UNIVERSITÀ DEGLI STUDI DI PADOVA

DNS DIPARTIMENTO DI NEUROSCIENZE

IEB 2019

# Symposium & 56th Inner Ear Biology Workshop

"Hearing research: from history into the future"

**7-10 SEPTEMBER 2019**
**PADUA, ITALY**

**Welcome to the Inner Ear Biology 2019**

Dear Friends and Colleagues,
I am delighted and honoured to welcome all of you to the 56th Workshop on Inner Ear Biology (IEB 2019) that will be held at the University of Padua from 7th to 10th September 2019.
The IEB Workshop will be preceded by a Symposium entitled "Hearing research: from history into the future", a historical excursus on hearing improvements.

The IEB Workshop annually gathers our community of biologists and otolaryngologists for a fruitful debate on translational medicine, that is on how basic and applied research may help solving clinical problems.
The University of Padua is known for the spirit of freedom in cultural expression which encouraged students and professors to establish the University in 1222 and is conveyed by its motto: "Universa Universis Patavina Libertas".

Notable scholars and professors of the University of Padua include Andrea Vesalio, Gabriele Falloppio, Girolamo Fabrici d'Acquapendente, Gian Battista Morgagni, Antonio Vallisneri and Galileo Galilei. The University of Padua takes pride in having established the first European botanical garden, the "Orto dei Semplici" (1545), the first permanent anatomical theatre in the world, the "Teatro Anatomico"(1595), and the first Italian University Library, the "Sala dei Giganti" (1629). Moreover, in 1678 the "Sacro Collegio dei filosofi e medici" (Holy University College of Philosophers and Physicians) awarded the first university degree in the world to a woman, the noble Venetian Elena Lucrezia Cornaro Piscopia.

On behalf of the Organizing Committee, I welcome you all to IEB 2019 workshop in Padua and hope that more and more scientists and physicians will join our scientific community in the coming years.

Alessandro Martini

**IEB 2019 - COMMITTEES**

### *Organizing Committee*

Alessandro Martini (Chairman)
Paolo Gasparini (Chairman)
Fabio Mammano (Chairman)
Laura Astolfi (Scientific Secretariat)
Roberto Bovo
Diego Cazzador
Erica Gentilin
Gino Marioni
Patrizia Trevisi
Elisabetta Zanoletti

### *Spoendlin Junior Award Committee*

Leila Abbas (Sheffield, UK)
Laura Astolfi (Padua, IT)
Roberto Bovo (Padua, IT)
Helena Caria (Lisbon, PT)
Cosimo De Filippis (Padua, IT)
Jose Antonio Lopez-Escamez (Granada, ES)
Anna Rita Fetoni (Rome, IT)
Giorgia Girotto (Trieste, IT)
Anthony W. Gummer (Tuebingen, DE)
Ito Juichi (Kyoto, JP)
Marlies Knipper (Tuebingen, DE)
Fabio Mammano (Rome, IT)
Rosamaria Santarelli (Padua, IT)
Verena Scheper (Hannover, DE)


# Symposium

**Saturday 7 September 2019**

## Fiera di Padova

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| 10.00<br>12.00 | Registration |

## Aula Magna, Bo Palace - University of Padova

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| 13.30 | Registration |

## SATELLITE SYMPOSIUM

### New Approaches for Improving Inner Ear Function

**Chairman: Alessandro Martini**

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| 14.30 | **The early days of dexamethasone-eluting electrode**<br>Alessandro Martini *(Padua, Italy)* |
| 14.45 | **Dexamethasone-eluting electrode /other drug delivery**<br>Verena Scheper *(Hannover, Germany)* |
| 15.00 | **Vestibular implants**<br>Angelica Peres Fornos *(Geneva, Switzerland)* |
| 15.15 | **Brief summary and closure**<br>Susanne Braun *(Starnberg, Germany)* |

15.30 – 15.45 Coffee break

## INNER EAR BIOLOGY SYMPOSIUM

### Hearing Research: From History into the Future

**Chairmen: Alessandro Martini, Paolo Gasparini, Fabio Mammano**

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| 15.45 | Welcome address | |
| 16.00 | **S1** | **Highlight in Human Cochlear Microanatomy**<br>Helge Rask-Andersen *(Uppsala, Sweden)* |
| 16.30 | **S2** | **Tailoring tissue remodeling during early inner ear development: together we can**<br>Isabela Varela-Nieto *(Madrid, Spain)* |
| 17.00 | **S3** | **Inner Ear Drug Delivery by Precise Perforation**<br>Anil K. Lalwani *(New York, USA)* |
| 17.30 | **S4** | **Central auditory prostheses: challenges and potentials**<br>Andrej Kral *(Hannover, Germany)* |
| 18.00 | **S5** | **Cochlear neuroregeneration: progressing towards the clinical use of human pluripotent stem cells**<br>Marcelo N. Rivolta *(Sheffield, UK)* |
| 18.30 | **S6** | **Padua, cradle of modern medicine**<br>Giorgio Zanchin *(Padua, IT)* |
| | | *ECM - for Italian participants only:*<br>*Compilazione del modulo della qualità percepita* |

## Opening Ceremony of the 56th Inner Ear Biology Workshop

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| 19.00 | **Welcome from the Rector**<br>*Magnificus Professor Rosario Rizzuto, University of Padova* |
| | **Elena Lucrezia Cornaro Piscopia Award Ceremony**<br>*Helen Cornaro was a Venetian philosopher of noble descent, who in 1678 became one of the first women to receive an academic degree from a University, and the first to receive a Doctor of Philosophy degree. The degree was conferred on 25 June 1678 in the Padua Cathedral, in the presence of the University authorities.*<br>*The Elena Lucrezia Cornaro Piscopia Awards honour outstanding women scientists from all over the world, whose studies are focused on Inner Ear Neurobiology and Developmental biology.* |

## Palazzo della Ragione, external arcade

**20.00 Welcome Reception**


# 56th Inner Ear Biology Workshop

## Fiera di Padova

**Sunday 8 September 2019**

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| 07.30 | Registration |

## SESSION I

**REGENERATION, STEM CELLS AND DEVELOPMENTAL BIOLOGY**

Moderators: *Leila Abbas, Anna Rita Fetoni*

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| 08.00 | **TL1** | **TARGET LECTURE**<br>**In vitro expansion of human fetal cochlear duct cells and differentiation into functional hair cells in 3D organoids**<br>*Marta Roccio [(1)], Silvia Erni [(1)], Carlotta Palaferri [(1)], Michael Perny [(1)], Megan Ealy [(3,4)], Hans Ruedi Widmer [(1)], Stefan Heller [(3)], Pascal Senn [(1,2)] – Bern, Switzerland [(1)]; Genève, Swizterland [(2)]; Stanford, CA – USA [(3)]; Springfield, MO – USA* |
| 08.25 – 10.10 | | **COMMUNICATIONS** |
| 08.25 | **C01** | **Comprehensive analyses of gene expressions in the developing primate cochlea**<br>*Makoto Hosoya, Masato Fujioka, Kaoru Ogawa - Tokyo, Japan* |
| 08.38 | **C02** | **Wnt Signalling Regulates the Formation of Inner Ear Sensory Organs by Antagonizing Prosensory signals**<br>*Magdalena Zak, Vincent Plagnol, Nicolas Daudet - London, UK* |
| 08.51 | **C03** | **Proteostasis is essential during cochlear development for neuron survival and hair cell polarity**<br>*Steve Freeman, Susana Mateo-Sanchez, Ronald Pouyo, Brigitte Malgrange, Laurence Delacroix - Liege, Belgium* |

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| 09.04 | **C04** | **Frizzled3 and Frizzled6 Cooperate with Vangl2 to Direct Cochlear Innervation by Type II Spiral Ganglion Neurons**<br>*Michael Deans - Salt Lake City, United States* |
| 09.17 | **C05** | **Differential expression of transmembrane channel-like protein 1 (TMC1) during fetal development of the human inner ear**<br>*E.S.A. van Beelen, W.H. van der Valk, J.C.M.J. de Groot, H. Locher, P.P.G. van Benthem - Leiden, Netherlands* |
| 09.30 | **C06** | **Single-cell transcriptomics of chick auditory epithelial cells during hair cell regeneration**<br>*Takayuki Nakagawa, Mami Matsunaga,Tomoko Kita, Ryosuke Yamamoto, Hiroe Ohnishi, Norio Yamamoto, Koichi Omori, Satoko Sakamoto, Akira Watanabe - Kyoto, Japan* |
| 09.43 | **C07** | **Evidence of intrinsic multi-potent/stem cells in the mature porcine cochlea**<br>*Desmond Nunez, Printha Wijesinghe, Boyuan Zheng, Elizabeth Hui, Germain Ho - Vancouver, Canada* |
| 09.56 | **C08** | **How the computational methods can contribute to better knowledge of the ear**<br>*Fernanda Gentil, Marco Parente, Carla Santos, Bruno Areias, Jorge Belinha, Renato Natal Jorge - Porto, Portugal* |

*Coffee station in the Exhibition Area from 10.00 to 11.30*

## SESSION II

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| **EAR PHYSIOLOGY AND AGING**<br>Moderators*: Ito Juichi, Fabio Mammano* | | |
| 10.10 | **TL2** | **TARGET LECTURE**<br>**The voltage and frequency dependence of prestin nonlinear capacitance is time-dependent**<br>*Joseph Santos-Sacchi, Winston Tan - New Haven, United States* |
| 10.30 – 13.00 | | **COMMUNICATIONS** |
| 10.30 | **C09** | **Emilin 2 promotes the stiffness and smooth mechanical gradient of the cochlear basilar membrane that is essential for fine, consistent, frequency resolution**<br>*Ian Russell [(1)], Victoria A. Lukashkina [(1)], Snezana Levic [(1)], Young-Wook Cho [(2)], Lily Ng [(2)], Douglas Forrest[(2)], Andrei N. Lukashin [(1)] - Brighton, UK [(1)]; Bethesda, Maryland, United States [(2)]* |
| 10.43 | **C10** | **Membrane traffic in the outer hair cell**<br>*Csaba Harasztosi, Anthony W. Gummer - Tübingen, Germany* |
| 10.56 | **C11** | **Correlation analysis of inner hair cell Ca2+ action potential activity and spontaneous Ca2+ signaling in non-sensory cells of the pre-hearing mouse cochlea**<br>*Federico Ceriani [(1)] - Stuart L. Johnson [(1)] - Aenea Hendry [(1)] - Bechara Kachar [(2)] - Walter Marcotti [(1)] - Fabio Mammano [(3)] - Sheffield, UK [(1, 2)]; Bethesda, MD, USA [(2)] - Padova, Italy [(3)]* |

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| 11.09 | **C12** | **Mechanical creep of the hair bundle is not correlated with Ca2+-dependent slow adaptation**<br>*Giusy Caprara, Andrew Mecca, Anthony Peng - Aurora, United States* |
| 11.22 | **C13** | **Age-related structural changes at auditory hair cell ribbon synapses: the role of cadherin23 and otoferlin**<br>*Didier Dulon - Bordeaux, France* |
| 11.35 | **C14** | **Second messengers regulate the sensitivity of cochlear hair cell mechanotransduction**<br>*Andrew Mecca, Anthony Peng - Aurora, United States* |
| 11.48 | **C15** | **Relevance of the presence of auditory nerve peripheral processes for the electrically evoked compound action potential**<br>*Dyan Ramekers, Henk Vink, Ferry Hendriksen, Huib Versnel - Utrecht, Netherlands* |
| 12.01 | **C16** | **G6PD overexpression protects from oxidative stress and ameliorates ARHL progression**<br>*Jose María Bermúdez Muñoz[(1)], Adelaida María Celaya [(1)]; Sara Hijazo[(1)], Manuel Serrano[(2] , Isabel Varela Nieto[(1)] - Madrid, Spain [(1)]; Barcelona, Spain [(2)]* |
| 12.14 | **C17** | **Inflammatory cytokines as diagnostic predictors of age-related hearing loss: cross-sectional investigation from the Great Age Study**<br>*Rodolfo Sardone, Petronilla Battista, Rossella Donghia, Marcello Chieppa, Madia Lozupone, Vito Guerra, Fabio Castellana, Letizia Pesole, Valeria Passalacqua, Antonio Lippolis, Domenico Scrutinio, Ilaria Bortone, Gianluigi Giannelli, Francesco Panza, Giancarlo Logroscino, Nicola Quaranta - Bari, Italy* |
| 12.27 | **C18** | **Age-related differences in the auditory temporal processing at peripheral and central levels in Fischer 344 rats**<br>*Jiří Popelář [(1)], Kateryna Pysanenko [(1)], Zbyněk Bureš [(2)], Daniel Šuta [(1], Natalia Rybalko [(1)],Tzai-Wen Chiu [(3], Yohan Bouleau [(4)], Didier Dulon [(4)], Josef Syka [(1)] - Prague, Czech Republic [(1]; Jihlava, Czech Republic [(2]; Hsinchu, Taiwan, Province of China [(3)]; Bordeaux, France [(4)]* |
| 12.40 | **C19** | **Hearing Loss in aging: does the shape of the audiogram predict perfusion changes in the primary auditory cortex?**<br>*Renzo Manara [(1)], Sara Ponticorvo [(1)], Davide Brotto [(2)], Arianna Cappiello [(1)], Sofia Cuoco [(1)], Donato Troisi [(1)], Claudia Cassandro [(1)], Marta John [(1)], Alfonso Scarpa [(1)], Ettore Cassandro [(1)] , Francesco Di Salle [(1)], Maria Teresa Pellecchia [(1)], Fabrizio Esposito [(1)] - Salerno, Italy [(1]; Padova, Italy [(2)]* |

13.00 - 13.15 **Group photo**

13.15 - 14.00 Lunch time

## SAELLITE SYMPOSIUM

| BEYOND THE DEVICE: NEW THERAPEUTIC APPROACHES FOR HEARING LOSS | |
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| 14.00 | **Cochlear Implants: the future is now**<br>*Denise Goldman (UK)* |
| 14.07 | **Understanding how drugs work in the cochlea – Pharmacokinetics studies**<br>*Manuel Manrique Rodriguez (Navarra, Spain)* |
| 14.27 | **The journey from preclinical to the clinic**<br>*Jonas Dyhrfjelds-Johnsen (France)* |
| 14.47 | **Conclusions**<br>*Denise Goldman (UK)* |

## SESSION III

| COCHLEAR IMPLANT, IMPLANTABLE PROSTHESIS AND DRUG DELIVERY SYSTEMS<br>Moderators: *Verena Scheper, Roberto Bovo* | | |
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| 15.00 | **TL3** | **TARGET LECTURE**<br>**Delivery of drugs to the entire cochlea without breaking its boundaries**<br>*Andrei Lukashkin [(1)], Ildar Sadreev [(2)], Natalia Zakharova [(3)], Yury Yarin [(4)], Ian Russell [(1)] - Brighton, UK [(1)]; Bristol, UK [(2)]; Uckfield, UK [(3)]; Dresden, Germany [(4)]* |
| 15.20 – 17.20 | | **COMMUNICATIONS** |
| 15.20 | **C20** | **Hearing preservation at low frequencies by insulin-like growth factor 1 in a guinea pig model of cochlear implantation**<br>*Norio Yamamoto [(1)], Kohei Yamahara [(1)], Koji Nishimura [(1)], Hideaki Ogita [(2)], Juichi Ito [(2)],Takayuki Nakagawa [(1)], Ichiro Furuta [(1)],Tomoko Kita [(1)],Koicih Omori [(1)] - Kyoto, Japan [(1)]; Moriyama, Japan [(2)]* |
| 15.33 | **C21** | **Advances in piezoelectric nanomaterials for cochlear stimulation**<br>*Serena Danti [(1)], Delfo D'Alessandro [(1)], Bahareh Azimi [(1)], Marco Onorati [(1)], Luisa Trombi [(1)],Laura Astolfi [(2)], Alessando Martini [(2)], Stefano Berrettini [(1)] - Pisa, Italy [(1)]; Padova, Italy [(2)]* |
| 15.46 | **C22** | **Hearing preservation of adult cochlear implant users with Partial Deafness – one year follow up after using steroids therapy**<br>Magdalena B. Skarzynska [(1)], Piotr H. Skarzynski [(2)], Bartlomiej Krol [(2)], Elzbieta Gos [(2)], Kamila Kordowska[(2)], Monika Boruta [(2)] - Henryk Skarzynski [(2)] - Kajetany, Poland [(1)] - *Warsaw/Kajetany, Poland [(2)]* |
| 15.59 | **C23** | **Surgical feasibility of localized therapeutic hypothermia application for preservation of residual hearing in cochlear implantation**<br>*Andrea Viziano [(1)], Enrique Perez [(2)], Zaid Al-Zaghal [(2)], Fred F. Telischi [(2)], Rachele Sangaletti [(2)], Weitao Jiang [(2)], W. Dalton Dietrich [(2)], Curtis King [(3)], Michael Hoffer [(2)], Suhrud Rajguru [(2)] - Rome, Italy[(1)]; Miami, FL, United States[(2)]; Seattle, WA, United States[(3)]* |

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| 16.12 | **C24** | **Cochlear implant perspective in congenital single side deafness: a temporal bone study**<br>*Eva Orzan, Giulia Pizzamiglio, Paola Staffa, Raffaella Marchi, Sara Ghiselli, Flora Maria Murru, Enrico Muzzi, Lucio Torelli, Massimo Gregori - Trieste, Italy* |
| 16.25 | **C25** | **Accelerated osteointegration of the titanium-implant coated with biocomponents, collagen/hydroxyapatite/bone morphogenetic protein-2, for bone-anchored hearing aid**<br>*ChulHo Jang [(1)], HyungJin Lee [(2)], GeunHyung Kim [(3)] - Gwangju, Republic of Korea [(1)]; Winston-Salem, United States [(2)]; Suwon, Republic of Korea [(3)]* |
| 16.38 | **C26** | **Outcomes after application of active bone conducting implants**<br>*Eleonor Koro [(1,2)], Mimmi Werner [(1,2)] - Umeå, Sweden [(1)]; Örnsköldsvik, Sweden [(2)]* |
| 16.51 | **C27** | **Sustained N-acetylcysteine delivery to the inner ear by poloxamer 407 hydrogels in a guinea pig model**<br>*Christoph Arnoldner, Julia Clara Gausterer, Nodir Saidov, Navid Ahmadi, Chengjing Zhu, Michael Wirth, Gottfried Reznicek, Franz Gabor, Clemens Honeder - Vienna, Austria* |

## POSTER SESSION

17.15 – 20.00
**Poster viewing and discussion**
Wine & Cheese

20.00 End of daily sessions

## Monday 9 September 2019

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| 07.30 | Registration |

## SESSION IV

### GENETICS OF DEAFNESS AND GENE THERAPY

Moderators: *Helena Caria, Giorgia Girotto*

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| 08.00 | **TL4** | **TARGET LECTURE**<br>**Mutations in PLS1 cause autosomal dominant non-syndromic hearing loss in three families of European ancestry**<br>*Anna Morgan [(1)], Daniel C. Koboldt [(2)], Elizabeth S. Barrie [(2)], Erin R. Crist [(2)], Gema García García [(3)], Massimo Mezzavilla [(1)], Flavio Faletra [(1)], Theresa Mihalic Mosher [(2)], Richard K. Wilson [(2)], Catherine Blanchet [(3)], Kandamurugu Manickam [(2)], Anne-Francoise Roux [(3)], Paolo Gasparini [(1)], Daniele Dell'Orco [(4)], Giorgia Girotto [(1)] - Trieste, Italy [(1)]; Columbus, Ohio, United States [(2)]; Montpellier, France [(3)]; Verona, Italy [(4)]* |

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| 08.20 – 10.30 | | **COMMUNICATIONS** |
| 08.20 | **C28** | **Gene Therapy of Usher Syndrome Type IC**<br>*Gwenaelle Geleoc [(1)], Carl Nist-Lund [(1)], Charles Askew [(2)], Christopher Tran [(3)], Alice Galvin [(1)], Abhilash Ponnath [(3)], Selena Heman-Ackah [(1)], Yukako Asai [(1)], Artur Indzykulian [(1)], Francine Jodelka [(4)], Michelle Hasting [(4)], Hamilton Farris [(3)], Frank Rigo [(5)], Jeffrey Holt [(1)], Jennifer Lentz [(3)] - Boston, United States [(1)]; Chapel Hill, United States [(2)]; New Orleans, United States [(3)]; Chicago, United States [(4)]; Carlsbad, United States [(5)]* |
| 08.33 | **C29** | **A rationally designed adeno-associated viral vector enables safe and efficient gene transfer to supporting cells in the mouse cochlea**<br>*Renjie Chai - Nanjing, China* |
| 08.46 | **C30** | **The distinct functions of TRIOBP-5: sculpting stereocilia rootlets and stiffening supporting cells**<br>*Shin-ichiro Kitajiri [(1)], Tatsuya Katsuno [(2)], Inna Belyantseva [(3)], Alexander Cartagena-Rivera [(3)], Keisuke Ohta [(4)], Shawn Crump [(5)], Ronald Petralia [(3)], Kazuya Ono [(2)], Risa Tona [(3)], Ayesha Imtiaz [(3)], Atteeq Rehman [(3)], Hiroshi Kiyonari [(6)], Mari Kaneko [(6)], Ya-Xian Wang [(3)], Takaya Abe [(6)], Makoto Ikeya [(2)], Cristina Fenollar-Ferrer [(3)], Gavin Riordan [(3)], Elisabeth Wilson [(3)], Tracy Fitzgerald [(3)], Kohei Segawa [(2)], Koichi Omori [(2)], Juichi Ito [(2)], Gregory Frolenkov [(5)], Thomas Friedman [(3)] - Matsumoto, Japan [(1)] - Kyoto, Japan [(2)] - Bethesda, United States [(3)] - Kurume, Japan [(4)] - Lexington, United States [(5)] - Kobe, Japan [(6)]* |
| 08.59 | **C31** | **Disruption of CLRN2, a gene encoding clarin-2, causes autosomal recessive hearing loss in humans and zebrafish**<br>*Barbara Vona [(1)], Neda Mazaheri [(2)], Reza Maroofian [(3)], Hela Azaiez [(4)], Kevin T. Booth [(4)], Kate Clancy [(5)], Gholamreza Shariati [(2)], Alireza Sedaghat [(2)], Ruben Stepanyan [(5)], Richard J. H. Smith [(4)], Thomas Haaf [(6)], Hamid Galehdari [(2)], Kumar N. Alagramam [(5)], Suhasini R. Gopal [(5)] - Tuebingen, Germany [(1]; Ahvaz, Iran (Islamic Republic of) [(2)]; London, United Kingdom [(3)]; Iowa City, United States [(4)]; Cleveland, United States [(5)]; Wuerzburg, Germany [(6)]* |
| 09.12 | **C32** | **Disease modelling and drug screening for GJB2 related hearing loss with iPS cells**<br>*Kazusaku Kamiya, Ichiro Fukunaga, Shori Tajima, Keiko Kanayama, Yoko Oe, Cheng Chen, Sayaka Ohta, Osamu Minowa, Katsuhisa Ikeda - Tokyo, Japan* |
| 09.25 | **C33** | **Synaptic Changes in Cochlear Hair cells of Tmc Mutant Mice**<br>*John Lee [(1)], Jeffrey Holt [(2)] Gwenaëlle Géléoc [(2)] - Cambridge, United States [(1)]; Boston, United States [(2)]* |
| 09.38 | **C34** | **Whole exome sequencing in Slovak patients with bilateral sensorineural hearing impairment**<br>*Zuzana Slobodova, Lukas Varga, Lucia Demesova, Ivica Masindova, Daniel Danis, Lenka Langova, Milan Profant, Daniela Gasperikova - Bratislava, Slovak Republic* |

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| 09.51 | **C35** | **Characterization of the genetic bases of hearing loss in an Italian cohort**<br>*Federica Cesca [(1)], Elisa Bettella [(1)], Roberta Polli [(1)] - Emanuela Leonardi [(1)], Maria Cristina Aspromonte [(1)] , Mariagrazia Bellini [(1)], Stefania Bigoni [(2)], Alberto Sensi [(3)], Pietro Scimemi [(1)], Rosamaria Santarelli [(1)], Alessandra Murgia [(1)] - Padova, Italy [(1)] - Ferrara, Italy [(2)] - Cesena, Italy [(3)]* |
| 10.04 | **C36** | **Enrichment of rare missense variants in OTOG gene in Familial Meniere disease**<br>*Pablo Román-Naranjo [(1)], Maria del Carmen Moleon [(1)], Alvaro Gallego-Martinez [(1)], Andrés Soto-Varela [(2] , Juan Carlos Amor-Dorado [(3)], Ángel Batuecas-Caletrio [(4)], Ismael Aran [(5)], Paz Perez-Vazquez [(6)], Jose Antonio Lopez-Escamez [(1)] - Granada, Spain [(1)]; Santiago de Compostela, Spain [(2)]; Ibiza, Spain [(3)]; Salamanca, Spain [(4)]; Pontevedra, Spain [(5)]; Oviedo, Spain [(6)]* |

*Coffee station in the Exhibition Area from 10.00 to 11.30*

## SESSION V

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| **NOISE INDUCED HEARING LOSS, OTOTOXICITY AND OTOPROTECTION**<br>Moderators: *Anthony W. Gummer, Laura Astolfi* | | |
| 10.20 | **TL5** | **TARGET LECTURE**<br>**Implications of compound screens for protection of mammalian hair cells from aminoglycoside ototoxicity**<br>*Allen F Ryan, Kwang Pak, Taylor Wyrick, Clara Draf,, Arwa Kurabi - La Jolla, United States* |
| 10.40 – 13.10 | | **COMMUNICATIONS** |
| 10.40 | **C37** | **MicroRNA Expression Changes in the Cochlear Nucleus and Inferior Colliculus after Noise-Induced Hearing Loss**<br>*Sohyeon Park, Myung-Whan Suh, Jun Ho Lee, Seung Ha Oh, Moo Kyun Park - Seoul, Republic of Korea* |
| 10.53 | **C38** | **Glucose supplementation and prevention of noise-induced hearing loss**<br>*Hao Xiong - Guangzhou, China* |
| 11.06 | **C39** | **Targeting cellular defensive response and inflammation in cancer cells and cisplatin-induced ototoxicity**<br>*Anna Rita Fetoni, Fabiola Paciello, Rolando Rolesi, Diana Troiani, Gaetano Paludetti - Rome, Italy* |
| 11.19 | **C40** | **Entry rate of gentamicin through the MET channels of outer hair cells varies with position along the cochlea**<br>Virginia Mahieu [(1)], Peter Steyger [(2)], Corne Kros [(1)] - *Brighton, United Kingdom [(1)]; Omaha, United States [(2)]* |
| 11.32 | **C41** | **Prevention of cisplatin ototoxicity: the role of nanoceria and dexamethasone**<br>*Erica Gentilin [(1)], Mariarita Candito [(1)], Edi Simoni [(1)], Serena Danti [(2)], Alessandro Martini [(1)], Laura Astolfi [(1)] - Padua, Italy [(1)]; Pisa, Italy [(2)]* |

