinea pigs were isoflurane-anesthetized. Acute cochlear implantation consisted of (1) retro-auricular bullostomy to expose the basal turn, (2) hand-drilling of 0.5- or 0.6-mm cochleostomy in the basal turn ~0.5 mm from the round window, (3) insertion (~4 mm) of a four-contact electrode array (Advanced Bionics). Before and after each of these steps, ECochG was performed using a golden-ball electrode placed in the round-window niche; after insertion array electrodes were additionally used. Acoustical stimulation consisted of pure tones ranging from 0.25 to 32 kHz. ECochG signals were analyzed in terms of amplitude, latency and threshold of cochlear microphonics (CM) and the compound action potential (CAP). Cochlear midmodiolar sections were histologically analyzed for structural integrity.
Results:. For seven animals the cochleostomy severely affected the CAP and CM, while for the other six animals the CAP and CM were mildly affected. Histology showed that the basilar membrane was severely affected in the first group, and unaffected in the second group. CAP threshold shifts varied from 10 to 60 dB at high frequencies for the first group, and from 0 to 20 dB for the second group. After electrode insertion the responses in both groups declined further: ~10 dB CAP threshold shift for the first group and 0-35 dB for the second group. CAP and CM amplitudes decreased according to threshold shifts. CAP latencies increased up to 0.5 ms across all frequencies. Threshold shifts were observed not only for high frequencies for which an effect was to be expected (considering the basal location of cochleostomy and electrode array), but also, albeit to a smaller extent, for the lower frequencies (1 kHz and below).
Conclusions: ECochG signals were affected by both cochleostomy and subsequent insertion of an electrode array. The extent of deterioration of the ECochG signals was associated with structural trauma to the cochlear base. Even though the cochleostomy was drilled in the basal turn and the electrode array did not reach beyond the basal turn, our data show that ECochG responses to the lower frequencies can be significantly affected as well. This implies that both cochleostomy and subsequent array insertion can affect the low-frequency residual hearing of CI recipients, even with relatively short arrays located basally in the cochlea.

Keywords: Electrocochleography, cochlear implant, cochleostomy, compound action potential, cochlear microphonics

03. Developmental biology

P2.03.01

31 - Diabetes & Hearing: link to a auditory neuropathy?

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Background: Currently, diabetes mellitus (DM) and hearing loss are two major adult public health problems worldwide. DM can cause serious health complications that involve multiple physiological systems like cardiovascular diseases or peripheral/vegetative neuropathy. The aim of this study is to examine, in two strains of mice, whether induced DM causes either vascular or neural consequences in peripheral auditory function.
Material and methods: Two strains of mice were used, both 5 weeks old at the beginning of the study, and followed during 12 weeks after the DM induction using a high dose of streptozotocin (STZ, 200mg/kg). The C57Bl/6JRj mouse which is known to develop early age-related hearing loss (from 2-3 months of age), and the CBA/JRj mouse which has shown stable hearing function over nearly 2 years. For each strain, groups of treated and control animals were phenotyped to assess the onset of DM: blood glucose level, body weight and peripheral neuropathy (nerve conduction velocity, NCV). In parallel to this follow-up, a hearing assessment was also conducted: distortion product otoacoustic emissions (DPOAE) and auditory brainstem response (ABR), at different frequencies: 8, 10, 16, 24 kHz and time points: 0, 4, 8, 12 weeks post-induction.
Results: During the 12 weeks of following (after STZ injection), and compare to the control group, DM is established in both two strains of mice and is associated with typical characteristics: high blood glucose level, polyuria, slight body weight decrease, decrease in NCV. No hearing complications (DPOAE, ABR) were identified for CBA/JRj mice with DM. High-frequency hearing loss was clearly shown and more significantly for C57Bl/6JRj mice with DM with increased hearing thresholds (DPOAE, ABR) and changes in ABR wave I morphology.
Conclusion: This study did not reveal any impairment of peripheral auditory function in CBA/JRj strain with confirmed DM. However, induced DM enhances the hearing loss classically associated with cochlear damage described in C57Bl/6JRj strain but which is associated with unsuspected functional auditory neural damages.

Keywords: diabetes mellitus, auditory neuropathy, auditory brainstem response, distortion product otoacoustic emissions, mice

04. Drug delivery system

P2.04.01

97 - Application of Acoustic Multi-Frequency Stimulation to Increase Apical Drug Concentrations after Intratympanic Injection in Guinea Pigs - a Pilot Study

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Background: Intratympanic injection has been evaluated for the delivery of various compounds into the cochlea due to the possibility to achieve high intracochlear drug concentrations and its low risk for systemic side effects. However, efficient drug delivery into the cochlear apex, and thereby the treatment of low frequency hearing loss, remains a challenging task and broadly researched issue. The natural barrier of the round window membrane (RWM) and the length of the cochlea impede the diffusion of drugs into the cochlear apex. In a recent study, the use of acoustic multi-frequency stimulation to elicit steady streaming, a process

accompanying different forms of fluid motions, was evaluated to improve intracochlear particle transport in silico. By adapting these results to the anatomy of an animal cochlea, improved drug delivery into the apical turn can be expected. Hence, in this study we assessed the use of multi-frequency stimulation to improve intracochlear drug distribution after intratympanic application of a commonly used steroid, triamcinolone-acetonide (TAAC), in a guinea pig model.
Methods: TAAC formulated in a thermoreversible hydrogel was intratympanically applied to the RWM of guinea pigs. Afterwards, the animals were divided into five different groups: two groups were exposed to the multi-frequency stimulation for 2.5 or 24 hours (100 or 85 dB), one group to the high frequencies of the stimulation sound for 24 hours (85dB), and two groups were used as controls without noise exposure for 2.5 or 24 hours. At the end of the observation period, perilymph was sequentially sampled from the cochlear apex and TAAC concentrations were measured using high performance liquid chromatography-mass spectrometry. Acoustic brainstem response audiometry (ABR) measurements were performed before application and sampling.
Preliminary Results: Both groups exposed to acoustic stimulation for 24 hours displayed higher intracochlear TAAC concentrations compared to the 24 hours control group. In contrast, after 2.5 hours of acoustic stimulation lower intracochlear TAAC concentrations were observed compared to the unexposed control group. Overall high inter-subject variances of TAAC concentration levels within the separate groups were noted. Furthermore, several animals displayed considerable ABR threshold shifts between intratympanic application and termination of the observation period.
Conclusions: In this pilot study, we confirm the feasibility of multi-frequency stimulation to influence intracochlear pharmacokinetics in an animal model. Most notably, we observed a tendency of increased TAAC concentrations in the apical perilymph after 24 hours of acoustic multi-frequency stimulation. Further investigations with adapted stimulation protocols and higher sample numbers may allow deeper insights into the potential positive effects of steady streaming.

P2.04.02

60 - Applying Glycol Chitosan Thermogel to increase the drug delivery into the inner ear

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Introduction: The conventional therapeutic strategy for inner ear diseases is mainly systemic administration. However, systemic administration is inevitably accompanied by the possibility of systemic toxicities and related adverse effects, as a high dosage is required to achieve and maintain the therapeutic drug concentrations in the inner ear; further, the blood-labyrinthine barrier interferes with efficient drug delivery to the inner ear.
Methods: We prepared a new injectable thermogel to enhance the efficiency of inner ear delivery of dexamethasone (DEX). Hexanoyl glycol chitosan (HGC) was synthesized and evaluated as an amphiphilic thermogel (T gel ~ 32 ◦ C) for use as a solubilizing agent as well as an injectable carrier for intratympanic delivery of the hydrophilic and hydrophobic forms of DEX.
Results: Various thermogel formulations with different drug types and concentrations were prepared, and their physicochemical and thermogelling properties were characterized by 1 H NMR, ATR-FTIR, and rheometer. They exhibited versatile release kinetics from several hours to more than 2 weeks, depending on drug type and concentration. Our formulations further showed good residual stability for more than 21 days without any cytotoxicity or inflammation in the middle and inner ear and could deliver a considerably high drug concentration into the inner ear.
Conclusion: HGC thermogel has great potential as an effective and safe formu lation for inner ear drug delivery.

Keyword: Glycol chitosan, Thermogel, Inner ear, Dexamethasone, Intratympanic delivery

P2.04.03

88 - Lack of NHE6 and inhibition of NKCC1 associated with increased permeability in blood labyrinth barrier-derived endothelial cell layer

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Acoustic trauma, autoimmune inner ear disease, and presbycusis feature loss of the integrity of the blood-labyrinth barrier (BLB). Normal BLB function depends on endothelial structural integrity, which is supported and maintained by tight junctions and adherens junctions within the microvascular endothelial layer. When these junctions are disrupted, vascular leakage occurs. Tight junctions and adherens junctions are functionally and structurally linked, but the exact signaling pathways underlying their interaction remain unknown. In addition, solute carriers (SC) are essential for optimal exchange through BLB. Previously, we found that SC family member, the sodium–hydrogen exchanger NHE6, was expressed in all wildtype cochlear tissues, and that Nhe6 knockout mice displayed moderate hearing loss. Moreover, NHE6 depletion affected Trk protein turnover and endosomal signaling. Here, we investigated whether NHE6 might impact BLB integrity. We found that Nhe6-knockout, BLB-derived endothelial cells showed reduced expression of major junctional genes: Tjp1, F11r, Ocln, Cdh5, and Cldn5. Co-culturing BLB-derived endothelial cells with pericytes and/or perivascular resident macrophage-like melanocytes in a transwell system showed that monolayers of Nhe6-knockout BLB-derived cells had lower electrical resistance and higher permeability, compared to wildtype endothelial monolayers. Additionally, another SC, NKCC1, which was previously linked to congenital deafness, was downregulated in our Nhe6-knockout mouse model. Blocking NKCC1 with a NKCC1-specific inhibitor, bumetanide, in wildtype BLB-derived endothelial cells also caused the downregulation of major junctional proteins, particularly Tjp1 and F11r, which encode the zonula occludens and junctional adhesion molecule-1 proteins, respectively. Moreover, bumetanide treatment increased cell permeability. In conclusion, we showed that the lack or inhibition of NHE6 or NKCC1 affected the permeability of endothelial BLB-derived cells. These findings suggested that NHE6 and NKCC1 could serve as potential targets for modifying BLB permeability to facilitate drug delivery across the BLB to the cochlea or to protect the cochlea from ototoxic insults.

Keywords: blood-labyrinth barrier, cochlea, NHE6/SLC9A6, hearing loss, NKCC1

05. Ear physiology

P2.05.01

93 - Insights into the ion permeation of the cochlear mechano-electrical transducer channels

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Transmembrane channel like -1 (TMC1) is the major component of the MET channel and is thought to form the ion pore. More than 60 human mutations of TMC1 have been reported to cause deafness. Based on its homology to TMEM16a, a calcium activated chloride ion channel, TMC1 is predicted to have ten transmembrane domains (TM) and the region of TM4-TM7 to outline the ionic pore. To assess the ionic permeation pathway of TMC1, we generated five murine models carrying Tmc1 single missense mutations. All TMC1 mutations in the present study, showed loss of hair cells and profound deafness after few weeks of age. We characterized the properties of the MET channels for each TMC1 mutants in the absence of TMC2 and prior to hair cell death.

The mutation, Tmc1 p.D528N, located on TM6 segment, induced the most severe change in ion conduction of the channel. A drastic reduction of the calcium permeability and a reduction of the single channel conductance was noted. We identified another mutation in TM6, Tmc1 p.E520Q, which reduced the single channel conductance.

TMC1 mutations in TM6-TM7 segment and TM7 segment (Tmc1 p.D569N and Tmc1 p.W554L) decreased the size of the maximal MET current without reducing the single channel conductance. A decreased expression of TMC1-containing channels at the mechano-transduction site, at the tip of the shorter stereocilia, was observed. We propose that this stretch of amino acids constitutes a site of interaction with the accessory protein LHFPL5. has been shown to be essential for trafficking of TMC1 to the mechano-transduction site.

TMC1 mutations in the TM4 domain (Tmc1 p.M412K, Tmc1 p.T416K) altered neither the maximum amplitude of MET current, nor the single channel conductance but reduced the calcium permeability. Despite large MET currents in these two mutants, hair cells degenerated after two weeks of age.
All TMC1 missense mutations tested supported the proposed view that TMC1 transmembrane domains 4 to 7 form the ion permeation pathway of the MET channel. Additional accessories proteins (TMIE LHFPL5) have been suggested to be integral parts of the MET channel complex. However, MET channel currents could still be detected in absence of these proteins indicating that they are not obligatory for the formation of the ion channel.

P2.05.02

173 - D-Serine wakes silent NMDA receptors at the mammalian cochlear ribbon synapse

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NMDA receptors (NMDARs) populate the complex between inner hair cell (IHC) and spiral ganglion neurons (SGNs) in the developing and mature cochlea. However, in the mature cochlea, activation of NMDARs is thought to mainly occur under pathological conditions such as excitotoxicity. Ototoxic drugs such as aspirin enable cochlear arachidonic-acid-sensitive NMDAR responses, and induced chronic tinnitus was blocked by local application of NMDAR antagonists into the cochlear fluids. We largely ignore if other modulators are also engaged. In the brain, D-serine is the primary physiological co-agonist of synaptic NMDARs. Whether D-serine plays a role in the cochlea had remained unexplored. We now reveal the presence of D-serine and its metabolic enzymes prior to, and at hearing onset, in the sensory and non-neuronal cells of the cochlea of several vertebrate species. In vivo intracochlear perfusion of D-serine in guinea pigs reduces sound-evoked activity of auditory nerve fibers without affecting the receptor potentials, suggesting that D-serine acts specifically on the postsynaptic auditory neurons without altering the functional state of IHC or of the stria vascularis. Indeed, we demonstrate *in vitro* that agonist-induced activation of NMDARs produces robust calcium responses in rat SGN somata only in the presence of D-serine, but not of glycine. Surprisingly, genetic deletion in mice of serine racemase (SR), the enzyme that catalyzes D-serine, does not affect hearing function, but offers protection against noise-induced permanent hearing loss as measured 3 months after exposure. However, the mechanisms of activation of NMDA receptors in newborn rats may be different from those in adult guinea pigs. Taken together, these results demonstrate for the first time that the neuro-messenger D-serine has a pivotal role in the cochlea by promoting the activation of silent cochlear NMDAR in pathological situations. Thus, D-serine and its signaling pathway may represent a new druggable target for treating sensorineural hearing disorders (i.e., hearing loss, tinnitus).

Keywords: NMDA receptors; acoustic trauma; tinnitus; D-serine; neuroprotection

06. Genetics of hearing loss and Gene therapy

P2.06.01

115 - Tailored WES data analysis and reanalysis in the Lebanese population and lessons learned

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Introduction: The recent implementation of next generation sequencing technologies significantly improved the molecular biology field. Indeed, it accelerated the linkage of diseases to their causative mutations while also allowing the identification of incidental findings that are of a medical value despite being unrelated to the

patient's condition. Besides, it sheds the light on private pathogenic variants frequent in consanguineous populations.
Methods: In this study, 500 Lebanese patients, presenting a wide spectrum of genetic disorders were referred to us for molecular diagnosis. Whole Exome Sequencing (WES) was performed. Incidental findings in 73 genes were evaluated as per the ACMG guidelines, in addition to the private Lebanese mutations listed in the CTGA database1.
Results: This allowed us to identify the causative mutations in nearly 51% of the cases, in line with other international studies. To improve the diagnostic yield, WES data, generated during the first 2 years of this study, was reanalyzed for all patients who were left genetically undiagnosed. Reanalysis, based on updated bioinformatics tools and novel gene discoveries, enabled us to increase the diagnostics yield to 57%. An association between the rate of positive diagnosis and the disease group was noted. Indeed, the highest diagnosis success rate corresponds to the group of congenital hearing and visual disorders (100%), followed by neuromuscular disorders (85%), metabolic and mitochondrial disorders (84.2%), bone diseases and leukodystrophy (75%), epilepsy (66.7%) and neurodevelopmental disorders (30.4%). In parallel, dominant actionable variants were found in 6% of our cohort where genes associated with dominant cardiac diseases were the most frequently mutated (in 2% of our cohort). Genetic predisposition to cancer was observed in 1% of the cases while 2.5% carry a recessive disease-allele.
Conclusions: In conclusion, the present work pinpoints the contribution of WES to an efficient genetic diagnosis and better clinical management. Lessons learned from WES reanalysis will also be shared.

Keywords: Whole Exome Sequencing, genetic diagnosis.