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| 11.45 | **C42** | **3R mouse model for cisplatin ototoxicity studies: platinum correlation with deafness**<br>*German Nacher-Soler, Francis Rousset, Marta Coelho, Karl-Heinz Krause, Pascal Senn - Genève, Switzerland* |
| 11.58 | **C43** | **Inhibition of the adenosine A2A receptor mitigates excitotoxic injury in organotypic tissue cultures of the rat cochlea**<br>*Srdjan Vlajkovic, Belinda Han, Shelly Lin, Kristan Espinosa, Peter Thorne - Auckland, New Zealand* |
| 12.11 | **C44** | **Opioid modulation of cochlear auditory responses in the rat inner ear**<br>*Enrique Soto, Teresa Ramírez, Rosario Vega - Puebla, Mexico* |
| 12.24 | **C45** | **A665-conjugated Acetylcysteine target prestin of outer hair cells with peptide hydrogel delivery preventing cisplatin-induced hearing loss**<br>*Jiaqi Pang, Hao Xiong, Xiaoding Xu, Yiqing Zheng - Guangzhou, China* |
| 12.37 | **C46** | **Loss of function mutation in the NADPH oxidase subunit p22phox prevents early onset hearing loss**<br>*Francis Rousset, German Nacher Soler, Marta Coelho, Sten Ilmjarv, Antoine Marteyn, Vivianne Kokje, Karl-Heinz Krause, Pascal Senn - Geneva, Switzerland* |
| 12.50 | **C47** | **Head-to-head comparison of different classes of otoprotectants against cisplatin-induced hearing loss in clinically relevant ex vivo models of hair cell, SGN and stria vascularis damage**<br>*Bonnie Jacques, Pranav Mathur, Phillip Uribe, Anne Harrop-Jones, Stephanie Szobota, Sairey Siegel, Kathie Bishop, Fabrice Piu, Alan Foster - San Diego, United States* |

13.10 - 13.20 **Spoendlin Award Ceremony**

*13.30 FREE AFTERNOON*

**Tuesday 10 September 2019**

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| 07.30 | Registration |

## SESSION VI

**PHYSIOPATHOLOGY OF AUDITORY PATHWAYS AND INNER EAR IMMUNOLOGY**

Moderators: *Marlies Knipper, Rosamaria Santarelli*

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| 08.00 | **TL6** | **TARGET LECTURE**<br>**Presence and characterization of cochlear mast cells**<br>*Agnieszka Szczepek, Heidi Olze, Alina Smorodchenko - Berlin, Germany* |
| 08.20 – 10.20 | | **COMMUNICATIONS** |
| 08.20 | **C48** | **Brain-derived neurotrophic factor in auditory brainstem controls central learning mechanisms and social behavior**<br>*Philipp Eckert [(1)], Philine Marchetta [(1)], Michael Walter [(1)], Marie Manthey [(2)], Wibke Singer [(1)], Michele Jacob [(2)], Lukas Rüttiger [(1)], Thomas Schimmang [(3)], Peter Pilz [(1)], Marlies Knipper [(1)] - Tuebingen, Germany [(1)]; Boston, United States [(2)]; Valladolid, Spain [(3)]* |
| 08.33 | **C49** | **Neural correlates of fine structure and temporal envelope in the human auditory nerve**<br>*Xavier Dubernard [(1)], Frederic Venail [(1)], Jean-Charles Kleiber [(2)], Arnaud Bazin [(2)], André Chays [(2)], Jean-Luc Puel [(1)], Jérôme Bourien [(1)] - Montpellier, France [(1)]; Reims, France [(2)]* |
| 08.46 | **C50** | **The development and subpopulation of tissue-resident macrophages in the mouse cochlea**<br>*Ippei Kishimoto, Takayuki Okano, Koichi Omori - Kyoto, Japan* |
| 08.59 | **C51** | **Decoding the auditory nerve and measuring the effect on speech-in-noise intelligibility of each known sensorineural pathology in the auditory periphery**<br>*Jacques Grange, John Culling - Cardiff, UK* |
| 09.12 | **C52** | **Vascular associations in the choroid plexus: do they matter for the auditory system?**<br>*Paola Perin, Victoria Barcio, Simone D'Onofrio, Stefano Scarpa, Roberto Pizzala - Pavia, Italy* |
| 09.25 | **C53** | **Absence of STAT1 predisposes mice to otitis-related hearing loss**<br>*Soledad Levano [(1)], Peter Kern [(1)], Ana Bento [(1)], David Bächinger [(2)], Arianne Monge Naldi [(2)], Daniel Bodmer [(1)] - Basel, Switzerland [(1)]; Zurich, Switzerland [(2)]* |
| 09.38 | **C54** | **Cell-free biological drug for the inner ear: Extracellular vesicles derived from mesenchymal stromal cells support the survival of spiral ganglion neurons**<br>*Jennifer Schulze [(1)], Athanasia Warnecke [(1)], Thomas Lenarz [(1)], Eva Rohde [(2)], Julia Hollerweger [(2)], Teresa Lassacher [(2)], Mario Gimona [(2)] - Hannover, Germany [(1)]; Salzburg, Austria [(2)]* |

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| 09.51 | **C55** | **Stress receptors in higher frontal brain regions influence auditory nerve function and auditory brainstem responses**<br>*Philine Marchetta, Philipp Eckert, Lukas Rüttiger, Wibke Singer, Marlies Knipper - Tübingen, Germany* |
| 10.04 | **C56** | **The acoustic challenge in school age children with mild hearing loss**<br>*Claudia Cassandro, Giulia Aschero, Valeria Landi, Silvano Lovallo, Alessandra Manassero, Diego Sammarco, Irene Vernero, Roberto Albera - Turin, Italy* |

*Coffee station in the Exhibition Area from 10.00 to 11.30*

## SESSION VII

**TINNITUS AND VESTIBULAR DISORDERS**

*Moderators:* Jose Antonio Lopez-Escamez, Cosimo De Filippis

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| 10.20 | **TL7** | **TARGET LECTURE**<br>**Tinnitus induced hyperexcitability in view of deafness and cochlear implants?**<br>Marlies Knipper [(1)], Pim Van Dijk [(2)], David Baguley [(3)], Lukas Rüttiger [(1)] - *Tübingen, Germany [(1)]; The Netherlands [(2)]; University of Nottingham, UK [(3)]* |
| 10.40 – 13.10 | | **COMMUNICATIONS** |
| 10.40 | **C57** | **Identification of functional biomarkers of tinnitus and tinnitus/hyperacusis in patients**<br>*Benedikt Hofmeier, Marlies Knipper, Lukas Rüttiger, Uwe Klose, Stephan Wolpert - Tübingen, Germany* |
| 10.53 | **C58** | **Psychiatric comorbidity in patients with tinnitus or auditory hallucination and sound therapy**<br>*Kensuke Kiyomizu, Takeshi Nakamura, Tetsuya Tono, Kensei Yoshida, Yasushi Ishida - Miyazaki, Japan* |
| 11.06 | **C59** | **Subclinical cochlear dysfunction in newly diagnosed relapsing- remitting multiple sclerosis**<br>*Massimo Ralli [(1)], Marco de Vincentiis [(1)], Arianna Di Stadio [(2)], Stefano Di Girolamo [(1)], Maria Albanese [(1)] - Roma, Italy [(1)]; Perugia, Italy [(2)]* |
| 11.19 | **C60** | **The proteome of the perilymph in relation to hearing loss in patients with vestibular schwannoma**<br>*Jesper Edvardsson Rasmussen, Per Olof Eriksson, Jonas Bergquist, Göran Laurell - Uppsala, Sweden* |
| 11.32 | **C61** | **Pharmacological Ablation of Vestibular Hair Cells or Ganglion Neurons to Generate a Model of Unilateral Vestibular Dysfunction**<br>*Koji Nishimura [(1)], Akiyoshi Yasumoto [(1)], Steven Meas [(2)], Hideaki Ogita [(3)] , Akiko Taura [(4,] , Juichi Ito [(3)], Koichi Omori [(1)] - Kyoto, Japan (1); Toronto, Canada (2); Moriyama, Japan (3); Ibaraki, Japan (4)* |

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| 11.45 | **C62** | **Ionic direct current stimulation results in spike-rate adaptation in vestibular afferents of the mouse crista in vitro**<br>*Marco Manca, Elisabeth Glowatzki, Dale Roberts, Gene Yevgeny Fridman, Felix Peter Aplin - Baltimore, United States* |
| 11.58 | **C63** | **Inefficient cranial venous outflow and increased CSF pulsatility in the Aqueduct of Sylvius in patients with Meniere's disease**<br>*Christian Contarino [(1)], Giuseppe Nicolò Frau [(2)], Sabino Walter Della Sala [(2)], Eleuterio F. Toro [(3)] - Delaware, United States [(1)]; Rovereto, Italy [(2)]; Trento, Italy [(3)]* |
| 12.11 | **C64** | **Biomarkers for the differential diagnosis of Vestibular Migraine and Meniere Disease**<br>*Marisa Flook [(1)], Lidia Frejo [(2)], Alvaro Gallego-Martinez [(1)], Eduardo Martin-Sanz [(3)], Marcos Rossi-Izquierdo [(4)], Juan Carlos Amor-Dorado [(5)], Andres Soto-Varela [(6)], Sofia Santos-Perez [(6)], Angel Batuecas-Caletrio [(7)], Juan Manuel Espinosa-Sanchez [(1)], Patricia Perez-Carpena [(1)], Marta Martinez-Martinez [(1)], Jose Antonio Lopez-Escamez [(1)] - Granada, Spain [(1)]; New York, United States [(2)]; Getafe, Spain [(3)]; Lugo, Spain [(4)]; Ibiza, Spain [(5)]; Santiago de Compostela, Spain [(6)]; Salamanca, Spain [(7)]* |
| 12.24 | **C65** | **Electrocochleography finding in Meniere disease after active pressure treatment**<br>*Edoardo Covelli, Maurizio Barbara, Simonetta Monini, Silvia Tarentini - Roma, Italy* |
| 12.37 | **C66** | **Anatomical, biochemical and behavioural characterization of a mouse model for Meniere's Disease**<br>*Anna Lysakowski [(1)], Maria Teresa Requena [(2)], Jacob Kulaga [(1)], Joseph Lesus [(1)], Jose Antonio Lopez Escamez [(3)] - Chicago, United States [(1)] - Edinburgh, UK [(2)] - Granada, Spain [(3)]* |
| 12.50 | **C67** | **Metabolomics in Meniere's disease: redox modulation by nutritional approaches with mushrooms**<br>*Maria Concetta Scuto, Angela Trovato Salinaro, Gabriele Di Rosa, Vittorio Calabrese, Luigi Maiolino - Catania, Italy* |
| | | *ECM - for Italian participants only:*<br>*Compilazione del modulo della qualità percepita* |
| 13.10-14.00 | | **IEB Business Meeting and Closing** |

# POSTER SESSION

## Sunday 8 September 2019

**From 17.15 to 20.00 hrs**
Poster viewing and discussion - Wine & Cheese

*The Moderators of the Workshop oral sessions will also moderate the poster session*

| Imaging and Anatomy | |
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| **P1** | **The innervation of the mammalian cochlea, an immunocytochemical study**<br>Linda Bieniussa, Johannes Völker, Kristen Rak, Rudolf Hagen - *Würzburg, Germany* |
| **P2** | **Abnormalities of the ear and far beyond: the long lasting Padua's experience on aural atresia**<br>Davide Brotto, Flavia Sorrentino, Ezio Caserta, Patrizia Trevisi, Silvia Montino, Anna Agostinelli, Roberto Bovo, Miriam Torsello, Diletta Giuntoli, Renzo Manara, Alessandro Martini - *Padua, Italy* |
| **P3** | **The vascularization of the labyrinth: an anatomical study**<br>Sebastiano Franchella, Giulia Ramacciotti, Diego Cazzador, Alessandro Martini, Antonio Mazzoni, Elisabetta Zanoletti - *Padua, Italy* |
| **P4** | **Hearing and cognitive impairment: a functional evaluation of associative brain areas in patients affected by Alzheimer's disease**<br>Emanuela Fuccillo, Agostino Chiaravalloti, Maria Ricci, Pier Giorgio Giacomini, Alessandro Martorana, Orazio Schillaci, Stefano Di Girolamo - *Rome, Italy* |
| **P5** | **The complexity of multidisciplinary evaluation of children with sensorineural hearing loss: three case reports**<br>Sara Ghiselli, Veronica Castro, Massimo Gregori, Irene Bruno, Raffaella Marchi, Stefano Pensiero, Maura Bin, Flavio Faletra, Giorgia Girotto, Paolo Gasparini, Eva Orzan - *Trieste, Italy* |
| **P6** | **Outcomes of Modified Canal Wall Down Mastoidectomy and Mastoid Obliteration Using Autologous Materials**<br>Bo Gyung Kim, Jong Dae Lee, Jae Yong Lee - *Bucheon, Republic of Korea* |
| **P7** | **Conformity between Magnetic Resonance Imaging and Surgery Outcome in Cholesteatomas**<br>Eleonor Koro, Emely Ögren, Mimmi Werner - *Umeå, Sweden* |
| **P8** | **Vascular network of the rat cochlear nuclei**<br>Paola Perin, Victoria Barcio, Simone D'Onofrio, Stefano Scarpa, Roberto Pizzala - *Pavia, Italy* |
| **P9** | **Relationship between the drainage patterns of the dural venous sinus and the affected side of sudden sensorineural hearing loss**<br>Woongsang Sunwoo - *Incheon, Republic of Korea* |

## Regeneration and Stem Cells

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| **P10** | **The Gunn Rat - A Model for Cell Transplantation Therapy in Auditory Neuropathy?**<br>Leila Abbas, Marcelo N. Rivolta - *Sheffield, United Kingdom* |
| **P11** | **Functional recovery of regenerating lateral line hair cells**<br>Ana Amariutei [(1)], Francesca De Faveri [(1)], Katherine Hardy [(1)], Aenea Hendry [(1)], Federico Ceriani [(1)], Walter Marcotti - *Sheffield, United Kingdom)* |
| **P12** | **Engraftment of human induced pluripotent stem cells (iPS) and guinea pig bone marrow-derived stem cells (MSC) into the cochlea of guinea pig**<br>Juichi Ito [(1)], Hideaki Ogita [(1)], Koji Nishimura [(2)], Hiroe Ohnishi [(2)], Akiko Taura [(3)] - *Moriyama, Japan [(1)]; Kyoto, Japan [(2)]; Ibaraki, Japan [(3)]* |
| **P13** | **Alteration in Atoh1 expression during the loss and regeneration of auditory hair cells in explant cultures of chick basilar papillae**<br>Mami Matsunaga - *Kyoto, Japan* |
| **P14** | **Examination of EYA4 gene mutation related hearing loss using the common marmoset (Callithrix jacchus) cochlea and patient-derived induced pluripotent stem cell (iPS cells)**<br>Saeko Matsuzaki, Masato Fujioka, Makoto Hosoya, Kaoru Ogawa - *Tokyo, Japan* |
| **P15** | **The transplantation of sphere-forming stem cells from the inner ear into cochlea**<br>Hideaki Ogita [(1)], Hiroe Onishi [(2)], Koji Nishimura [(2)], Juichi Ito [(1)] - *Shiga, Japan [(1)]; Kyoto, Japan [(2)]* |
| **P16** | **Improvement of Otic Induction from Human Induced Pluripotent Stem Cell**<br>Hiroe Ohnishi [(1)], Desislava Skerleva [(1)], Hideaki Okuyama [(1)], Norio Yamamoto [(1)], Juichi Ito [(2)], Koichi Omori [(1)], Takayuki Nakagawa [(1)] - *Kyoto, Japan [(1)]; Shiga, Japan [(2)]* |
| **P17** | **Stimulation of spiral ganglion neurons cultured in vitro with a global electro-magnetic field**<br>Viktorija Radotić; Jelena Žarković, Ana Bedalov, Damir Kovačić - *Split, Croatia* |
| **P18** | **Selective induction of cochlear hair cells from human induced pluripotent stem cells**<br>Tsubasa Saeki, Makoto Hosoya, Masato Fujioka, Kaoru Ogawa, Hideyuki Okano - *Tokyo, Japan* |
| **P19** | **Exogenous BDNF and NT-3 in mouse explant cultures: Only a neural survival factor or also promoting axonal outgrowth?**<br>Dominik Schmidbauer [(1)], Stefan Fink [(2)], Francis Rousset [(3)], Marcus Müller [(2)], Pascal Senn [(3)], Rudolf Glückert [(1)] - *Innsbruck, Austria [(1)]; Tübingen, Germany [(2)]; Geneva, Switzerland [(3)]* |
| **P20** | **The efficacy and safety of Wnt and Notch-signaling modulators in the cochlea**<br>Rana Yadak, Ferry Hendriksen, Dyan Ramekers, Huib Versnel, Robert Stokroos, Louise Straatman - *Utrecht, Netherlands* |

## Developmental Biology

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| **P21** | **Identification of TMCC2 as novel hair cell marker and characterization of antibodies for phenotypic study of hair cell deficits**<br>Ranju Kumari, Piotr Kazmierczak - *Warsaw, Poland* |
| **P22** | **The inhibition of endogenous ceramide kinase alters the morphogenesis of the chicken inner ear primordium**<br>Yolanda Leon, Marta Magariños, Isabel Varela-Nieto - *Madrid, Spain* |
| **P23** | **Elucidating pathological mechanisms of hearing loss induced by hypothyroidism using Duox2 mutant mice**<br>**Sera Park, Jinwoong Bok, Jae-Young Choi - Seoul, Republic of Korea** |

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| **P24** | **Analysis of auditory system of mice lacking brain-specific angiogenesis inhibitor 3 (Bai3)**<br>Chika Saegusa, Wataru Kakegawa, Eriko Miura, Takanori Nishiyama, Makoto Hosoya, Kaoru Ogawa, Michisuke Yuzaki, Masato Fujioka - *Tokyo, Japan* |
| **P25** | **Shh Signaling Pathway Role in the Differentiation of Mouse Pluripotent Stem Cells into Inner Ear Organoids**<br>Elham Salehisiavashani, Farideh Moinvaziri, Ali Sharifi-Zarchi, Hossein Baharvand - *Tehran, Islamic Republic of Iran* |
| **P26** | **Expression of Carbonic Anhydrase 13 in the Developing Mouse Cochlea**<br>Yuki Tamaki, Hiroe Onishi, Ryosuke Yamamoto, Koichi Omori, Takayuki Nakagawa, Norio Yamamoto - *Kyoto, Japan* |
| **P27** | **Cochlear Implant-Based Electrical Stimulation Modulates Neural Stem Cell-Derived Neural Regeneration**<br>Mingliang Tang, Rongrong Guo, Menghui Liao - *Nanjing, China* |
| **Ear Physiology** | |
| **P28** | **Otolin-1 as a possible Biomarker for Inner Ear Disease?**<br>Emilio Avallone, Heike Schmitt, Giorgio Lilli, Athanasia Warnecke, Anke Lesinski-Schiedat, Thomas Lenarz, Kerstin Willenborg - *Hannover, Germany* |
| **P29** | **Medial olivocochlear and middle-ear reflex: is it possible to differentiate?**<br>Thamara Suzi Dos Santos, Pierrick Bordiga, Paul Avan, Fabrice Giraude - *Clermont Auvergne, France* |
| **P30** | **Dominant deafness due to a point mutation in TMC1**<br>Robert Fettiplace, Maryline Beurg, Amanda Barlow - *Madison, WI, United States)* |
| **P31** | **Variation of intercellular K+ concentration at the mouse vestibular Type I hair cell-calyx synapse can contribute to afferent signaling**<br>Roberta Giunta (1), Paolo Spaiardi (1), Elisa Tavazzani (1), Marco Manca (1), Giancarlo Russo (1), Ivo Prigioni (1), Gerardo Biella (1), Stuart Johnson (2), Walter Marcotti (2) - Sergio Masetto (1) - *Pavia, Italy (1); Sheffield, United Kingdom (2)* |
| **P32** | **Biophysical Model of Synaptic Transmission at the Vestibular Hair Cell Calyx**<br>Aravind Chenrayan Govindaraju (1), Imran Quraishi (2), Anna Lysakowski (3), Ruth Anne Eatock (3), Robert M Raphael (1) - *Houston, TX, United States (1); New Haven, CT, United States (2); Chicago, Il, United States (3)* |
| **P33** | **Changes in intracellular pH, Na+, and Cl- induced by hydrogen sulfide in outer hair cells**<br>Narinobu Harada, Yukari Ito, Atsufumi Kawabata - *Osaka, Japan* |
| **P34** | **Piezoelectric Vibrator-Stimulated Potential and Heart Rate Accelerations Detected from the Fetus**<br>Rina Mastuoka, Sinyoung Lee, Miho Sato, Remi Motegi, Yota Shimanuki, Misato Kasai, Kazusaku Kamiya, Atsuo Itakura, Takuji Koike, Katsuhisa Ikeda - *Tokyo, Japan* |

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| **P35** | **Distribution of Na/K-ATPase Subunits and Voltage-Gated Ion Channels in the Human Cochlea and Auditory Nerve - A Study Using Super Resolution Microscopy with Special Reference to Cochlear Implantation**<br>Wei Liu, Niklas Danckwardt-Lilliestrom, Charlotta Kämpfe Nordström, Helge Rask-Andersen - *Uppsala, Sweden* |
| **P36** | **The Role of D1-like and D2-like Dopamine Receptors in Lateral Olivocochlear Efferent Function**<br>Jingjing Wu [(1)] - Marco Manca [(1)] - Pankhuri Vyas [(1)] - Kushal Sharma [(2)] - Eunyoung Yi [(2)] - Elisabeth Glowatzki [(1)] - *Baltimore, MD, United States [(1)]; Muan, Republic of Korea [(2)]* |
| **P37** | **Simultaneous detection of Ca2+ signaling and ATP release in the developing cochlea**<br>Flavia Mazzarda [(1)], Annunziata D'Elia [(2)], Adele De Ninno [(1)], Gaia Ziraldo [(1)], Veronica Zorzi [(1)], Chiara Nardin [(3)], Chiara Peres [(3)], Francesco Chiani [(2)], Francesca Romena Bertani [(1)], Luca Businaro [(1)], Roberto Massari [(2)], Alessandro Soluri [(2)], Anna Maria Salvatore [(2)], Fabio Mammano [(3)] - *Rome, Italy [(1)]; Monterotondo, Italy [(2)]; Padua, Italy [(3)]* |
| **P38** | **Digital hearing: biophysical modeling of auditory processes**<br>Evgeniy L. Ovchinnikov, Danil S. Tarasenko, Anas M. Alkurdi - *Samara, Russian Federation* |
| **Aging** | |
| **P39** | **Gender effect, quality of life and genetic biomarkers in a Portuguese sample with ARHL with or without tinnitus**<br>Haula Haider, Ganna Matskul, Diogo Ribeiro, Sara Ribeiro, Assunção O'Neill, Graça Fialho, João Paço, Helena Caria - *Lisbon, Portugal* |
| **P40** | **Neuro-otological treatment for patients with dementia and hearing loss in unique psychiatric hospital**<br>Kensuke Kiyomizu [(1)], Takeshi Nakamura [(1)], Tetsuya Tono [(1)], Yasushi Ishida [(1)], Kensei Yoshida [(1)], Sho Kanzaki [(2)] - *Miyazaki, Japan [(1)]; Tokyo, Japan [(2)]* |
| **P41** | **Inhibition of DRP-1-dependent mitophagy promotes cochlea hair cell senescence and exacerbates AHL**<br>Hanqing Lin, Hao Xiong, Zhongwu Su, Jiaqi Pang, Lan Lai, Yiqing Zheng - *Guangzhou, China* |
| **P42** | **Life-long-lasting functional damage of the inner ear cells induced by P-type Ca2+-ATPase mutations**<br>Osamu Minowa [(1)], Takashi Daiho [(2)], Kazuo Yamasaki [(2)], Hiroshi Suzuki [(2)], Toshihiko Shiroishi [(3)], Atsushi Yoshiki [(3)], Tetsuo Noda [(1)], Nagomi Kurebayashi [(1)], Takashi Murayama [(1)], Kazusaku Kamiya [(1)], Yasushi Okazaki [(1)], Katsuhisa Ikeda [(1)] - *Tokyo, Japan [(19 ; Asahikawa, Japan [(2)]; Tsukuba, Japan [(3)]* |
| **P43** | **Deficiency of mitochondrial tRNA modification causes early spiral ligament damage**<br>Toru Miwa - *Osaka, Japan* |
| **P44** | **Cx26 partial loss causes accelerated presbycusis by redox imbalance and dysregulation of Nfr2 pathway**<br>Anna Rita Fetoni [(1)], Veronica Zorzi [(2)], Fabiola Paciello [(1)], Gaia Ziraldo [(2)], Chiara Peres [(2)], Marcello Raspa [(2)], Ferdinando Scavizzi [(2)], Anna Maria Salvatore [(2)], Giulia Crispino [(2)], Gabriella Tognola [(3)], Giulia Gentile [(4)], Antonio Gianmaria Spampinato [(4)], Denis Cuccaro [(4)], Maria Guarnaccia [(4)], Giovanna Morello [(4)], Guy Van Camp [(5)], Erik Fransen [(5)], Marco Brumath [(6)], Giorgia Girotto [(6)], Gaetano Paludetti [(1)], Paolo Gasparini [(6)], Sebastiano Cavallaro [(4)], Fabio Mammano [(7)] - *Rome, Italy [(1)]; Monterotondo, Italy [(2)]; Milan, Italy [(3)]; Catania, Italy [(4)]; Antwerp, Belgium [(5)]; Trieste, Italy [(6)]; Padua, Italy [(7)]* |

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| **P45** | **Age-related alterations in the hearing function of Fischer 344 rats**<br>Kateryna Pysanenko, Natalia Rybalko, Zbyněk Bureš, Jiří Popelář, Josef Syka - *Prague, Czech Republic* |
| **P46** | **Degradation of cochlear gap junction is a crucial pathogenesis in age related hearing loss**<br>Shori Tajima, Kazusaku Kamiya, Katsuhisa Ikeda - *Tokyo, Japan* |
| **P47** | **The potential effect of food intake in gaining or reducing an incidence of hearing impairment after middle age among Japanese community dwellers**<br>Yasue Uchida [(1)], Rei Otsuka [(2)], Saiko Sugiura [(3)], Yuki Kato [(1)], Yukiko Nishita [(2)], Hiromi Ueda [(1)], Fujiko Ando [(1)], Hiroshi Shimokata [(4)] - *Nagakute, Japan [(1)]; Obu, Japan [(2)]; Toyota, Japan [(3)]; Nisshin, Japan [(4)]* |

## Cochlear Implant and Implantable Prosthesis

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| **P48** | **Band-limited Chirp-evoked Compound Action Potentials in Guinea Pigs: Feasibility for Cochlear Implantation Monitoring**<br>Youssef Adel [(1)], Jochen Tillein [(2)], Hannah Petzold [(1)], Tobias Weissgerber [(1)], Uwe Baumann [(1)] - *Frankfurt am Main, Germany [(1)]; Innsbruck, Austria [(2)]* |
| **P49** | **Cochlear implants in elderly population: results and benefits**<br>M. Negri [(1)], Paola Benincasa [(1)], Silvia Palma [(1)], M. Guida [(2)], S. Tassi [(1)] - *Modena, Italy [(1)] ; Parma, Italy [(2)]* |
| **P50** | **Working memory function in children with unilateral hearing loss using a bone anchored hearing implant: a case control study**<br>Antonietta De Lucia, Valentina Ippolito, Sabina Garofalo, Antonio della Volpe - *Naples, Italy* |
| **P51** | **VSB surgery for conductive and mixed hearing loss - the most sensitive predictable factor for speech perception with VSB**<br>Katsumi Doi, Hajime Koyama, Daisuke Nagatomi - *Osaka, Japan* |
| **P52** | **Restoring hearing can affect the degraded cognitive domains in older patients**<br>Anna Rita Fetoni [(1)], Giorgia Rossi [(1)], Francesca Brigato [(1)], Roberta Anzivino [(1)], Walter Di Nardo [(1)], Guido Conti [(1)], Camillo Marra [(1)], Valeria Guglielmi [(1)], Francesca Forli [(2)], Giada Giuntini [(2)], Gaetano Paludetti [(1)], Stefano Berrettini [(2)] - *Rome, Italy [(1)] ; Pisa, Italy [(2)]* |
| **P53** | **Defined BDNF and NT-3 Growth Factor Cocktail Enhanced Neurite Outgrowth in a Postnatal Day 7 (adult-like) Murine In Vitro Spiral Ganglion Explant Model**<br>Stefan Fink, Hubert Löwenheim, Marcus Müller - *Tübingen, Germany* |
| **P54** | **Cochlear duct length measurements in congenital single side deafness**<br>Giulia Pizzamiglio, Massimo Gregori, Flora Maria Murru, Paola Staffa, Raffaella Marchi, Sara Ghiselli, Enrico Muzzi, Lucio Torelli, Eva Orzan – *Trieste, Italy* |
| **P55** | **Does proteome analysis of human perilymph give information about hearing performance after cochlear implantation?**<br>Heike Schmitt, Andreas Pich, Martin Durisin, Athanasia Warnecke, Nils Prenzler, Thomas Lenarz - *Hannover, Germany* |

## Drug Delivery Systems

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| **P56** | **Effect of alginate-cell based drug-delivery systems on the insertion forces of CI-electrodes**<br>Jana Schwieger [(1)], Silke Hügl [(1)], Michael M. Gepp [(2)], Thomas Lenarz [(1)], Thomas Rau [(1)], Verena Scheper [(1)] - *Hannover, Germany [(1)]; Sulzbach, Germany [(2)]* |

## Genetics of Deafness and Gene Therapy

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| **P57** | **GWAS meta-analysis points to new loci for normal hearing function and age-related hearing loss**<br>Marco Brumat [(1)], Anna Morgan [(1)], Massimiliano Cocca [(1)], Massimo Mezzavilla [(1)], Daniela Toniolo [(2)], Guy Van Camp [(3)], Erik Fransen [(3)], Ginevra Biino [(4)], Umberto Ambrosetti [(2)], Sara Ghiselli [(1)], Paolo Gasparini [(1)], Giorgia Girotto [(1)] - *Trieste, Italy [(1)]; Milan, Italy [(2)]; Antwerp, Belgium [(3)]; Pavia, Italy [(4)]* |
| **P58** | **Genetic mutations in Non Syndromic Deafness patients, clinical cases of Varese**<br>Adelaide Bussini, Chiara Pessania, Francesco Pallotti, Rosario Casalone, Annalisa Meli, Eliana Cristofari - *Varese, Italy* |
| **P59** | **Audiological phenotype of GJB2 gene variants in Slovakia**<br>Lucia Demešová, Lukáš Varga, Zuzana Slobodová, Martina Škopková, Milan Profant, Daniela Gašperíková - *Bratislava, Slovakia (Slovak Republic)* |
| **P60** | **Genetic analysis of hearing loss: some relevant cases from Campania Region (Southern Italy)**<br>Carmela Monzillo, Domenico D'Errico, Rita Malesci, Pasqualina Apisa, Gennaro Auletta, Carla Laria, Angelo Sarnelli, Letizia Cruoglio, Elio Marciano, Annamaria Franze' - *Naples, Italy* |
| **P61** | **AAV mediated whirlin gene therapy for the treatment of DFNB31 and USH2D**<br>Hannah Goldberg [(1)], Yukako Asai [(1)], Bifeng Pan [(1)], Kevin Isgrid [(2)], Jun Yang [(3)], Wade Chien [(1)], Gwenaelle Geleoc [(1)] - *Boston, MA, United States [(1)]; Bethesda, MD, United States [(2)]; Salt Lake City, UT, United States [(3)]* |
| **P62** | **Gene therapy in a mouse model of genetic hearing loss**<br>Kyu-Yup Lee, Min-A Kim, Un-Kyung Kim - *Daegu, Republic of Korea* |
| **P63** | **Identification of a potential founder effect of a novel PDZD7 variant involved in moderate-to-severe sensorineural hearing loss in Koreans**<br>Sang-Yeon Lee, Byung Yoon Choi - *Seoul, Republic of Korea* |
| **P64** | **Rare KCNQ4 variants found in public databases underlie impaired channel activity that may contribute to hearing impairment**<br>Haiyue Lin, Hye Ji Choi, Jee Eun Yoo, Seung Ho Shin - *Seoul, Republic of Korea* |
| **P65** | **Cochlear Nerve Hypotrophy and Degeneration in a Mouse Model of Charcot-Marie-Tooth Disease Type 2E**<br>Kye Hoon Park [(1)], Young-Jun Chung [(1)], Hunki Paik [(2)], Ronald KH Liem [(2)], Ana H Kim [(2)] - *Cheonan, Republic of Korea [(1)]; New York, NY, United States [(2)]* |

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| **P66** | **Functions of NEDD4-2 in the auditory portion of mouse inner ear**<br>Lionel Ronald Pouyo, Laurence Delacroix, Brigitte Malgrange - *Liège, Belgium* |
| **P67** | **The creation of LOXHD1b gene knockout zebrafish**<br>Shunsuke Tarumoto, Youichi Asaoka, Kazuma Sugahara, Yoshinobu Hirose, Makoto Seiki, Hiroshi Yamashita - *Yamaguchi, Japan* |
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UNIVERSITÀ DEGLI STUDI DI PADOVA

DNS DIPARTIMENTO DI NEUROSCIENZE

Symposium &
56th Inner Ear Biology Workshop
"Hearing research: from history into the future"
*Chairs: Alessandro Martini, Fabio Mammano and Paolo Gasparini*

IEB 2019

7-10 SEPTEMBER 2019 - PADUA, ITALY

# ABSTRACTS

**Saturday 7 September 2019**

## INNER EAR BIOLOGY SYMPOSIUM

## "Hearing Research: From History into the Future"

# S1

**Highlight in Human Cochlear Microanatomy**

Helge Rask-Andersen [(1)] - Wei Liu [(2)] - Anneliese Schrott-Fischer [(3)] - Rudolf Glueckert [(3)]
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**Introduction**

We studied the human cochlear microanatomy using correlative electron microscopy and super resolution structured illumination immunohistochemistry in rare surgical specimens after ethical permission and patient approval. It allowed unprecedented structural and molecular preservation. Obtained data gave detailed information of the structure and molecular constituents of the human cochlea and how it relates to animal models. Results gained over a thirty-year period are presented with clinical correlations.

**Methods**

Super-resolution microscopy (SR-SIM) was performed at Uppsala SciLife national facilities (http://www.scilifelab.se/#). SR-SIM gave a lateral precision of 80 nm. Transmission, electron microscopy was performed at the ENT-Department in Uppsala using a JEOL 100SX microscope. Scanning electron microscopy (FE-SEM) was performed at the Department of Cell Biology in Innsbruck.using a ZEISS DSM982 Gemini field emission electron microscope at 5 kV. Maximum resolution was estimated to 2 nm. Coating with gold-palladium was performed to a nominal depth of 10-12 nm.

**Results**

The ion channel maschinery in the lateral cochlear wall ("battery") essential for mechano-electric transduction was analyzed. Na/K-ATPase, NKCC1, Kir4.1, Cx26, Cx30, Kv7.1, isolator protein Claudin-11 were reproduced at the nanoscopic level. Resident macrophages (IBA1) were widely distributed in the connective tissue, neurons and supporting cells. Spectacular cellular interactions were unveiled. The human inner ear including the 8$^{th}$ nerve seem to be controlled by a resident macrophage system.

**Conclusion**s

Electron microscopy and super-resolution microscopy were used to study the molecular expression and structure of the human cochlea. A neuro-immune axis may exist that can be essential for auditory nerve preservation and neuro-inflammation. Its roles in sensorineural hearing loss, cochlear implantation and cell renewal need further clarification. An immunologic key player may be the endolymphatic sac.

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## S2

**Tailoring tissue remodeling during early inner ear development: together we can**

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The understanding of the embryonic processes that lead to the formation of the adult organs is the base and major instrument for the progress of regenerative medicine. The embryonic development of organs requires tissue reshaping that is highly regulated at the genetic level. During the earliest stages of otic development, the otocyst undergoes rounds of cell proliferation to grow a spherical structure formed by a pseudostratified epithelium, which contains most of the molecular cues to form the adult inner ear.
Apoptosis has been proposed as the singular cellular process that sculpts the shape of the inner ear. However, recent evidences suggest that it is coordinated with other tissue-remodeling events in an increasing number of embryonic tissues. Cell senescence participates in tissue repair, cancer, aging, and, more recently, it has also been associated with embryonic morphogenesis. Senescent cells show a highly regulated temporal pattern in the developing vertebrate inner ear. Being more abundant in the dorsal otocysts where the primordium of the endolymphatic duct is being formed. Transforming growth factor β2 (TGF β2), its receptors and downstream targets drive the spatiotemporal senescent response leading to morphogenesis and cell differentiation.
Finally, otocyst remodeling requires metabolic energy for the elimination of damaged organelles and apoptotic cells. Autophagy catabolic pathways keep energy homeostasis by directing cargo to the endolysosomal system and actively participate in the elimination of death cells.
In summary, autophagy, apoptosis and cell senescence have distinct and complementary roles during early otic morphogenesis. Cell senescence and apoptotic cell death coordinately contribute to tissue remodeling along the development of the vertebrate inner ear.

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## S3

**Inner Ear Drug Delivery by Precise Perforation**

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Protected by one of the hardest bones in body, the cochlea is nearly an impenetrable structure frustrating both bacteria and clinician trying to gain access to it. As a result, a means for reliable delivery of agents into the inner ear for therapeutic purposes remains a formidable challenge. No method currently exists to provide effective and precisely dosed delivery of therapeutics to the inner ear without risking permanent damage to the patient's hearing. We believe that an elegant solution to overcome the difficulties of intracochlear delivery is to use microneedles to facilitate reliable and predictable intracochlear delivery across the RWM without anatomic or functional damage. Intracochlear drug administration has been shown to be superior to transtympanic injection and results in significantly higher and less variable drug levels. In addition, there is a much smaller concentration gradient from base to apex, as is typical of transtympanic injection, resulting in a more even distribution of material. With the availability of a reliable method of inner ear delivery, targeted delivery to hair cells, spiral ganglion neurons or other intra-cochlear structures could be accomplished for the treatment of variety of auditory and vestibular disorders such as sudden or progressive SNHL, Ménière's Disease, and tinnitus.

## S4

**Central auditory prostheses: challenges and potentials**

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Central auditory prostheses have been used for hearing restoration in subjects not eligible for cochlear implants. The most successful has been the brainstem implant. However, few is known about the physiological mechanisms of central stimulation, particularly about the differences to cochlear stimulation. Auditory midbrain and cortex stimulation have so far not provided sufficient success. We focused on the central auditory microstimulation and compared the responses in the auditory cortex to stimulation of the midbrain (Quass et al., 2018, Brain Stim) and cortex (Voigt et al., 2017, Brain Stim) to acoustic responses.
The results have shown several differences in responsiveness compared to acoustic stimulation, particularly a smaller dynamic range. Current focusing approaches, effective in cochlear implants, proved an unsuccessful strategy with central auditory implants, with no significant effect on spread of excitation due to the intimate contact of the electrode and the stimulated neurons (Quass et al., 2018). Furthermore, stimulation in the midbrain generated an asymmetric spread of excitation in the cortex. With cortical microstimulation we could identify the high stimulation currents used in previous studies as one main problem (Voigt et al., 2017, Brain Stim; Voigt et al., 2019, J Neural Eng), causing an artificial activity in the whole cortical column. Using low current stimulation, particularly in the main input layer of the cortex, layer IV, evoked focused responses that could be integrated into ongoing processing in a more natural way (Voigt et al., 2018, J Neurosci). Such stimulation could even be used to boost induced cortical activity, a footstep of corticocortical interactions.
The present data indicate that microstimulation in the central auditory system is a promising technique if stimulation is adapted to the needs of the central auditory structures.

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## S5

**Cochlear neuroregeneration: progressing towards the clinical use of human pluripotent stem cells**

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The only treatment for profound neurosensory hearing loss available today is the application of an electronic prosthesis, the cochlear implant. This device converts a sound stimulus into an electrical signal, functionally replacing the sensory hair cells. However, patients must have a working auditory nerve in order to convey the information to the brain. Thus, this is not a suitable therapeutic option for everyone. Up to now, no biological regenerative solution to restore any part of the cochlea is clinically available.
Our lab has been exploring the use of stem cells in general, and human pluripotent stem cells in particular, to develop a regenerative strategy to repair 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. Currently, 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. Ongoing studies using qPCR for human-specific sequences show that cells do not spread systemically and are not detected in other organs.
We have also developed a new gerbil model with a two-pronged sensorineural hearing loss - auditory neuropathy is induced with topical ouabain and subsequently the hair cells are lesioned with a kanamycin/furosemide treatment. To recapitulate cochlear implantation, we are using a fully-implantable rodent stimulator in which the electrode is activated by a magnetic field. Initially, we implanted animals in which only the hair cells were damaged. Brainstem evoked responses were obtained after electrode stimulation, and animals showed behavioural changes compatible with auditory responses. We are now combining the cochlear implant prototypes with the rebuilding of the auditory nerve using hONPs. As before, these progenitors were produced using a hESC line carrying a fluorescent gene that is expressed by the otic lineages, allowing for the purification of the otic cells prior to transplantation. Histology suggests that the transplanted cells survive and differentiate in the implanted animals, with neural fibres tracking towards the implant. These developments further support the potential use of hESC-derived ONPs for the restoration of the cochlea and should facilitate their clinical use.

## S6

**Padua cradle of modern medicine**

Giorgio Zanchin (1)
*Padua University Medical School (1)*

During late Middle Age, in Europe different factors contributed to a renewed attention focused upon the physical world in opposition to theological themes, in particular the rediscovery of the so called "physical" writings of Aristotle. As an expression of these naturalistic interests in the anatomical field, during the 1300s the dissection of the human body began to be performed in the Bologna-Padua area. The renaissance of medical studies gave exceptional results in Padua, such as the birth of modern anatomy with the publication of the Fabrica (1543) by Andreas Vesalius, the method of clinical instruction at the bedside of the patient by Giovanni Battista da Monte (1489–1551). Girolamo Fracastoro, in his De contagione et contagiosis morbis (1546), was the first to hypothesize the presence of “seminaria morbi”, foreseeing the microbial theories established only three centuries later. Moreover, the foudation of the Botanical Garden (1545) by Francesco Bonafede allowed the “ostensio simplicium”, that is the demonstration and the study for therapeutic purpose of real plants, the naturalistic counterpart of the “lectura simplicium”, i.e. the theoretical description of the subject. At the end of the century Fabrici d’Acquapendente gave outstanding contributions renovating the embryological studies and describing the venous valves. The first permanent anatomical theatre that Fabrici erected in 1594, became the model of the demonstrative teaching of anatomy in Europe, since similar structures were to be built by pupils returning from their medical studies in Padua to universities such as Leiden, Copenhagen, Basel.

A second flourishing season of our Medical School took place in the 18th century, with the foundation of Occupational Medicine by Ramazzini (De morbis artificum diatriba, 1700), and with the shift from humoral galenic medicine to solidistic medicine, through the introduction of the anatomo-clinical method by Morgagni (De sedibus et causis morborum per anatomen indagatis, 1761).

For the facts considered above, there is general consensus among medical historians on the pivotal role played by the Padua Medical School in the development of medical knowledge and a prominent historian of Medicine, Henry Sigerist, quoted our University as “the cradle of modern medicine”.

**Sunday 8 September 2019**

# 56th Inner Ear Biology Workshop

## ORAL COMMUNICATIONS

## SESSION I

### REGENERATION, STEM CELLS AND DEVELOPMENTAL BIOLOGY

## TL1

**In vitro expansion of human fetal cochlear duct cells and differentiation into functional hair cells in 3D organoids**

Marta Roccio [1,2] - Silvia Erni [1,2,3] - Carlotta Palaferri [1,2,3] - Michael Perny [1,2,3] - Megan Ealy [6,7,8] - Hans Ruedi Widmer [2,5] - Stefan Heller [6,7] - Pascal Senn [1,2,4]

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Sensory hair cells located in the organ of Corti are essential for cochlear mechanosensation. Their loss is irreversible in humans resulting in permanent hearing loss. The development of therapeutic interventions for hearing loss requires fundamental knowledge about similarities and potential differences between animal models and human inner development as well as the establishment of human cell based-assays.
We have carried out a systematic analysis of the fetal human inner ear in a temporal window spanning from week 8 to week 12 post conception, when cochlear hair cells become specified. We analyzed gene and protein expression of the developing cochlear duct, the spiral ganglion and vestibular tissue. We have identified surface markers for the cochlear prosensory domain, namely EPCAM and CD271, that allow to purify postmitotic hair cell progenitors. When placed in culture, in three-dimensional organoids, these regained proliferative potential and at the same time maintained marker expression and features of the native tissue. We could further differentiate these tissue resident progenitors into hair cell-like cells *in vitro*, displaying expression of hair cell markers such as BRN3C and MYO7A, F-Actin and Espin positive hair bundles. The competence of the generated hair cells to uptake dyes such as FM1-43 and aminoglycosides antibiotics such as gentamycin, indicates active mechano-electrical transduction channels, and points to the fact that they could be used to test ototoxicity and regeneration. Expansion and differentiation yields from fetal tissue are still not suitable for drug screening purposes. We are now analyzing new approaches to overcome these limitations, exploiting neonatal rodent sensory epithelia for testing.
These results provide a foundation for comparative studies with otic cells generated from human pluripotent stem cells and for establishing novel cell based platforms for drug validation.

## C01

**Comprehensive analyses of gene expressions in the developing primate cochlea**

Makoto Hosoya [1] - Masato Fujioka [1] - Kaoru Ogawa [1]

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Considering regenerative therapy, understanding of organ development and tissue generation is important, because re-track developmental steps are one of the promising ways for regeneration. Moreover, recently pluripotent stem cell based cell therapy has been developed and expected to be a feasible therapeutic way by replacing damaged cells to new targeted cells induced from pluripotent stem cell including embryonic

stem cells or induced pluripotent stem cells (iPS cells). Detailed developmental information about the targeting organ or cells are required for inducing well differentiated cells efficiently.
Much of the understanding of the cochlear development has been derived from rodent model. These knowledges from mouse model are useful for understanding basic our human cochlear development and would be beneficial for realizing hearing regenerative therapy. However, there are several gap for applying rodent model to human. First, it is known that some mouse model fail to reproduce human congenital hearing loss model. This means that the factor involving the cochlear development have different rules between the rodent and human at least in some cases. Secondly, it was known that for inducing cochlear hair cells from embryonic stem cells or iPS cells, there was no bipotent induction method and we have to choose different ways for mouse cell or human cells. This fact suggests that there might be some different factors involving each developmental steps. Therefore, it is important for understanding human cochlear development. However, there are more limited information about human cochlear development, especially from the molecular biology view point, because of rarity of chance to assess human fetal sample or ethical hardness in some country. Moreover if human fetal samples were available, it would be suitable for only anatomical or histopathological analysis and not to be used for molecular biological analysis for tissue preparation problems.
Therefore, we used non-human primates to investigate the development of hearing organs. In particular, we were interested in a small New World monkey species, the common marmoset (Callithrix jacchus), which has been investigated for hereditary hearing loss research in this field. Moreover, now genetic modification is possible in the common marmoset we can use this species as a non-human primate model for the developmental analysis.
Here, we report comprehensive analyses of gene expressions in the developing primate cochlea of common marmoset and comparing the developmental stages between the common marmosets, mice and human for basic analyses toward inner ear regeneration in human.

## C02

**Wnt Signalling Regulates the Formation of Inner Ear Sensory Organs by Antagonizing Prosensory signals**

Magdalena Zak [(1)] - Vincent Plagnol [(2)] - Nicolas Daudet [(1)]
*University College London, Ear Institute, London, United Kingdom [(1)] - University College London, Genetics Institute, London, United Kingdom [(2)]*

The inner ear is composed of the auditory organ responsible for sound detection and the vestibular system providing the sense of balance and acceleration. The mechanisms controlling their embryonic formation in the early otocyst remain unclear. Our recent work has shown that several of the sensory organs arise by progressive segregation from a common ‘pan-sensory’ domain, in which Notch signalling propagates prosensory identity by lateral induction. Wnt/β-catenin signalling is another pathway previously implicated in the development of the sensory organs, but its role in the sensory organ formation and whether it interacts with Notch in this context is unknown. Here, we show that in the developing inner ear Wnt signalling gradually expands in the dorsal part where it forms a gradient of Wnt activity. Our functional experiments in vivo revealed that Wnt signalling promotes non-sensory fate and the progression of Wnt activity coincides with the loss of neurosensory competence in the dorsal otocyst. Furthermore, Wnt antagonises Notch signalling and restricts the spatial pattern of Notch activity in the otocyst. These results suggest that Wnt signalling is part of the mechanism regulating formation and positioning of prosensory domains. RNA-Seq and bioinformatics analyses suggest that Wnt controls neurosensory specification by regulating the expression of proneural genes and inhibitors from bHLH and SRY-boxes families. Further investigation will show whether some of these genes are part of the mechanisms by which Wnt regulates prosensory organ formation in the otocyst.

## C03

**Proteostasis is essential during cochlear development for neuron survival and hair cell polarity**

Steve Freeman [(1)] - Susana Mateo-Sanchez [(1)] - Ronald Pouyo [(1)] - Brigitte Malgrange [(1)] - Laurence Delacroix [(1)]
*University of Liege, GIGA Neurosciences - Developmental Neurobiology Unit, Liege, Belgium [(1)]*

Protein homeostasis is essential to cell function, and a compromised ability to reduce the load of misfolded and aggregated proteins is linked to numerous age-related diseases, including hearing loss. Here, we show that altered proteostasis consequent to Elongator complex deficiency also impacts the proper development of the cochlea and results in deafness. In the absence of the catalytic subunit Elp3, differentiating spiral ganglion neurons display large aggresome-like structures and undergo apoptosis before birth. The cochlear mechanosensory cells are able to survive to proteostasis disruption but suffer defects in polarity and stereociliary bundle morphogenesis. We demonstrate that protein aggregates accumulate at the apical surface of hair cells, where they cause a local slowdown of microtubular trafficking, altering the distribution of intrinsic polarity proteins LGN, G□$_{i3}$ and aPKC and affecting kinocilium position and length. Alleviation of protein misfolding using the chemical chaperone 4-phenylbutyric acid during embryonic development ameliorates hair cell polarity in Elp3-deficient animals. Our study highlights the importance of developmental proteostasis in the cochlea and unveils an unexpected link between proteome integrity and polarized organisation of cellular components.