References:
1Bizzari S et al., Genes (Basel). 2021 Sep 27;12(10):1518. doi: 10.3390/genes12101518

P2.06.02

166 - ATP1A3 gene is responsible for isolated and syndromic auditory neuropathy (CAPOS syndrome)

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CAPOS syndrome combines progressive hearing loss (auditory neuropathy type, (AN)), optic atrophy, hypotonia, and cerebellar ataxia. The disorder is described as appearing in childhood, with acute episodes of febrile neurological deterioration resembling encephalitis.
We conducted a cohort study of 39 families (43 patients) with isolated (73%) or syndromic (27%) AN without cochlear nerve malformation. Their DNA was analyzed by Next Generation Sequencing using a panel of 216 genes involved in isolated or syndromic deafness.
Four unrelated patients had the same heterozygous pathogenic variant of the ATP1A3 gene, c.2452G>A, p.(Glu818Lys), already reported as responsible for CAPOS syndrome (OMIM-601338). The diagnosis of the hearing loss was made in post-lingual period from 5 to 12 years old. The deafness progressively worsened with very low word recognition (10%) despite a classical hearing aid. A single or bilateral cochlear implantation allowed recovering a word recognition score close to 100% (up to 12 years post-implant). Two patients have never had any of the febrile episodes classically described. Optic nerve damage was not present in two patients, one of whom was 16 years old. The ataxia described in the CAPOS syndrome is attributed to cerebellar damage but the implication of a vestibular deficit was present in 2/3 of the patients tested.
We have identified the ATP1A3 p.(Glu818Lys) variant in patients with isolated neuropathy with or without inaugural febrile episodes. Balance disorders could involve peripheral vestibular damage. Cohort studies should confirm efficacy in auditory perception in these patients.

P2.06.03

137 - Non-syndromic or syndromic hearing loss? Our experience with the challenge of non-syndromic mimics

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Introduction: Hearing loss (HL), both syndromic (SHL) and non-syndromic (NSHL), is the most common sensory disorder characterized by a high genetic and clinical heterogeneity. To date, 124 genes have been reported as causative of NSHL, and more than 400 syndromes are associated with SHL. In the past years the introduction of next generation sequencing into the diagnostic routine allowed for the discovery of another subcategory, the one of non-syndromic mimics (NSMs). NSMs typically present as isolated hearing loss but as the patient ages other phenotypes become evident. Thus, an early diagnosis is fundamental for a precise care of the patients.
Materials & Methods: In our Institute we employed a multi-step approach to characterize HL patients. In particular, after a deep clinical evaluation, we perform *GJB2* and *STRC* analyses and, in case of negative results, we proceed with Whole Exome Sequencing (WES).
Results: During the last two years, we collected 104 patients affected by HHL negative to *GJB2* and *STRC* analyses; in particular 68 were clinically classified as NSHL and 36 as SHL. WES analysis allowed us to identify the final molecular diagnosis for 41,9% of patients. Interestingly, after the genetic tests, 9 out of 68 patients (13%) initially classified as NSHL, have been molecularly re-classified as SHL and further clinical assessment have been performed. Genes involved were: *USH2A* (2 patients), *CDH23, GPR98, GATA3* (2 patients), *HARS2* and *KARS1* (2 patients). Clinical re-evaluation after the diagnosis permitted us to determine that the two patients diagnosed as Usher type 1 have developed a motor delay due to vestibular areflexia and the two patients with *KARS1* pathogenic variant showed behavioral abnormalities and hyperactive behavior. On the contrary the patients diagnosed as Usher type 2 and the patients with *GATA3* and *HARS2* pathogenic variants still did not show other symptoms.
Conclusion: In conclusion, clinical re-evaluation of 9 patients with NSMs showed that some of them had subtle syndromic findings, while confirmed that none of them met criteria for the clinical suspicion of the associated syndrome at the first genetic evaluation. WES analysis permitted us a very early diagnosis of SHL and to set up the correct follow-up. These children needed to rule out complementary laboratory or imaging tests and to be evaluated by other specialists. When a NSM is diagnosed, post-test genetic counseling is really important to discuss the genetic testing results and their implications, both for the patient and for the family.

Keywords: Hearing loss; Syndromic deafness; Non-syndromic deafness; Non-syndromic mimics

P2.06.04

170 - MTTL1 gene variant m.A3243G in Slovak cohorts of hearing loss and diabetic patients: expect the unexpected

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Background: Mitochondrial DNA (mtDNA) mutations account for up to about 5% of hereditary hearing loss cases and certain pathogenic variants, of which m.A3243G is the most common one, do also contribute to rare monogenic form of diabetes (MIDD – Maternally Inherited Dabetes and Deafness) or neurological disease (MELAS - Mitochondrial Encephalopathy, Lactic Acidosis, and Stroke-like episodes). Different proportion of the mutated mtDNA (heteroplasmy) among the affected tissues leads to variability in the clinical manifestation and severity of the phenotype.
Aim: The aim of the presented study was to establish the prevalence of m.A3243G variant in large cohorts of hearing impaired and diabetic patients in Slovakia.
Patients and methods: Probands (n=5787) were recruited via three independent nationwide studies on genetics of hearing loss (n=1030) and diabetes (n=4757). DNA from peripheral blood was tested for the

presence of m.A3243G variant using qPCR method. Audiological and other clinical data of the identified mutation carriers were also collected for phenotype evaluation.
Results: To date, we identified 18 probands/families harbouring the m.A3243G variant (0.31%). The prevalence was higher in the hearing loss group (8/1030, 0.78%) than in the diabetes group (10/4757, 0.21%). Heteroplasmy levels from peripheral blood ranged between 1% and 62 % (preliminary data). The symptoms became manifest in the fourth decade of life in the majority of the affected subjects with MIDD phenotype or isolated hearing loss / diabetes, but as early as in the second decade in the probands with MELAS phenotype. However, we observed high phenotype variability among the mutation carriers and coincidences with other symptoms which could lead to misdiagnosis. Four patients were identified as cochlear implant recipients.
Conclusion: The diagnostic yield was higher in the deafness group than in the diabetes group. It is in agreement with the fact that hearing impairment is genetically determined more often that diabetes. Implementation of rigorous inclusion criteria requiring presence of both diabetes and hearing loss may lead lower detection rate due to different or incomplete phenotype manifestation.

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Key words MIDD, MELAS, mtDNA

P2.06.05

123 - Syndromic and non-syndromic hearing loss: identification and functional characterization of putative novel splicing variants

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Introduction: Hereditary hearing loss (HL) is a heterogeneous disorder that affects over 5% of the world's population. To date, more than one hundred genes have been associated to syndromic and non-syndromic HL forms and the majority of cases follow an autosomal recessive inheritance pattern. Considering the genetic heterogeneity of HL, the application of Next Generation Sequencing technologies (both Whole Exome Sequencing and Whole Genome Sequencing) has facilitated and accelerated the identification of novel pathogenetic variants, including deep intronic or splicing mutations that can occur in both exon and introns and disrupt canonical splice sites or splicing regulatory sequences. Here we describe four heterozygous variants with potential effect on splicing, recently identified in patients affected by syndromic and non-syndromic HL: *OTOF* c.5533+13G>T (*de novo* mutation; temperature-sensitive HL), *LOXHD1* c.5085+970T>C (only one deep intronic variant described for this gene until now), *OTOGL* c.6754+4A>C and c.448C>T, p.(Arg150Trp) (very few variants described for this gene) and *PPP1R12A* c.792+3A>C (*de novo* mutation; syndromic HL with genital abnormalities).
Methods: We used *in silico* bioinformatic algorithms to predict the impact of selected variants on pre-mRNA splicing and then, since we were unable to obtain additional samples for the referred patient, we functional characterize the effect of variants by employing splicing minigene assays: the region of interest (exon and surrounding introns) both in wild-type (WT) and mutated form, was cloned into an exon-trapping vector and transfected into HEK-293T cells. After expression in cells, we compared RT-PCR fragments generated from minigene spliced RNA of WT and mutant constructs to verify whether the identified variant influences the mRNA sequence and leads to the formation of alternative transcripts.
Results: We assessed *in silico* splicing score for *OTOGL* c.6754+4A>C and *PPP1R12A* c.792+3A>C donor splice site (5'ss) variants and for *OTOF* c.5533+13G>T, that is not strictly located in the 5'ss, but within flanking nucleotides (<20bp from intron-exon boundary). We determined that they were considered as "disruptive" according to thresholds applied to SpliceAI and, therefore, we started to better characterize these variants through minigene assays. We are also testing the spliceogenic effect of the exonic variant that we have found in the *OTOGL* gene, that could alter an exonic splicing regulatory sequence (enhancer or silencer). We will also further investigate the deep intronic variant identified in *LOXHD1* gene in order to verify if this intronic region could have the potential to be included as pseudo-exon.
Conclusions: Advances in sequencing technologies, *in silico* predictive models and the functional characterization of specific splicing variants pave the way for their improved detection and interpretation. All together these results, revealed that splicing alterations are an important mechanism of disease-causing mutations in the context of hearing loss increasing the diagnostic yields for patients and their families.

Keywords**:** HL, NGS technologies, splicing mutation, minigene assay.

P2.06.06

89 - Integrating optimized surgery and behavioral testing for efficient and safe adeno-associated virus gene therapy development for inner ear disorders

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Hearing loss affects approximately 466 million people worldwide. A genetic cause can be identified in 60% of the cases of hearing loss in multiplex families, and more than 120 genes have been associated with non-syndromic hearing loss in humans. Current treatment options are still primarily restricted to either corticosteroid, sound amplification, or cochlear implants. Adeno-associated virus (AAV)-mediated gene therapy is a promising approach for treating genetic hearing loss. In this context, Sensorion has developed full in-house cross-functional in vitro / in vivo R&D platforms to optimize new therapeutic candidates development for hereditary monogenic forms of deafness.

To produce translationally-relevant proof of concept data, mouse models should be treated after P12 to correspond to a post-natal intervention in human. Injection through the round window membrane (RWI) increases the risks of short and long-term damage to inner ear structures, as commonly reported by ABR threshold elevations of around 30dB. This is especially true in mature organs with less tolerance to stress. Surgery-induced damages could thus be counterproductive when assessing therapeutic candidates in mouse models, leading to the non-selection of a product with high potential otherwise. ABR recordings are not predictive of efficient auditory processing. Therefore, ideally, the development of gene therapy drug candidates for the treatment of inner ear pathologies should also include behavioral evaluation to complement audiological assessment.

To be clinically relevant, we injected P14 wild-type (WT) mice with recombinant AAV vectors by RWI. The inner ear morphology, hair cell survival and transduction efficiency were evaluated by immunohistochemistry. The impact of the surgery on hearing was assessed by auditory brainstem response (ABR) measurements weeks after injection. ABR recordings (including threshold and wave analyses) were performed in a bilateral open-field configuration. ABR recordings were completed by startle reflex tests, predictive of efficient hearing integration, to assess complex auditory integrative responses.

Our data demonstrate that the RWI procedure is mastered at Sensorion, enabling safe surgery, therapeutically relevant AAV transduction efficiency with no impact on hearing according to ABR threshold determination, wave I latency and amplitude analyses nor on inner ear morphology and hair cell survival. ABR recordings were well correlated with behavioral data, indicating that the startle reflex test can be routinely applied.

To conclude, integrating optimized surgery and behavioral testing advantageously supports the development of efficient and safe AAV gene therapy development for inner ear disorders notably. It provides more translationally relevant proof-of-concept and efficacy studies, for a lasting positive impact on people afflicted with inner ear disorders.

Keywords: gene therapy, audiometry, startle reflex, inner ear injection

P2.06.07

172 - A disease-associated mutation in thyroid hormone receptor α1 causes hearing loss and sensory hair cell patterning defects in mice

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Resistance to thyroid hormone due to mutations in *THRA*, the gene encoding the thyroid hormone receptor α (TRα1), is a genetic disease that displays substantial variability in its clinical presentation. Mutations affecting TRβ1 and TRβ2 cause deafness in mice and have been associated with deafness in humans, but hearing deficits have not been reported for patients with mutations in *THRA*. To test whether TRα1 affects hearing function, we used mice heterozygous for a frameshift mutation in *Thra* that is very similar to human *THRA* mutations (*Thra*$^{S1/+}$ mice) and reduces tissue sensitivity to thyroid hormone. Compared to wild-type littermates,

Thra$^{S1/+}$ mice displayed moderate high-frequency sensorineural hearing loss as juveniles and showed increased age-related hearing loss. Ultrastructural examination revealed aberrant orientation of ~ 20% of sensory outer hair cells (OHCs), together with an increase in the numbers of mitochondria with fragmented morphology and of autophagic vacuoles in both OHCs and auditory nerve fibers. Molecular dissection of the OHC lateral wall components revealed that the K + channel Kcnq4 was aberrantly targeted to the cytoplasm of OHCs in the mutants. In addition, cochleae from the mutants showed increased oxidative stress, autophagy, and mitophagy associated with an increase in age-related cochlear cell damage, demonstrating that TRα1 is required for proper development of OHCs and for maintenance of OHC function. These findings suggest that patients with *THRA* mutations may present under-diagnosed, mild hearing loss and may be more susceptible to age-related hearing loss.

07. Imaging and Anatomy

P2.07.01

13 - A deep learning approach to quantify auditory hair cell survival

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The sensory cells of the cochlea, the auditory hair cells (HCs), are among the most vulnerable structures in the inner ear and commonly lost in sensorineural hearing loss. Currently, there are no therapeutic options to restore these HCs. Therefore, the study of molecular mechanisms to promote hair cell survival and regeneration is an important area of hearing research. An important and widely used model to investigate HCs is the in vitro culture of the neonatal Organ of Corti (OC), since it allows the study of HCs in their anatomical microenvironment. In this in vitro culture system, HC survival is commonly assessed. HC counting is performed by manual counting in many laboratories. However, manual counting is time consuming and inter-rater reliability might be a concern. The objective of our study was to test a deep learning approach to quantify HC survival in neonatal in vitro OC cultures. By using StarDist, a publicly available plugin for Fiji (Fiji is just ImageJ), we trained and validated a custom deep learning model. We trained and validated our model in both control and damaged (gentamicin and cisplatin exposed) OC explants. We show that our custom StarDist model reliably reproduces manual counts. Our described method is easy to implement and a custom model can be trained by researchers for their own needs. To conclude, we show that deep learning is a valuable approach to quantify auditory HC survival and provide a method that can be entirely performed in Fiji by using the publicly available plugin StarDist. Therefore, our described deep learning approach facilitates the study of HCs in OC cultures.

Keywords: Hair Cell Counting, Hair Cell Quantification, Deep Learning, Neural Network, Segmentation

10. Noise induced hearing loss

P2.10.01

155 - The interplay between sensory and cognitive neurodegeneration: cochlear vulnerability to noise exposure in a model of Alzheimer's disease.

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The strong correlation between peripheral hearing loss and Alzheimer's Disease (AD) has been widely documented, however, the exact relationship between environmental 'risk factors' (as noise exposure) and AD

is not fully understood and more research is needed. Our recent findings demonstrated that exposure to noise in the pre-symptomatic phase in a mouse model of AD (3×Tg AD mice) accelerated cognitive decline, exacerbating auditory cortex and hippocampal dysfunctions (Paciello et al., 2021).
Here, we wondered if cochlear structures in this model of neurodegenerative disease could be most vulnerable to noise insult and if an altered cochlear processing could be associated with central brain dysfunctions.
To this aim, we exposed 2 months of age WT (BL6129 Sv mice) and 3×Tg AD mice to repeated noise sessions (pure tone of 100 dB, 100 kHz, 60 min/day for 10 consecutive days) and we analyzed cochlear samples 4 months after noise exposure (corresponding to 6 months of age), a critical time point in which we previously found accelerated cognitive decline in 3xTg-AD mice exposed to noise. We monitored auditory thresholds after noise exposure by measuring the Auditory Brainstem Responses (ABR). Morphological evaluations were performed to assess damage in cochlear structures (hair cells, spiral ganglion neurons, afferent nerve fibers). Also, analyses of AD hallmarks (increased Tau and APP phosphorylation, inflammatory markers and oxidative stress makers) were carried out.
Our findings showed that 3xTg-AD cochleae are more vulnerable to noise insult compared to WT mice. Indeed, 4 months after noise exposure (corresponding to 6 months of age), morphological analyses showed a significant neuronal damage (reduction of spiral ganglion neuron viability and primary afferent fibers) in noise-exposed animals compared with age-matched not-exposed animals, indicating a deafferentation process induced by noise. Western Blot results displayed an increase of phosphorylation levels of Tau and APP proteins following acoustic trauma, probably mediated by Cdk5 up-regulation. Also, an increase of oxidative stress and inflammatory markers expression (TNFα and NF-κB) confirmed the high susceptibility of cochlear tissue to noise-insult in the neurodegenerative model.
In conclusion, our findings demonstrates that 3×Tg AD mice are more vulnerable to cochlear damage induced by noise exposure, and supports the hypothesis that exogenous risk factors, as noise exposure, can target both sensory and cognitive neurodegenerative processes, exacerbating AD pathology.

11. Otoprotection

P2.11.01

119 - Mitochondrial derived peptide in the inner ear

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Introduction: Mitochondrial derived peptides (MDP) are a class of small open reading frame encoded polypeptides. Three members of MDP are recognize, including humanin (HN) and small HN-like peptides (SHLP1-6) encoded by the 16S rRNA, and the mitochondrial open reading frame of the 12S rRNA type c (MOTS-c) encoded by the 12S rRNA. The protective role of humanin and MOTS-c in age-related disease and under various cellular stresses has been demonstrated.1,2 Furthermore, MOTS-c has been reported to translocate to the nucleus and to regulate the transcription of stress-responsive genes.3 On the other hand, humanin can also exert tumor-promoting effect.4 The involvement of MDP in the inner ear remains to be investigated. We therefore examined the effect of exogenous humanin and MOTS-c in explants of the organ of Corti exposed to gentamicin.
Methods: We use a rat ototoxic model in which the isolated organ of Corti is exposed to gentamicin at a concentration sufficient to maintain 50% hair cell survival. Exogenous MDP was administered before and together with gentamicin. Hair cell survival was determined by phalloidin staining. Gene and protein expression of MDP was assessed by real-time PCR and Western blot, respectively.
Results: Humanin and MOTS-c showed a significant protective effect on hair cell survival. Currently, we are analyzing the transcripts and protein expression of endogenous and exogenous MDP.
Conclusions: Our preliminary data suggest that MDPs exert a protective function in gentamicin-induced hair cell damage and may be a novel therapeutic agent.

Keyword: Mitochondrial derived peptides, gentamicin, organ of Corti

References:
1. Kim, S.J., et al. Mitochondrial-derived peptides in aging and age-related diseases. Geroscience 43, 1113-1121 (2021).

2. Wu, D., Kampmann, E. & Qian, G. Novel Insights Into the Role of Mitochondria-Derived Peptides in Myocardial Infarction. Front Physiol 12, 750177 (2021).
3. Kim, K.H., Son, J.M., Benayoun, B.A. & Lee, C. The Mitochondrial-Encoded Peptide MOTS-c Translocates to the Nucleus to Regulate Nuclear Gene Expression in Response to Metabolic Stress. Cell metabolism 28, 516-524 e517 (2018).
4. Moreno Ayala, M.A., et al. Humanin Promotes Tumor Progression in Experimental Triple Negative Breast Cancer. Sci Rep 10, 8542 (2020).

12. Ototoxicity

P2.12.01

87 - Effects of corticosteroids on survival and neurite length of spiral ganglion neurons in vitro

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Introduction: Corticosteroids are widely used in Otology for the treatment of a variety of inner ear diseases such as Menière's Disease and sudden sensorineural hearing loss and even during cochlear implantation to prevent loss of residual hearing. They have anti-inflammatory and immunomodulatory effects but they also do alter ion and fluid homeostasis. But most importantly, they may have significant toxic effects on auditory neurons. In this study, we therefore tested four different clinically applied corticosteroids (fludrocortisone, triamcinolone, dexamethasone, and prednisolone) for their effects on the survival rate and neurite length of spiral ganglion neurons (SGN) in vitro.
Methods: The SGN were isolated from neonatal (P3-5) Sprague-Dawley rats followed by a mechanical and enzymatical dissociation. For all experiments, SGN were pre-cultured with 10 % fetal calf serum (FCS) for 24 h. For each of the four corticosteroids (fludrocortisone acetate, TriamHEXAL® [triamcinolone acetonide], Fortecortin®Inject [dexamethasone 21-phosphat disodium salt] and Prednisolut® [prednisolone succinate]), a dose-response curve was determined. In a second setting, the inhibitors mifepristone and spironolactone were used to investigate their inhibitory effect to reverse the gluco- and mineralocorticoids. After 48 h of cultivation with corticosteroids (and inhibitors), SGN were fixed, stained and the survival rate and neurite length of the SGN were determined.
Results: A concentration for normal survival of SGN (related to the positive control: treatment with 10 % FCS) and one for putative toxic effects indicated by lower survival rates of SGN (related to the negative control) was identified for each of the corticosteroids. Treated with a concentration of 0.4 mg fludrocortisone, SGN showed normal survival rates. A concentration of 4.0 mg resulted in lower SGN survival and could be considered as toxic. We identified for triamcinolone 1.0 mg and 4.0 mg, for dexamethasone 0.005 µg and 0.010 µg and for prednisolone 0.25 mg and 1.5 mg as concentrations for normal and lower SGN survival, respectively. Specifically for prednisolone we observed that increasing concentrations resulted in subsequently reduced neurite lengths in a dose-dependent manner. Both inhibitors were not able to reverse the reduced SGN survival after treatment with the respective high concentration of each corticosteroid.
Conclusion: The results indicated that – depending on the used corticosteroid – there is only a small therapeutic window with respect to avoid possible toxicity on SGN. This should be considered when corticosteroids are applied during cochlear implantation to maintain functional residual hearing.

Keywords: corticosteroids, SGN survival, residual hearing

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P2.12.02

154 - Evaluation of NADPH oxidase 3 as drug target in a novel 3R model of cisplatin ototoxicity

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Cisplatin is a lifesaving chemotherapeutic drug with marked ototoxic side effects. Cisplatin-induced hearing loss affects a significant part of cancer surviving patients and is an unmet clinical need with important socio-economic consequences. Cisplatin ototoxicity has been demonstrated to involve a strong oxidative stress component. The reactive oxygen species generator NADPH oxidase isoform NOX3 is strongly and specifically expressed in the inner ear and may contribute to cisplatin ototoxicity. The objective of this study was therefore to evaluate whether mice deficient for NOX3 would be protected from cisplatin-induced hearing loss. Unfortunately, in current pre-clinical animal models of cisplatin ototoxicity, which are mainly based on systemic delivery, important morbidity is observed leading to premature sacrifice or death. This methodology not only raises obvious animal welfare concerns, but also increases the number of animals employed in ototoxicity studies to compensate for dropouts related with early sacrifice. To overcome these important limitations, we previously developed a local delivery model based on the application of a cisplatin solution directly into the otic bulla through a retroauricular approach. Based on this novel preclinical model of cisplatin ototoxicity devoid of systemic toxicity, we have evaluated hearing of NOX3 deficient mice. Cochlear platinum concentration was measured by mass spectrometry and hearing threshold by ABR measurements. The ratio between cochlear platinum concentration and hearing loss was compared between NOX3-deficient and wild type littermates, providing an easy and objective readout. The preliminary results showed a lower susceptibility of NOX3 deficient animals to cisplatin when compared to the WT littermates, characterized by a significantly higher EC50 platinum dose. Histological correlates, including hair cell and spiral ganglion neurons counting are currently being investigated. In conclusion, our newly developed local cisplatin model allowed to assess cisplatin susceptibility in NOX3 deficient mice and to demonstrate that NOX3 is a relevant target for the prevention of cisplatin ototoxicity.

P2.12.03

142 - Development of a cisplatin-induced hearing loss (CIHL) rat model for preclinical efficacy assessment

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Introduction: Cisplatin is a widely used antineoplastic drug used in the treatment of many cancers. However, its use is often associated with serious side effects, such as progressive and irreversible hearing loss. The development of experimental animal models is essential to understand the mechanisms of cisplatin's ototoxicity and to develop effective treatments. In animals, a single-bolus injection of cisplatin can result in high mortality rates and/or health issues, making it difficult to assess potential therapies. To date, few treatments have shown efficacy against cisplatin-induced hearing loss (CIHL). Among them, chemoprotective agent sodium thiosulfate (STS) has demonstrated efficiency in CIHL both in animals and patients and represents an otoprotective reference agent.
Methods: The goal of these experiments was to develop an acute rat model of cisplatin-induced ototoxicity in a short time frame to mimic the effects observed in patients. Therefore, this model must present hearing loss at high frequencies with no to low moribundity and mortality. To develop the most appropriate model, 2 doses of cisplatin were tested, 10 and 13 mg/kg, administered as a single intraperitoneal infusion at T0 in male Wistar rats. To limit health issues, specific care was provided to the animals treated with cisplatin (hydration, food supplementation). DPOAE amplitudes and ABR thresholds were measured at baseline and T+3DAYS to evaluate hearing impairments.
In the most suitable model (10 mg/kg), the protective effects of STS (2 000 mg/kg) were assessed when administered one hour after cisplatin infusion. The functional measures, ABR and DPOAE, were correlated with histological analyses, IHC and OHC counts.
Results: Both doses, 10 and 13 mg/kg, induced important hearing loss, demonstrated by a significant increase of ABR thresholds and a decrease of DPOAE amplitudes. However, health issues were observed in animals at the higher dose, while the animals treated with the lower dose showed no clinical signs. Therefore, no further

analyses were performed on the 13 mg/kg treated group, and the 10 mg/kg treated group was selected. In this group, a loss of the outer hair cells (OHC) was observed at the base of the cochlea. The administration of STS at T+1HOUR completely reversed the hearing and cellular loss.
Conclusion: To conclude, a single administration of cisplatin at 10 mg/kg induces important hearing loss without deleterious effects on health, reflecting CIHL observed in clinic, and consisting in a suitable model to test the protective effects of drugs against cisplatin's ototoxicity.

Keyword : Cisplatin, ototoxicity, ABR, DPOAE, hair cell, sodium thiosulfate

13. Pharmacology of inner ear

P2.13.01

135 - Effectiveness of Various Treatments for Sudden Sensorineural Hearing Loss—A Retrospective Study

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Background: A retrospective clinical study was conducted to compare the effectiveness of different pharmacological and non-pharmacological regimens for treating sudden sensorineural hearing loss (SSNHL).
Methods: Adult patients (n = 130) diagnosed with sudden sensorineural hearing loss (SSNHL) and hospitalized between 2015 and 2020 were enrolled in this study. Depending on the treatment regimen applied, patients were divided into five groups. Inclusion criteria were as follows: (i) hearing loss of sudden onset; (ii) hearing loss of at least 30 dB at three consecutive frequencies; (iii) unilateral hearing loss; (iv) age above 18 years. Exclusion criteria were as follows: (i) no follow-up audiogram; (ii) bilateral hearing loss; (iii) recognized alternative diagnosis such as tumor, disorder of inner ear fluids, infection or inflammation, autoimmune disease, malformation, hematological disease, dialysis-dependent renal failure, postdural puncture syndrome, gene-related syndrome, mitochondrial disease; and (iv) age below 18 years.
Results: Complete recovery was found in 14% of patients (18/130) and marked improvement was found in 6% (8/130), giving an overall success rate of 20%. The best results were obtained in the second group (i.e., patients given intratympanic glucocorticoid + prolonged orally administered glucocorticoid) where the success rate was 28%. In general, the older the patient, the smaller the improvement in hearing, a correlation that was statistically significant.
Conclusions: In treating SSNHL, the highest rate of hearing recovery—28%—was in the group of patients given intratympanic corticoid plus prolonged treatment with orally administered glucocorticoid.

Keywords: sudden sensorineural hearing loss; dexamethasone; prednisone

14. Physiopathology of Auditory Pathways

P2.14.01

51 - Central stress receptors differentially participate in auditory nerve response though cGMP signaling cascade

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Corticosteroid treatment has been the most common used therapy for a wide variety of hearing disorders. Conversely, aging, acoustic trauma or stressful conditions impair the hearing function. The mechanism behind the opposing stress effects on hearing is still unclear. The stress response is induced under challenging

conditions via increased release of glucocorticoids (GCs), which is controlled by the hypothalamic-pituitary-adrenal (HPA) axis. There are two glucocorticoid receptor type mineralocorticoid receptors(MR) and glucocorticoid receptors(GR), which facilitate learning and memory as well as attention to respond to the novel situation, memory consolidation, and long-term adaptation. These stress receptors, MR and GR, are also expressed in the auditory system where they are found in the inner ear and central auditory nuclei. The MR and GR have been shown to differentially impact on auditory function. The conditional deletion of central stress receptors under the CamKII-a promoter showed alteration in auditory nerve response as well as changes in the inner hair cell ribbon numbers. MR-deletion results in reduced and slower auditory nerve activity while GR-deletion markedly increased activity along the entire auditory pathway. This appears to be a feature of top-down regulatory mechanisms of the central stress responses on peripheral processing of auditory information. The interaction between the periphery and central auditory and associated centers (e.g., hippocampus, limbic system) is still elusive. First molecular results suggest that this interaction seems to be translated by cGMP dependent signaling cascades. Since we observe differential impact of MR and/or GR deletion on guanylyl cyclase expression level. This is in line with the previous findings in which an otoprotective effect of cGMP has already been shown in terms of age-dependent hearing loss and traumatic acoustic events. It has already been shown that PDE9A- inhibitor improves cognitive function, LTP, synaptic plasticity and auditory gating deficiency. In this regard, we suggest that inhibition of cGMP degradation by PDE9A may be beneficial for central auditory processing, including memory-associated adaptation.

Keywords: stress, auditory nerve function, cGMP signaling, PDE9Ai, mineralocorticoid-receptor, glucocorticoid-receptor, cognition, memory.

Acknowledgements
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P2.14.02

29 - Distortion Product Otoacoustic Emissions correlate to the putamen DATscan signal in Parkinson's Disease

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Background and objective: In previous studies, a decay of the cochlear function was assessed in Parkinsonian patients compared to age-matched controls. A lateralization effect was also observed in the peripheral auditory function, with lower Distortion Product Otoacoustic Emissions (DPOAE) levels on the side more affected by motor symptoms. The hypothesis is made here that a correlation should be found between the DPOAE level of the side more affected by motor symptoms and the contralateral DATscan signal at the putamen nucleus.
Methods: 29 patients (14 males, 15 females, average age = 63 years) were enrolled in this study, with a diagnosis of Parkinson's disease (PD), made at the Center for Neurological Disorders of the University of Rome Tor Vergata. All the patients underwent DATscan according to standard guidelines after the injection of 185± 5 MBq of 123-I Ioflupane (Datscan®). Dopaminergic system impairment was detected in all the subjects by semi-quantitative analysis of functional data. The auditory function was assessed by means of pure tone audiometry (PTA) and DPOAEs.
As the PD patients are generally elderly subjects, and further affected by the auditory effects of PD, the use of a sensitive customized acquisition and analysis system was necessary for measuring the weak DPOAE signal. Effective noise insulation was obtained by using an audiometric booth, earmuffs and ear-tips. High-resolution DPOAE spectra were measured using two slow chirps as primary stimuli, with an instantaneous frequency ratio r=1.22. A rejection algorithm was applied in real time to discard only the frames of each chirp affected by artefacts and/or excess noise, preserving the rest of the chirp to optimize the SNR. By selecting the distortion DPOAE component, using time-frequency filtering, a significant increase of the SNR (6 to 12 dB) was obtained. The filtered signal was analyzed in third-octave frequency bands in the range 1150-4610 Hz.
The statistical analysis was performed by means of the R software (version 4.1.2, R Foundation for Statistical Computing, Vienna, Austria). Mixed effect linear model regressions for repeated measures were used. Correlation analysis was used in order to investigate the possible relationship between DPOAE and DATscan uptake in basal ganglia.

Results: A statistically significant association ($p < 0.05$) between the distortion DPOAE level and the DATscan at the putamen nucleus contralateral with respect to the motor symptoms was found, with 4 frequency bands between 1452 and 3360 Hz included in the analysis, [regression coefficient = 3.3 (dB/putamen units), $p = 0.029$, repeated measure ANOVA $p = 0.045$].
Conclusions: This study supports this hypothesis as the DPOAEs amplitudes are positively correlated at a statistically significant level to the DATscan. Therefore, the auditory function seems to be affected by dopamine depletion at peripheral level. These preliminary results must be confirmed on a larger sample of PD patients.

15. Regeneration and Stem cells

P2.15.01

83 - Drug repositioning to find potential drug candidates for inner ear protection against noise induced hearing loss: the use of Pharos

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Introduction: The most crucial step for the development of new drugs is the discovery of a target gene, protein or enzyme. However, even if the target genes, proteins or enzymes are known, the financial costs, the amount of time required to develop new molecular entities or biologics to novel drugs and approved drugs may be discouraging. Drug repositioning is the process of identifying and developing new uses for existing drugs that are outside the scope of the original medical indication. Increased safety deriving from lessons learned in clinical trials, accelerating the time for drug development, achieving sustainability, saving resources and improving animal welfare and reducing the financial burden are among the advantages of drug repositioning. The growing field of inner ear regeneration and protection is no exception to these trends, as the multitude of different regulatory molecules highlighted by the preclinical research is waiting for a clinical translation. A way to facilitate the research for potential drug candidates is represented by Pharos, an integrated web-based informatics platform for analysis of data aggregated by the Illuminating the Druggable Genome (IDG) program, collating data on human proteins, disease and phenotype associations and hundreds of thousands ChEMBL compounds.
Methods: We applied the Pharos software to find available molecules that could influence the genes involved in the protection pathways induced by the intracochlear infusion of mesenchymal stromal cells (MSC) in a mouse model of noise induced hearing loss. Treatment with MSC has already been proved as protective against tissue damage induced by sound and a variety of ototoxins. Analysis of cochlear transcriptome after MSC infusion resulted in an up-regulation of genes related to immune modulation, hypoxia response, mitochondrial function and regulation of apoptosis; conversely, there was a down-regulation of genes related especially to synaptic remodelling, calcium homeostasis and the extracellular matrix. The statistically most relevant transcriptome changes were matched with Pharos to identify small molecules able to influence the expression of the involved genes.
Results: Using Pharos, we identified 46 potential drug candidates to modulate the identified protection pathways, especially the downregulation of transthyretin and GABA neurotransmission.
Conclusion: Pharos represents a valuable tool for refining research of potential clinical drug candidates in inner ear protection in humans. Its use helps to quickly highlight already available drugs that could be tested for their ability to modulate the discovered protective and regenerative pathways of the cochlea, thus streamlining the clinical translation of the most recent research developments in inner ear biology.

Keywords: cochlear transcriptome; hearing loss; hearing protection; noise trauma; drug repositioning; Pharos.