## C04

**Frizzled3 and Frizzled6 Cooperate with Vangl2 to Direct Cochlear Innervation by Type II Spiral Ganglion Neurons**

Michael Deans [(1)]
*University of Utah, Otolaryngology, Salt Lake City, United States [(1)]*

Type II spiral ganglion neurons provide afferent innervation to outer hair cells of the cochlea and are proposed to have nociceptive functions important for auditory function and homeostasis. These neurons are anatomically distinct from other classes of spiral ganglion neurons because they extend a peripheral axon beyond the inner hair cells that subsequently makes a distinct 90 degree turn towards the cochlear base. As a result, patterns of outer hair cell innervation are coordinated with the tonotopic organization of the cochlea. Previously we demonstrated that peripheral axon turning is directed by a non-autonomous function of the core planar cell polarity (PCP) protein VANGL2. Here we demonstrate that the Frizzled (FZD) receptors encoded by *Fzd3* and *Fzd6* similarly regulate axon turning, are functionally redundant with each other, and genetically interact with *Vangl2* to guide this process. FZD3 and FZD6 proteins are asymmetrically distributed along the basolateral wall of cochlear supporting cells, and are required to promote or maintain the asymmetric distribution of VANGL2 and CELSR1. These data indicate that intact PCP complexes formed between cochlear supporting cells contribute to the non-autonomous regulation of axon pathfinding during cochlear innervation. Consistent with this hypothesis, in the absence of PCP signaling type II SGN peripheral axons turn randomly and often project towards the cochlear apex. Additional analyses of *Porcn* mutants in which WNT secretion is reduced suggest that non-canonical WNT signaling establishes or maintains PCP signaling in this context. A deeper understanding of these mechanisms is necessary for repairing auditory circuits following acoustic trauma or promoting cochlear re-innervation for the success of regeneration-based deafness therapies.

## C05

**Differential expression of transmembrane channel-like protein 1 (TMC1) during fetal development of the human inner ear**

E.S.A. van Beelen [(1)] - W.H. van der Valk [(1)] - J.C.M.J. de Groot [(1)] - H. Locher [(1)] - P.P.G. van Benthem [(1)]
*Leiden University Medical Center, Otorhinolaryngology, Leiden, Netherlands [(1)]*

Inner ear hair cells convert mechanical stimuli into electrical signals. Mechanoelectrical transduction (MET) is mediated by mechanosensitive ion channels located in the tips of stereocilia. It has recently been confirmed that the transmembrane channel-like protein 1 (TMC1) is a pore-forming component of the MET channel of both cochlear and vestibular hair cells. Mutations in the *TMC1* gene cause sensorineural hearing loss, but no vestibular dysfunction, in mice and humans.

The aim of the present study was to investigate the onset of expression of the TMC1 protein and its distribution during embryological development of the human inner ear.
Human fetal inner ears were obtained at different fetal ages (gestational weeks 12-16), decalcified and embedded in paraffin. Some specimens were cleaved along a midmodiolar plane followed by dissection of the membranous labyrinth including the cochlear duct and the vestibular organs. In addition, human adult vestibular organs were obtained during vestibular schwannoma surgery. As positive controls, microdissected cochlear quarter turns from postnatal mice were used. TMC1 expression was visualized by means of an indirect immunofluorescent technique in paraffin sections and in dissected whole mounts. Hair cells were identified using phalloidin staining.
The TMC1 protein is present in the immature stereociliary bundles in the human fetal cochlea and its intracochlear distribution demonstrates a spatiotemporal gradient. To the best of our knowledge, this is the first study to demonstrate the presence and distribution of TMC1 during human inner ear development.

# C06

**Single-cell transcriptomics of chick auditory epithelial cells during hair cell regeneration**

Takayuki Nakagawa [1] - Mami Matsunaga [1] - Tomoko Kita [1] - Ryosuke Yamamoto [1] - Hiroe Ohnishi [1] - Norio Yamamoto [1] - Koichi Omori [1] - Satoko Sakamoto [2] - Akira Watanabe [2]
*Kyoto University Graduate School of Medicine, Otolaryngology, Kyoto, Japan [1] - Kyoto University, CiRA, Life Science Frontiers, Kyoto, Japan [2]*

The limited capacity of mammalian cochleae for hair cell regeneration is closely related to the difficulty in the treatment of sensorineural hearing loss. Aiming to explore key molecules for hair cell regeneration in mammals, the regeneration process in the avian auditory epithelia has been investigated. However, investigations of avian basilar papillae have revealed few information that could be utilized for realizing hair cell regeneration in mammals. To overcome this issue, we planned single-cell transcriptomics of chick basilar papillae during hair cell regeneration.
First, we aimed to establish a hair cell regeneration model of chick auditory epithelia using explant cultures. Exposure to streptomycin for 2 days caused nearly total hair cell loss. After additional culture for 4 days, newly generated, but immature, hair cells appeared in explant cultures. EdU incorporating assay demonstrated that new hair cells were generated through direct conversion of supporting cells, not through proliferation of supporting cells. We also examined chick auditory epithelium explants that were cultured without the exposure to streptomycin. In specimens after 6 day-culture, newly generated, immature hair cells appeared in explant cultures. These findings suggest that hair cell regeneration via direct conversion of supporting cell occurred in explant cultures of chick auditory epithelia, which is the main pathway for hair cell regeneration. In addition, spontaneous replacement of hair cells occurred in chick auditory epithelia in culture condition unlike in vivo.
We then conducted single-cell RNA sequencing of intact and cultured chick auditory epithelia with or without exposure to streptomycin using C1 Single-Cell Auto Prep system. Prior to library preparation of the cells, we prepared single-cell suspensions from auditory epithelial cells. Gene expression analysis of cell-type specific markers identified cell clusters, corresponding to supporting cells and hair cells. We also identified the candidate trans-differentiating cells from supporting cells to hair cells. The results provided a new insight into the molecular basis of hair cell regeneration through direct conversion of supporting cells to hair cells in chick auditory epithelia.

**Keywords**: hair cell regeneration; chick basilar papilla; single-cell RNA sequencing

# C07

**Evidence of intrinsic multi-potent/stem cells in the mature porcine cochlea**

Desmond Nunez [1] - Printha Wijesinghe [1] - Boyuan Zheng [1] - Elizabeth Hui [1] - Germain Ho [1]
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**Background**
Cochlear hair cell loss in humans which underlies many forms of hearing loss is accepted as irreversible in contrast to other animals such as birds. Stem/progenitor cells have been identified in the mature mammalian vestibule but not in the mature cochlea. This study characterizes cells derived from the cochleae of mature pigs (Sus Scorfa) and determines the persistence of inner ear cell characteristics across several cell culture passages.

**Methods**
Twenty-four temporal bone labyrinth capsules were harvested from 12 adult pigs, age range 15-19 weeks (*Sus scrofa*). The cochlea duct was extracted piecemeal and macerated under microscopic control. Two-thirds of the resulting tissues were collected in 15ml sterile tubes containing 10ml Dulbecco's phosphate-buffered saline (DPBS) to which 2ml of 0.25% trypsin-EDTA was added prior to incubation at 37$^{o}$C for 20-25 minute. The remaining tissue was used for RNA extraction. The cells were then placed in a growth medium consisting of Dulbecco's Modified Eagle Medium (DMEM) enriched with 10% Fetal Bovine Serum (FBS) on a cell culture plate in an incubator at 37$^{o}$C and 5% $CO_2$ for 3-4 days. When cell growth reached greater than 80% confluence, the cells were washed with DPBS, then trypsinized using 250µl 0.25% trypsin-EDTA per well for 5 minutes. One-third portion of the passage 1 (P1) cell pellet was re-suspended in fresh growth medium and placed in $T_{25}$ vented culture flasks for the generation of the next passaged cells (P2 cells). Phase-contrast microscopy, Scanning electron microscopy (SEM), fluorescence immunocytochemistry and quantitative reverse transcription polymerase chain reaction (RT-qPCR) were used to characterize the cells.

**Results**
Primary cultures were heterogeneous and formed monolayers. Sphere forming cells were identified on phase-contrast microscopy. SEM demonstrated that the sphere-forming cells displayed stemcell ultrastructural features. Hair cell markers myosin VIIa and prestin; and stem –cell markers nestin and Sox2 were identified on fluorescence immunocytochemistry across all passages. Nestin gene expression level was high in the harvested cochlear tissue and persisted through passages tested up to P6. Gene expression levels of supporting cell marker cytokeratin 18 and hair cell markers myosin VIIa and prestin were significantly positively correlated [p=0.005, Spearman correlation (τ) =0.943].

**Conclusion**
Inner ear hair cell and supporting cell characteristics persist across passages up to P6. Innate otic stem/progenitor cells exist in the mature porcine cochlea.

**Keywords:** Porcine, inner-ear, multi-potent, stem/progenitor cell

# C08

**How the computational methods can contribute to better knowledge of the ear**

Fernanda Gentil [(1)] - Marco Parente [(2)] - Carla Santos [(2)] - Bruno Areias [(2)] - Jorge Belinha [(3)] - Renato Natal Jorge [(2)]
*Escola Superior de Saúde - ESS, IPP, Porto, Portugal [(1)] - FEUP, INEGI, Porto, Portugal [(2)] - IPP, ISEP, Porto, Portugal [(3)]*

The biomechanical behavior of the human ear can be studied using mathematical approaches, such as the finite element method. This methodology allows to perform simulations to evaluate the influence of sound transmission through all way of the ear, since the sound is selected by the outer ear to the inner ear.
The finite element method is currently the preferred method for the numerical solution of partial differential equations, and it is used in several fields of the biological sciences. Due to its large applicability and efficiency, there are several works where this methodology is applied in different specialties, to analyze loads, strains or displacements, as in the case of the biomechanical study of the ear. From CT images, three-dimensional accurate computational models are created. The technique employs a set of calculations of numerical analysis for solutions of kinematic (displacements and rotations) and mechanical aspects (strength, stress, pressure, deformation). The use of these computer models enables the motion analysis of all ossicular chain points at any moment.

More specifically, these are the following steps performed in this work:
1. From CT images, it was taken the geometry of the tympanic membrane and ossicles.
2. The respective discretization into finite elements, was performed.
3. The appropriate material properties were assigned.
4. The boundary conditions were defined.
5. Several static and dynamic studies of the behavior of the model were made.
6. Calculation of the umbo and stapes footplate displacements.

CAD software was used to treat each of the parts that compose the human ear. The model was composed of

the external auditory canal, eardrum, ossicles, two muscles, six ligaments, two joints, the temporal bone and the cochlea.
The model was simulated numerically with the Abaqus software.
The frequency band used in this study was between 100 Hz and 10 kHz.
After the construction of the model representative of the normal ear, the results can be compared with other models illustrating some ear diseases, such as eardrum perforations, tympanosclerosis, otosclerosis, tumors, as well as inner ear disorders. Other application can be related with prosthesis replacement in the middle ear (stapes prosthesis or all ossicular chain), as well as a simulation of electrode insertion in cases of cochlear implant. These models are created according to the specification of the surgical techniques described by various authors.

The aim of this study is to analyse different situations to improve the understanding of ear pathologies and its rehabilitation.
In cases of eardrum perforations, we can conclude, for example, that different sizes of eardrum perforations result in different displacements, being smaller with bigger perforations. When simulating otosclerosis, it can be concluded that there was a decrease of the displacements for all frequencies, with the increase of stiffness of annular ligament.

With this technique it is possible to promote the best possible performance of prostheses in the ear, in order to know the best results for different diameter and weight of these prosthesis.
The present study allows to conclude that computational methods can help the audiological evaluation/rehabilitation of the ear.

**Keywords**: Biomechanics,Ear,Computational,Finite Element Method

## SESSION II

### EAR PHYSIOLOGY AND AGING

# TL1

**The voltage and frequency dependence of prestin nonlinear capacitance is time-dependent**

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*Yale University, Surgery, Neuroscience, Cellular and Molecular Physiology, New Haven, United States [(1)]*

Prestin (SLC26a5) underlies outer hair cell (OHC) mechanical activity (eM). The response is driven by voltage resulting from prestin's voltage-dependent conformational changes that couple into length changes of the cell. These conformational changes are measurable as an electrical correlate of eM, i.e., nonlinear capacitance (NLC), which is maximal at Vh, the voltage where prestin charge is distributed equally across the OHC lateral membrane.
We have recently found that NLC and eM show low pass behavior that counters current dogma. NLC and eM exhibit multi-exponential components, and the frequency response is dependent on holding voltage relative to Vh, that is, it is voltage-dependent (Santos-Sacchi and Tan, JNeuro, 2018).
We have also shown that NLC is not stationary in time following a voltage step (Santos-Sacchi et al., JPhysiol, 1998). An apparent shift in Vh occurs over the time course of the step duration. For a simple two state Boltzmann process, the ratio of forward to backward transition rates between the states sets the location of Vh along the excitable voltage range. Thus, we hypothesize that the frequency response of prestin NLC alters during prolonged voltage steps. Here we directly test this idea.
We used macro-patches ( ~4 □m inner pipette tip diameter; seal > 3G□) of guinea pig and mouse lateral membrane under voltage clamp, where voltage control is excellent. Voltage chirp arrays (32 contiguous chirps, 4096 pts each at 10 us sampling; 10 mV peak) were summed with 20 mV step offsets from -160 to +160 mV). Stray and linear capacitive currents were removed by subtracting the AC response at +160 mV, where NLC is absent. The resulting nonlinear capacitive currents were used to solve for membrane capacitance with the dual sine-technique (Santos-Sacchi, JBiophys, 2004) at harmonic frequencies, and Boltzmann fits were made to extract the parameters Vh, Qmax, and z. Measures were made from 390 to 19000 Hz.
We find that the voltage and frequency dependence of NLC changes over the course of step durations. Notably, a low frequency component of NLC diminishes over time. Vh shifts positively across all frequencies, but is fairly stable across frequency. *z*, a measure of voltage sensitivity, is smaller at early times, but decreases across frequency at all durations. Importantly, our data illustrate that experimental approaches that average eM, NLC, or possibly even in vivo electrophysiological correlates of OHC activity in order to enhance signal-to-noise will miss important aspects of OHC performance.
**Key words**: Outer hair cell, prestin, capacitance

*(Supported by NIH-NIDCD R01 DC016318 and R01 DC008130.)*

# C09

**Emilin 2 promotes the stiffness and smooth mechanical gradient of the cochlear basilar membrane that is essential for fine, consistent, frequency resolution**

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*University of Brighton, Sensory Neuroscience Research Group, School of Pharmacy and Biomolecular Sciences, Brighton, United Kingdom [(1)] - National institutes of Health, Laboratory of Endocrinology and Recent Biology, NIDDK, Bethesda, Maryland, United States [(2)]*

Perception of the component frequencies in sound is fundamental to hearing and depends upon frequency-specific mechanical responses at defined locations along the length of the cochlear basilar membrane (BM). Mechanosensory outer hair cells transduce and amplify these responses and through interaction with other major non-cellular cochlear matrices excite inner hair cells, which signal their responses to fibers of the auditory nerve that convey frequency place-specific information to the brain. Each frequency component of

incoming sound has its own sweet-spot on the BM, thereby creating a tonotopic frequency-to-place map. Perceptual frequency discrimination requires fine resolution of this map but little is known of intrinsic molecular features that demarcate the place of response on the BM. We report that protein emilin 2 organizes the filamentous architecture in the extracellular BM. The filamentous organization of the BM appeared less orderly in *Emilin2*-/- mice than in wild-type mice when analyzed by a novel form of polarized light microscopy that takes advantage of the anisotropy of the refractive index (birefringence) of structures. Mechanical and electrophysiological measurements reveal that *Emilin2*-/- mice display broadened mechanical and neural frequency tuning with multiple response peaks that are shifted to lower frequencies than normal and sensitized low frequency tails. Thus, emilin 2 promotes the stiffness and smooth mechanical gradient of the BM that is essential for the precise and consistent frequency resolution of the mammalian hearing system.

## C10

**Membrane traffic in the outer hair cell**

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Coated and uncoated vesicles have been shown both at the apical (Kachar *et al.*, 1997) and synaptic (Nadol, 1983; Siegel & Brownell, 1986) poles of hair cells. However, the physiological relevance of endocytic activity is still in debate. Intense endocytic activity was demonstrated in isolated (Meyer *et al.*, 2001) and in-situ (Griesinger *et al.*, 2004) outer hair cells (OHCs) using the fluorescence plasma membrane marker FM1-43, in combination with live confocal imaging. Membrane bound vesicles endocytosed at the cuticular plate are transcytosed to the basolateral membrane and along the central strand towards the nucleus (Griesinger *et al.*, 2004; Kaneko *et al.*, 2006). A local double-barrel perfusion technique has been developed to visual uptake and transport from either pole in the same cell (Harasztosi *et al.*, 2018). It was demonstrated that endocytic activity at the synaptic pole and basoapical trafficking is more intense than at the cuticular plate and trafficking towards the synaptic pole (Harasztosi *et al.*, 2018; Harasztosi & Gummer, 2019). The aim of the present study was to identify the different types of endocytic activities and trafficking mechanisms in OHCs.

FM1-43, as plasma membrane marker, was applied locally to OHCs isolated from the adult guinea-pig cochlea. Confocal laser scanning microscopy was used to visualize live cell labelling. Phenylarsine oxide, a blocker of pinocytosis and phagocytosis, and concanavalin-A, an inhibitor of clathrin-mediated process-were used to identify the different types of endocytosis. The presence of kinesin, dynein and myosin VI dependent trafficking mechanisms, were tested in both the apicobasal and basoapical directions using inhibitors of monastrol, 2,4,6-triiodophenol or ciliobrevin-D.
Both endocytosis blockers, concanavalin-A and phenylarsine oxide, reduced the dye uptake in both the infracuticular and synaptic regions. In the case of apicobasal traffic, 2,4,6-triiodophenol significantly reduced the speed of vesicle traffic. However, neither monastrol nor ciliobrevin-D had statistically significant effect on the speed. In the case of basoapical traffic, all the three applied inhibitors, monastrol, ciliobrevin-D and 2,4,6-triiodophenol, significantly reduced the speed of vesicle traffic.

These data demonstrate the presence of both clathrin-mediated and non-clathrin-mediated processes at both poles of OHCs. However, the contribution of these processes to the total endocytic activity at the opposite poles of the cell do not appear to be equal. Data also imply myosin VI dependent trafficking in both the apicobasal and the basoapical directions, but that kinesin- and dynein-dependent processes participate only in the basoapical vesicle traffic.

**Keywords:** Cochlea, Endocytosis, Pinocytosis, Transcytosis, FM dyes

# C11

**Correlation analysis of inner hair cell Ca2+ action potential activity and spontaneous Ca2+ signaling in non-sensory cells of the pre-hearing mouse cochlea**

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Spontaneous $Ca^{2+}$-dependent action potential (AP) activity in inner hair cells (IHCs) and $Ca^{2+}$ waves in non-sensory cells in the greater epithelial ridge (GER) were recorded from the apical coil of the immature mouse cochlea (P4-P5) bathed in a perilymph-like extracellular solution. By combining $Ca^{2+}$ imaging and cell-attached patch clamp recordings, we confirmed that the majority of the observed IHCs $Ca^{2+}$ transients represent the optical readout of AP activity. Of note, IHCs fired $Ca^{2+}$ APs also in the complete absence of detectable $Ca^{2+}$ activity in the GER. In these conditions, the IHC-IHC average correlation index, *c*, was not significantly different from 0, indicating that IHCs generated APs independently of each other. Patch clamp recordings from IHCs isolated from the rest of the sensory epithelium confirmed that this firing activity is an intrinsic property of immature IHCs. Both $Ca^{2+}$ waves in the GER and IHC synchronous large-amplitude $Ca^{2+}$ transients were abrogated by P2Y and P2X receptor antagonists suramin (200 μM) and PPADS (100 μM), as well as by thapsigargin (10 μM), whereas ryanodine (50 μM) was not effective. However these drugs failed to suppress $Ca^{2+}$ APs in IHCs. In contrast, the latter were obliterated by nimodipine (50 μM), whereas $Ca^{2+}$ waves in the GER were able to propagate in the presence of this L-type $Ca^{2+}$ channels inhibitor.
When bursts of IHC AP activity were detected in the presence of GER $Ca^{2+}$ waves, AP frequency, *c* index and burst duration increased significantly compared to the condition when no waves were observed. The average longitudinal and radial dimensions of the GER area showing significant correlation with AP activity of a given IHC were 310 ± 49 μm and 120 ± 11 μm, respectively ($n$ = 7 cochleae). Together, these results indicate that GER $Ca^{2+}$ waves promote synchronization of IHC AP activity by variable amounts, depending on wave extension.
Of note, IHC depolarization under whole cell patch clamp conditions triggered $Ca^{2+}$ signals in nearby non-sensory cells, with a delay that was proportional to the distance from the stimulated IHC. This results is consistent with the observation that a fraction of the $Ca^{2+}$ waves appeared to depart from the IHC region, pointing towards the bulk of the GER. Therefore there appears to be reciprocal relationship between $Ca^{2+}$ signals generated by IHCs and by non-sensory cells of the GER, each of which can be detected in the absence of the other.

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# C12

**Mechanical creep of the hair bundle is not correlated with Ca2+-dependent slow adaptation.**

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Hair cells of the inner ear are the sensory cells of the auditory and vestibular system that transduce mechanical forces arising from sound waves and head movement. Deflection of the hair bundle, a crosslinked and staircased cluster of stereocilia on the apical surface of a hair cell, gates mechanically sensitive ion channels (MET channels) to start the mechanotransduction process. The mechanotransduction complex is built around the tip-link, an extracellular filament that connects the top of shorter stereocilia with the side of the next taller row. One important property of the kinetics of mechanotransduction currents is a decline in current during a sustained displacement stimulus, a process called adaptation. Adaptation increases the dynamic range of the system and optimizes the system's sensitivity. Adaptation can be divided into multiple different components. The molecular mechanism underlying the $Ca^{2+}$-dependent slow (motor) adaptation is hypothesized to be the motor model of adaptation. In the model, unconventional myosin-1c (Myo1c) climb along the sides of stereocilia to generate resting tension in the tip link. Upon stimulation of the hair bundle, $Ca^{2+}$ influx through mechanotransduction channels causes unconventional Myo1c to release tip-

link tension by sliding down the sides of the stereocilia, thus allowing mechanotransduction channels to close (Holt, et al., 2002; Howard & Hudspeth, 1987). A key finding that led to the climbing and slipping model is a mechanical creep that correlated with observed slow adaptation in the current during a force step stimulation of the hair bundle using a flexible fiber (Howard & Hudspeth, 1987). However, this correlation has not been rigorously tested. Using rat and gerbil auditory and vestibular hair cells, high-speed imaging, and pharmacology, we investigated the mechanisms of slow adaptation and the mechanical creep. Our results confirmed that slow adaptation is dependent on $Ca^{2+}$ influx and requires myosin motor activity. However, the mechanical creep of the bundle is not correlated with the amount of the adaptation in cochlear or vestibular hair cells. Additionally, when the Myo1c (the putative adaptation motor) activity is blocked in vestibular hair cells, the mechanical creep is unaffected. Our results show that the current motor model cannot be the molecular mechanism behind the $Ca^{2+}$-dependent slow adaptation, and we require new molecular models of slow adaptation in hair cells.

**Keywords**: Mechanotransduction, motor model, bundle creep, adaptation.

# C13

**Age-related structural changes at auditory hair cell ribbon synapses: the role of cadherin23 and otoferlin**

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Hearing relies on faithful synaptic transmission at the afferent ribbon synapses contacting the cochlear inner hair cells (IHCs). At these glutamatergic synapses, IHCs have a unique presynaptic organelle, the synaptic ribbon, which is essential for aggregating synaptic vesicles and organizing $Ca^{2+}$ channels at the active zone. The morphological and functional changes occurring in the organization of the IHC synaptic ribbons during development and aging are not fully understood. Here, we characterized the age-related changes in the IHC synaptic ribbons of C57BL/6J mice, a strain which is known to carry a cadherin23 mutation and experiences early hearing loss with age. In these mice, we found a progressive decrease in the number of synaptic ribbons per IHCs with aging, starting at postnatal day 30 (P30) and reaching up to 50 % loss in middle age mice at P365. In parallel, the size of the remaining ribbons progressively get larger to reach a nearly three-fold volume increase at P360. Remarkably, a deletion of the *Otof* gene, encoding otoferlin, a hair cell specific excytotic $Ca^{2+}$ sensor, produced a much early and accelerated loss of IHC ribbons with aging in C57BL/6J mice, with a 50 % loss by P60. The ribbons of IHCs lacking otoferlin also become larger with aging, but with the particularity to have a very compact spatial distribution at the IHC synaptic basal plasma membrane, where F-actin was found disrupted. Taken together, these results suggested that cadherin23 and otoferlin are important for the development and maintenance of the synaptic ribbons in IHCs, in particular for the small synaptic ribbons which are known to be associated with the postsynaptic afferent auditory nerve fibers having high spontaneous firing rate and encoding low-threshold sound intensity.

# C14

**Second messengers regulate the sensitivity of cochlear hair cell mechanotransduction**

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Hair cells of the inner ear play a critical role in auditory transduction by converting mechanical information from sound vibrations into electrical signals through a process termed mechano-electrical transduction (MET). In response to high intensity sound, MET saturates, leading to calcium excitotoxicity, hair cell death, and permanent hearing loss. Since inner ear hair cells do not regenerate, we aim to improve our understanding of the basic molecular mechanisms which regulate the MET process towards the goal of developing new preventative strategies for acoustic trauma.
Previous data suggests that the cyclic adenosine monophosphate (cAMP) second messenger may shelter hair cell function during acoustic overexposure by modulating the sensitivity of the MET channel. In turtle hair cells, pharmacological upregulation of cAMP produces a rightward shift of the hair bundle displacement vs. MET current relationship (called the activation curve), which shifts the set point of the channel beyond the range of stimuli that would normally saturate MET. (Ricci & Fettiplace 1997). In rodents subjected to acoustic trauma, inhibition of phosphodiesterases (PDEs), which increases intracellular cyclic nucleotides, promotes faster recovery from temporary hearing threshold shifts and increases hair cell survival (Jaumann et al., 2015). Additionally, a loss of function mutation in adenylate cyclase 1 (*ADCY1*), the enzyme that catalyzes cAMP formation, causes hearing loss in humans. An equivalent mutation in zebrafish hair cells abolishes

FM1-43 dye uptake, suggesting a loss of resting MET and that cAMP is required for establishing the baseline sensitivity of the MET channel (Santos-Cortez et al., 2014). Together, these data suggest that cAMP regulates the operating point of the MET channel, where a basal level of cAMP is required for resting mechanosensitivity and elevated cAMP shifts the channel to a position of lower sensitivity to prevent saturation. However, a cAMP-based mechanism of MET channel regulation has yet to be described in mammalian hair cells.
Here, we tested the hypothesis that cAMP modulates the set point and dynamic range of the MET channel in outer hair cells (OHCs) of the rat cochlea. We combined patch-clamp electrophysiology, force-controlled hair bundle stimulation, and a novel state-of-the-art high-speed imaging system to measure the sensitivity of the MET channel in response to pharmacological manipulations of cAMP and calcium signaling. We found that cAMP analogs produced a rightward shift and an increased width of the MET activation curve, confirming the presence of a cAMP-based mechanism of MET regulation in mammals. Secondly, the magnitude the of cAMP-induced shift decreased with lower intracellular calcium buffering, suggesting a potential interplay between calcium and cAMP signaling in regulation of activation curve position. Consistent with this observation, cAMP analogs significantly increased the extent and rate of a calcium-dependent slow adaptation process which correlated with the shift in activation curve set point. Together, our findings demonstrate that cAMP and intracellular calcium regulate the sensitivity of mammalian MET by modulating the resting position and operating range of the MET channel. We propose that cAMP may be a promising molecular target for novel preventative strategies for acoustic trauma.

**Keywords**: mechanotransduction, physiology, calcium, cAMP

# C15

**Relevance of the presence of auditory nerve peripheral processes for the electrically evoked compound action potential**

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The auditory nerve generally degenerates after profound sensorineural hearing loss or deafness. The extent of degeneration partly determines the hearing performance of deaf patients using a cochlear implant (CI). Electrically evoked compound action potentials (eCAPs) may be applied to assess the condition of the nerve. The eCAP depends, among others, on the number of surviving spiral ganglion cells (SGCs). However, the contribution of peripheral processes (PPs) of the SGCs to the eCAP is unclear (Ramekers et al., Hear. Res., 2015). Therefore, we examine the role of the PPs in eCAPs recorded in a large number of deafened guinea pigs, including animals treated with neurotrophic factors, in which the proportion of PPs relative to SGCs varies.
Guinea pigs were deafened by systemic administration of kanamycin and furosemide. A large portion of these animals was treated with neurotrophic factors two weeks following onset of deafness. Neurotrophins were either delivered by means of a mini-osmotic pump, which provides complete protection of SGCs in the basal and middle cochlear turn (Ramekers et al., J. Neurosci., 2015) or by gelfoam placed on the round window, enabling the neurotrophins to protect SGCs in the basal turn (Havenith et al., Otol. Neurotol., 2015). Remaining animals received control treatment. All animals received a 4-electrode array (MED-EL) in the basal turn. Various stimulation paradigms of eCAP recordings were applied, including varying the inter-phase gap (IPG) of single pulses which have been reported to correlate with SGC survival (Ramekers et al., JARO, 2014). The PPs and SGCs were quantified for each animal and normalized to the outcomes in normal-hearing animals.
The PP/SGC ratio globally varied between 0.5 and 1.5, with lower values often found in cochleas treated with brain-derived neurotrophic factor using the osmotic pump. Among the basic eCAP outcome measures, the amplitude of the N1 peak increased with PP/SGC ratio, whereas N1 latency and eCAP threshold did not show clear relationships with the PP/SGC ratio.
Our preliminary analyses indicate that the presence of PPs is relevant for the eCAP. We may conclude that either spike initiation occurs at the PP, or that spikes are initiated at the soma and PP presence merely reflects a healthy and functional SGC.

**Keywords**: Spiral ganglion cell, eCAP, Cochlear Implant, Sensorineural hearing loss, Guinea pig

# C16

**G6PD overexpression protects from oxidative stress and ameliorates ARHL progression**

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Ageing of the auditory system is associated with the incremental production of reactive oxygen species (ROS) and the accumulation of oxidative-derived damage in macromolecules, which contribute to cellular malfunction, compromise cell viability and, finally, causes functional decline. The cellular detoxification power partially relies in NADPH production, which serves as cofactor for the activity of major cellular antioxidant enzymes. NADPH is mainly produced by glucose-6-phosphate dehydrogenase (G6PD), an enzyme that catalyzes the rate-limiting step in the pentose phosphate pathway. We show here that the transgenic mouse *G6PD*-Tg, which shows enhanced NADPH production along life, maintains lower auditory thresholds than wild type mice during ageing. *G6PD* overexpression preserves irreplaceable cochlear cell populations, thus *G6PD*-Tg mice exhibit higher number of inner and outer hair cells (OHC), more widespread OHC innervation and higher number of synapses per IHC than wild type mice. Transcripts for antioxidant enzymes and pro-apoptotic proteins levels were increased and reduced respectively in 3-month-old *G6PD*-Tg. Accordingly, tyrosine modification by nitration in proteins and mitochondrial damage was reduced in 9-month-old *G6PD*-Tg compared with wild type mice. As well, as lesser TUNEL positive apoptotic cells were detected in whole mount preparations in *G6PD*-Tg mice. Interestingly, *G6PD* overexpression turned out to trigger an inflammatory response effectively resolved without cellular damage or macrophage infiltration in the cochlea. In conclusion, we propose that NADPH overproduction from an early stage is an efficient mechanism to maintain the balance between the generation of ROS and the cell detoxification power along ageing and, therefore to prevent hearing loss progression.

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# C17

**Inflammatory cytokines as diagnostic predictors of age-related hearing loss : cross-sectional investigation from the Great Age Study**

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**Background**

Age related hearing loss (ARHL) or presbycusis is a chronic disorder that consists in a physiological alterations of the central and peripheral auditory system that occurs with aging and that lead to hearing loss and difficulty in understanding spoken language. In recent years there have been a series of evidences that have linked the age related hearing loss with numerous aging disorders as cerebrovascular diseases and physical frailty. Serum markers of systemic inflammation it is well known how increase with age and have been associated with cardiovascular outcomes, frailty and all-cause mortality.

**Objective**

The aim of this study was to determine if circulating markers of inflammation, namely the cytokines interleukin-6 (IL-6), interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α) and the acute phase protein C-reactive protein (CRP) are associated with age related hearing loss and which of those cytokines could be the best diagnostic predictors hearing impairment.

**Methods**

Participants were randomly selected from sub-sample of the GreatAge Study, a population based cohort study on aging, conducted in Castellana Grotte a town in the south of Italy only on 65+ residents of the town.Peripheral ARHL was defined with a pure tone average (PTA) threshold greater than 40 dB hearing level (HL) in the better ear. The speech discrimination score (SDS) was defined as the percentage of

phonetically balanced Italyn words recognition, in a list of 10, at 30 dB sound pressure level. The age related central auditory processing disorder (CAPD) was diagnosed with the Synthetic Sentences Identification with Ipsilateral Competitive Message (SSI-ICM) only in normal to mild hearing loss participants (PTA < 40 dBHL in the better ear). Cytokines (high sens. CRP, TNF-α,IL-6,IL-1β), was assayed in the serum of participants at the first day of enrollment with ILLUMINA technology with double check output method.

**Results**
The study sample included 783 participants with 322 males (41.13%) and 461 females(58.87%). The mean age was 69.67±6.8 years, the median educational level is of 6 years.The overall prevalence of peripheral ARHL and CAPD were 12.52% (98) and 9.45% **(74),** respectively. IL-6 is strongly associated with the presence of CAPD (OR 1.20, CI 1.15-1.25) and not with the peripheral ARH and it is also shown as powerful diagnostic classificator for for CAPD on the ROC analysis (AUC = 0.81, CI 0.75 to 0.88, cut-off >3.9).

**Conclusions**
The high prevalence of central and peripheral presbycusis has now shown an urgent need to show simple biomarkers to be used in clinical practice, which can predict the trajectories of pathological outcomes associated with hearing impairment such as dementia or physical frailty. The use of an inflammatory cytokine such as IL6 could open the door to pathways of diagnosis and personalized therapy of hearing impairment, specifically to monitor hearing restoration outcomes. Furthermore, although this study does not show causal inferences, it is possible that the strong association between systemic inflammation and central hearing impairment may represent an interesting basis for future studies both in the diagnostic and intervention field on the interaction between hearing loss and neuro-inflammation.

# C18

**Age-related differences in the auditory temporal processing at peripheral and central levels in Fischer 344 rats**

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In the fast aging rat strain Fischer 344 (F344) basic parameters of the hearing function and auditory temporal processing at peripheral and central levels were compared by recording of auditory brainstem responses (ABRs) and middle latency responses (MLR). The response characteristics were evaluated in adult (5-11 months old) and old (23-32 months old) F344 rats under various background noise levels. In addition, neuronal responses were correlated with results of the morphological analysis of the inner ear. The hearing thresholds based on ABR evaluation were not different between the adult male and female F344 rats. In old females, the ABR thresholds were 20-30 dB higher than those measured in adult rats, while in old males the ABR thresholds were 10-20 dB higher than those measured in old females. The ABR and MLR amplitudes were significantly smaller in old rats in comparison with adult rats. Auditory temporal processing was tested using stimulation with a pair of clicks with variable interclick interval (ICI). Since the characteristics of temporal processing were similar in both F344 males and females, the data obtained in both genders were analysed together. Under silent conditions both ABR and MLR amplitudes to the second click in a pair reached the amplitude elicited by the first click at the same ICI in both adult and old animals. The differences between auditory temporal processing at the periphery and in the central auditory structures occurred when click pairs wer presented together with a background broad band noise (BBN). Amplitudes of both ABR and MLR were suppressed by BBN more efficiently in adult animals than in old rats. Whereas the ABR amplitudes to second click reached the amplitude elicited by the first click at similar ICI as in the silent conditions in both adult and old animals, the functions of the MLR amplitudes at ICI values under BBN in adult and old rats were different. In old rats the MLR amplitudes to the second click increased with increasing ICI values much faster at higher BBN levels and reached the MLR amplitudes to the first click stimulation at significantly shorter ICI compared to adult rats. The results demonstrate more pronounced age-related dysfunction of the auditory temporal processing at cortical level in the presence of the BBN that is present also in human presbycusis.

# C19

**Hearing Loss in aging: does the shape of the audiogram predict perfusion changes in the primary auditory cortex?**

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Age-related hearing loss (HL) can be related to brain dysfunction or structural damage which can in turn result in cerebral metabolic/perfusion abnormalities. Recent studies suggest that not only absolute pure tone audiometry but also the shape of the audiogram might be relevant to predict cerebral blood flow reductions in the primary auditory cortex of HL patients

To investigate this aspect, we revised the audiograms of 55 HL patients (not involved in previous MRI study) and subdivided patients in two groups using k-means clustering based on the area of right/left audiograms (defined as Low area HL (LHL) and High area HL (HHL) groups).

Pseudo-continuous arterial spin labeling (ASL) and T1-weighted MRI (at 3 Tesla) were performed in 62 HL patients (age range 47-77 years, pure tone average HL>50 dB). Patients were separated in two HL groups (LHL and HHL). Twenty-eight normal hearing (NH) subjects (age-range 48-78 years) were also investigated. Cerebral blood flow and gray matter volume were analyzed in the cortical volume to assess group differences. No significant differences in either global or local atrophy were detected between groups but the HL group. A significant difference was found in the auditory cortex only when comparing the NH and the HHL groups (cluster in the right primary auditory cortex, $p<0.05$ cluster level corrected in the auditory cortex mask). In addition, when extracting the mean values among all subjects in this cluster, a significant negative correlation was found with the slope between 2-4 KHz of both the right ($p=0.0053$) and left ear ($p=0.019$) in the HHL group. The regional cerebral blood flow was correlated positively to the regional gray matter volume ($p=0.02$) and negatively to the audiogram steepness in HL subjects (right ear: $p=0.02$, left ear: $p=0.02$). HHL (but not LHL) patients exhibited negative correlation in CBF vs. Steepness (S) of the audiograms

The observed cortical pattern of perfusion reduction suggests that neuronal metabolism can be related to HL before the recognition of brain structural damage. This also illustrates the potential of ASL-MRI to contribute early functional markers of reduced central processing associated with HL. Patients with higher areas of the HL audiogram, i.e. patient with more severe hearing loss, also exhibit a negative correlation between perfusion and audiogram steepness. Future studies may benefit from patients' stratification in low and high areas of the audiogram rather based on pure tone audiometry.

## SESSION III

### COCHLEAR IMPLANT, IMPLANTABLE PROSTHESIS AND DRUG DELIVERY SYSTEMS

# TL3

**Delivery of drugs to the entire cochlea without breaking its boundaries**

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The mammalian cochlea is one of the least accessible organs for drug delivery. Systemic administration of many drugs, notably the most frequently used corticosteroids and aminoglycoside antibiotics, is severely limited by the blood-brain barrier. Local intratympanic administration into the middle ear would be a preferable option in this case and the only option for many old and newly emerging classes of drugs and therapies including local anaesthetics, antioxidants, apoptosis inhibitors, neurotransmitters and their antagonists, monoclonal antibodies, growth factors, signalling pathway regulators and genetic material. Intratympanic administration of drugs relies on their remaining in contact with the cochlear round window membrane long enough to allow their diffusion into the cochlea fluid. The ability of drugs to pass through the round window does not, however, lead to their effective distribution along the long and narrow cochlear spiral. This slow technique leads to steady-state, base-to-apex concentration gradients that still are orders of magnitude and well outside the therapeutic windows for many drugs. Here we present a cochlear drug delivery method which permits reliable, rapid and uniform, drug distribution along the entire length of the intact cochlea. The method utilizes specific cochlear geometry and hydrodynamics. Our preliminary experiments using salicylate as a model drug with well-established physiological effect demonstrate the exceptionally high efficiency of the method which permitted drug delivery into the cochlear apex within minutes without disrupting cochlear boundaries. In the short term, the presented method should lead to significant improvements in the efficacy and reliability of currently employed drugs for the treatment of sensorineural hearing loss, Menière's disease, noise-induced hearing loss, tinnitus and autoimmune inner ear disease. In the longer term, the outcomes of our research should facilitate new and novel ways of approaching the treatment of inner ear disorders since we have overcome the challenge of delivering of therapeutics along the entire cochlea. Our method will enable researchers to concentrate on other aspects of drug development.

# C20

**Hearing preservation at low frequencies by insulin-like growth factor 1 in a guinea pig model of cochlear implantation**

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The hybrid or electric-acoustic stimulation cochlear implant is indicated in patients with a residual hearing at low frequencies. It provides electric and acoustic stimulation for compensating for high- and low-frequency sounds, respectively. However, the implantation procedure damages the cochlea, resulting in loss of the residual-hearing and diminished effects of the acoustic-hearing in several patients. To prevent hearing loss after implantation, corticosteroids have been used clinically although their effects are limited. As an alternative to corticosteroids, insulin-like growth factor 1 (IGF1) has shown potent effects in various types of cochlear injury. In this study, the effects of IGF1 on hearing preservation were examined after cochlear implantation to a normal-hearing guinea pig model. The electrode was inserted through the round window membrane of guinea pigs with the application of a gelatin-sponge soaked with IGF1 or saline. The auditory brainstem response (ABR) was recorded pre-operatively, immediately after cochlear implantation, and 7, 14, 28, and 56 days after electrode insertion. In comparison to the control group, the IGF1-treated group showed better hearing preservation at low frequencies, 7 days after surgery. IGF1 application was effective at low frequencies (2 and 4 kHz) throughout the period of examination. Histological studies revealed that outer hair cell numbers, in the IGF1-treated group, were maintained in the cochlear region responsible for low-

frequency hearing (upper midbasal turn) and that there was less fibrous tissue formation around the electrode. Both the outer hair cell counts and the extent of fibrosis significantly correlated with the ABR threshold shifts at low frequencies. These results indicate that IGF1 might attenuate loss of low-frequency hearing after cochlear implantation, suggesting its possible clinical use in soft surgeries involving cochlear implants with electric-acoustic stimulation for hearing preservation.

**Keywords**: hearing preservation, cochlear implant, IGF1

# C21

**Advances in piezoelectric nanomaterials for cochlear stimulation**

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**Introduction**

The present study aims to develop significant advances in the implementation of a new concept of cochlear implant that improves the quality of life and hearing, and reduces the current expected costs for patients suffering from profound-to-severe hearing loss. New generation cochlear implants could exploit piezoelectric materials, if the sensitivity of existing materials will be sufficiently enhanced. Our hypothesis was to increase the material performance thanks to nanotechnology, which allows the improvement of existing polymeric materials through structural and morphological features, thus also favoring the material/neuron interface. Electrospinning is a versatile method to produce ultrafine fibers which can provide structural features for cell adhesion and growth.

**Materials and methods**

This report describes the fabrication and characterization of piezoelectric electrospun poly (vinylidenefluoride-*co*-trifluoroethylene) P(VDF-TrFE) scaffolds for neural stimulation. The effects of collector velocity on fiber morphology, crystalline phase, mechanical properties, piezoelectric properties and cell behavior on the scaffolds were investigated. Ototoxicity was studied with OC-k3 cells. Therefore, human induced pluripotent stem cells (iPSCs) were cultured on these fibers. We thus differentiated human iPSCs into cortical neurons on random and aligned fiber meshes according to a dual-SMAD inhibition protocol. At day 76, neurons were fixed and immunostained for βIII-tubulin (yellow) and counterstained with DAPI, and observed via Confocal Laser Scanning Microscopy (CLSM). Finally, first experiments of piezoelectric device insertion were performed in cadaveric rat cochleae by electrospinning P(VDF-TrFE) fibers onto a nylon wire.

**Results and discussion**

By increasing the collector velocity to 4000 rpm, the fiber diameter reduced and the mutual fiber alignment increased, corresponding to enhanced mechanical properties and piezo-active β-phase content. However, as a consequence of the diverse mechanical properties of random and aligned fibers, which ultimately affected the piezoelectric properties, randomly-oriented fibers exhibited higher spontaneous polarization ($V_{out}$ and $d_{31}$ piezoelectric coefficient) than aligned ones. Therefore, randomly-oriented fiber scaffolds may exert electric signal perceived by cells even in rest conditions (without application of an external mechanical stress), whereas aligned fiber scaffolds may exert topographic guidance. P(VDF-TrFE) nanofibers were not ototoxic to OC-k3 cells. Furthermore, both on randomly-oriented and aligned fibrous scaffolds, iPSCs showed an excellent capability of neurogenic differentiation. CLSM imaging indicated the presence of well differentiated neurons on the scaffolds. Axons on aligned fiber scaffold followed the parallel fibers, while axons were randomly arranged on random fiber scaffolds. These findings suggested that mesh morphology and remnant piezoelectricity played a determinant role on iPSC neurogenic differentiation. The first insertion trial in a cadaveric cochlea highlighted the importance of finding a proper size support for fiber application.

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# C22

**Hearing preservation of adult cochlear implant users with Partial Deafness – one year follow up after using steroids therapy**

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**Introduction**

Cochlear implantation is now considered to be the 'gold stan-dard' treatment method for hearing impairment. One of the approaches to improving hearing function following cochlear implantation has been the use of corticosteroids. Although the systemic route of drug administration, via the oral, intravenous (IV), and intramus-cular (IM) routes, might cause serious adverse effects due to the high drug doses required, these routes are considered to be the most convenient and reliable methods of first-line drug administration for the treatment of the inner ear disorders.

**Objective**

The objective of this prospective clinical study was to assess the influence of different regimens of steroid therapy on preservation of hearing following cochlear implantation

**Material and methods**

Into the study we included participants aged ≥18 years, with hearing sound levels in the range of 10-120 decibels (dB) and sound frequencies of 125-250 hertz (Hz); sound levels of 35-120 dB and frequencies of 500-1,000 Hz; sound levels of 75-120 dB and frequencies of 2,000-8,000 Hz. Study exclusion criteria included diseases with contraindications for steroid therapy or medications that increased the effects of steroids. Patients were qualified to cochlear implantation and were divided into three treatment groups: IV steroid therapy (standard steroid therapy): combined oral and IV steroid therapy (prolonged steroid therapy); and a control group (cochlear implantation without steroid therapy). Hearing preservation was established by pure tone audiometry based on the pre-operative and postoperative average hearing thresholds according to the formula developed by the HEARRING Network

**Results**

According to Hearing Preservation classification patients from the prolonged steroid therapy group achieved the best results of hearing preservation. The complete hearing preservation index was observed in the highest percentage of patients from this group. The dispersion of measured values was lesser in comparison with other subgroups, showing the stability of obtained results.

**Conclusions**

Combined oral and IV steroids therapy enables stabilization of hearing thresholds to the biggest extent and, by doing this, preserves hearing in adult patients with partial deafness who underwent cochlear implantation.

**Keywords:** hearing preservation; cochlear implantation; steroids

# C23

**Surgical feasibility of localized therapeutic hypothermia application for preservation of residual hearing in cochlear implantation**

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**Background**

Cochlear implantation surgery often results in a loss of residual low-frequency hearing, which can be related to insertion trauma-related damage to hair cells or blood-labyrinth barrier. In preclinical studies, we have shown that local application of controlled, mild therapeutic hypothermia can be a promising hearing

preservation strategy. This custom-designed system does not require modifications in the surgical approach and is acutely delivered during surgery. The present study investigated the feasibility using this approach to deliver local hypothermia in patients undergoing implantation using anatomical and radiologic measurements and experimental outcomes in cadaveric human temporal bones .

**Methods**
Ten human cadaveric temporal bones were scanned with micro-computed tomography: various anatomical features involving the facial recess and measurements predicting round window (RW) visibility were characterized. For each bone, the standard facial recess and myringotomy surgical approaches were used to deliver hypothermia. The St. Thomas Hospital classification (STH) was used to record the degree of RW visibility with and without placement of the hypothermia probe. This grading was repeated following further drilling for optimizing visualization of the RW; when needed, an independent cochleostomy was performed. To devise the effects of hypothermia application to the surgical field, temperatures were recorded by thermistors placed inside the RW, at RW niche, over the lateral semicircular canal and the supero-lateral mastoid edge.

**Results**
There was no significant correlation between degree of RW visualization using STH and the radiologic measurements. The average facial recess area was 13.87 ± 5.52mm$^2$ (range of 8.44 to 24.28 mm$^2$). The introduction of the cooling probe through either approach did not impede visualization of the RW or cochleostomy as determined by STH grading. The average maximum temperature decrease, using the facial recess approach, was 4.57 ± 1.68 °C at RW, while the myringotomy approach produced a mean cooling of 4.11± 0.98°C at RW. Overall, no significant differences were found between the two probe approaches with the degree or rates of cooling, and the temperature profile did not show any variation between different techniques for any of the recorded surgical landmarks. EAC angle was correlated to maximum cooling in facial recess approach but not in the myringotomy approach.

**Conclusion**
Using our customized delivery system, local application of therapeutic hypothermia for residual hearing preservation during CI surgery was demonstrated to be clinically feasible through both the facial recess and myringotomy approaches, without limiting optimal surgical visualization. Our findings and approach can also be extended to pediatric CI, where preservation of residual hearing may greatly aid early language development and speech perception.

**Keywords:** Therapeutic hypothermia, cochlear implant, residual hearing, electrode-induced trauma.

## C24

**Cochlear implant perspective in congenital single side deafness: a temporal bone study**

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Today, 43% of children with unilateral hearing loss (UHL) are identified prior to 6 months of age by means of the newborn hearing screening testing. In terms of the outcomes, we know that about 80% of children with UHL are going to have academic difficulty, will require grade repetition or will need additional support or assistance.
Although general awareness on congenital UHL issue has increased over the last 20 years, we have not yet agreed on treatment approaches that would be capable to effectively lower the incidence of the abovementioned problems. In this regards, cochlear implantation as a treatment for UHL is increasing, but little is known about the factors that could affect outcomes, and what are the risks of insufficient cochlear implant results in cases where hearing is normal in one ear.
The overall goal of our research was to in depth investigate the frequency and characteristics of inner ear anomalies in congenital UHL, and thereby help determine anatomic and physiopathologic factors that can be relevant to decisions and expectations regarding cochlear implantation in this population. The structures of the inner ear were analyzed by CT (Computed Tomography) and MR (Magnetic Resonance) images in 39

children affected by isolated congenital UHL. Anatomical measures were compared with the normal contralateral ear and a control group.
The results of the study can be of help in developing recommendations and guidelines for diagnosing and selecting the most appropriate aid treatment in case of congenital UHL.

**Keywords**: unilateral hearing loss; cochlear implant; inner ear malformations.

# C25

**Accelerated osteointegration of the titanium-implant coated with biocomponents, collagen /hydroxyapatite/bone morphogenetic protein-2, for bone-anchored hearing aid**

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**Background & Objective**
Early osteointegration after implantation is difficult in some patients with thin bone thickness, poor bone quality, or inadequate bone and metabolic disease. According to reported histologic studies of explanted BAHA implants, spontaneously lost implants showed no adequate signs of osteointegration. To our knowledge, no previous studies have examined the use of BMP-2/collagen-coated titanium-implants with BAHA for evaluating osteointegration. The purpose of this study was to determine the osteogenesis of BMP-2/ collagen-coated implants in the BAHA-attract system both in vitro and in vivo.

**Materials and Methods**
We coated hydroxyapatitite (HA)/bone morphogenetic protein-2 (BMP-2)/collagen on the implant screw. The surface was observed by scanning electronmicroscopy. To evaluate the osteogenesis induced by the modified titanium implant, we conducted in vitro test using mouse preosteoblast (MC3T3-E1). Live/dead cell assay and cell proliferation using MTT were performed. Alkaline phosphatase and alizarin-S were stained. The three group implant screws (no coated: control, collagen coated: group I, BMP-2/collagen coated: group II) were implanted in Zealand white rabbits. were used for in vivo study. All rabbits were administered fluorescent bone labels (Sigma- Aldrich) for qualitative evaluation of bone formation. Calcein (blue) was administered at 4 weeks, and oxytetracycline hydro- chloride (green) at 8 weeks, and alizarin (red) at 10 weeks. After 12 weeks, tibia bones were embedded glycol methacrylate solution after fixation process. HE and MT stain were performed.

**Results**
The optical density (OD) of group-II was significantly higher than those of the other scaffolds ($p < 0.05$). The number of live cells cultured in group-II was significantly greater than for control and group-I. The result of DAPI-Phalloding stain means that the group-II is associated with meaningfully higher metabolic activities than the control and group-I due to the topological property of the surface roughness due to the HA/BMP-2 particles and biochemical properties induced by the collagen. We observed that calcium mineralization, which was observed in the optical images as dark-red intensity, was significantly higher in group-II, which agreed with the ALP activity results. The control group did not show active initiation of bone formation, but most of the peri-implant spaces were occupied mainly by bone marrow tissue according to light microscopic observation. In group-I, collagen also enhanced new bone formation around the implant compared to in the control group. However, MT staining revealed markedly enhanced peri-implant osteogenesis in the group-II. Based on the histomorphometric analysis, significantly higher osteogenesis of the peri-implant in group-II was observed, compared to in the other two implant groups.