P2.15.02

54 - Survival of LGR5-positive supporting cells in the cochlea of adult mice 28 days after ototoxic trauma

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Background: Sensorineural hearing loss is mainly caused by irreversible damage to sensory hair cells (HCs). A subgroup of supporting cells (SCs) in the cochlea express the leucine-rich repeat-containing G-protein coupled receptor 5 (LGR5), a marker for tissue-resident stem cells. These LGR5+ SCs could potentially be used as an endogenous source of stem cells for regeneration of HCs to treat hearing loss and deafness. We have recently described that LGR5+ SCs survive one week after ototoxicc treatment (Smith-Cortinez et al., 2021). However, it is still unknown whether LGR5+ SCs from the deafened mouse cochlea retain regenerative potential or if they are present in the long term after deafening. Here, we will evaluate long-term (i.e., 28 days) survival of LGR5+ SCs in adult deafened cochlea of Lgr5GFP transgenic mice and determine the regenerative potential of these LGR5+ SCs.
Methods: Adult (postnatal day 30-50) normal-hearing Lgr5-eGFP-IRES-creERT2 heterozygous (Lgr5GFP) and deafened Lgr5GFP female and male mice were used. Animals were deafened with a single dose of furosemide (100 mg/kg i.v.) and kanamycin (700 mg/kg s.c. males, 900 mg/kg s.c. females). Before, 7 and 28 days after deafening, auditory brainstem responses (ABRs) were recorded. Cochleas were harvested to characterize mature hair cells and LGR5+ SCs by immunofluorescence microscopy and quantitative reverse transcription PCR (q-RT-PCR).
Results: As previously described, we found survival of LGR5+ SC in the third row of Deiters' cells; minor loss of inner hair cells (IHCs) and complete absence of outer hair cells (OHCs) one week after deafening in adult Lgr5GFP mice. The q-RT-PCR expression profile showed up-regulation of Lgr5 in the deafened cochlea, and downregulation of Prestin compared to the normal-hearing cochlea. Twenty eight days after ototoxic trauma, LGR5+ SCs were present in the third row of Deiters' cells and in inner pillar cells; the majority of IHCs were still present but expressed less myosin7A compared to normal-hearing littermates and there was partial OHC loss.
Conclusions: The presence of LGR5+ cells in the adult mouse cochlea demonstrates potential endogenous cochlear stem cells with regenerative capacities in adulthood. Furthermore, these LGR5+ SCs do survive an ototoxic trauma even 28 days after deafening. To our knowledge, this is the first study showing increased Lgr5 expression after deafening in the adult mouse cochlea. This might be a result of ototoxicity-induced LGR5+ cell proliferation, which will be further explored and objectified in a future study.

Key words: LGR5+ supporting cells; adult mammalian cochlea; hearing loss; inner ear regeneration; ototoxicity.

References

Smith-Cortinez N, Yadak R, Hendriksen FGJ, Sanders E, Ramekers D, Stokroos RJ, Versnel H, Straatman LV. LGR5-Positive Supporting Cells Survive Ototoxic Trauma in the Adult Mouse Cochlea. Front Mol Neurosci. 2021 Oct 5;14:729625

16. Tinnitus

P2.16.01

4 - Pulsatile tinnitus due to dural arterio-venous fistula

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Introduction: Pulsatile tinnitus is perceived as a rhythmic pulsing and can be experienced as a thumping or whooshing sound. About 3% of tinnitus patients experience this type of tinnitus.
Case Report I: A 28-year-old woman visited a tertiary referral center with pulsatile tinnitus of left ear for 1week. The audiology test and physical examination were no significant findings. On temporal bone MRI including

angiography, she was found dural arteriovenous fistula (dAVF) in the left transverse sinus. She transferred to neurosurgery for performing embolization of dAVF. After surgery, her symptom was completely disappeared.
Case Report II: A 35-year-old man with pulsatile tinnitus of right ear for 3 weeks visited the outpatient department. The physical examination, audiology tests, and even temporal bone CT were no significant findings. On temporal bone MRI including angiography, he was found dural arteriovenous fistula (dAVF) in right clivus and jugular foramen. After explaining the situation to the patient, he was transferred to neurosurgery for surgical intervention.
Discussion: In these cases, two types of dAVF were identified as the cause of pulsatile tinnitus. We reported rare vascular tinnitus of two cases with literature review.

17. Vestibular disorders

P2.17.01

25 - Expression of Autoantibodies and Complements in Acute Peripheral Vestibulopathy

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Introduction: The etiology and pathophysiology of acute peripheral vestibulopathy are largely unknown. The purpose of this study is to evaluate the expression of the autoantibodies and complements in patients with acute peripheral vestibulopathy.
Materials and Methods: We checked anti-ds-DNA, rheumatoid factor, anti-phospholipid IgG and IgM, anti-nuclear antibody (ANA), C3, C4 in 72 patients who were diagnosed as acute peripheral vestibulopathy on physical examination and the caloric test. The results of the patients with unilateral acute peripheral vestibulopathy were compared to those of the patients with bilateral acute peripheral vestibulopathy.
Results: Twelve patients (16.6%) in anti-ds-DNA, 4 patients (5.5%) in C3, 10 patients (13.8%) in C4, 2 patients (2.7%) in anti-phospholipid IgG and 13 patients (18%) in antinuclear antibody (ANA) showed abnormal findings among patients with acute peripheral vestibulopathy. There was no difference in the manifestation of the autoantibodies and complements between the patients with unilateral and bilateral acute peripheral vestibulopathy.
Conclusion: The autoimmune diseases may be one of etiologic factors in acute peripheral vestibulopathy

19. Miscellaneous

P2.19.01

94 - The pathophysiology of hearing loss in congenital cytomegalovirus: from a direct cytopathic effect towards associated inflammatory-based damage

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Introduction and aim: Congenital cytomegalovirus (cCMV) is recognized as an important cause of neurodevelopmental impairment and the major cause of non-hereditary congenital sensorineural hearing loss (SNHL). Large retrospective studies have contributed to a thorough description of the clinical features and epidemiology of this complex disease. Both the onset as evolution of hearing loss may be highly variable: SNHL may be present at birth, develop in early infancy or be progressive or fluctuating. About 10% of cCMV-infected newborns experience hearing loss in the absence of any other symptom. In otherwise symptomatic children the occurrence of hearing loss is even higher. Despite the high prevalence, the exact mechanism of cCMV-related hearing loss remains unclear.
Study design: A review of the literature on the pathophysiology of cCMV related inner ear damage was performed. Studies investigating human temporal bones as well as animal models were consulted.
Results: The first reports suggested a cytopathic effect of the virus leading to an altered auditory function. Over the years, a shift has taken place towards associated virus-induced inflammation. Several inner ear structures have been found to be damaged: hair cells, stria vascularis, scala vestibuli, scala tympani, Reissner's membrane etc. Researchers suggested hearing loss being caused by synaptopathy, loss of outer hair cells, endolymphatic hydrops, degeneration of spiral ganglion neurons, auditory neuropathy or metabolic

dysregulation of the inner ear. However, it remains unclear which inner ear structure is primarily affected, possibly resulting in secondary degeneration of other cochlear structures. Additionally, the importance of particular pro-inflammatory cytokines, apoptosis, aberrant endocochlear potential, or oxidative stress remains contradictory. Even as the lack of a straightforward translation to the clinical findings. Progressive or fluctuating hearing loss is hypothesized to be due to reactivation of a latent strain. The severity of hearing loss would rather be associated with the extent of the inflammatory auto-immune response rather than the amount of viral load.
Conclusions: Recently, the focus of cCMV-related hearing loss has been laid on the inflammatory response rather than direct cytopathic effect of the virus. However, neither the first site of inner ear damage nor the consecutive cascade has been revealed. This study provides a clear overview of described hypotheses of cCMV-related hearing loss and the possible patterns of CMV dissemination. Particular attention has been given to each individual inner ear structure. Additional studies using animal models are required to gain insights into the pathophysiology of cCMV-related hearing loss. Thorough understanding may provide targets for novel therapy.

Keywords: Congenital cytomegalovirus, hearing loss, pathophysiology, human, animal model.

P2.19.02

139 - Corticosterone modulates protein expression in murine inner ear explants

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Introduction: Clinical observations suggest the link between stress and different hearing conditions, such as tinnitus. Additionally, acute stress altered auditory processing (1, 2). To determine how acute stress impacts the cochlea, we have adopted a model of short pulse corticosterone (primary stress hormone of rodents) used to study stress-induced changes in organotypic cultures of the mouse brain (3, 4) in the cochlear explants. Subsequently, we have analyzed the changes in the cochlea's glutamate receptor subunits in the inner ear after exposure to corticosterone.
Methods: Cochlear explants were prepared from C57BL/6 mice (P4-5) of both genders (total n=88). The explants were briefly (20 minutes) exposed to 100 nM corticosterone to mimic acute stress, followed by a wash and 24h tissue culture without corticosterone. All experiments were performed at the same time of the day (11 AM) to address diurnal variation in corticosterone levels. We used Western blot and enzyme-linked immunosorbent assay (ELISA) to measure the levels and concentration of glutamate receptor subunits (GluR1-4). Statistical analyses were performed using IBM SPSS.
Results: Our first observation was that the expression of glutamate receptors in the untreated cochleae is sex-dependent. The male animals had a significantly higher concentration of GluR2 in the membranous cochlea than female animals ($p< 0.01^{**}$), and there were no such differences in the levels of GluR1, GluR3, or GluR4. Our second observation was that brief exposure to corticosterone increases the concentration of GluR2 in females ($p<0.05^{*}$) but not in male C57BL/6 mice.
Conclusions: The present study indicates that acute stress might modify the cochlear glutamate receptor subunits on the protein level. Changes are gender-dependent. Obtained results are consistent with the clinical observations, such as stress and tinnitus affecting more women than men (5).

Keyword: acute stress, inner ear, corticosterone

References
1. Maxwell CR, Ehrlichman RS, Liang Y, Gettes DR, Evans DL, Kanes SJ, et al. Corticosterone modulates auditory gating in mouse. Neuropsychopharmacology. 2006;31(5):897-903.
2. Stevens KE, Bullock AE, Collins AC. Chronic corticosterone treatment alters sensory gating in C3H mice. Pharmacol Biochem Behav. 2001;69(3-4):359-66.
3. Karst H, Karten YJ, Reichardt HM, de Kloet ER, Schütz G, Joëls M. Corticosteroid actions in hippocampus require DNA binding of glucocorticoid receptor homodimers. Nat Neurosci. 2000;3(10):977-8.
4. Karst H, Berger S, Erdmann G, Schütz G, Joëls M. Metaplasticity of amygdalar responses to the stress hormone corticosterone. Proc Natl Acad Sci U S A. 2010;107(32):14449-54.
5. Hasson D, Theorell T, Bergquist J, Canlon B. Acute stress induces hyperacusis in women with high levels of emotional exhaustion. PLoS One. 2013;8(1):e52945.

POSTER SESSION 3

01. Aging

P3.01.01

68 - Audiometric markers of cochlear synaptopathy and speech in noise deficits in humans

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Aging people often experience difficulties in perceiving speech in a noisy environment, even without elevated audiometric thresholds associated with cochlear synaptopathy. The development of poor supra-threshold speech processing during aging in humans has recently been associated with progressive cochlear synaptopathy. It is crucial to understand the impact of cochlear synaptopathy on speech coding to develop effective therapeutic interventions.
Here, we examine young, middle-aged, and elderly individuals with and without hearing impairment for characteristic features of cochlear synaptopathy. We present first data of a larger clinical study in which we gain indications of speech comprehension problems through the comparison of different audiometric and psychoacoustic techniques with speech in quiet and speech in noise tests in subjects of different age. We compare Pure Tone audiometry (PTA), DPOAE (IO, DP-Gram, Level-Maps), ABR, ASSR, Depression (Yesaga, Becks), Oldenburger Satz Test (OLSA), psychoacoustic test of speech in noise, Mini Mental State Examination and Custom Questionnaire in normal-hearing and hearing-impaired people of different age.
We discuss the results in the context of the opportunity to use objective functional audiometric biomarkers for speech discrimination disorders in the future.

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P3.01.02

110 - Neuropathy of the inner ear due to plasma membrane Ca2+-ATPase mutations

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Introduction: It remains unclear which mechanism underlies the onset and development of human age-dependent hearing loss or presbycusis. It also controversial whether a rodent model of age-dependent hearing loss can be applied to human. Using a forward genetics procedure, we isolated four Pmca2 mutant mouse lines, each of which had a different missense mutation in Pmca2 and a distinct phenotype. We found large differences in the age-dependent ascending rate of ABR thresholds and period of hair cell/SG cell disappearance between the mutant mice. Age-dependent phenotypic progression in different mouse allelic mutants [1,2,3] and a variety of diagnostics for patients with the de-novo mutations in human paralogues have

been reported [4]. These observations suggest that intracellular Ca2+ status plays a latent role in deafness progression.
Methods: We screened mice for startle responses evoked by a click box and then measured auditory brainstem responses (ABR) [5]. The isolated mutants were further subjected to comprehensive screening to confirm phenotypically that they did not have other traits, which is a typical characteristic of human diagnostic-type non-syndromic deafness. Hearing function and morphological analysis were performed successively by performing DPOAE measurements and histological analysis, including immunofluorescence microscopy. To detect possible defects in hair cell calcium-ion exporting activity, Ca decay assays of cells expressing the mutant product were performed.
Results: Functional and histological analyses showed that the early-phase of impairment could be distinguishable from the late-phase, during which hair cells and SG cells are impaired and completely obliterated. The clear differences in phenotype between the four mutants appear to be due to differences in Ca2+-pump activities of P-type Ca2+-ATPase, the product of the Pmca2 gene.
Conclusions: Assuming that the biomolecular pathway leading to age-dependent emergence of hearing loss phenotypes can be divided into two axes (functional impairment and cell death process), clarifying these axes may facilitate the development of an appropriate model of progressive hearing loss in human. Ca ion is generally thought to act as a second messenger in multiple signaling pathways over very short time periods; however, it might also act via an as-yet-unknown pathway over life-long periods. The status of intracellular Ca-mobilization could act as a timer. To investigate these axes in vitro, we have established a cell culture system in which the expression of each mutated protein is controlled so that its amount and localization is the same between the mutants. Molecular analysis of this system is expected to reveal the latent basis of the long term effects in the mutants.

Key words; forward genetics, calcium signaling, hair cell, SG cell

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Reference;
[1] Spiden SL, PLOS Gen. (2008)
[2] Bortolozzi M, JBC. (2010)
[3] Watson CJ, Hear Res. (2013)
[4] Smits JJ, Hum. Gen. (2019)

P3.01.03

3 - A Nationwide Population-based Study of Association between Age-related Hearing Loss and Cognitive Disorder in Korea

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Hearing loss and cognitive disorder are worldwide public-health concerns. There are studies that suggest there may be a relationship between hearing loss and cognitive disorder, but there have been no large scale cross-sectional epidemiologic studies of a representative sample of the entire national population to look at this possible association. The aim of this national population-based retrospective study was to investigate the relationship between hearing loss and cognitive disorder in South Korea using data from the Korean Health Insurance claims database during 2009–2015. We analyzed cross-sectional data of 66-year-olds who completed the Korea National Health and Nutrition Examination Surveys. Among the 1,815,835 participants at the age of 66, the prevalence of unilateral hearing loss was 5.84% and that of bilateral hearing loss was 3.40%. The normal cognitive group was 86.35% and the high-risk group for cognitive disorder was 13.65%. The bilateral hearing loss group had the highest percentage of subjects who responded “sometimes or frequently” to all five questionnaires about cognitive disorder compared to the normal hearing or unilateral hearing loss group. After adjustment for gender, smoking status, alcohol intake, exercise, income, diabetes, hypertension, dyslipidemia, and depression, the hazard ratio (HR) of cognitive disorder was 1.183 (95% confidence interval [CI], 1.163-1.203) for bilateral hearing loss and 1.141 (95% CI, 1.126-1.156) for unilateral hearing loss compared to the normal cognitive group. The HR for the bilateral hearing loss compared with normal hearing or unilateral hearing loss group was increased in all five questionnaires about cognitive disorder after adjustment for confounders. Hearing loss has a significant effect on cognitive function in the Korean population. In our study, the bilateral hearing loss group showed poorer cognitive function than did the unilateral hearing loss group.

02. Cochlear implant and implantable prosthesis

P3.02.01

163 - The effectiveness of targeted electrical stimulation via cochlear implant on tinnitus perceived loudness

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Introduction: The cause of tinnitus improvement in cochlear implant (CI) users is not understood. On the basis that a spatially limited dysfunction in the auditory pathway could cause tinnitus, we used single-channel stimulation to evaluate any variation of tinnitus perceived loudness and identify the cochlear regions involved.
Methods: It was an observational prospective case-crossover study. After the first mapping, 21 adults with unilateral CI and chronic tinnitus expressed their tinnitus loudness based on the VAS score (0-10) at baseline (L0), during a 10 second single-channel stimulation with C-level of electric current (L1) and 30 minutes after CI activation (L2). Tinnitus reduction [RT = (L0 – L1) x 100 / L0] > 50% was considered significant.. VAS outcomes were compared between baseline (L0) and (each) single-channel stimulation (L1), to find the channel with the greatest RT (suppressive channel-SC), whose frequency range revealed the cochlear region involved. Seven patients with asymmetric hearing loss underwent the pitch-matching test to identify the actual frequency evoked by the SC. We compared selective (L1) and non-selective (L2) intracochlear stimulation using paired T-test. Preoperative THI score was compared to those at 1, 6 and 12 months with paired T-tests to evaluate long-term tinnitus perception.
Results: We observed a significant reduction of tinnitus loudness during the experimental procedure (L0(6.4±2.4) vs. L1(1.7±2.7), p=0.003). 15/21 patients (71.4%) had a significant (RT>50%) and selective improvement, reporting a mean L1 of 0.4±2.0 (p=0.0001). In 10/15 (66.6%) patients the SC was in the apical turn, within 1000 Hz; in 5/15 patients (33.4%) within 4000 Hz. The cochlear region 125-313Hz was the most affected by tinnitus improvement (p=0.0074). Targeted stimulation was more effective than non-selective stimulation (L1 vs. L2 (4.3±2.5), p=0.0022). In 3/7 patients the perceived pitch did not fall within the SC frequency ranges. All patients with selective attenuation described tinnitus as monotone. Patients with non-selective attenuation had polyphonic tinnitus and better THI results after one year.
Conclusions: Targeted intracochlear electrical stimulation improved chronic tinnitus perception especially in monotone tinnitus and the apical region was mainly involved. Our results provide new insights into pathophysiological mechanisms of tinnitus and targets for innovative therapeutic strategies.

Keywords: cochlear implant, tinnitus, intracochlear electrical stimulation, cochlear regions, pitch match

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P3.02.02

44 - Anti-inflammatory coated cochlear implants in guinea pigs

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Introduction: Many people around the world suffer from sensorineural hearing loss. If the auditory nerve is intact, a cochlear implant (CI) is a possible treatment. After CI implantation connective tissue around the electrode array starts to grow. This leads to poorer signal transduction and increased impedances at the electrode contacts. Locally applied anti-inflammatory drugs may reduce the fibrosis. In this study, Poly-L-lactid acid (PLLA) coatings of CI electrode arrays releasing diclofenac or the immunophilin inhibitor MM284 were investigated for their efficacy to reduce connective tissue growth in vivo.

Methods: Cochlear implants with 4 contacts were implanted in guinea pigs for 28 days. The animals were divided into 4 groups (control (no coating), PLLA, PLLA with diclofenac, PLLA with MM284). Hearing thresholds were measured on day 0 before cochlear implantation. Cochleostomy and multiple insertions during implantation were used to provoke connective tissue growth. Impedances were measured daily for 14 days and additionally on days 21 and 28. On day 28, hearing thresholds were determined, blood and perilymph were collected and the animals were perfused. The cochleae were removed, fixed in paraformaldehyde (PFA) and decalcified in ethylenediaminetetraacetic acid (EDTA). After optical clarification of the sample and staining for vimentin, cochleae were scanned for autofluorescence and the vimentin signal.
Results: On day 0, impedances were initially high (7 to 10 kOhms), but then decreased on day 1 to about 4 to 6 kOhms. Subsequently, a continuous increase was observed at all 4 contacts in all groups. The groups treated with diclofenac and immunophilin inhibitor MM284 showed a slower impedance increase in the first 7 days compared to the control group. At day 28, impedances are nearly similar in all groups (10 to 14 kOhms). On the implanted side, the animals showed significant hearing loss with a threshold shift of more than 50 dB whereas the contralateral side remained unaffected. Histological analysis reveals great variability in tissue growth, confined to a small layer around the array or massively extending onto the apex, regardless of the anti-inflammatory coating.
Conclusion: The results indicate that diclofenac and immunophilin inhibitor MM284 reduce connective tissue growth in the first days after CI implantation. However, based on the impedances a long-term effect was not observed.

Keywords: anti-inflammatory, diclofenac, MM284, guinea pig, cochlear implant coating

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03. Developmental biology

P3.03.01

79 - Epithelial formations in the developing human vestibular system: Newly identified dark cell areas

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Inner ear endolymph homeostasis relies on the function of specialized epithelial and stromal cells expressing a large variety of essential ion pumps, ion channels and gap junctions in their cell membranes. In the vestibular system, dark cells and subepithelial melanocytes work intimately together and form a functional unit essential for potassium recycling. These dark cell areas are found adjacent to the sensory areas of the utricle and ampullae of the semicircular canals. Some earlier studies observed morphologically similar epithelial formations in other areas of the vestibular system, but their functional significance was not unraveled.
To investigate the distribution and functional significance of these epithelial formations in the developing human inner ear, we collected embryonic and fetal human inner ears from W8-W17. Deparaffinized sections were immunostained for markers known to be specific for dark cells (the Na/K-transporting ATPases ATP1A1, ATP1B2, Barttin CLCNK-type chloride channel accessory beta subunit, SLC12A2 solute carrier, and melanocytes (Melan-A/MART 1).
We could corroborate earlier observations that epithelial formations morphologically similar to dark cell areas are located at the junction between the utricle and common crus as well as the junction of the inferior utricular sinus and the ampulla of the posterior and horizontal semicircular canal in the developing human inner ear. Furthermore, we demonstrated that these epithelial formations express proteins involved in endolymph production and that melanocytes line the epithelium in a manner similar to that seen in the dark cell areas of the utricle and ampullae. These findings led us to believe we identified new dark cell areas in the developing human vestibular system.

04. Drug delivery system

P3.04.01

32 - PLLA-coating of cochlear implant electrode arrays to release anti-inflammatory substances

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Cochlear implants are the most effective treatment option for hearing loss. Opening of the cochlea and insertion of the electrode promotes formation of fibrous tissue around the electrode array. Among current strategies to reduce this tissue formation are deposition of steroids in the cochlea during cochlea implantation and slow release of dexamethasone (DMS) from the silicone body of the electrode array. The aim of the current study is to combine this slow release with a faster release of anti-inflammatory substances from a polymeric surface coating. To achieve this, otherwise approved drugs such as diclofenac were tested in vitro for application to cells from the inner ear, release characteristics and the influence of the coating on electrode contact impedances.

Diclofenac, dexamethasone and enalapril were tested on freshly isolated spiral ganglion neurons (SGN), cultured for 48 hours. Cells were stained immunocytochemically before evaluation of cell survival and neurite length. For drug release measurements, PLLA coated samples (Ø = 6 mm) were placed in 1 ml fresh artificial perilymph and released drugs were quantified by HPLC. Impedance measurements of flat rectangular silicone samples coated with 10 µm PLLA and loaded with 10 % or 20 % diclofenac, were measured for 24 hours in 0.9 % NaCl solution without and for another 24 hours with pulsatile electrical stimulation. Strategies to remove the coating from contacts were developed and transferred to animal electrodes.

At concentrations of 2x10-4 mol/l, surviving SGN were barely found with all three substances. Survival increased to about 100 % at a concentration of 8x10-6 mol/l for DMS and diclofenac and remained stable for lower substance concentrations. Using enalapril, the highest survival of SGN with about 76.8 % was achieved at a concentration of 8x10-6 mol/l. In contrast, neurite length was not affected for all substances. PLLA coating reduced the release of DMS from the silicone carrier whereas incorporation of diclofenac enhanced the DMS release slightly again. For the release of diclofenac, a significantly higher burst release was detected for samples containing 20 % diclofenac. Incorporation of 10 % diclofenac into a PLLA coating results in initial impedances of >10 MΩ whereas with 20 % diclofenac initial impedances were between 1 MΩ and 10 MΩ. For both concentrations, impedances could drop under electrical stimulation to below 10 kΩ or remain at >1 MΩ. Measured values after removal of the coating were slightly increased compared to uncoated electrode contacts.

Diclofenac might be suitable for application in the cochlea. All PLLA-coatings serve as insulator. This can be overcome by using removable masking on the contacts during the coating.

Keywords: PLLA coating, spiral ganglion neuron, impedance measurements, cochlear implant, diclofenac

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P3.04.02

61 - Increasing drug delivery to the inner ear using junctional regulation of round window membrane

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Introduction: Delivery of substances into the inner ear via local route is increasingly being used in clinical treatment, Studies have Studies have focused on methods to increase permeability through the round window membrane (RWM) and enhance drug diffusion into the inner ear.

Methods: we employed the medium chain fatty acid caprate, a biologically safe, clinically applicable substance, to modulate tight junctions of the RWM.

Results: Intratympanic treatment of sodium caprate (SC) induced transient, but wider, gaps in intercellular spaces of the RWM epithelial layer and enhanced the perilymph and cochlear concentrations/uptake of

dexamethasone. Importantly, dexamethasone co–administered with SC led to significantly more rapid recovery from noise–induced hearing loss at 4 and 8 kHz, compared with the dexamethasone-only group.
Conclusion: Junctional modulation of the RWM by SC enhances dexamethasone uptake into the inner ear, thereby hastening the recovery of hearing sensitivity after noise trauma.

Keyword: Round window membrane, Juctional regulation, Sodium caprate

P3.04.03

102 - Development of a 3D-Printed Round Window Niche Implant for Controlled Drug Delivery *in vivo*

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Introduction: Pharmacotherapy of inner ear disorders is challenging due to poor drug availability to this protected compartment with systemic administration. When being applied in the bulla, most drugs can diffuse directly through the round window membrane (RWM) into the cochlea at high doses without risking adverse side effects. Today's intratympanic delivery systems are applying the drug relatively uncontrolled. We aim to develop an individualized drug eluting round window niche implant (RNI) to achieve a controlled and atraumatic drug delivery. This concept must at first be developed and tested in animal models before being transferred into clinical application. We chose the guinea pig, an established animal model in hearing research, and designed, manufactured and tested a RNI for this animal model.
Methods: Modeling**:** Compared to humans, the guinea pig round window niche has no individuality. Therefore, we created a one-size-fits-all RNI model to reduce the variabilities in the animal model. The average RNI model was based on the mean values of four Dunkin-Hartley guinea pigs. The temporal bones were scanned by Micro-CT (Xtreme CTII, Scanco Medical) and the data were exported as DICOM (digital imaging and communications in medicine) files which can be used for segmenting and establishing models in 3D Slicer™ (http://www.slicer.org). A handle that additionally illustrates the orientation of implantation and keeps the RNI in situ was added.
Manufacturing: The average model was exported as a STL (standard tessellation language) file and printed by a 3D-Bioplotter (EnvisionTEC, Germany) using UV silicone (EnvisionTEC, Germany) with and without 1% dexamethasone and containing an x-ray dense wire. RNI were sterilized and the accuracy and precision of printing were checked. The implantability and attachment to the RWM were evaluated in fresh guinea pig cadavers (n = 6) performing histology and µCT imaging.
Results: The printing of the RNI was precise and accurate. The designed model fitted in all tested guinea pig niches. The implantation shows that the RNI stayed in situ, had no structural damage by processing for histology and was attached to the RWM.
Conclusions: The developed individualized RNI shows a good suitability as a precise RWM drug delivery system in guinea pigs. For further exploration and improvement, drug release studies and in vivo applications are being performed.

Keywords: round window niche implant; drug delivery; 3D printing; animal model

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P3.04.04

66 - Investigation of Inner Ear Drug Delivery in Piglets with a Cochlear Catheter as a Representative Model for Human Cochlear Pharmacokinetics

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Background: Animal models are indispensable in translational hearing and vestibular research. Yet, the small inner ear dimensions and other anatomical features in rodents poorly correlate with their human counterparts. The translational gap between these species may be bridged using larger animal models such as non-human primates, however, their use is challenging and impeded by administrative, regulatory, and financial hurdles. Other easily accessible large animal models with more human-like inner ear dimensions are scarce. Therefore,

we carried out an in-depth evaluation of the inner ear anatomy and pharmacokinetics in piglets to assess their potential use in inner ear research.
Methods: Prior to in vivo experiments, anatomical landmarks were identified and a step-by-step surgical approach for inner ear drug delivery and perilymph (PL) sampling was established in the cadaver model. In vivo, a novel CE-marked cochlear catheter was inserted intraoperatively through the porcine round window membrane (RWM) and fluorescein isothiocyanate-dextran (FITC-d) was applied via micropump infusion. Sequential apical PL sampling was performed after FITC-d application and concentration levels were determined with fluorometric measurements. Additionally, intraoperative acoustic compound action potentials and cochlear microphonics were measured to determine cochlear function before and after compound administration. Extracted inner ears were scanned via micro-CT to determine exact cochlear dimensions.
Results: The established endaural surgical approach enabled replicable RWM delivery and sequential PL collection. Analysis of PL obtained two hours after RWM application revealed high FITC-d concentrations in samples corresponding to the apex. Interestingly, a stapes-venting group showed a more uniform distribution with a ten times lower maximum FITC-d concentration when compared to a non-venting group. Longer observation times demonstrated a more homogenous distribution of the FITC-d throughout the cochleae with concentration levels comparable to the short-term observed stapes-venting group. Additionally, mid-modiolar sections in micro-CT scans revealed a relatively prominent basal cochlear turn with a rapid decline of the scala tympani volume from the second cochlear turn onward. Hearing measurements after cochlear catheter insertion revealed increased thresholds compared to baseline measurements.
Conclusions: Inner ear delivery of FITC-d or other agents with a novel CE-marked cochlear catheter is feasible in piglets. The obtained pharmacokinetic insights represent important findings regarding the compound distribution within human-like cochleae as the porcine inner ear dimensions largely match the human anatomy. Due to the widespread availability, fewer ethical concerns, and their easy handling, these animals should be assessed in a variety of translational hearing research settings to confirm their potential as an alternative to non-human primates.

05. Ear physiology

P3.05.01

171 - The Exocytotic Characteristics of Mature Mammalian Vestibular Hair Cells

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Introduction: Balance and gaze rely on the faithful and rapid signalling of head movements by vestibular hair cells (VHCs) to primary sensory neurons. There are two types of VHCs in mammals, type-I and type-II. While type-I VHCs are contacted by a giant afferent nerve terminal, called a calyx, that encloses their basolateral membrane almost completely, type-II cells are innervated by multiple bouton afferent terminals. In both VHC types, glutamate exocytosis is triggered by Ca2+ influx through voltage-gated CaV1.3 Ca2+ channels. While signal transmission in type-I and type-II VHCs involves the Ca2+-dependent quantal exocytosis of glutamate at specialised ribbon synapses, type-I cells are also believed to exhibit a non-quantal mechanism that increases the reliability and the speed of signal transmission. However, the reliance of mature type-I hair cells on non-quantal transmission remains unknown.
Methods: In this study we investigated synaptic vesicle exocytosis in mature mammalian utricular hair cells using whole cell patch-clamp recording of Ca2+ currents and changes in membrane capacitance (?Cm). Signal transfer from type-I cells to the calyceal afferent terminal was measured by cell attached recording of action potentials in the calyx in response to VHC depolarisation with an endolymphatic low Ca2+ solution.
Results: We found that mature type-II hair cells responded to depolarisation with Ca2+-dependent exocytosis that showed a high-order dependence on Ca2+. By contrast, the Ca2+ current in type-I cells was approximately four times smaller and exocytosis was around ten times smaller than that observed in type-II cells. While type-II VHCs showed kinetically distinct pools of synaptic vesicles in response to increased stimulus duration, the responses of type-I cells remained comparatively small with a single pool of vesicles.
In VHCs of CaV1.3 (CaV1.3-/-) knockout mice both the Ca2+ current and exocytosis were largely absent in both type-I and type-II cells. In otoferlin (Otof-/-) knockout mice the Ca2+ currents were similar to control cells but synaptic vesicle exocytosis was largely absent.

Even though Ca2+-dependent exocytosis was small in control type-I hair cells, or absent in CaV1.3-/- and Otof-/- mice, these cells were able to drive action potential activity in the postsynaptic calyces.
Discussion: These findings show that mature type-I VHCs have a much smaller Ca2+-dependent exocytosis than type-II cells. The large vesicle pools in type-II cells would facilitate sustained transmission of tonic or low-frequency signals, whereas the restricted vesicle pool size in type-I cells, together with their rapid non-quantal mechanism, could specialise these large calyceal synapses for high-frequency phasic signal transmission.

06. Genetics of hearing loss and Gene therapy

P3.06.01

136 - Molecular bases of hearing loss in Campania region (Italy)

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Introduction: Sensorineural hearing loss (SNHL) is one of the most common sensory deficits in both childhood (0.5-1.5/1000) and adulthood, affecting approximately 466 million people worldwide and more than half of the population over 60. of age, with an enormous burden on the National Health Services both from an organizational and cost point of view.
It is estimated that 60% of sensorineural hearing loss can be traced back to purely genetic causes, due to mutations of about 150 known genes, demonstrating the extreme heterogeneity of this sensory disorder.
Among these genes, several connexins have been associated with various diseases including sensorineural hearing loss. where some mutations in connexin (Cx) 26 contribute to over 50% of the incidence of non-syndromic deafness in different human populations.
Methods: Generally, for each affected subject, our molecular analysis begins with the sequencing of the two main genes involved, namely GJB2/GJB6. If negative, based on the patient's medical history, the molecular analysis is extended to a panel of different disease genes and in some cases a CGH array analysis and an exome analysis. For all new variations identified, if possible, functional and/or bioinformatic analyses are carried out to demonstrate their involvement in the pathology.
Results: The study undertaken for years by our operating unit in the Campania region (Regional Reference Center for Neonatal Hearing Loss Screening) has allowed us to carry out an accurate neonatal screening on patients with sensorineural hearing loss. Our database to date consists of several "new mutations" on genes known in the literature for their implication in the etiology of the disease. For the most interesting mutations, an in-depth molecular study has been launched.
Conclusion: Our study contributes to providing useful information for understanding the etiopathogenetic mechanisms underlying the development of the main forms of sensorineural hearing loss.