**Conclusion**
From these results, collagen/HA/BMP-2- coated implant can enhance the peri-implant osteogenesis of the BAHA-attract system, although this study period did not allow the observation of long-term effects.

# C26

**Outcomes after application of active bone conducting implants**

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**Background**
A bone conducting implant is a treatment option for individuals with conductive (CHL) or mixed hearing loss (MHL) who do not tolerate regular hearing aids, and for individuals with single-sided deafness (SSD).
An active bone conducting implant (ABCI) was introduced in 2012 with the indications CHL, MHL and SSD, and it is still the only ABCI available. With complete implantation of the active transducer and sequent intact skin, there is a decrease in infections, skin overgrowth and implant losses, all common disadvantages with earlier passive bone conducting implants. Our Ear, Nose and Throat Department, a secondary care center for otosurgery that covers a population of approximately 365,000 inhabitants, was approved to operate ABCIs in 2012.

**Objectives**
Evaluation of audiological and subjective outcomes with ABCIs.

**Method**
A cohort study with retrospective and prospective data collection. The first twenty consecutive patients operated with an active bone conducting implant were asked for informed consent. Main Outcome Measures: Pure tone and speech audiometry and the Glasgow Benefit Inventory (GBI).

**Results**
Seventeen patients accepted to participate and fifteen were able to complete all parts. Six patients had conductive or mixed hearing loss. In this group the pure tone audiometry tests are comparable with an average functional hearing gain of 29.8 dB HL. With bilateral hearing, the mean Word Recognition Score (WRS) in noise was 35.7% unaided and 62.7% aided. Ten patients had the indication single-sided deafness. With the hearing ear blocked, the pure tone average was > 101 dB HL, compared to 29.3 dB HL in sound field aided. With bilateral hearing, the mean WRS in noise was 59.7% unaided and 72.8% aided. The mean of the total GBI score was 42.1 in the group with conductive or mixed hearing loss and 20.6 in the group with single-sided deafness.

**Conclusions**
The patients benefit from their implants in terms of quality of life, and there is a substantial hearing gain from the implant for patients with conductive or mixed hearing loss. Patients with single-sided deafness benefit less from the implant than other diagnoses but the positive outcomes are comparable to other options for this group.

**Keywords:** active hearing implant; audiometry; bone-conduction implant; hearing loss; single-sided deafness;

# C27

**Sustained N-acetylcysteine delivery to the inner ear by poloxamer 407 hydrogels in a guinea pig model**

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**Introduction**
Cisplatin is used in the treatment of various types of cancer and can cause a dose-related, bilateral high-frequency hearing loss. Amongst other side-effect combating strategies, thiol-containing antioxidants such as N-acetylcysteine, D-Methionine, and thiosulfate emerged as promising candidates in terms of antioxidant-mediated protection from cisplatin-induced ototoxicity. Systemic administration of such compounds could potentially counteract the tumoricidal effect of cisplatin. Therefore, intratympanic injection of antioxidants, which results in a restricted systemic uptake of the drugs, whilst intracochlear drug levels are substantially

higher, is preferable. Formulations for intratympanic injection should maximize the contact time between the active compound and the membranes of the inner ear and need to be controlled for osmolality and pH properties. This study therefore focused on (i) the evaluation of concentration-time profiles of N-acetylcysteine in perilymph, cerebrospinal fluid and plasma after intratympanic administration, (ii) the influence of a thermoreversible poloxamer 407 hydrogel on N-acetylcysteine pharmacokinetics, and (iii) the development of a pH- and osmolality-adjusted formulation for intratympanic N-acetylcysteine delivery.

**Materials and Methods**
49 female albino guinea pigs were randomized into a group receiving a single intratympanic injection of a 4% N-acetylcysteine poloxamer 407 hydrogel or into a group receiving a 4% N-acetylcysteine solution. 8 animals served as untreated controls. N-acetylcysteine levels in perilymph, cerebrospinal fluid and plasma were measured over a period of 24 h. Samples were taken at 1, 3, 6, 12 and 24 h (poloxamer 407 hydrogel group) and at 1, 6 and 24 h (solution group) post injection, and analyzed by high performance liquid chRometography-tandem mass spectrometry.

**Results**
Intratympanic application of the 4% N-acetylcysteine poloxamer 407 hydrogel resulted in a 4-fold higher perilymph area under the concentration-time curve (0-24 h) than topical administration of the equally concentrated N-acetylcysteine solution. Plasma N-acetylcysteine levels were comparable. N-acetylcysteine concentrations in the cerebrospinal fluid remained below the level of detection (5 ng/ml) in both treatment groups. N-acetylcysteine-containing formulations applied to the middle ear were isohydric and osmolality was reduced by up to 200 mosmol/kg compared to equally concentrated formulations used in previous studies.

**Conclusion**
We were able to demonstrate that the intratympanic application of poloxamer 407 hydrogels increases and sustains N-acetylcysteine delivery to the inner ear. Because of the low plasma N-acetylcysteine concentrations after topical application and the physiological pH and osmolality of the hydrogel, the risk of local side effects as well as the potential to interfere with the antineoplastic effects of cisplatin have been minimized.

**Keywords**: N-acetylcysteine, cisplatin, otoprotection

## Monday 9 September 2019

## SESSION IV

### GENETICS OF DEAFNESS AND GENE THERAPY

# TL4

**Mutations in PLS1 cause autosomal dominant non-syndromic hearing loss in three families of European ancestry**

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**Introduction**

Non-syndromic hearing loss (NSHL) is a common sensory disorder characterized by high clinical and genetic heterogeneity. In the last decade, the use of next-generation sequencing (NGS) technologies has proved to be an effective strategy for the molecular diagnosis of NSHL leading to the identification of ~115 genes and 170 loci. Nevertheless, almost half of patients submitted for genetic testing fail to receive a conclusive molecular diagnosis, suggesting that new genes still need to be identified.

**Methods**

Three families of European ancestry, affected by autosomal dominant NSHL (ADNSHL) and negative to mutations in already known deafness genes have been recruited in different research centers: IRCCS Burlo Garofolo in Trieste (Italy), Nationwide Children's Hospital in Columbus, Ohio (US) and Centre of Reference for Genetic Sensory diseases, CHU in Montpellier, (France).

Families have been analyzed by whole exome and whole genome sequencing. Sequencing data have been filtered according to allele frequency reported in public databases (MAF<0.0001), pathogenicity and pattern of inheritance. A population structure analysis to further prioritize the candidate genes was performed evaluating the signature of natural selection in European populations and constraint metrics (i.e. observed/expected missense and loss of function ratios and the RVIS score).

*In silico* mutagenesis and protein modelling have been performed to investigate the pathogenic mechanism of the identified variants.

**Results**

After sequencing data analysis, three novel likely-pathogenic missense variants in *PLS1* gene (NM_002670.2) have been identified: c.805G>A, p.(E269K); c.713T>G, p.(L238R); c.383T>C, p.(F128S). All variants have been detected at the heterozygous state and segregated with the ADNSHL phenotype within the families.

*PLS1* encodes plastin-1, also known as fimbrin, one of the most abundant actin-bundling proteins of the stereocilia and known for causing hearing loss in mice (PMID: 25124451). According to our gene prioritization framework, *PLS1* revealed signatures of natural selection in European populations, in addition to a low observed/expected ratio of both missense and loss of function mutations and the lowest RVIS score compared to other candidates genes. *In silico* protein modelling demonstrated that all variants affect the actin-binding domain 1 (ABD1), a domain which binds one actin monomer in the filament, and predicted an overall destabilization of the protein structure. In particular, the modelling suggests a perturbation of the structural stability of the whole ABD1 via disruption of an essential electrostatic interaction (p.(E269K)) or hydrophobic core network (p.(F128S) and p.(L238R)), that may result in a reduced protein's ability to bind F-actin.

It is possible to speculate that these changes may eventually cause an abnormal stereocilia formation , leading to the hearing defect identified in all patients.

**Conclusions**
Present results, i.e. genomic data of three independent ADNSHL families of European ancestry, *Pls1*$^{-/-}$ mice phenotype, the demonstration that *PLS1* is under selection and the protein modelling results, provide compelling evidence that *PLS1* is required for normal hearing and that its alteration in humans leads to ADNSHL. Screening of additional families will be performed to further evaluate the overall contribution of this gene to the etiopatogenesis of ADNSHL and to lay the foundation for possible innovative therapeutic approaches.

# C28

**Gene Therapy of Usher Syndrome Type IC**

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Usher syndrome type 1 is associated with congenital sensorineural hearing loss, vestibular areflexia and progressive retinitis pigmentosa (RP). A recessive *USH1C* c.216G>A mutation, originally identified in French-Acadian patients, creates a cryptic splice site which reduces production of harmonin. Harmonin is essential for normal inner ear hair cell development and function. Our goal is to identify novel biological tools to treat auditory and vestibular deficits associated with this mutation.
Adeno-associated virus (AAV) gene augmentation therapy is a promising approach to target recessive mutations of genes expressed in the inner ear. Lentz et al. (2013) have also demonstrated that systemic injection of antisense oligonucleotides (ASO) in *Ush1c* c.216AA mouse mutants partially restores auditory and vestibular function. In this report, we describe and compare results obtained with local deliveries of either therapeutic to the inner ear through the round window membrane.
Our work shows that both treatments lead to recovery of *Ush1c* gene and protein expression along with restoration of hair cell mechanotransduction. This cellular level repair promotes increased hair cell survival, rescues complex auditory function, and recovers hearing and balance behavior to near wild-type levels. The data represent unprecedented recovery of inner ear function. Comparative benefits and shortcomings of both treatments will be discussed.

# C29

**A rationally designed adeno-associated viral vector enables safe and efficient gene transfer to supporting cells in the mouse cochlea**

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Hearing loss is the most common sensory disorder world-wide, affecting over 6.8% of the world's population[1]. While gene therapy has recently emerged as a promising strategy for the treatment of inherited diseases like hearing loss, it is dependent on the identification of gene delivery vectors[1]. Adeno-associated viral (AAV) vector-mediated gene therapy has been approved in the US for treating patients with a rare inherited eye disease[2]. However, for hearing loss, it remains constrained by a lack of safe and efficient vectors that can target the diverse types of inner ear cells. A target of particular interest is the supporting cells (SCs) where important key deafness genes are expressed and which has shown promise for regenerating hearing-critical hair cells (HCs). Here, we identify the rationally designed AAV-inner ear (AAV-ie) vector for gene delivery in mouse inner ear cochlea. Our results show that AAV-ie transduces SCs with high efficiency *in vivo*, representing a vast improvement over conventional AAV serotypes. Furthermore, we find that after AAV-ie-mediated transfer of Atoh1 gene, which plays a key role in HC fate determination[2], many SCs trans-differentiated into new hair cells *in vivo*. This research represents the first successful identification of a viral vector that targets SCs, and the first AAV vector to be used for efficient HC regeneration. We anticipate AAV-ie will play a key role in future treatment of human hearing loss.

# C30

**The distinct functions of TRIOBP-5: sculpting stereocilia rootlets and stiffening supporting cells**

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TRIOBP bundles actin filaments to generate the dense rootlet structure of hair cell stereocilia. TRIOBP has several alternative splice isoforms. Mutations that simultaneously ablate the functions of TRIOBP-4 and TRIOBP-5 cause a complete loss of rootlets resulting in profoundly deaf humans and mice. However, the mechanisms regulating the formation of stereocilia rootlets by each TRIOBP isoform remain unknown. As the first step to examine this issue, we studied individually the functions of TRIOBP-4 and TRIOBP-5. Because the amino acid sequence of TRIOBP-4 is entirely included in the sequence of TRIOBP-5, it is not possible to generate a TRIOBP-4 specific antibody. So, an EGFP-TRIOBP-4 transgenic mouse was engineered. We found that TRIOBP-4 is expressed predominantly in the rootlet segment located within the stereocilia core, while TRIOBP-5 is expressed in the rootlet segment located in the cuticular plate. We also engineered two different TRIOBP-5 specific knockout mouse models that have dysmorphic rootlets, which are abnormally thin within the cuticular plate. This rootlet dysmorphology is likely to contribute to progressive deafness recapitulating the human phenotype. A NanoSPD assay, which utilizes a myosin-10 motor for a nanoscale pulldown to detect protein-protein interactions, showed homo-oligomerization of TRIOBP-5, suggesting that TRIOBP-5 oligomers are essential for maintaining mature shape of rootlets. In addition to the rootlets, TRIOBP-4 and TRIOBP-5 are expressed in Deiters' cells. Atomic Force Microscopy analyses demonstrated decreased Deiters' cell-stiffness in the absence of TRIOBP-5. This study reveals unique mechanisms of action of TRIOBP-4 and TRIOBP-5 within the inner ear sensory and non-sensory cells.

# C31

**Disruption of CLRN2, a gene encoding clarin-2, causes autosomal recessive hearing loss in humans and zebrafish**

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Non-syndromic hearing loss (NSHL) is the most common hereditary sensory disorder that affects one to two per 1000 newborns. Hereditary hearing loss is characteristically heterogeneous in both clinical and genetic aspects. Up to 80% of NSHL follows an autosomal recessive mode of inheritance. Out of the currently identified genes that are associated with autosomal recessive forms of NSHL, roughly 80% of these genes have been identified by studying families with consanguineous unions.

Homozygosity mapping of an extended consanguineous Iranian family segregating autosomal recessive sensorineural NSHL revealed a 15.2 Mb run of homozygosity on chromosome 4p15.32p15.1. Exome sequencing identified a novel homozygous missense variant contained within the locus affecting the gene clarin-2 (*CLRN2*) that was predicted to be pathogenic via *in silico* pathogenicity prediction programs. Segregation of the variant in 14 recruited family members was concordant with autosomal recessive hearing loss. The missense variant was predicted to activate an exonic splice enhancer site. *In vitro* analyses using mini-gene assays revealed two splicing products; one of which was the normally spliced product, the other disclosed defective splicing leading to intron retention and a shift in the reading frame. The mutant Clrn2*-YFP protein was transiently expressed in zebrafish hair cells and it failed to localize to the hair bundle or plasma membrane.

The clarin-2 (*CLRN2*) gene encodes a putative four transmembrane protein closely related to tetraspanins. Its amino acid sequence in the human, mouse, and zebrafish is well conserved and mutations in the paralogous gene, clarin-1 (*CLRN1*), have been causally linked to hearing loss in humans (Usher syndrome IIIA, USH3A), mice, and zebrafish.

To investigate the functional consequence of disrupted clarin-2, we generated zebrafish carrying a *clrn2* knockout (KO) allele using CRISPR/Cas9 technology. *clrn2*$^{+/KO}$ zebrafish were normal at all ages; however, *clrn2*$^{KO/KO}$ larvae exhibited poor startle response (poor hearing) and a balance defect 6 days post fertilization. Hair cells in *clrn2*$^{KO/KO}$ zebrafish displayed abnormal morphology with short and disrupted hair bundles and a significantly reduced number of mature ribbon synapses. The mechanotransduction function of *clrn2*$^{KO/KO}$ neuRomest hair cells, as reflected by microphonic potentials, was significantly attenuated. Consistent with the *clrn2*$^{KO/KO}$ phenotype, we showed that *clrn2* mRNA is restricted to hair cells within the inner ear. Additionally, Clrn2-YFP fusion protein expressed in zebrafish hair cells localized to the hair bundle and plasma membrane. These data demonstrate that clarin-2 is an essential hair cell protein, and its defect results in loss of hearing and balance in zebrafish.
Our results associate *CLRN2* with autosomal recessive NSHL and link disruption of CLRN2 to the pathophysiology of hearing loss.

**Key words**: Autosomal recessive hearing loss, Clarin-2 (*CLRN2*), CRISPR/Cas9, novel gene discovery, zebrafish

# C32

**Disease modelling and drug screening for GJB2 related hearing loss with iPS cells**

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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 cases in some populations. 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*, 124(4):1598–1607, 2014). Cochlear CX26-gap junction plaque (GJP)-forming cells such as cochlear supporting cells are thought to be an important therapeutic target for the treatment of hereditary deafness. We demonstrated that gene therapy was one of the effective treatment to restore the disrupted GJPs in GJB2 related hearing loss (Iizuka, *Hum Mol Genet.* 2015, 24(13):3651-61.). To develop the effective and feasible therapy for GJB2 associated hearing loss, restoration of GJP macromolecular complex using drugs or gene therapy vectors are expected to rescue the hearing function of 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. Furthermore, these disease model cells from CX26-deficient mice recapitulated the drastic disruption of GJPs, the primary pathology of GJB2-related hearing loss (Fukunaga, *Stem Cell Reports*, 7(6), 1023-1036, 2016). To establish the disease model cells from the patients with GJB2 related hearing loss, we developed human iPS cells from the patients with Japanese and east Asian typical GJB2 mutations, GJB2 V37I, G45E/Y136X and 235delC. As the patients with these three homozygous mutations shows different hearing levels (mild, severe, and profound hearing loss respectively), the disease model cells from these patient derived iPS cells can be used to analyze different degree of GJB2 related hearing loss among most typical

GJB2 mutations in Japan and east Asia. The methods for high throughput screening using these model cells has been established and assessed by the functional assays.
These iPS cell derived disease models and the screening systems should be useful for establishing the drugs and gene therapy vectors that target GJB2-related hearing loss.

## C33

**Synaptic Changes in Cochlear Hair cells of Tmc Mutant Mice**

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*Tmc1* and *Tmc2* form sensory transduction channels in auditory and vestibular hair cells. Mice with targeted deletion of *Tmc1 (Tmc1Δ)* exhibit deafness while those lacking both (*Tmc1Δ;Tmc2Δ),* lack sensory transduction, are deaf and have vestibular dysfunction. Single-channel properties also differ between TMC1 and TMC2. Hair cells that express TMC2 have higher calcium selectivity and higher single-channel conductance than those that express TMC1. While hair bundle morphology and function, and hair cell survival have been assessed in *Tmc* mutant mice, consequences of absent and/or impaired sensory transduction on the development and/or maintenance of ribbon synapses have not been examined. Cochlear and vestibular hair cells utilize ribbon synapses to mediate synchronous release of glutamate-filled vesicles for temporally precise transmission of auditory information. Absent or altered numbers of synaptic ribbons contribute to abnormal synaptic transmission, impaired speech-in-noise discrimination, and perceptual anomalies like tinnitus. The purpose of this study was to determine if synaptic differences exist between wild-type mice and mutant mice that lack *Tmc1*, *Tmc2* or both across development. Characterizing the consequences of impaired/absent sensory transduction on ribbon synapses will be informative when assessing the effectiveness
of restoring sensory transduction via gene therapy.

Cochlea from wild-type, *Tmc1Δ, Tmc2Δ,* and *Tmc1Δ;Tmc2Δ* mice were harvested at various time points (P7, P10, P14, P21, P28) and immunostained with antibodies to C-terminal binding protein 2 (CtBP2), glutamate receptors (GluA2/3), and myosin7a (Myo7a). Each cochlea was microdissected and frequency maps were generated using apex-to-base length measurements. Confocal z-stacks of the 8.0, 11.3, 16.0, 22.6, and 32.0kHz regions were obtained and image stacks were analyzed in Imaris, an image analysis software. 3-D projections were generated and the average number of synapses per inner hair cell were counted using the Imaris “Spot Detection” feature.

Significant differences in the number of synapses in *Tmc1Δ;Tmc2Δ* mice were evident at several developmental time points, suggesting TMC channels may be required for normal development and maintenance of synapses. Synapse counts in *Tmc1Δ* mice resembled those in *Tmc1Δ;Tmc2Δ* mice, while counts in *Tmc2Δ* mice were similar to those of WT mice. Our observations suggest that absence of TMC1 but not TMC2 leads to variations in the number of synaptic puncta. We hypothesize that sensory transduction in developing hair cells of the mouse is required for normal development and maintenance of cochlear synapses.

## C34

**Whole exome sequencing in Slovak patients with bilateral sensorineural hearing impairment**

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**Background**
The genetic heterogeneity of sensorineural hearing loss (HL) is a major hurdle to the discovery of disease-causing variants. Whole exome sequencing (WES) is a method that allows for an efficient analysis of all known genes associated with hereditary hearing loss.

**Aim**
Using WES to identify new genetic causes of hearing impairment in the Caucasian and Rome populations in Slovakia, where standard genetic tests performed so far have not elucidated the etiology of deafness.
**Patients and methods**
Since 2010, we have been conducting a nationwide screening of sensorineural HL patients. Currently, we register 1,369 individuals, of which 1,071 are probands and their family members with this disease. We selected 77 individuals from 44 families, including 34 of Caucasian and 10 of Rome ethnicity. WES was implemented by Beijing Genomics Institute (Hong Kong) and Theragen BioInstitute (South Korea). The data obtained were subsequently bioinformatically processed (using virtual panel of sensorineural HL) in the Diabgene Laboratory.

**Results**
Using WES, we identified 18 probands of Caucasian ethnicity and their 20 family members with HL (another 4 family members are expected to have a later onset of HL) harboring 11 pathogenic variants and 10 likely pathogenic variants responsible for hearing impairment. 13 variants have not yet been described in the literature. The detected variants were found in genes *COCH*, *COL4A5*, *CREBBP*, *EDNRB*, *EYA4*, *ILDR1*, *LOXHD1*, *MYO6*, *MYO15A*, *OTOA*, *OTOG*, *P2RX2*, *SIX1*, *TECTA, TMC1* and *TMPRSS3*. We did not identify any causal variants in the Rome ethnicity.
**Conclusion**
Using WES, we detected the genetic etiology of hearing impairment in 53% probands of Caucasian ethnicity. WES in this ethnicity dramatically increases the detection of pathogenic variants in known genes associated with HL.
**Key words**: bilateral sensorineural hearing loss, whole exome sequencing, Caucasian and Rome ethnicity

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# C35

**Characterization of the genetic bases of hearing loss in an Italyn cohort**
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Approximately 1-2/1000 children are born with bilateral permanent sensorineural hearing loss. About 50% of these recognize a genetic cause which may be syndromic (25%) or isolated/non-syndromic (75%).
Non-syndromic hearing loss (NSHL) is genetically heterogeneous with more than 160 loci described in humans and 100 genes so far identified.
The goal of our work was to develop advanced molecular tools, with high diagnostic rate to investigate the genetic bases of HL in a Caucasian population mainly of pediatric age.

We designed and developed a customized NGS targeted panel containing 59 carefully prioritized genes strongly associated, in Caucasians, with NSHL or with the two most frequent syndromes with onset as seemingly isolated deafness (i.e. Pendred and Usher).
The Ion Torrent PGM™ platform and a customized bioinformatics pipeline have been used for the analysis of DNA samples from clinically highly selected subjects negative for the frequently mutated *GJB2* mutations and *GJB6* deletions.
This targeted panel has been tested in 189 subjects, reaching a conclusive diagnostic result in 79 individuals, with a diagnostic yield of 42%. In 73 cases (38.6%) only monoallelic mutations in autosomal recessive genes were detected, and 33 cases (17.4%) resulted negative. In 4 cases the characterization of variants of uncertain significance is ongoing.

In 21 of the positive subjects (27%) we identified mutations in genes involved both in non-syndromic hearing loss (NSHL) and Usher syndrome (*ADGRV1, CDH23, MYO7A, PCDH15, USH1C, USH2A*) making this the second most frequent cause of HL after mutations in the GJB2/GJB6 genes. We found in these genes 13 novel likely pathogenic mutations, of which one splice-site mutation was characterized at the RNA level.

The single most frequently mutated gene in our cohort was *CDH23* with a total of 12 pathogenic mutations identified, of which 5 novel ones.

We achieved a diagnosis of Usher syndrome type 1 in three subjects (3 y.o.; 6 y.o.; 16 y.o.) with congenital profound hearing loss, retinal anomalies/retinitis pigmentosa and/or history of motor delay, who carried mutations in *CDH23, MYO7A* and *PCDH15*. Usher syndrome type 2 was diagnosed in a 12 y.o. boy referred for congenital bilateral mild hearing loss and subsequently found to have early signs of retinal alteration.
Testing cases with family history of deafness allowed us to provide dual diagnoses, as the case of a novel *EYA4* mutation found to co-segregate in two individuals of the same family, with a novel *PAX3* gene mutation.

Our NGS analysis combined with a very careful data processing allowed the identification of a homozygous gene deletion of *OTOA* in two dizygotic twins and three homozygous and two heterozygous deletion of the *STRC* gene, the latter found in-trans with known *STRC* pathogenic variants
Our targeted panel, coupled with a solid bioinformatics pipeline, has proven a sensitive molecular tool that, provided a careful clinical selection offers a high diagnostic yield; this allows to reach precise early diagnosis and, particularly in view of the high prevalence of mutations in Usher genes, provides important prognostic and follow-up information for affected individual and better counseling for their families.

# C36

**Enrichment of rare missense variants in OTOG gene in Familial Meniere disease**

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Meniere's disease (MD) is a rare inner ear disorder characterized by sensorineural hearing loss (SNHL), episodic vertigo and tinnitus. Epidemiological studies indicate that MD is more common in certain European descendent populations, suggesting a genetic predisposition. Most of MD patients are sporadic, although familial clustering is observed in 8-9% of sporadic cases, supporting also a genetic background. MD phenotype varies widely, and it usually overlaps with migraine and different autoimmune disorders.
Familial MD (FMD) follows an autosomal dominant pattern of inheritance with incomplete penetrance. Different whole exome sequencing (WES) based studies have described several genes which could explain FMD. Single nucleotide variants (SNV) in DTNA, FAM136A, PRKCB, DPT and SEMA3D were identified in 4 different families, however, these SNV have not been replicated yet neither in other affected families nor sporadic MD (SMD) cases.
The current approach of "one variant-one disease" must be changed to explain the incomplete penetrance or variable expressivity observed in MD. In fact, applying more complex genetic models, a recent study with SMD cases reported an excess of missense SNV in several genes linked with SNHL, such as *GJB2*, *SLC26A4* or *USH1G*. This study supports the possibility of multiallelic inheritance in MD.
In this study we have investigated the genetic background of FMD by analyzing 46 families by WES. A single rare variant analysis (SRVA) and a gene burden analysis (GBA) focusing on SNHL genes were conducted. For SRVA, we selected exonic and nonsynonymous SNV with a minor allele frequency (MAF) <0.001 and a Combined Annotation Dependent Depletion Score (CADD) >15 to identify rare variants that have strong effects on FMD. For GBA, we applied a MAF cutoff <0.05 to simulate variants interactions. Two datasets were used as reference to compared observed MAF in our MD cohort: the non-Finnish European population (NFE) from ExAC and the Spanish population from the Collaborative Spanish Variant Server (CSVS). Variants not described in these reference datasets were discarded to minimize false calls and population-specific variants. Odds ratio with 95% confidence interval were calculated for each gene. One-sided p-values were obtained and corrected for multiple testing by the total number of variants found in each gene following the Bonferroni approach.

A total of 5136 SNV located in SNHL genes were considered for conducting a SRVA in FMD cases. After filtering and prioritizing, only 52 rare SNV variant remained, but only one SNV was shown in more than one family. This rare variant (rs552304627), located in *OTOG* gene, was detected in two families and it is likely pathogenic according to the American College of Medical Genetics and Genomics guidelines. Following the GBA pipeline, we found an enrichment of rare missense variants in the *OTOG* gene compared with reference populations. A total of 14 families out of 46 showed, at least, a rare missense SNV in this gene. The *OTOG* gene encodes otogelin, a tectorial membrane protein which has been previously associated with deafness and imbalance. These results suggest a key role of otogelin in inherited deafness in MD.

## SESSION V

### NOISE INDUCED HEARING LOSS, OTOTOXICITY AND OTOPROTECTION

# TL4

**Implications of compound screens for protection of mammalian hair cells from aminoglycoside ototoxicity**

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**Background**
The complexity of cellular processes involved in stress and damage responses makes identification of protective interventions challenging. Signaling within cells can be compared to a microprocessor, with input from many cellular events and pathways contributing to decisions that may, or may not, lead to damage and/or cell death. The resulting difficulty in predicting effective interventions has led to the use of high-throughput screens to identify compounds with potential therapeutic benefit for many conditions, without an *a priori* hypothesis based on a proposed mechanism. Damage to hair cells (HCs) from ototoxic agents is likely to be similarly complex. It would therefore be advantageous to screen mammalian HCs, especially cochlear outer HCs which are the most sensitive to damage, against large numbers of compounds. This would potentially identify new therapeutics, compare efficacy across compounds, and identify novel cellular processes involved in HC damage. Unfortunately, high-throughput screening is not practical for mammalian HCs.

**Methods**
As an alternative, we developed a medium-throughout, *in vitro* assay based on micro-explants of the neonatal murine organ of Corti, which can screen up to a few hundred compounds for HC protection against toxins. The explants consisted of approximately 20 inner HCs and 60 outer HCs from the basal or middle turns of mice expressing GFP restricted to HCs. Explants are treated with 200 µM gentamicin plus one of three concentrations of a library compound, in triplicate. No treatment, gentamicin alone or compound alone are employed as controls. HCs are imaged and quantified daily. This system has been used to screen antioxidant, kinase inhibitor, autophagy, phosphatase inhibitor and protease inhibitor compound libraries.
**Results**
The screens have yielded new information on the complexity of ototoxic HC damage mechanisms. They also provide data on the relative effectiveness of intervening at various points in the damage process. Moreover, because the damage conditions are uniform, they identify relative effectiveness across compounds. Finally, they have identified a number of novel HC protectants.
**Conclusions**
The micro-explant screens have identified critical checkpoints in the damage process where intervention is likely to be maximally effective. We also found that not all compounds with similar targets provide the same level of HC protection, identifying optimal protectants. The results can be used to develop novel therapeutic strategies.
*(Supported by grants from the US National Institutes of Health/NIDCD and the Veterans Administration.)*
(Dr. Ryan is a co-founder of Otonomy, Inc. The company played no part in the research presented.)

# C37

**MicroRNA Expression Changes in the Cochlear Nucleus and Inferior Colliculus after Noise-Induced Hearing Loss**

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**Objectives**
Noise exposure is one of the most common causes of hearing loss. Exposure to loud noise leads to secondary changes such as reduced synapses, degeneration of auditory nerve fibers and reorganization of Cochlear Nucleus (CN) and Inferior Colliculus (IC), which might induce the neural plasticity in central auditory pathway. MicroRNAs (miRNAs) are important regulators of biological processes such as cell differentiation, proliferation

and survival by silencing the complementary sequences within mRNA molecules. This study is aimed to identify the role of miRNAs in neural plasticity of the central auditory pathway after noise-induced hearing loss.

**Methods**
The 6 weeks old Sprague-Dawley rats were divided into four groups. 1 Day control group (n=12), 3 Days control group (n=12), 1 Day after noise exposure group (n=12), 3 Days after noise exposure group (n=12). The animals were exposed to white band noise (2-20kHz) at 115 dB for 2 hours. Auditory Brainstem Response (ABR) threshold, amplitude of Wave II and IV and the IV-II latency was evaluated. A morphological evaluation of organ of corti was performed by H&E staining. Also, the whole mounting was executed for hair cell counting by phalloidin staining. Microarray analysis of the miRNAs in the CN and IC was performed at EBIOGEN Inc. using Affmetrix miRNA 4.0 GeneChip. RT-qPCR was carried out for a validation of candidate miRNAs.
**Result**: At 3 days after the noise exposure ABR threshold shift was significantly lower than the 1 Day group at all three frequencies ($p<0.001$). There was no siginificant difference at IV-II latency, but the amplitude of Wave II were significantly larger at all frequencies in 3 Days group than 1 Day group ($p<0.001$). Organ of corti on the basal turn exhibited abnormality and the number of surviving outer hair cells demonstrated significant differences on basal and middle turn area between control groups and hearing loss groups. Total 10 and 13 Candidate miRNAs for each CN and IC were selected by fold change of normalized intensity values of 1.5 with $p<0.1$. from microarray analysis and the RT-qPCR validation of candidate miRNAs narrowed them to 5 miRNAs; miR-200b-3p, 183-5p, 411-3p, 20b-5p, 377-3p and 3 miRNAs; miR-92a-1-5p, 136-3p and 26b-5p.

**Conclusion**
Considering the given results, even the short term acoustic stimulation can cause miRNA expression changes in CN and IC, which also might induce plasticity in central auditory pathway. The progress in ABR amplitude of Wave II, which represents CN suggested the possibility of change in CN. Based on the microarray analysis and RT-qPCR, miR-200b-3p, 183-5p, 411-3p, 20b-5p, 377-3p might have the key roles in neuroplasticity of central auditory pathway. Pathway analysis of the 5 candidate miRNAs that found within the Kyoto Encyclopedia of Genes and Genomes (KEGG) were MAPK signaling pathway, axon guidance, and TGF-beta signaling pathway and so on. Further study that also validates the candidate miRNAs by using miRNA oligomers is required, but theses candidate miRNAs could be useful for early diagnosis of hearing loss and treatment for acute hearing loss.

# C38

**Glucose supplementation and prevention of noise-induced hearing loss**

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Long-term high sugar intake increases the risk for obesity, diabetes, cardiovascular diseases and auditory degeneration. In contrast, recent research reveals that a high-sugar diet improves survival in flies on challenge with oxidative stress. Here, we investigated the effect of glucose supplementation on noise-induced hearing loss in CBA/J mice. Mice that were supplemented with a high-concentration of glucose showed less cochlear hair cell and synapse loss and better auditory response thresholds following noise exposure in comparison with controls. Mechanistically, glucose supplementation shortly before noise exposure activated anti-oxidant enzymes and reduced oxidative stress in the cochlea. Our findings reveal that energy availability is a key determinant for prevention of noise-induced hearing loss.

# C39

**Targeting cellular defensive response and inflammation in cancer cells and cisplatin-induced ototoxicity**

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Platinum-based agents, such as cisplatin, form the mainstay of currently used chemotherapeutic regimens for several malignancies, however, the main limitations to the clinical usefulness of cisplatin are the incidence of chemoresistance and its ototoxic side effects. Research addressed to overcome cisplatin

limitations has been focused on the development, as an adjuvant therapy, of safe molecules able to counteract chemotherapy-associated organ toxicity and to promote tumor toxicity and chemosensitisation. In this study we used two different polyphenols, curcumin and ferulic acid, as adjuvant chemotherapeutics evaluating *in vivo* their antioxidant effects in protecting against cisplatin-induced ototoxicity and targeting *in vitro* the transcription factors involved in tumor progression and cisplatin resistance in head and neck squamous cell carcinoma. We showed that both polyphenols, at the effective dosage, show antioxidant and oto-protective activity in normal cisplatin-stressed cells by up-regulating Nrf-2/HO-1 pathway and downregulating p53 phosphorylation. However, only curcumin is able to influence inflammatory pathways counteracting NF-κB activation. In cancer cells, curcumin converts the anti-oxidant effect into a pro-oxidant and anti-inflammatory one exerting permissive and chemosensitive properties, by targeting the cisplatin chemoresistant factors Nrf-2, NF-κB and STAT-3 phosphorylation. Ferulic acid shows a biphasic response: it is pro-oxidant at lower concentrations and anti-oxidant at higher concentrations promoting chemoresistance. Thus, polyphenols, mainly curcumin, targeting ROS-modulated pathways may be a promising tool for cancer therapy thanks to their biphasic activity of antioxidant in normal cells undergoing stressful conditions and pro-oxidant in cancer cells, probably involving an interplay among the key factors Nrf-2, NF-κB, STAT-3 and p53.

**Keywords**: cochlea, antioxidants, chemoresistance, polyphenols, head neck cancer, personalized medicine

# C40

**Entry rate of gentamicin through the MET channels of outer hair cells varies with position along the cochlea**

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There is evidence for a gradient in single-channel conductance of the mechano-electrical transducer (MET) channels of outer hair cells (OHCs) along the length of the cochlea, with conductance getting larger towards the basal end (Ricci et al 2003 Neuron 40:983-990, Beurg et al 2006 J Neuroscience 26(43):10992-11000, Lelli et al 2009 J Neurophysiol 101:2961-2973). Support for this notion also comes from evidence for a visible gradient in the passage of large fluorescently labelled peptides through the channels (Desmonds et al 2014 ARO Abs:150), which may indicate a gradient in the pore diameter, increasing from apex to base.
However, a gradient in drug-induced toxicity of OHCs is observed even for smaller molecules such as the aminoglycoside antibiotics (Forge and Schacht 2000 Audiol Neurootol 5:3-22), with apical cells being relatively insensitive compared to basal. We present data that for one such antibiotic, gentamicin, there is a difference in the entry rate into the cytoplasm through the MET channel between apical and basal OHCs of early postnatal mice, which correlates with the gradient in the OHC MET channel conductance. This suggests that not only does the pore diameter of the MET channel vary, but so does its electrical profile and topology. Furthermore, we present data indicating that the fluorescently conjugated gentamicin-Texas Red (GTTR) binds more strongly to the MET channel, with a ten-fold lower half-blocking concentration than native gentamicin.

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# C41

**Prevention of cisplatin ototoxicity: the role of nanoceria and dexamethasone**

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Cisplatin is a chemotherapic drug currently used to treat many human cancers, which use is associated with numerous side-effects among which ototoxicity. Many studies have demonstrated that cisplatin damages hair cells, spiral ganglion neurons and stria vascularis, leading to hearing impairments. Understanding

cisplatin mechanism of action will allow the development of effective treatment to counteract or prevent hearing loss. It is known that cisplatin ototoxicity is caused by inflammation, oxidative stress and DNA damage that lead inner ear cells to death mainly through apoptosis.
The aim of the study was to evaluate the effects of dexamethasone, a widely employed anti-inflammatory drug, and nanoceria, a new catalytic antioxidant, to prevent cisplatin ototoxicity.
The effects of pre-treatment with different doses of dexamethasone or nanoceria were tested on an inner ear mouse cell line derived from the organ of Corti (OC-k3), treated with cisplatin for 24 and 48h.At the end of the incubation times, treatment outcomes were evaluated by cell viability, cell morphology, antioxidant defence system and production of reactive oxygen species (ROS), and release of inflammation markers.
The results showed that cisplatin reduces OC-k3 cell viability, alters cell morphology and antioxidant defense system, increases ROS production and the release of pro-inflammatory cytokine (interleukin-6, IL-6). Dexamethasone and nanoceria slightly altered the cell physiological features of organ of Corti cells.
Nevertheless, both agents partially protected OC-k3 cells from cisplatin toxicity. In particular, dexamethasone partially prevented cisplatin toxicity on cell viability, cell morphology and IL-6 release. While nanoceria reduced the effects of chemotherapic drug on cell viability, cell morphology and endogenous antioxidant alteration.
Overall, these data support the use of dexamethasone and nanoceria to protect auditory cells from damages caused by exposure to cisplatin, possibly preventing hearing loss.

## C42

**3R mouse model for cisplatin ototoxicity studies: platinum correlation with deafness**

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Prevailing pre-clinical models to study ototoxic side effects of cisplatin relies on high or repeated intraperitoneal (IP) doses of this chemotherapeutic. As result, mice suffer from high systemic toxicity, leading to premature sacrifice or death before obtaining a robust hearing loss model. As an alternative, we proposed a local delivery model, in which a small volume of cisplatin was directly delivered into the otic bulla through a retroauricular route. Only one ear was operated in each animal, allowing us to have a contralateral control. Consistently, mice did not show visible stress or pain symptoms, showing near complete weight recovery 3 days following the surgery. Furthermore, after cisplatin delivery a significant auditory threshold shift, was recorded by auditory brainstem response (ABR), also showing a significant decrease of the number of ribbon synapsis (up to 40% in the basal turn of the cochlea). Oppositely, saline delivery did not show any detrimental effects. To further characterize our model, mass spectrometry analysis was performed to compare the platinum concentration in several organs (including the inner ear, liver and kidney). Following systemic injection of cisplatin (10mg/kg IP), we observed strong accumulation of cisplatin in the liver, kidneys and both inner ears. By contrast, platinum was only detected in the operated ear of locally injected animals, demonstrating the absence of systemic toxicity. Interestingly, the results showed a clear relationship between platinum fixation and auditory threshold shifts ($r^2 = 0.3491$; $p = 0.0005$).
In conclusion, our novel local cisplatin delivery model induces robust hearing loss without significant morbidity and mortality. Histomorphologically, the functional loss is mainly due to the toxicity at the synaptic connections between hair cells and auditory neurons. This 3R model offers the possibility to address long-term effects of cisplatin on the cochlea without significant morbidity, which is not possible with current systemic models.

**Keywords**: Local delivery, 3R, Synaptic degeneration, Cisplatin, Mass Spectrometry.

# C43

**Inhibition of the adenosine A2A receptor mitigates excitotoxic injury in organotypic tissue cultures of the rat cochlea**

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The primary loss of the glutamatergic afferent nerve synapses due to noise or ageing (cochlear neuropathy) is thought to occur as a result of glutamate excitotoxicity. This primary neural injury has considerable clinical significance as it compounds the injury profile and may lead to more significant central effects presenting as difficulties in speech discrimination in noisy conditions (also known as hidden hearing loss). Currrently, there is no treatment for hidden hearing loss. Our previous studies in mice with genetic deletion of adenosine $A_{2A}$ receptor ($A_{2A}R$) have demonstrated better preservation of cochlear afferent synapses and the minimal loss of spiral ganglion neurons after noise exposure compared to wildtype mice. This has informed our current targeted approach to cochlear neuroprotection based on pharmacological inhibition of the $A_{2A}R$. In the current study we have used organotypic tissue culture of the Wistar rat cochlea at postnatal day 6 (P6) to model excitotoxic injury. Cochlear explants were subjected to a combined NMDA (0.5 mM) / kainic acid (0.5 mM) or NK treatment for 2 hours in culture conditions with or without addition of clinically approved $A_{2A}R$ antagonist istradefylline (200 nM). The explants were fixed with 4% paraformaldehyde18 hours post-NK treatment and labelled with β-tubulin (labels afferent processes of spiral ganglion neurons), myosin VIIa (labels hair cells), PSD95 (labels post-synaptic density protein) and CtBP2 (labels synaptic ribbons in the inner hair cells). The binary image of the β-tubulin projection was used to analyse the density of afferent processes within several regions of interest underneath the inner hair cells (IHC). The presence of paired (functional) afferent synapses was determined by identifying the co-localisation of CtBP2 and PSD95 immunofluorescence. The excitotoxic injury was characterised by a reduction in the density of cochlear afferents immediately after NK treatment and loss of afferent synapses in the presence of intact sensory hair cells. The injury reached the peak at 18 hours post-NK treatment. The administration of istradefylline reduced deafferentation of inner hair cells and stimulated regeneration of afferent synapses after excitotoxic injury. As $A_{2A}R$ are predominantly expressed post-synaptically at the IHC-auditory nerve synapse, inhibition of the $A_{2A}R$ may dampen the post-synaptic glutamate receptor sensitivity to excessive ligands, interact with various proteins to internalise and thus reduce the number of post-synaptic receptors, or alter the transport of ions to reduce activation of post-synaptic neurons. This study thus provides evidence that $A_{2A}R$ inhibition promotes cochlear recovery from excitotoxic injury, and may have implications for the treatment of cochlear neuropathy and prevention of hidden hearing loss. This study was supported by Eisdell Moore Centre, New Zealand.

**Key words**: glutamate excitotoxicity, cochlear synaptopathy, cochlear explant, adenosine $A_{2A}$ receptor, istradefylline

# C44

**Opioid modulation of cochlear auditory responses in the rat inner ear**

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The auditory system has an extensive efferent innervation, which contributes to processes of control and regulation of the afferent input. The expression of receptors to various neurotransmitters and neuropeptides in the inner ear has been described, among which endogenous opioid receptors are found. The role of opioid receptors in the cochlea is not yet fully defined, it has been reported that opioid agonists and antagonists modulate the response to auditory stimuli and in clinical practice multiple cases have been reported in which the consumption of opioid derivatives induce sensorineural hearing loss. In this work we evaluated the effects of acute treatment with morphine, fentanyl, tramadol and naloxone, in the afferent response through auditory brain stem potentials (ABR) and the compound action potential (CAP) recording, as well as the role of the cochlear amplifier by recording the distortion products otacoustic emissions (DPOAE). Adult Long-Evans rats of the strain CII/ZV weighing 180-220 gr were used. For the ABR recording drugs were administered intravenously. For the CAP and DPOAE drugs were applied by direct perfusion in the middle ear. The opioid agonists produced a consistent increase in the amplitude of the PI component of the ABR and of the N1-P1 amplitude of the CAP. Naloxone produced no change in the ABR and a reduction of the CAP N1-P1. Also, opioid agonists induced an increase in the amplitude of the DPOAE and naloxone

decreased its amplitude. These results show that the opioid receptor activation modulates cochlear response at both the afferent response to acoustic stimuli, and also at the cochlear mechanics as revealed by DPOAE changes. In this work we determined the effects of opioids on ABR, DPOAE and CAP responses across a wide range of stimulus frequencies and amplitudes, it presents an important step in understanding how opioid modulation of auditory responses may contribute to the auditory processing and to sensorineural hearing loss produced by opioids.
This study was supported by grant from Vicerrectoria de Investigación y Estudios de Posgrado (VIEP-BUAP grants to ES) and grant from Consejo Nacional de Ciencia y Tecnología (CONACyT), Fronteras de la Ciencia 1544. TGR was supported by CONACyT fellowship 230020.

**Keywords:** hearing loss, morphine, tramadol, fentanyl

# C45

## A665-conjugated Acetylcysteine target prestin of outer hair cells with peptide hydrogel delivery preventing cisplatin-induced hearing loss

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### Background
The delivery of treatment agents to inner ear with drug delivery system (DDS) has been under investigation to overcome the limitations of the conventional therapeutic agents in curing or alleviating the cisplatin ototoxicity.

### Methods
In the present study, a novel targeted **Acetylcysteine** (NAC) - loaded DDS, A665-NAC, was constructed for prevention from cisplatin-induced hearing loss. A665 peptides specifcally bind to prestin, which is limited to the outer hair cells (OHCs). HEI-OC1 and cisplatin-treated mice (3.5 mg/kg *4 days for three cycle, intraperitoneal) were used as in vitro and in vivo models for investigating the targeting and protective efficiency
against cisplatin.

### Results
As expected, compared to random peptide-NAC (RP-NAC), A665-conjugated Cy5.5-labeled NAC showed active targeting to OHCs. Furthermore, A665-conjugated Cy5.5-labeled NAC could be signifcantly internalized by HEI-OC1 cells via the A665–prestin interaction. This facilitated the uptake of cells pretreated with A665-NAC, followed by the cisplatin-treated group, which led to enhanced cell viability, reduced apoptotic properties, and decreased reactive oxygen species levels as compared to cells pretreated with NAC or RP-NAC, 1 hours in advance of cisplatin treatment. In cisplatin-treated mice, pretreatment with A665-NAC with peptide hydrogel effectively preserved OHCs and showed signifcant hearing protection at 8, 16 and 32 kHz as compared to
pretreatment with saline, NAC, or RP-NAC formulation.

### Conclusion
This OHC-targeting DDS provides a novel strategy for NAC application that can be potentially used to combat cisplatin ototoxicity.

**Keywords:** A665 peptide, Acetylcysteine, peptide hydrogel, prestin, cisplatin ototoxicity

# C46

## Loss of function mutation in the NADPH oxidase subunit p22phox prevents early onset hearing loss

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Oxidative stress is thought to contribute to the ageing process and, in particular, to play a key role in age-related hearing loss, a condition with important social and medical consequences. The Reactive Oxygen Species (ROS) generating NADPH oxidases (NOX), in particular NOX3, have been implicated in inner ear redox pathology. Here we investigate the impact of the deficiency of $p22^{phox}$ (a common subunit of NOX1,2,3,4) in a mouse strain prone to early onset age-related hearing loss (A/J genetic background). In a first series of experiments, we compared hearing thresholds and cochlear histologies of $p22^{phox}$-deficient

mice to wild type littermates. Our data show early and progressive hearing loss in WT A/J mice, initially at high frequencies, but rapidly propagating to lower frequencies resulting in an almost complete hearing loss at 6 months of age. Our results define the onset of presbycusis in WT A/J mice at 6 weeks of age, with predominant signs of synaptic and post-synaptic damage, including decrease in auditory neurons density, loss of synaptic ribbons, and decrease in ABR wave 1. These signs of cochlear damage were markedly attenuated in p22phox-deficient mice; which, by contrast to the WT littermates, exhibited relatively stable hearing threshold over time. To further understand the nature of the protection observed in p22phox mice, we performed transcriptome analysis in cochlea from 6 weeks old mice. In p22phox-deficient animals, we observed a decreased expression of genes linked to excitotoxicity. In vitro analysis of auditory neurons confirmed a decreased activity of the excitatory pathway and decreased in glutamate-induced cell death in the absence of p22phox. In this study, we provide an entirely novel observation: absence of the NOX1-4 subunit p22phox provides a strong protection from age-related hearing loss. Our results show that NADPH oxidase activity contributes to age-related hearing loss through enhanced excitotoxic damage to auditory neurons, thereby providing a novel insight into pathomechanisms of presbycusis.

## C47

**Head-to-head comparison of different classes of otoprotectants against cisplatin-induced hearing loss in clinically relevant ex vivo models of hair cell, SGN and stria vascularis damage**

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Hearing loss is a serious side effect of many platinum-based chemotherapies that affects a large proportion of cancer patients. In the cochlea, hair cells (HCs), spiral ganglion neurons (SGNs) and the marginal cells of the stria vascularis are most susceptible to cisplatin-induced damage. This ototoxicity is thought to result from the formation of DNA crosslinks and enhanced levels of reactive oxygen species which activate cell death pathways. Several strategies have been identified which may prevent this type of cell death, including channel blockers, anti-apoptotic compounds or antioxidants/ROS inhibitors. Here we developed clinically relevant ex vivo models of cisplatin-induced hearing loss (CIHL), which included assays for HCs, SGNs and marginal cells, to use as screening tools to determine the relative therapeutic potential of various classes of otoprotectants.

P2-P4 Sprague-Dawley rat pups were used for all experiments. Whole-organ cochlear explants were established, then treated for 24-72 hours under varied conditions (including cisplatin concentration, cell culture media components, and cisplatin sources and excipients) to determine optimal culture conditions. To evaluate otoprotectants, cochlear explants were established then pre-treated for 1.5 hours in various concentrations of candidate compounds prior to co-incubation with cisplatin for 48-72 hours. Explants were then fixed and stained for HC and SGN markers and cells were quantified. To evaluate protection of the stria vascularis, dissociated marginal cells were expanded in culture for 48-72 hours, then were similarly pre-incubated in candidate otoprotectants, with subsequent co-incubation with cisplatin for up to 24 hrs. Strial cultures were stained for marginal cell identification and caspase activation.

Here we characterized ex vivo models of mild to severe CIHL using rat cochlear explants to assess HC, SGN, and marginal cell damage, and several classes of compounds were found to be protective of these cell types at clinically feasible doses. This protection was dose-responsive in most cases, although varied depending on the concentration and degree of cisplatin damage. Interestingly, the specificity of HC, SGN and marginal cell protection varied greatly depending on the otoprotectant class with some classes showing better protection of one cell type than another. The well-characterized antioxidant D-methionine, used as a reference compound, was found to be protective against various degrees of cisplatin-induced damage in all cell types, but showed slightly less protection of SGNs than HCs at equivalent doses. This demonstrates that optimal otoprotective strategies should take into account the diverse cellular pathologies associated with CIHL. The lead otoprotective candidates identified from these experiments are being evaluated in clinically relevant in vivo models of CIHL.