Keywords: RRC, Campania region, Sensorineural hearing loss, CGH array, NGS analysis, Exome analysis.

P3.06.02

140 - Overcoming the limits of Whole Exome Sequencing: the application of Whole Genome Sequencing for the molecular diagnosis of Hearing Loss

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Introduction: Hereditary hearing Loss (HHL) is the most prevalent sensory disorder and is characterized both by a high genetic and clinical heterogeneity with more than 400 syndromes and ~124 genes reported as causative of non-syndromic forms. In the last few years, the diagnosis of HHL improved with the introduction

of Whole Exome Sequencing (WES), highlighting the complexity underlying these phenotypes. However, due to technological limitations, WES is not always successful in the detection of disease-causing variants. In this context, Whole Genome Sequencing (WGS) proved to be a promising tool, complementing WES with information on structural rearrangements, intron and regulatory regions, therefore improving the diagnosis of complex cases.
Aim: This study aims to the detection of structural variants (SV) in a selected cohort of ten HHL patients negative to WES and processed with WGS, using a bioinformatic approach.
Methods: FastQ files, obtained from WGS of HHL patients, underwent a first quality control using FastQC (v0.11.9) software, in order to trim adaptor sequences and check raw sequences integrity. Alignment to the Human Reference Genome build 38 (GRCh38p13) was then carried out using bwa (v2.1) software. Total coverage of aligned files was calculated using mosdepth (v0.3.3) and SV calling was performed using three different software: Manta (v1.6.0), Delly (v0.9.1) and Smoove (v0.2.8). Resulting VCFs files from each software were then merged using Survivor (v1.0.7) software, including only variants >30 bp called at least by two software. Finally, merged VCFs files were annotated using svpack software.
Results: Sequenced patients were classified as follows: 1) six patients carrying a single mutation in autosomal recessive HL genes; 2) four patients selected for a clear history of familial HHL and similar audiological phenotypes. Our approach led to the detection of several structural rearrangements within a set of HHL genes. Concerning 1), a ~46 kb inversion in the *TSPEAR* gene has been detected in Patient 3. As regards 2), a deletion of ~5 kb in *TJP2* and an inversion of ~7 kb in the *PTPRQ* gene, were detected in Patient 1; moreover, a suspect inter-chromosomal translocation in *CLIC5* and a ~7 kb deletion in *CCDC50* were detected in Patient 2.
Conclusion: These data, for the first time, highlight the possible use of WGS in complementation to WES in the diagnosis of complex cases of HHL. Next steps will involve the investigation of promotors and regulatory regions in addition to the implementation of variant annotation using splicing *in silico* predictors. Moreover, an experimental validation will be carried out in order to prove the potential of this new diagnostic approach.

Keywords: Whole Genome Sequencing, Molecular Diagnosis, Hearing Loss

P3.06.03

130 - There's more behind Hereditary Hearing Loss: molecular and phenotypic expansion of *PPP1R12A*-related disorder

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Background: Heterozygous variants in *PPP1R12A* have recently been associated with Genitourinary and/or/brain malformation syndrome (MIM: #618820), a condition characterised by abnormal internal and external genitalia, structural renal abnormalities, brain malformations (i.e. abnormalities of the corpus callosum and cortical dysplasia), eye and skeletal anomalies. Patients also present with variable degrees of developmental delay and intellectual disability. So far, only 12 patients have been reported in literature and none presented Hearing Loss (HL). We hereby report the first Italian patient affected by *PPP1R12A*-related disorder, also presenting HL and we discuss the possibility that deafness may represent a phenotypic expansion of the clinical features associated with this newly discovered disease.
Case report: A twelve-year-old boy born from non-consanguineous healthy parents presented to our attention with sensorineural HL, right megaureter and ureterocele. He firstly underwent a detailed dysmorphology assessment which revealed the presence of relative macrocephaly, frontal bossing, broad eyebrows, and posteriorly rotated ears. Afterwards, a hearing evaluation was performed by pure tone audiometry, which showed a bilateral, symmetric, profound sensorineural HL. Moreover, brain and inner ear imaging bilaterally detected a common cavity, i.e. a cochlear malformation characterised by the presence of a single round chamber representing both the cochlea and the vestibule. The lateral and posterior semicircular canals (SCCs) also appeared to be involved in the malformation, while the superior SCC seemed structurally normal. In order to complete the boy's clinical evaluation, an ophthalmological examination was performed, revealing severe unilateral myopia of the right eye; an abdominal ultrasound confirmed the presence of pelvicaliectasis whereas an echocardiography resulted normal. The deep clinical evaluation led to a clinical diagnosis of syndromic HL.

The patient underwent first-tier genetic testing, which included *GJB2* analysis, *STRC* copy number variants evaluation and *SLC26A4* sequencing and resulted all normal. A trio-based WES analysis was performed and led to the identification of a novel heterozygous splice-region variant (c.792+3A>C) predicted as deleterious within *PPP1R12A* (*NM_002480.3*).
Conclusion: Among the clinical features typical of *PPP1R12A*-related disorder, our patient presented urogenital abnormalities and eye involvement but did not present brain malformations or intellectual disability. Moreover, he presented bilateral sensorineural HL, which has never been reported in other patients. This might be due to the limited number of *PPP1R12A*-mutated individuals described so far and the report of additional cases is instrumental in achieving a full delineation of *PPP1R12A*-related phenotypic spectrum. Overall, our findings support the hypothesis that *PPP1R12A*-related disorder is a clinically heterogeneous condition and bilateral sensorineural HL could represent an expansion of the clinical phenotype associated with this disease.

Keywords: *PPP1R12A*, Syndromic Hearing Loss, Phenotypic expansion

P3.06.04

125 - Pendred syndrome and related phenotypes: the definition of a molecular diagnosis

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Introduction: Patients clinically diagnosed with Pendred syndrome (PDS) always display sensorineural hearing loss (SNHL) and enlarged vestibular aqueduct (EVA), but other inner ear malformations, such as IP-II, goitre, and eventually hypothyroidism can also be detected. PDS is inherited with autosomal recessive patterns, and the major gene involved is *SLC26A4*. However, other genes, such as *FOXI1* or *KCNJI0,* and the "CEVA haplotype" may also play a role. Nevertheless, PDS is characterized by high clinical and genetic heterogeneity. The majority of patients displaying PDS related symptoms do not receive a molecular diagnosis suggesting that other genetic contributions to PDS still need to be discovered. Thus, we aim to 1) define a molecular diagnosis for subjects displaying PDS-related phenotypes, 2) define new genotype-phenotype correlations in the carriers of *SLC26A4* mutations 3) suggest novel candidate genes.
Methods: We set up a multi-step approach that includes several clinical examinations (i.e. audiological and radiological evaluations and thyroid function assessments) and genetic tests, such as:
1)Whole-Exome Sequencing (WES), focusing on known PDS and HL causative genes.
2)Multiplex ligation-dependent probe amplification analyses (MLPA), to evaluate insertion/deletion within *SLC26A4*
3) Sanger sequencing of the twelve variants of the CEVA haplotype.
Finally, Whole-genome sequencing (WGS) was performed for selected negative patients.
Results: The careful clinical examination led to the selection of 50 patients (mean age 15 years old), all displaying bilateral SNHL and EVA. Interestingly, IP-II was detected in 35/50, while thyroid dysfunctions were identified in three patients older than 14 years old. The first round of genetic testing has been completed for 24 patients, leading to the definition of a molecular diagnosis for 5 of them by identifying pathogenic variants within *SLC26A4*. As regards the other subjects, five resulted heterozygous for a *SLC26A4* variant. Interestingly, we defined a consistent genotype-phenotype correlation for those ten patients, such as a characteristic audiometric profile. Further, WES analysis highlighted a novel HL candidate gene, *MYO5C*, which we suggest should be further evaluated in other PDS patients. Finally, the carriers of heterozygous variants within *SLC26A4* underwent WGS, searching for a possible second allele, and the data are under investigation.
Conclusions: In conclusion, we enrolled a large cohort of PDS patients with clearly defined phenotypical characteristics and set up a multi-step strategy (WES, MLPA, CEVA analysis, WGS) to genetically characterize them. This successful approach has already led to the discovery of consistent genotype-phenotype correlations for carriers of *SLC26A4* variants and the identification of a novel candidate gene.

Keywords: Pendred syndrome, Whole-exome sequencing, genotype-phenotype correlation, Whole-genome sequencing

P3.06.06

34 - AAV2/7 is a Promising Vector to Transduce Spiral Ligament Fibrocytes in a Save and Efficient Way

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Background: Inner ear gene therapy is a promising approach to restore sensorineural hearing loss, for which several gene therapy applications have been studied and reported in preclinical animal studies. Although gene delivery by using adeno-associated viral vectors (AAV) is considered as the best option, most animal studies to date have only injected viral vectors into neonatal ears to effectively transduce inner and outer hair cells. In this study, our aim is to inject AAV2/7 through the posterior semicircular canal (PSC) in adult mice in order to assess safety, immunogenicity and transduction efficiency in the fibrocytes of the spiral ligament.
Methods: Six-months old C57BL/6NTac-Cdh23<ahl+em3H>/H mice received an injection with a CMV-eGFP-T2A-FfLuc AAV2/7 vector (4.50E+12 vg/ml) through the PSC approach in the left ear. Hearing assessment involved distortion product otoacoustic emission (DPOAE) and auditory brainstem response (ABR) measurements at baseline and four days after injection. In addition, in vivo bioluminescence imaging (BLI) was performed to follow up transduction efficiency up to 25 days post-injection. After the experiment mice were euthanized to perform immunohistochemistry to visualize eGFP expression in the spiral ligament and assess inner ear inflammation.
Results: DPOAE and ABR measurements revealed that injection of AAV2/7 in the inner ear of adult mice has no negative influence on cochlear functioning as hearing function was completely preserved in all injected animals after four days. Furthermore, in all mice, BLI signal was observed in the region of the left ear indicating efficient transduction of inner ear cells.
Conclusions: As these preliminary results are highly promising, we will inject more mice with AAV2/7 and assess hearing function, inner ear inflammation and transduction efficiency at different time points, up to three months.

Keywords: inner ear inflammation, transduction efficiency, AAV, PSC injection, gene therapy

07. Imaging and Anatomy

P3.07.01

108 - Transmission electron microscpy cochlear images in deficiency of mitochondrial tRNA modification mice

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Introduction: Mitochondrial dysfunction is considered to be associated with aging and age-related hearing loss. However, the detailed mechanism and pathophysiology of hearing loss remain unknown.
Transfer RNAs (tRNAs) contain a wide variety of posttranscriptional modifications that are important for accurate decoding. Mammalian mitochondrial tRNAs (mt-tRNAs) are modified by nuclear-encoded tRNA-modifying enzymes (Wei, 2013). Cdk5 regulatory subunit-associated protein 1 (cdk5rap1) is responsible for 2-methylthio (ms2) modifications of mt-tRNAs. Deficiency in ms2 modification markedly impaired mitochondrial protein synthesis. This resulted in respiratory defects in cdk5rap1 knockout (KO) mice.
We reported the influence of a mitochondrial dysfunction caused by the ms2 modifications of mt-tRNAs on age-related change in vivo and in vitro. Herein, we investigated the morphology of cochlea to determine the influence on age-related hearing loss.
Materials and Methods: Each stage cochlea of cdk5rap1KO and hetero mice was dissected, and demonstrated transmission electron microscopy (TEM) .
Results: TEM showed that mitochondria in each parts in cochlea ballooned and mitochondrial cristae was diminished from postnatal 3 months in KO mice. It was earlier than those in hetero mice.

Conclusions: Our previous study and these results suggest that ms2 modifications of mt-tRNAs may induce mitochondrial damage and oxidative stress in the spiral ligament and accelerated their aging, thereby causing aging-hearing loss. In this study, mitochodria in hair cells and spiral ganglion as well as spiral ligament were the same finidings.

Key Word: cdk5rap1, age-related hearing loss, cochlea

P3.07.02

65 - Positron emission tomography-based in-vivo imaging of tissue responses in the cochlear implanted guinea pig

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Introduction: The cochlea implant (CI) performance varies widely between patients. Reasons for this can be inflammation due to the insertion trauma and a foreign body reaction against the electrode array surface, leading to encapsulation. The poor accessibility of the cochlea makes it a black box. Up to now, it is not possible to ascertain tissue reactions due to the implant and thus to determine a need for treatments. This deficit could potentially be overcome by the non-invasive positron emission tomography (PET)/computed tomography (CT) imaging using specific radiotracers.
Methods: Normal hearing guinea pigs were implanted unilaterally with a CI after initially setting an electrode insertion trauma (EIT). Subsequently, PET/CT imaging with F-18-FDG (F-18 fluorodeoxyglucose) was performed under general anesthesia at different time points after CI-surgery: for acute reactions at days 7, 14, and 21 (CI acute) and for chronic reactions after 1 year (CI chronic). As reference, non-implanted animals (nCI) were scanned as well. F-18-FDG was chosen as tracer indicating an increased metabolic activity of cells, e.g. in the case of inflammation. Volumes-of-interest (VOIs) were defined in the CT data around the cochlea on either side to evaluate the respective mean standardized uptake value (SUVs) from the corresponding co-registered PET image.
Results: The VOIs sizes (voxels) of the analyzed cochleae did virtually not differ between implanted (2289 ± 167) and non-implanted (2301 ± 126) animals. In contrast, the SUVs were significantly increased in implanted cochleae (0.953 ± 0.196) compared to non-implanted cochleae (nCI: 0.636 ± 0.066 [Mann–Whitney U test, $p < 0.001$]). A comparison of the F-18-FDG SUVs at the different time points after CI-surgery showed significant differences between implanted and non-implanted cochleae (CI acute: 0.897 ± 0.027, CI vs. nCI: $p < 0.05$; CI chronic: 1.008 ± 0.293, CI vs. nCI: $p < 0.01$ [ANOVA]). However, the values between implanted ears in acute and chronic phase did not differ significantly (CI acute vs. CI chronic).
Conclusions: Our results demonstrate the feasibility to use F-18 FDG-PET/CT imaging to detect acute and chronic inflammatory tissue responses in the guinea pig cochlea by increased radiotracer uptake after CI-insertion with EIT. This non-invasive in-vivo-imaging technique allows the longitudinal observation of ongoing processes in the implanted cochlea and thus possibly gives us key information in this current diagnostic dilemma. Further preclinical studies with more specific radiotracers (e.g. targeting specifically fibrosis or activated inflammatory cells) and the translation to patients may improve our understanding of the implanted inner ear and thereby paving the way for interventional treatments of CI-patients.

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09. Nanotechnologies

P3.09.01

27 - Efficiency of a dexamethasone nanosuspension as an intratympanic injection for acute hearing loss

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Objectives: In this study, we investigated whether dexamethasone, a hydrophobic drug, can be made into a stable nanosuspension solution, and whether this dexamethasone nanosuspension solution has a higher drug delivery efficiency to cochlea than the hydrophilic dexamethasone sodium phosphate (Dex-SP).
Material and methods: Nanocrystals of dexamethasone were produced by the NUFSTM (Nanoparticulation using fat and solid lipid) method, a patented method of Biosynectics, inc. Excipients were added to keep nanocrystals suspended in water. Three kinds of nanosuspensions (NUFS A, NUFS B, and NUFS C) were made by varying the composition of this excipient. We examined the safety and efficacy of nanosuspensions in in vitro and in vivo experiments.
Results: The size of dexamethasone nanocrystals in three kinds of nanosuspensions were approximately between 250 and 350 nm. When observed at room temperature for up to 8 hours, all three solutions apparently maintained a suspension state, but the particle size of NUFS C increased to about 1000 nm over time. In the in vitro toxicity assessment, cytotoxicity was not observed when three solutions were treated in HEI-OC1 cell line up to 100 ug/ml. The concentrations of dexamethasone in the perilymph after middle ear drug injection, were examined up to 24 hours, and three groups of nanosuspensions showed significantly higher drug concentration than that of Dex-SP in the result at 6 hours. In addition, interestingly, the concentration of dexamethasone in the tissue of cochlea of NUFS group, was 26-fold higher than that of Dex-SP at 6 hours. In the evaluation of drug efficacy, NUFS B showed better phosphorylation of glucocorticoid receptors than Dex-SP in both in vitro and in vivo, and, in the ototoxic animal model, it showed significantly better hearing protection effect than Dex-SP against ototoxic drugs. In safety evaluation, it showed no toxicity at concentrations up to 20 mg/mL in an in vivo test.
Conclusion: A nanosuspension of dexamethasone was able to deliver dexamethasone to the cochlea very safely and efficiently and showed potential as a formula for intratympanic injection. In addition, it can be applied in studies on the delivery of various hydrophobic antioxidants to treat acute hearing loss.

10. Noise induced hearing loss

P3.10.01

122 - The Time Course of Monocytes Infiltration After Acoustic Overstimulation

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Background: Cochlea macrophages regulate cochlea inflammation and may harbors the potentials to protect hearing function from injury, including acoustic overstimulation. Cochlea macrophage numbers increase at 3–7 days after acoustic stimulation. However, the exact timing of macrophage infiltration and maturation from inflammatory monocytes is unclear. Furthermore, neutrophils may also be involved in this process. Therefore, in this study, we investigated time-dependent immune cell infiltration, macrophage transformation, and neutrophil involvement following acoustic stimulation.
Methods: This study, time-dependent immune cell infiltration, macrophage modification, and neutrophil invasion after acoustic stimulation. Flow cytometry and immunofluorescence were performed on C-X3-C motif chemokines. RNA sequencing and quantitative polymerase chain reaction were performed to identify differentially expressed genes. Imaging data were acquired via in vivo imaging of the collecting vein. The cochlea of anesthetized CX3CR1GFP/+ mice was surgically exposed and the stapes artery was ligated by injecting Texas-red conjugated dextran (500 μg/animal) into the retro-orbital sinus. The stapedial artery was ligated, and the bony capsule of the basal turn was carefully drilled until the collecting venule could be visualized by optical microscopy. Then, a two-photon microscope was used to acquire imaging data.

Results: In conclusion, our findings showed that inflammatory monocytes in the bloodstream penetrate into the lower part of the spiral ligament within 1-2 days after acoustic hyperstimulation.
Neutrophils are not the main phagocyte type in the cochlea in this process. Infiltrating monocytes were transformed into macrophages by upregulating CX3CR1 and downregulating Ly6C within 5 days of acoustic hyperstimulation.
Conclusions: In consideration of these results, treatment to control the immune response after noise-induced hearing loss should be planned quickly and carefully according to the target process to be controlled.

Keywords:
acoustic overstimulation, noise-induced hearing loss, cochlea, macrophage, monocyte, neutrophil

11. Otoprotection

P3.11.01

45 - The effect of TrkB-selective agonist monoclonal antibody M3 on structure and function of the auditory nerve in deafened guinea pigs

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Introduction: The auditory nerve degenerates following severe damage to the organ of Corti including loss of hair cells. For optimal hearing performance with a cochlear implant (CI), a healthy auditory nerve is essential. In numerous animal studies it has been established that TrkB and TrkC agonists, such as brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3), can reduce degeneration of spiral ganglion cells (SGCs) after severe loss of cochlear hair cells. In the present study, we investigated the efficacy of the TrkB-selective monoclonal antibody agonist M3 in a guinea pig model of acquired deafness, following the reported beneficial effects of this molecule in rat models of cochlear synaptopathy ex vivo (Szobota et al., 2019, PLoS One 14, e0224022).
Methods: Thirty-one guinea pigs were ototoxically deafened. Two weeks thereafter a gelatin sponge, soaked in one of three solutions, was placed on the perforated round window membrane (RWM; Vink et al., 2020, Brain Sci. 10, 787). These solutions were: 1) 7 mg/ml M3 in buffer (n=10), 2) 0.7 mg/ml M3 in buffer (n=11) or 3) buffer alone (negative control, n=10). The experimenters were blinded with regard to the treatment. Four weeks after treatment, the animals received a CI (MED-EL, Innsbruck, Austria) and electrically evoked compound action potential (eCAP) recordings were performed to assess nerve responsiveness. Specifically, we analyzed the eCAP inter-phase gap (IPG) effect indicative of neural health (Ramekers et al., 2015, J. Neurosci. 35: 12331–12345). Following animal termination, the cochleae were harvested and survival of the SGC somata and their peripheral processes (PPs) was quantified.
Results: The guinea pigs exposed to the 0.7 mg/ml M3 dose showed significantly more SGC survival in the basal turn of the cochlea of the treated ear than in that of the untreated contralateral ear. For the 7 mg/ml dose, a similar but smaller trend was observed. No differences were observed between ears in the control group. Enhancement of PP survival in the M3 groups was present but smaller than observed for the SGC somata. Regarding function, no differences in IPG effect were observed among the three groups.
Conclusions: Administration of M3 via the RWM in this guinea pig model of acquired deafness provided a protective effect on SGCs. While M3 enhanced SGC survival, this was not translated into functional preservation through eCAP assessment. In a previous study (Vink et al., 2020), we demonstrated that treatment with the naturally occurring TrkB agonist BDNF resulted in a clear IPG effect in addition to SGC survival only in the basal turn. Thus, in these experiments, M3 had less effect than BDNF in promoting functional survival.

Keywords: Auditory nerve, neurotrophic treatment, cochlear implant, eCAP

12. Ototoxicity

P3.12.01

118 - mTORC2 inhibitor JR-AB2-011 on drug-induced ototoxicity in HEI-OC1 cells

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Introduction: Sensorineural hearing loss (SNHL) results from damage to the cochlea, auditory nerve, or central auditory nervous system. Hearing loss is often progressive and irreversible, as adult mammalian cochlear cells are not thought to regenerate and not replaced after damage. The development of an effective treatment for SNHL relies on understanding the molecular mechanisms of inner ear cell function and damage. The mechanistic target of rapamycin (mTOR) pathway appears to regulate hair cell regeneration in the adult cochlea. The mTOR kinase is present in two distinct multiprotein complexes, mTORC1 and mTORC2, defined by unique subunits, RAPTOR and RICTOR, respectively. While mTORC1 regulates cell growth and autophagy, mTORC2 primarily regulates cell proliferation and survival. Recently, the mTORC1 pathway has been associated with age-related hearing loss. Our group has also discovered that the mTORC1 signaling pathway is involved in regulating sensory hair cell survival after aminoglycoside exposure. To better understand the role of mTORC2 in sensory hair cell loss, the investigation of targeted blockade of mTORC2 may provide insights into this molecular mechanism. A new small molecule JR-AB2-011 has been recently reported, which seems to specifically inhibit mTORC2 in glioblastoma cell lines by blocking the interaction of RICTOR and mTOR. The aim of this study was to test the effects of JR-AB2-011 on HEI-OC1 cell survival when co-treated with the ototoxic drugs gentamicin and cisplatin.
Methods: We used a HEI-OC1 cells that were exposed to JR-AB2-011, gentamicin or cisplatin alone and JR-AB2-011 in combination with gentamicin or cisplatin for 24h. We analysed the cells by measuring cell viability and cytotoxicity. Protein expression was assessed by Western blot.
Results: After testing different concentrations of gentamicin and cisplatin, we found that 10 mM gentamicin and 40µM cisplatin showed around 50% cell viability rate. These concentrations were used for further experiments. Cells exposed to different concentrations of JR-AB2-011 (0.1, 1 and 10µM) were not affected, except at the higher concentration of JR-AB2-011 the ATP content was lower than control. When the cells were co-exposed with JR-AB2-011 and gentamicin or cisplatin, there were no significant differences in cell viability and cytotoxicity, when compared to gentamicin or cisplatin alone at all concentrations tested. The phosphorylation of AKT at Ser473, the most used readout of mTORC2 activity, remained almost unchanged in the JR-AB2-011 exposed samples relative to the control samples.
Conclusions: Based on our data, we can conclude that mTORC2 inhibitor JR-AB2-011 had no apparent effect on cell viability of the HEI-OC1 cell line or on activation of AKT at the concentrations used and at the time points tested. We did not observe any effect in the auditory cell line either alone or in combination with the ototoxic drugs cisplatin or gentamicin.

P3.12.02

147 - Ototoxic effects of drugs used in Covid-19 therapies in comparison to well-known ototoxic agent Cisplatin in male CBA JRj mice

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Background: Many medications are under investigation as novel therapies to treat COVID-19. Among them, Hydroxychloroquine, Azithromycine, and Colchicine have been identified as potentially being ototoxic. The aim of this study was to determine if these drugs, administered similarly to clinic protocol have any effects on hearing. Cisplatin was used for comparative purposes.
Methods: Male CBA/JRj mice were randomly divided into six groups: one sham group, five groups treated with either Cisplatin (2.5 mg/kg), Cisplatin 3 mg/kg), Hydroxychloroquine (62 mg/kg), Azithromycin (51.5 mg/kg), Colchicine (0.1 mg/kg).
DPOAE at 4, 8, 16, 24 and 32 kHz and ABR at 4, 8, 16, 25 and 32 kHz were measured at baseline, T+10DAYS, and T+38DAYS.
Results: The animals of the Sham group did not exhibit any change of DPOAE amplitudes at all frequencies. At T+10DAYS, no significant change of DPOAE amplitudes was observed in all treated groups. In the Cisplatin 2.5 mg/kg treated group, a significant decrease of DPOAE amplitudes was observed at 8, 16, 24, and 32 kHz at T+38DAYS compared to the Sham group and at 16, 24, and 32 kHz compared to their baseline values. In

the Hydroxychloroquine treated group, no significant decrease of DPOAE amplitudes was observed compared to the Sham group at any time point. In the Azithromycin and Colchicine treated groups, no significant decrease of DPOAE amplitudes was observed compared to the Sham group, except at 32 kHz at T+38DAYS.
The animals of the Sham group did not exhibit any change of ABR thresholds. At T+10DAYS, no significant change of ABR thresholds was observed in all treated groups. In the Cisplatin 2.5 mg/kg treated group, a significant increase of ABR thresholds was observed at all frequencies compared to the Sham group and baseline at T+38DAYS only. In the Hydroxychloroquine treated group, no significant difference of ABR thresholds was observed compared to the Sham group and compared to baseline. In the Azithromycin treated group, a significant increase of ABR thresholds was observed at 4 and 25 kHz compared to the Sham group and compared to baseline at T+38DAYS only. In the Colchicine treated group, a significant increase of ABR thresholds was observed at 32 and 45 kHz compared to the Sham group at T+38DAYS
Conclusion: =Hydroxychloroquine did not demonstrate ototoxic effects in these experimental conditions in mice. After 10 days of administration, Colchicine and Azithromycin induced hearing impairments 4 weeks after the end of treatment (T+38DAYS), demonstrating ototoxic effects occurring late after the treatment regimen. ABR threshold shifts were observed up to 20 dB only for high (32 and 45 kHz) in the Colchicine group and up to 30 dB at all frequencies, except for 4 and 25 kHz, in the Azithromycin group, both corresponding to mild hearing loss.
Cisplatin did not induce any measurable hearing loss after the first cycle of administration (T+10DAYS), while increases of ABR thresholds, up to 60 dB at all frequencies, and decreases of DPOAE amplitudes, up to 30 dB only at high frequencies, were observed after the third cycle (T+38DAYS), demonstrating significant hearing loss induced by cyclic Cisplatin treatment.

13. Pharmacology of the inner ear

P3.13.01

134 - The Clinical Effect of Steroid Therapy on Preserving Residual Hearing after Cochlear Implantation with the Advanced Bionics HiRes Ultra 3D Cochlear Implant System

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Background: The main aim of this study was to assess the clinical effectiveness of two different schemes of administration of steroids ((1) dexamethasone administered intravenously in comparison with (2) combination of steroid treatments: orally administered prednisone and intravenously administered dexamethasone) in comparison with a control group (no steroid administration) on hearing preservation (HP) in patients who underwent an Advanced Bionics cochlear implantation.
Methods: Thirty-five adult patients met the inclusion criteria. All patients were randomly divided into three subgroups depending on the scheme of steroid administration: (1) the first subgroup with only intravenously administered dexamethasone (0.1 mg per kg body weight twice a day for three days), (2) the second subgroup with a combination of methods of administration of steroids (intravenous and oral steroid therapy (dexamethasone, 0.1 mg/kg body weight twice a day plus prednisone, 1 mg/kg weight once a day for three days before surgery and after administration of dexamethasone (4th, 5th, 6th day) and after this time the dose of prednisone was reduced)) and (3) the third subgroup without steroid therapy (control group). The results were measured by pure tone audiometry (PTA) in three periods: (1) before implantation, (2) during activation of the processor (one month after implantation), and (3) 12 months after activation. Patients' hearing thresholds before implantation were on average 82 dB HL, 77 dB HL, and 88 dB HL, respectively.
Results: The majority of the patients from the first subgroup had hearing preserved partially (77.8%). A similar result was observed in the second study group (oral + i.v.) (partial hearing preservation was found in 61.5% of the participants). The opposite was true in the control group; a plurality of control patients (38.5%) had no measurable hearing 12 months after the activation of the processor.
Conclusions: Pharmacological treatment consisting of the administration of steroids in patients who had undergone cochlear implantation with the Advanced Bionics HiRes Ultra 3D cochlear implant system may be beneficial for preserving residual hearing in patients.

Keywords: cochlear implantation; steroid administration; partial deafness treatment; hearing implants; dexamethasone; prednisone

14. Physiopathology of Auditory Pathways

P3.14.01

53 - Involvement of hearing system in type 1 diabetes mellitus

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Diabetes mellitus is one of the most common metabolic disease. Hearing loss in the type 1 diabetic patients is described, but the characteristics (e.g. which frequencies are involved, inner ear or central structures are involved more) isn't cleared yet. Hypoxia, ischemia, reactive species formation and oxidative stress can lead to apoptotic and necrotic death of hair cells and spiral ganglion cells. It is well known that diabetes mellitus plays an important role in microvascular disorders and neuropathy.
42 T1DM (type 1 diabetes mellitus) patients and 25 healthy controls participated in our study. The cases were subdivided into two groups regarding their ages: 19-39 years of age and 40-60 years of age. Physical examination, impedance audiometry, hearing threshold measurement, otoacoustic emission measurement and acoustically evoked brainstem response registration were performed by the T1DM patients and healthy controls. We tried to identify the effect of diabetes on cochlear and retrocochlear structures.
In the 19-39 years old group there was no notable difference between the T1DM and control patients by incidence.(15,4% and 16,6% respectively). Among the 40-60 years old people in the type 1 diabetes group was the hearing impairment more common (75%) than in the control group (15,4 %)The tendency of mean thresholds of PTA (pure tone audiometry) was higher in diabetics than in controls, with results being statistically significant in the following frequencies: 19-39 years old group: 500-4000Hz right ear, 4000Hz left ear, 40-60 years old group: 4000-8000 Hz both ears. OAEs (otoacoustic emission) were in general lower in both younger and older patients with T1DM as compared to the respective controls. In the younger T1DM group only at 8000 Hertz on the left side was a significant ($p<0,05$) difference by otoacoustic emissions. The aged patients with type 1 diabetes had significantly less otoacoustic emissions at 8000 Hz on the right side ($p<0,01$) and at 4000-6000-8000 Hertz on the left side, ($p<0,05$, $p<0,01$, $p<0,05$ respectively) than healthy controls. According to ABR (auditory brainstem response) latencies and wave morphologies, a possible retrocochlear lesion arose in 15 % of the young and 25 % of the aged diabetic patients. We found that type 1 diabetes causes hearing loss, mostly on the higher frequencies.
According to our results alterations of the hearing system in diabetic patients can be detected. We found that cochlear and retrocochlear part of the hearing system is involved in the pathomechanism. Overall, it seems that regular hearing screening could be useful in type 1 diabetic patients to evaluate the early hearing impairment.

Keywords: type I diabetes mellitus, vasculopathy, neuropathy

P3.14.02

80 - Machine-Learning-Based Audio Algorithms for Cochlear Synaptopathy Compensation: Which Speech Features are Enhanced?

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Introduction: Since 2009 we have known that cochlear synaptopathy (CS) is a new hearing loss pathology in which the synapses to the auditory nerve (AN) are damaged, impacting the encoding of speech in everyday listening conditions and the comprehension of speech in noise. Since that time, we have learned more about this pathology, but it is not clear how we can compensate for CS using audio-based treatments such as hearing aids or augmented hearing algorithms. Auditory models have been used for decades to develop audio signal processing algorithms in hearing aids. Typically, the difference signal between a normal-hearing (NH) and hearing-impaired (HI) model is used to design such algorithms, but only recently machine-learning (ML) methods have made their entry in this field. In this work, we look at how auditory models can help in the design of such ML-based audio signal processing algorithms that specifically compensate for CS, and we investigate

which sound and speech features are modified when letting the ML-algorithms decide the most-optimal solution.
Methods: We used a biophysically-inspired auditory model, in a differentiable convolutional neural network (CNN) description (CoNNear), to train different ML-based algorithms that maximally restore CS-affected auditory-nerve (AN) responses, using the same CNN encoder-decoder architecture but constraining their training using different loss functions. The NH CoNNear model parameters were adjusted to obtain individualized HI models simulating different degrees of outer hair cell loss and/or CS-related AN fiber loss. Based on the reference NH model and a HI model, we used backpropagation to design different ML-based audio signal processing algorithms that optimally compensate for CS.
Results: After training, we processed pure tone stimuli and a battery of words in quiet and noise to evaluate the auditory feature restoration capabilities of the ML-algorithms using transfer functions and auditory model simulations. These results showed enhanced AN responses to both low- and high-frequency pure tones, and to vowels in quiet and noise, but responses were usually not restored to the NH-level. Consonant enhancement was only obtained when using a loss function with a low AN response threshold. The algorithms generally sharpened the onset response to speech and improved the stimulus dynamic range. In an unconstrained operation, the ML-algorithms added more energy to the higher frequencies, degrading speech quality and intelligibility.
Conclusion: In this work we learned that ML-based audio signal processing algorithms are able to compensate for simulated affected AN responses related to CS. The constraints in the loss functions of the trained algorithms cause differences in restored auditory features to compensate for CS. In future work, we will objectively assess the effect of these compensation algorithms on sound quality and speech intelligibility in clinical experiments.

Acknowledgements: Work supported by ERC-StG 678120.

Keywords: cochlear synaptopathy, hearing-aid processing, machine-learning

15. Regeneration and Stem cells

P3.15.01

112 - An inner ear organoid model of Meniere disease

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Background: Familial Meniere's disease (FMD) is an inner ear disorder characterized by sensorineural hearing loss, episodic vertigo and tinnitus and it is observed in 5–15% of MD cases. By whole-exome sequencing, we identified two heterozygous single-nucleotide variants in *FAM136A* and *DTNA* genes in a Spanish family with three affected cases in consecutive generations, highly suggestive of autosomal-dominant inheritance. We have generated an Induced Pluripotent Stem Cell line (iPSC) from a FMD patient with both mutations and differentiated them into inner ear organoids (IEO).
Methods: Peripheral blood mononuclear cells from this FMD patient were reprogrammed and characterized. Briefly, the cell line included genetic analysis of DTNA and FAM136A variants, Short Tandem Repeats profiling (STR), expression of pluripotency-associated factors and differentiation studies *in vitro*. To begin the differentiation to IEO, hPSC were aggregated and treated with extracellular matrix proteins to promote epithelialization. Then, by recapitulating signaling pathway activation and attenuation during inner ear development we modulated signaling pathways inducing sequential formation and subsequent self-guided morphogenesis to form sensory epithelia containing hair cells and supporting cells, as well as neurons forming synapses with the hair cells. IEO were characterized by their capacity of expressing PAX2, SOX2 (otic progenitors) by day 40 and then MYO7a, ATOH1 and TUBB3 (mature IEO) after day 50.
Results: First, we confirmed the presence of *DTNA* and *FAM136A* variants by Sanger sequencing. The cell line silenced the expression of exogenous transgenes and activated the expression of the endogenous pluripotent transcription factors (SOX2, REX1, NANOG and OCT4). Importantly, cells showed normal karyotype (46, XX) and the expression of the pluripotent markers SSEA4, Tra1-60 and Tra1-81 was confirmed by flow cytometry and Confocal imaging. Finally, to demonstrate its capacity to differentiate into the three germ layers we performed an embryoid bodies (EBs) formation assay. EBs derived from this cell line showed specific expression of representative markers of the three germ layers: ectoderm (β3-Tubulin), mesoderm (Vimentin)

and endoderm (Cytokeratin CKAE1-AE). IEO were found to express PAX2 and SOX2 between days 20-40 using Confocal imaging and IEO showed high levels of MYO7a (FC= 4.3); ATOH1 (FC=54.1) and TUBB3 (FC=13.4) expression when compared to the hPSC, demonstrating their capacity to differentiate into inner ear tissue. Likewise, DTNA and FAM136A had higher expression in the IEO (FC= 15.1 and 1.6, respectively). Western blot supported the above results.
Conclusion: Both DTNA and FAM136A are expressed in inner ear tissue-like organoids. Further experiments are needed to define which cell types involve their expression in human inner ear tissue.

Funding
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P3.15.02

116 - Proof of principle of extracellular vesicle application in a cochlea implantation trauma model with guinea pigs preventing fibrosis and residual hearing loss

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Introduction: There is still no class of inner ear drugs available for preventing inner ear damage or for treating hearing loss and associated conditions. The implantation of the cochlear implant (CI) with direct electrical stimulation of the auditory neurons (or other hearing aids for sound amplification) remains as the only intervention for restoring hearing. Unfortunately, inserting the electrode array induces trauma leading to immunological and foreign body reactions compromising the performance of the CI. Application of extracellular vesicles (EVs) derived from bone marrow and umbilical cord mesenchymal stem cells (MSC) are a promising tool for the modulation of pathways and targeting cochlear cells in order to prevent or treat immunological processes. In a previous study we have shown the efficacy of EVs in spiral ganglion cell (SGC) cultures and first in vivo tests in mice indicate that EVs attenuate threshold shifts and protect hair cells after noise trauma [1].
Methods: As proof of principle of an EV application associated with CI- insertion trauma, we implanted a group of guinea pigs (n= 8) with a CI right after administration of research grade EVs isolated from human umbilical cord MSC (UC-MSC- EVs). The control group (n=11) was implanted with a CI exclusively. Hearing was observed pre and post implantation and at the day of sacrifice after 4 weeks via ABR and CAP (auditory brainstem response and compound action potential). Weekly impedance measurement and confocal laser scanning microscopy (CLSM) on optical cleared whole cochleae were conducted monitoring fibrosis development.
Results: Our findings revealed no negative effect and the tendency of protection of residual hearing after UC-MSC-EVs administration 4 weeks post implantation in the EV group compared to the control group (10 dB at 32 kHz). The extension of fibrosis within the cochlea in the EV group was statistically less compared to the control group.
Conclusions: EV application associated with cochlear implantation seems to be a safe and solid combination to prevent post implantation trauma and preserve residual hearing. For precise immune modulation to achieve a targeted inner ear cell protection or even restauration further investigations are needed.

Keywords: implantation trauma, residual hearing, hearing loss, hearing preservation

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Reference: [1] A. Warnecke, J. Harre, H. Staecker, N. Prenzler, D. Strunk, S. Couillard-Despres, P. Romanelli, Julia Hollerweger, T. Lassacher, D. Auer, K. Pachler, G. Wietzorrek, U. Köhl, T. Lenarz, K. Schallmoser, S. Laner-Plamberger, C. S. Falk, E. Rohde, M. Gimona. 2020. “Extracellular Vesicles from Human Multipotent Stromal Cells Protect against Hearing Loss after Noise Trauma in Vivo.” Clinical and Translational Medicine 10(8)

16. Tinnitus

P3.16.01

168 - Neuroprotective Effect of Valproic Acid on Salicylate-Induced Tinnitus

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Objective: Valproic acid (VPA) is widely used for the management of bipolar disorder, epilepsy, and migraine headaches, and is known to regulate NMDA receptor activity.
Materials and Methods: To investigate the effects of VPA on the expression of ARC, which is a neuronal immediate early gene, TNFa, and NR2B gene and protein, as well as on the levels of p-CREB in the SH-SY5Y cell line and rat cortical neurons. For the in vivo study, we used gap-prepulse inhibition of the acoustic startle reflex (GPIAS) and measured the auditory brainstem level (electrophysiological recordings of auditory brainstem responses; ABR). Furthermore, we examined NR2B expression in the auditory cortex to evaluate whether VPA could reduce salicylate-induced behavioral disturbances as well as the expression of NR2B. Additionally, we evaluated regional 18F-2-fluoro-2-deoxy-D-glucose (18F-FDG) uptake in the auditory cortex using micro-positron emission tomography (PET) imaging.
Results: We observed increased expression of NR2B and its related genes TNFa and ARC, increased intracellular ROS levels, and induced expression of cleaved caspase-3. These salicylate-induced changes were attenuated in the neuronal cell line SH-SY5Y and rat cortical neurons after VPA pretreatment. Together, these results provide evidence of the beneficial effects of VPA in a salicylate-induced temporary hearing loss and tinnitus model.
Conclusion: In the present study, our results revealed that VPA attenuated salicylate-induced auditory dysfunction.

17. Vestibular disorders

P3.17.01

120 - Saccades Matter: Reduced need for caloric testing of cochlear implant candidates by joint analysis of v-HIT gain and corrective saccades

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Objectives: The video head impulse test (v-HIT) is a quick, non-invasive and relatively cheap test to evaluate vestibular function compared to the caloric test. The latter is, however, needed to decide on the optimal side to perform cochlear implantation to avoid the risk of inducing a bilateral vestibular areflexia. This study evaluates the effectiveness of using the v-HIT to select cochlear implant (CI)candidates who require subsequent caloric testing before implantation, in that way reducing costs and patient burden at the same time.
Study Design: Retrospective study using clinical data from 83 adult CI-candidates, between 2015 and 2020 at the Leiden University Medical Center.
Materials and Methods: We used the v-HIT mean gain, MinGain_LR, the gain asymmetry (GA) and a newly defined parameter, MGS (Minimal Gain & Saccades) as different models to detect the group of patients that would need the caloric test to decide on the ear of implantation. The continuous model MGS was defined as

the MinGain_LR, except for the cases with normal gain (both sides ≥0.8) where no corrective saccades were present. In the latter case, MGS was defined to be 1.0 (the ideal gain value).
Results: The receiver operating characteristics curve showed a very good diagnostic accuracy with an area under the curve (AUC) of 0.81 for the model MGS. The v-HIT mean gain, the minimal gain and GA had a lower diagnostic capacity with an AUC of 0.70, 0.72 and 0.73, respectively. Using MGS, caloric testing could be avoided in 38 cases (a reduction of 46%), with a test sensitivity of 0.9 (i.e., missing 3 of 28 cases).
Conclusions: The newly developed model MGS balances the sensitivity and specificity of the v-HIT better than the more commonly evaluated parameters such as mean gain, MinGain_LR and GA. Therefore, taking the presence of corrective saccades into account in the evaluation of the v-HIT gain can considerably reduce the proportion of CI-candidates requiring additional caloric testing.
Keywords: sensorineural hearing loss, cochlear implant, cochlear implantation, candidacy criteria, vestibular outcome, caloric test, v-HIT, vestibular areflexia.

References:
Jacobson GPS, N. T. Balance Function Assessment and Management. 2nd ed. San Diego, CA (2016). p. 905
Janky KL, Patterson J, Shepard N, Thomas M, Barin K, Creutz T, et al. Video Head Impulse Test (vHIT): the role of corrective saccades in identifying patients with vestibular loss. Otol Neurotol . (2018)
West N, Klokker M, Caye Thomasen P. Vestibular screening before cochlear implantation: clinical implications and challenges in 409 cochlear implant recipients. Otol Neurotol . (2020)

P3.17.02

30 - The infra-red utilization in therapy of Ménière disease.

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Introduction: The therapy of the Ménière Disease is one of the main problems of the Otoneurologist. Prevention of vertigo crisis, unpleasant and debilitating for the patient, is an outstanding issue in clinical practice, also considering the variability and unpredictability of the vertigo crisis. Up to now no significant news have been produced in therapy, pharmacological and also from surgical point of view except for vestibular neurectomy, that is a very invasive surgical act, not well accepted by the patient. Starting from the above mentioned problems, considering that the hypertension of endolynph may be related to an alterate equilibrium with hematic circulation (excess of filtration or not-correct re-absorption), we assumed that the pathogenesis of the hydrope may be related to a non-correct hematic vessel meccanism. And so we tried to improve the hematic circulation, by using infra-red rays applied on the vetrebral column. The main goal of this study is to verify the possibility of reducing number and intensity of vertigo crises, by using infra-red rays.
Methods: 46 patients suffering from Ménière Disease have been submitted to this study. The crises frequency ranged between 1 per 2-3 months and 2-3 per week. All patients showed typical symptomatological triad (vertigo, with severe neurovegetative sympthoms, hypoacusia and tinnitus). The treatment consisted in exposing the vertebral column to a IR rays source. Time exposure varied between 30 and 45 minutes. The treatment was performed once a week for a full period of 12-15 weeks.
Results: In every case in a first step all patients reported a significative decrease of vertigo intensity and successively, just a dizziness sensation remained, that was progressively disappearing with the treatment prosecution. Until the end of the treatment equilibrium was restored in over than 90% of the patients.
Conclusions: We can reasonably conclude that the IR-treatment is able to restore endolymph pressure just improving the hematic circulation and so reducing intensity and frequency of vertigo crises.

Keywords: Ménière Disease, Vertigo, Therapy, Infra-red rays.

19. Miscellaneous

P3.19.01

159 - A metabolomics-based approach to understanding the association between Sensorineural Hearing Loss and Mild Cognitive Impairment

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Sensorineural Hearing Loss (SNHL) is a major sensory disability in adulthood, and the prevalence of age-related hearing loss (ARHL) is rising with increasing life expectancy. Recent studies have found an important association between both peripheral and central ARHL and cognitive frailty, leading to assumption that such hearing impairment may be associated with an augmented risk of Mild Cognitive Impairment (MCI) and dementia. MCI is a disorder related to a decline in cognitive ability that exceeds normal age-related changes but is not severe enough to meet the diagnostic criteria for dementia. To what specific mechanisms the association between hearing loss and an adverse cognitive state is due is still not entirely clear: in this context, metabolomics may represent a new key to the study and understanding of this connection. Such omics science is indeed concerned with the qualitative and quantitative characterization of metabolites in a biological sample, which collectively constitute the metabolome. From a dynamic point-of-view, the metabolome is the final result of the interaction between the genome, the transcriptome, the proteome and environmental factors, and is therefore the closest to reflecting the phenotype of a biological organism. Therefore, metabolomics in this case could help us establish a distinct "metabolomic fingerprint" to better understand the onset and causes of this condition; moreover, it could improve knowledge of the pathogenesis of this disease, thus helping in the diagnosis or prognosis of SNHL. Here, we describe a multicentre observational study based on an untargeted metabolomics approach with the aim of identifying metabolic alterations associated with MCI and non-MCI in patients with SNHL. A total of 25 SNHL participants aged 40-65 years will be enrolled, as well as 20 healthy matched controls. Metabolomic analyses will be performed on blood and saliva samples with single quadrupole mass spectrometry techniques coupled with gas chromatography (GCMS) and with high-resolution mass spectrometry techniques (ICR-FTMS). The ensemble machine learning algorithms will be then essential to study the resulting data in order to identify metabolites relevant for class discrimination; hence, metabolites obtained from the comparisons will be used to study the metabolic pathways involved and thus to understand whether and how their alteration may be involved in the onset of the disease. Metabolic profiling and pathway analysis in people with SNHL may therefore be essential to find useful biomarkers for early diagnosis and possibly help future research to develop preventive treatments for this condition. The identification of metabolites involved in the development of MCI in subjects with hearing impairment in middle age could help early therapy ,avoiding the copresence of HL and MCI that could accelerate cognitive decline.

Keywords: sensorineural hearing loss, mild cognitive impairment, metabolomics

P3.19.02

50 - Penetrating the pars petrosa: investigating human migration using strontium isotope analysis in archeological inner ears

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Strontium isotope analysis can be applied to the calcined human otic capsule in the petrous bone to gain information on childhood mobility in archaeological and forensic contexts. However, only a thin layer of the otic capsule, the inner cortex, demonstrates virtually no remodelling and would be suitable for obtaining childhood

ratios, while the external cortex (the layer directly surrounding the inner cortex) and other parts (i.e. the apex) of the petrous bone have higher bone turnover rates and may exhibit different 87Sr/86Sr. Applying our improved sampling method, calcined petrous bone from ten cremation deposits are sampled for strontium isotope analysis, thereby sampling both the inner and external cortex and the apex of two petrous bones. For comparison, diaphysis and rib fragments from each cremation deposit were also sampled. Forty percent (4/10) of the calcined petrous bones show marked differences in 87Sr/86Sr (0.00035-0.00065) between the inner and external cortex. One of the apices yielded a 87Sr/86Sr that differed >0.0009 from any of the other samples from the same cremation deposit. While the differences in 87Sr/86Sr between the inner and external cortex could be explained by dissimilar bone turnover rates, the large difference in 87Sr/86Sr between the apex and the rest of the elements may be attributed to other factors, such as diagenesis. Our study highlights the problematic nature of the external cortex and the apex, suggesting that despite being calcined, diagenesis may influence 87Sr/86Sr ratios in general but especially in the apex and that more research is needs to be undertaken by sampling various locations of the petrous bone to further improve our understanding of bone turnover and diagenesis in calcined petrous bones.

P3.19.03

113 - Adaptation method of a speech test for children 3-7 years into a different language

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Early detection of hearing loss in children has an utmost importance due to consecutive impaired speech development. Our scope was to adapt a German speech audiometry test for children (MATCH) to Hungarian language. The test is a supra threshold, age- appropriate, using 26 nouns, which as previous studies have shown, are most likely to be part of the lexicon of children aged 2 years and older. The test is a picture-pointing task with a four- option non-forced choice method. The first issue was that not all pictures were easily recognized by Hungarian children. We performed an online study among children aged 3-7 to test the identification of each test picture. Another difficulty was that the simple translation of the vocabulary did not correspond to the language specific phoneme distribution. Some words had to be changed to achieve a better phoneme arrangement. After recording and setting up the vocabulary by a professional female voice we performed the standardization studies on normal hearing children in three age groups. Each group included 30 subjects. The validation studies were performed with 15 subjects having hearing loss. Each subject was tested previously with play audiometry, tympanometry, otoacoustic emissions and auditory steady-state response (ASSR) as well. We provide insight and point out some pitfalls in adapting a speech test to a different language.

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