**Keywords:** cisplatin, stria vascularis, marginal cells, hair cells, spiral ganglion neurons

## Tuesday 10 September 2019

## SESSION VI

### PHYSIOPATHOLOGY OF AUDITORY PATHWAYS AND INNER EAR IMMUNOLOGY

# TL6

**Presence and characterization of cochlear mast cells**

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Mast cells (MC) are potent regulators and orchestrators of the immune, cardiovascular and neuronal responses. Several decades ago, the presence of mast cells was described in the endolymphatic sac. However, the existence of MCs in the cochlea was not observed or reported. In our present work, we revealed the presence of MCs in the cochleae of mice and rats. MCs were localized in the modiolus, spiral ligament and in the scala vestibuli. The identity of MCs in the cochlear cryosections and explants of both species was confirmed with avidin and toluidine blue (histochemical) and immunofluorescence methods (anti-cKit-CD117). Furthermore, we demonstrated that the cochlear MCs contain chymase, tryptase and interleukin 13 (IL-13). Moreover, we show that the average number of cochlear MCs decreases after birth of mice and rats, whereas the size of MCs increases during that time. The presence of MCs in the cochlea shades new light on some of the inner ear conditions such as Meniere's disease, Autoimmune Inner Ear Disease (AIED) or sudden sensorineural hearing loss (SSHL). Future functional studies are on the way to expand and translate this new basic knowledge to clinics.

# C48

**Brain-derived neurotrophic factor in auditory brainstem controls central learning mechanisms and social behavior**

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Brain-derived neurotrophic factor (BDNF), a key modulator of synaptic plasticity is predicted to locally control cortical receptive field maturation and memory with sensory experience. BDNF`s main function for the adult central nervous system performance starts with sensory experience when local cortical BDNF is assumed to regulate the excitability of cortical circuits (Hong et al., 2008), a process leading to enhanced cortical auditory resolution. In the auditory system BDNF become gradually upregulated in cochlear neurons or glial cells and the ascending auditory path between P4 and P14 (Singer et al., 2014). Aiming to get a first insight in BDNF`s function from hearing onset onwards, *Bdnf* was deleted in lower brain level regions under the promoter of the paired-box transcription factor *Pax2* (Zuccotti et al., 2012; Chumak et al., 2016).
A striking role of BDNF in Pax2-expressing cells in the auditory brainstem revealed essential for rapid auditory processing but also executive memory-linked functions and normal social behavior.

**Key words**: BDNF, rapid auditory processing, memory & learning, parvalbumin

# C49

**Neural correlates of fine structure and temporal envelope in the human auditory nerve**

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**Introduction**

The temporal cues contained in a sound can be decomposed into a fine structure (fast oscillation close to the central frequency of the sound) and a temporal envelope (low frequency amplitude modulation). Animal experiments show that the auditory nerve fibers encode the fine structure by generating action potentials at each sound wave cycle (phase locking on the fine structure) while the time envelope is coded by the modulation of the discharge rate (phase locking on the temporal envelope). Although auditory perception (including speech understanding) is based on both fine-structure and temporal envelope indices, few studies have investigated their neural correlates in the human auditory nerve.

**Material and Methods**

The electrical activity of the auditory nerve was measured during cerebellopontine angle surgery. The sounds were delivered in a closed field (Etymotic ER1) and the auditory nerve activity was measured using a ball electrode (Ø1.6 mm, Inomed) connected to a Grass P511 amplifier. The generation and acquisition of the signals was entirely processed by a NI-PXI 4461 device controlled by a LabVIEW interface (National Instrument). The neuronal indices of fine structure and temporal envelope were measured in response to pure tones (fine structure only), and narrow band of noise (presence of a fine structure and a temporal envelope). This study was performed in patients with normal hearing or hearing loss (ClinicalTrials.gov Identifier: NCT03552224).

**Results**

In response to low frequency tones, the electrical signal recorded in normal hearing subjects clearly demonstrates phase locked activity with the fine structure of the stimulus. The synchronization index calculated from a Fourier analysis of the nerve response is maximum at 700 Hz and declines progressively towards the high frequencies. Beyond 2 kHz, it is impossible to observe a phase-locked response. Surprisingly, a robust phase-locked response is observed in subjects in the 500-1000 Hz range even in case of hearing loss up to 60-70 dB SPL. In response to narrow band of noise, the neural response to envelope fluctuations is observed in normal hearing patients but disappears drastically in patients even with moderate hearing loss (~30 dB SPL).

**Conclusion**

These data show that the neural coding mechanisms of fine structure and temporal envelope can be studied in humans using intraoperative monitoring of the auditory nerve. Results suggest that the coding of the temporal envelope is more vulnerable to hearing loss than the fine structure coding. This approach allows to investigate how an impaired coding of the temporal envelope induces speech perception deficits, especially in patients with preserved fine structures coding.

**Keywords** : human auditory nerve, temporal envelope, fine structure

# C50

**The development and subpopulation of tissue-resident macrophages in the mouse cochlea**

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**Background**

The inner ear was once believed to be “immune-privileged”, but recent studies have demonstrated presence of immune-competent cells in the cochlea, which are referred to as tissue-resident macrophages in the cochlea. Some reports have revealed the functional aspects of them such as cell clearance in adult mice, however, the roles of cochlear macrophages are currently almost unknown. Our ultimate goal is to reveal the role of tissue-resident macrophages in the cochlea. Colony stimulating factor 1 (Csf1) is a growth factor that

regulates the survival, proliferation and differentiation of the mononuclear phagocyte lineage. In this study, we first show their distribution and *in-situ* proliferative capacity in wildtype mice from embryonic through adult stage, and then shows the results of experiments using Csf1 receptor (Csf1r) null mice.

**Methods**
The temporal bones of mice, between embryonic day (E) 9.5 and postnatal day (P) 21, were dissected out, and the specimens were perfused with 4% paraformaldehyde in phosphate buffer overnight and cryoprotected with 30% sucrose. Specimens were prepared for cryostat sections (10 µm in thickness) and the midmodiolar sections were provided for the fluorescent immunohistochemistry. We used anti Iba-1 antibody and CD11b antibody as surface markers of macrophage and monocytes which is a precursor of macrophage, respectively. We performed quantitative analysis of cochlear macrophage distribution, assessment of tissue-specific alteration in macrophage density, and assessment of *in-situ* proliferation activity by Ki67 and Phospho-Histone H3 (pHH3) immunostaining.

**Results**
Iba1 positive macrophages were not found around the otocyst at E9.5, but found at E10.5, which demonstrates the emergence of cochlear macrophages. In the embryonic stage, Iba-1-positive cochlear macrophages were distributed in the whole mesenchyme, and after the early postnatal stage, they were distributed, mainly in the spiral ganglion (SG), the spiral ligament (SL), and the stria vascularis (SV). The cell density of macrophage in the SG increased as the developmental stage progressed, while in the SV it had a peak around P3. On the other hand, the macrophages in the SV were not observed since P3. The evaluation for *in-situ* proliferative activity with quantitative analysis showed less than 1 % and up to 40 % of cochlear macrophages were positive for Ki67 and pHH3, respectively, which shows they have *in-situ* proliferative capacity. CD11b-positive monocytes distribution in the cochlea of Csf1r null mice was similar to that of wildtype mice. On the other hand, most of the Iba-1 positive cochlear macrophages disappeared in in the cochlea of Csf1r null mice and a few of them were still present on the intraluminal surface of the perilymphatic space and in the mesenchyme around the modiolus.

**Conclusion**
The cochlear macrophages appear at E10.5 around the otocyst, and afterward become matured during embryonic and early postnatal stages while changing their distribution in the cochlea. Some cochlear macrophages have *in-situ* proliferation capacity, which suggests that cochlear macrophages can be maintained by local proliferation in the steady state. There are two distinct subpopulations of cochlear macrophage, which are dependent on and independent of Csf1 signaling in their development.

# C51

**Decoding the auditory nerve and measuring the effect on speech-in-noise intelligibility of each known sensorineural pathology in the auditory periphery**

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Speech intelligibility relies on the faithful coding of the spectro-temporal modulations that carry speech information. Given the limited dynamic range (30 dB) of auditory nerve (AN) fibres found in conventional measures of rate-level functions (RLFs), optimal speech coding relies on the auditory periphery adapting to changes in contextual sound level, ideally to an extent preventing AN-fibre saturation.
Here, we demonstrate that (1) acoustic and medial-olivocochlear reflexes enhance AN adaptation to context level so as to prevents the saturation of AN fibres and associated information loss, (2) the absence of efferent pathways and/or the loss of outer haircells cause speech-signal degradation, mostly due to the saturation of high-spontaneous-rate fibres, (3) general deafferentiation (via synaptopathy, inner haircell loss or loss of AN fibres), a reduction in endocochlear potential or inner haircell otoferlin depletion all elevate the internal noise floor and degrade the signal, as these pathologies can all cause, amongst other problems, stochastic under-sampling (Lopez-Poveda 2013 & 2014).
Human context-level adaptation was predicted using a physiological model of the auditory periphery (MAP, Meddis, 2006). RLF predictions were extracted from the modelled response of AN fibres for a 50 ms probe period, immediately following a 400 ms precursor that set context level. Probe and context levels were independently varied (0-100 dB range) for tone/noise bursts and adaptation exceeding 0.5 dB/dB was predicted. Disabling efferent reflexes halved the amount of adaptation, to the same level as previously measured in anaesthetized Guinea Pig (Wen et al., 2009). Thus, around half of the adaptation predicted by

MAP emerges from efferent reflexes. Our predictions further suggest that human AN fibres could remain unsaturated in context levels up to 100 dB, well above natural speech levels.
Speech intelligibility in noise was measured in normal-hearing listeners attending to speech passed through a vocoder based on MAP (30,000 modelled AN fibres). Listeners´ speech-reception thresholds (SRTs) were compared between vocoded and unprocessed conditions at 65 dB (speech + noise) presentation level. The vocoded conditions had efferent reflexes disabled, enabled or enabled with an additional expansion. ´Vocoded´ SRTs were 4.7 dB, 2.6 dB and only 1.6 dB higher than ´unprocessed´ SRTs, respectively. These findings support a key role of efferent-based context adaptation in the optimal neural coding of speech modulations in quiet or in noise, validate a novel vocoder concept and perhaps suggest an alternative way to consider loudness coding. Additional pathologies were individually modelled and tested for psychophysically, for good measure.

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# C52

**Vascular associations in the choroid plexus: do they matter for the auditory system?**

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Hearing is known to be modulated by several vascular and inflammatory factors, affecting both the ear and central auditory system. Therefore, understanding the interaction of auditory structures with vascular and neuroimmune structures is essential.
The study of those interactions requires the visualization of macroscopic structures at cellular or subcellular resolution, which has been made possible by an increasing number of techniques for fluorescent labeling of whole clarified organs [Mano et al. 2018].
Using a whole clarified rat brainstem/temporal bone, we have previously shown that the 4$^{th}$ ventricle choroid plexus (CP) contacts the surface of the dorsal cochlear nucleus (DCN) and the dural fold covering the endolymphatic duct [Perin et al. 2019]. Since the CP is one of the main players in neuroimmune exchanges [Kaur et al. 2016], it is likely that these contacts are involved in immune communication, either in physiological or pathological conditions.
The CP displays a simple histology (blood vessels surrounded by a loose stRome and simple cuboid epithelium) but a very complex geometry (tortuous, irregular diameter vessels and stRome, sheet-like or villous local organization [Marquez et al. 2017]). Regional variations in the CP architecture have been observed in several species (including man) by SEM and vascular corrosion casts. In particular, peculiar vascular associations (artero-venous shunts [Sisson 1969], garland capillaries around arteries [Weiger et al. 1986]) have been hypothesized to be related to mechanisms for concentration or removal of local factors [Cserr 1971]. However, these studies did not allow a complete enough reconstruction of the vascular networks to build quantitative models for blood flow and blood-CSF diffusion in these structures.
In the present work, we collected the microanatomical data that will be subsequently used for modeling studies. In particular, we labeled capillaries (with collagen IV) and arteries (with SMA) in healthy adult rats, in the region encompassing the CP velar/cerebellar attachment, up to the DCN and other auditory structures.
Each half of the 4$^{th}$ ventricle CP displays an overall S shape, with a medial body directed anteriorly, a diagonal arm directed postero-laterally and a lateral expansion directed anteriorly.
Villi reaching the ventricular floor are only present in the diagonal arm, whereas the medial and lateral regions are compact. These villi contact the DCN surface, which carries no superficial arteries or veins (two large parallel vessels are flanking the nucleus, which is vascularized from deep branches).
In the lateral CP expansion, a single artery follows its entire length and emerges from the anterior tip, where it connects to a large venous vessel external to the plexus. The latter (which contains little but not negligible smooth muscle) receives small veins from the cochlear nuclei and continues to the temporal bone

periosteum. At the junction between diagonal and lateral regions, the CP artery is surrounded by a garland of capillaries.
These arrangements suggest the presence of local regulatory mechanisms for CP-derived factors diffusing in the region surrounding the DCN surface.

## C53

**Absence of STAT1 predisposes mice to otitis-related hearing loss**

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Signal transducer and activator of transcription 1 (STAT1) is known to be an important player in inflammatory responses. Inflammatory response is one of the common effects of ototoxicity. Our group and others reported that STAT1 knockout hair cells are less sensitive to ototoxic agents *in-vitro*. Therefore, we evaluated the cochlear function of wild type and STAT1 knockout mice via auditory brain stem response (ABR). Wild type and STAT1 knockout mice between 3 and 18 weeks were examined by ABR and histological methods. We found that ABR responses were affected in STAT1 knockout mice with some cases of bilateral and unilateral hearing loss. The degree of hearing loss was mild to severe. Pathological status correlated with ABR threshold elevation. STAT1 knockout mice in comparison to wild type showed an increased accumulation of inflammatory cells, cell debris and thickened middle ear epithelium after histopathologic examination. Otitis incidence did not increase with age, rather it seems that the mice experienced recurrent episodes of otitis.
These findings suggest that STAT1 ablation confers an increased susceptibility to otitis media leading to hearing impairment. STAT1 knockout mice could be used as a model to study the inflammatory mechanism of otitis and might help to develop therapeutic strategies against hearing loss associated with otitis media.

## C54

**Cell-free biological drug for the inner ear: Extracellular vesicles derived from mesenchymal stRomel cells support the survival of spiral ganglion neurons**

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**Overview**
In cochlear implantation, various experimental approaches for regenerative therapies such as the administration of neurotrophic factors via osmotic pumps or cells secreting neurotrophic factors from the implant surface have been developed. A novel therapeutic approach is the application of extracellular vesicles (EVs) for immune-modulatory and regenerative therapies. EVs are secreted by a variety of cell types and contain cell-specific combinations of bioactive molecules including proteins (enzymes, growth factors and cytokines), lipids and miRNAs. Therefore, EVs from cells that are already used for cell therapies, such as mesenchymal stRomel cells (MSCs), are promising for novel regenerative therapies in the inner ear. The aim of the present study was to investigate the neuroprotective effect of MSC-derived EVs on the survival rate of spiral ganglion neurons (SGNs).

**Methods**
EVs were isolated from human bone marrow and human umbilical cord MSCs. SGNs were isolated from neonatal (P3-5) Sprague Dawley rats and were enzymatically and mechanically dissociated. Isolated SGNs were cultivated in the presence of EVs at different concentrations and under control conditions. After 48h of cultivation, the SGNs were fixed, stained with an anti-neurofilament antibody and the neuronal survival rate as well as the neuronal morphology were determined. Moreover, the regenerative effect was investigated by measuring the length of the neuronal outgrowth.

**Results**
Addition of MSC-derived EVs from both bone marrow and umbilical cord significantly increased the survival

rate of SGNs compared to the positive control (BDNF; brain-derived neurotrophic factor). All EV concentrations used in this study resulted in a significant, dose-dependent increase in the survival rate. In addition, the number of bipolar neurons was increased.

**Conclusion**

The administration of EVs presents several advantages over the application of the intact parental cells with regard to safety and availability. Clinical application of EVs appears more feasible in the inner ear than administration of stem cells. The observed enhanced neuronal survival and regeneration is a promising first step in the evaluation of the suitability of EVs to protect the residual hearing of patients after cochlear implantation. Further investigations will concentrate on the identification of molecular pathways that are regulated by EVs in the inner ear to advance EVs to novel cell-free biological therapeutics.

**Keywords**: Spiral ganglion neurons, extracellular vesicles (EVs), cell-free therapy

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# C55

**Stress receptors in higher frontal brain regions influence auditory nerve function and auditory brainstem responses**

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Systemic corticosteroids have been the mainstay of treatment for various hearing disorders for more than 30 years. Accordingly, numerous studies have described glucocorticoids (GCs) and stressors to be protective for the auditory organ during damage situations associated with a variety of health conditions, including noise exposure. Conversely, stressors are also predictive risk factors to promote hearing disorders, as for instance negatively influence tinnitus symptoms.

How both of these contrasting stress actions are linked has remained elusive. The two different stress receptors mineralo- (MR) and glucocorticoid receptors (GR) translate the physiologic stress responses.

We induced a deletion of the conditional individual stress receptors upon tamoxifen inducible MR/GRCaMKIIαCre KO mice. This leads to selective deletion of the stress receptors in the frontal brain regions (hippocampus) within less than 4 weeks after tamoxifen treatment in adult mice.

We analyzed consequences of the acute deletion of MR/GR pre and post acoustic trauma using DPOAE, ASSR, ABR (with click, noise, pure tone) and analyzed suprathreshold waves as well as performing immunohistochemistry staining of specific markers in cochlea and brain tissue.

We surprisingly found evidence that acute central deletion of MR/GR in frontal brain regions exhibit a top down influence on auditory fiber processing.

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

# C56

**The acoustic challenge in school age children with mild hearing loss**

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The acoustic challenge is a combination of individual hearing ability and external acoustic characteristics (including speech quality and background noise). What happens when one or more of these characteristics are lacking? For example, in a mild hearing loss in school age children? A mild hearing loss, while not impeding the acquisition of language, makes it difficult or inadequate or, in the case of late onset, it can deteriorate. Children may have difficulties in verbal recognition both in quiet and noise conditions and above all, in difficult listening conditions where background noise and reverberation are present with an increase of listening effort. The lack of therapeutic approach, especially if deafness has occurred at an early age and with progressive characteristics or in conjunction with other conditions (mental insufficiency, bilingualism in deficiency situations) can cause different effects on curricular learning. We propose a review of the literature and our experience on the management of mild hearing loss.

## SESSION VII

### TINNITUS AND VESTIBULAR DISORDERS

# TL7

**Tinnitus induced hyperexcitability in view of deafness and cochlear implants?**

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Subjective tinnitus is the conscious perception of sound heard in the absence of physical sound sources. Most tinnitus literature argue for increased central spontaneous firing rates or central hyper-excitability following peripheral deafferentation leading to tinnitus percept through a homeostatic response (or central neural gain). In alternative views, tinnitus is linked to elevated cortical synchronicity, to disrupted interaction of frontostriatal with auditory-sensory regions or to a reinforcement of a `tinnitus precursor` through attention or fear. We here suggest that elevated hyper-excitability in tinnitus is the result of an incapacity to generate homeostatic adjustments due to loss of signals in noise. This alternative view of origin of elevated hyper-excitability in tinnitus results from reflecting excitability stages between (i) congenital deafness that describe a low prevalence of tinnitus (ii) acquired deafness that describe a high prevalence of tinnitus and (iii) cochlear implant electrodes (used to restore hearing) that can to some degree reduce tinnitus.

# C57

**Identification of functional biomarkers of tinnitus and tinnitus/hyperacusis in patients**

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**Introduction**
Tinnitus is as a symptomatic malfunction of our hearing system, where phantom sounds are perceived without acoustic stimulation.

**Material & Method**
In recent years, we have developed a fingerprint for tinnitus and recently hyperacusis using a combination of behavior animal models for tinnitus/hyperacusis and electrophysiological as well as molecular approaches in the peripheral and central auditory system. The characteristic features that distinguished equally hearing impaired animals with and without tinnitus or hyperacusis are described (Möhrle et al., 2019, Hofmeier et al., 2018, Knipper et al 2013; Rüttiger et al 2013, Singer et al 2013). We aimed to test the knowledge for patients with tinnitus only and tinnitus with co-occurrence of hyperacusis.

**Results**
Here we present a clinical pilot studies in hearing-impaired subjects with and without tinnitus in comparison to tinnitus with co-occurrence of hyperacusis. We use audiometric measurements, the analysis of body fluids, and functional magnetic resonance tomography (fMRI) analyzing evoked BOLD fMRI and resting state r-fcMRI.

**Conclusion**
The results in defined patient groups are discussed in the context of previous findings gained in animals.

**Keywords**: Tinnitus, Hyperacusis, ABR, fMRI

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# C58

**Psychiatric comorbidity in patients with tinnitus or auditory hallucination and sound therapy**

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**Objective**
We reported psychiatric (Psy) comorbidity (818/1183, 69.1%) in patients with dizziness. In this study, we investigated about tinnitus or auditory hallucination.

**Methods**
The subjects were 230 patients (93 men, 137 women) with tinnitus and 11 patients (4 men, 7 women) with auditory hallucination. Patients were diagnosed using ICD-10.

**Results**
Psy comorbidity was revealed in 179 (77.8%) with tinnitus. Of 171 patients, various types of Psy disorders (D) were found, such as anxiety or panic D (F41) in 85 (47.4%), mood D (F3) in 42 (23.4%), adjustment D or post-traumatic stress D (F43) in 5 (2.9%), other neurotic D (F48) in 7 (4.1%), organic mental D (F0) in 17 (9.4%) and schizophrenia (F2) in 9 (5%). Eleven patients with auditory hallucination suffered from schizophrenia in 9 cases and dementia in 2 cases.

**Conclusions**
We believe that collaboration between psychiatrists and otolaryngologists in the hospital and/or doctors in local area can improve the mental condition and the quality of life of patients who are suffering from tinnitus or dizziness with psychiatric comorbidity.

**Keywords**: psychiatric comorbidity, collaboration, sound therapy

# C59

**Subclinical cochlear dysfunction in newly diagnosed relapsing- remitting multiple sclerosis**

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**Background**
Hearing impairment in multiple sclerosis has long been considered a process mainly related to the central auditory system. However, increasing evidence also suggests a peripheral involvement of the inner ear. The objective of this study was to investigate subclinical cochlear dysfunction and possible correlation with disease severity in untreated newly diagnosed multiple sclerosis patients using transient-evoked and distortion-product otoacoustic emissions.

**Methods**
Forty patients with newly diagnosed relapsing-remitting multiple sclerosis, clinically normal hearing and no brainstem lesions (study group) and forty matched controls (control group) were included in the study. All subjects had a routine audiological evaluation including history and clinical examination, pure tone audiometry, acoustic immittance test, auditory brainstem response and otoacoustic emissions recording. Self-administered questionnaires were used to evaluate self-perception of hearing disability.

**Results**
Auditory brainstem response and pure tone audiometry thresholds resulted within normal range in all patients. The amplitudes of transient-evoked and distortion-product otoacoustic emissions responses were significantly reduced at 1000, 1500, 2000 and 3000 Hz in the study group compared to the control group.

**Conclusions**
This study shows decreased otoacoustic emission amplitudes in untreated multiple sclerosis patients with clinically normal hearing and no brainstem demyelinating plaques, suggesting a subclinical cochlear impairment. This alteration may represent an early sign of peripheral hearing damage, suggesting a role for

otoacoustic emissions in the early diagnosis of cochlear dysfunction in multiple sclerosis patients. However, given that otoacoustic emissions primarily reflects cochlear function, and that the wave I of the auditory brainstem responses was spared, the evidence supporting a peripheral involvement of acoustic pathways due to multiple sclerosis can only be hypothetically attributed to an early subclinical involvement of outer hair cells.

## C60

**The proteome of the perilymph in relation to hearing loss in patients with vestibular schwannoma**

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**Introduction**

The mechanisms causing sensorineural hearing loss in patients diagnosed with vestibular schwannoma (VS) are unknown and at present we have no tools to foresee future hearing function after initial diagnosis. This complicates the optimal choice of treatment for VS. The options available are surgical resection, stereotactic radiation or “a wait and scan” regime.

In our previous descriptive study on the proteome of perilymph in VS patients, we showed in an explorative analysis a correlation between low expression of alfa-2-HS-glykoprotein (Fetuin-A) and hearing loss. It is also known that perilymph from patients with VS has higher protein concentration than normal. Our hypothesis is that VS influences specific cellular and extracellular processes in the inner ear, not only affecting the vestibulocochlear nerve.

**Aim**

The aim of this study was to assess the relation between hearing loss and the proteome of perilymph.

**Patients and Methods**

Patients: Twenty-nine patients were included in the analysis. All patients were confirmed to have VS on histopathological examination.

Audiometry: The hearing thresholds were defined as pure tone average on 500, 1000, 2000, and 4000 KHz (PTA4) measured in both ears. Patients were grouped according to PTA4 of the tumour affected ear in the hearing categories functional (≤49dB) and non-functional (≥50dB).

Mass spectrometry: All perilymph samples were analysed in an LTQ-Orbitrap mass spectrometer. Spectrum data files were analysed with the MaxQuant software using Andromeda search engine and the UniProt reference human proteome.

Statistics: External expert bioinformatics consults assisted with analysis of protein expression, primary component analysis and further pathway analysis between patients groups. The difference in PTA4 between tumour-affected and unaffected ear was used for comparison between sensorineural hearing loss and expression of perilymphatic Fetuin-A.

The study was approved by the regional ethics committee in Uppsala (ref 2013/255) and followed the rules of the declaration of Helsinki.

**Results**

Preliminary results showed a significant difference in expression of Fetuin-A in the functional hearing and non-functional hearing groups. The results are currently being processed and will be presented in more detail at the conference.

# C61

**Pharmacological Ablation of Vestibular Hair Cells or Ganglion Neurons to Generate a Model of Unilateral Vestibular Dysfunction**

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Vestibular dysfunction is a significant clinical problem with limited regenerative therapeutic options. Hence, new therapeutic strategies for the recovery of vestibular function need to be investigated. However, there are a lack of appropriate animal models demonstrating this pathology. A model of unilateral vestibular dysfunction, as opposed to the bilateral form, can be useful for the investigation of functional recovery. There are two primary methods to acquire this phenotype; either through loss of hair cells or neurons. An aminoglycoside-induced method to eliminate hair cells was reported previously, however in vivo vestibular function was not always assessed. Furthermore, a model of vestibular neuron ablation can be studied in addition to the model of hair cell loss. A model of auditory neuropathy has been previously generated by applying ouabain to the round window of the gerbil cochlea. Ouabain is a well-known cardiac glycoside that specifically binds to Na+, K+-ATPase and blocks its activity. We hypothesized that we can ablate vestibular ganglion neurons in addition to spiral ganglion neurons by local application of ouabain into the inner ear. The aim of this project is to generate a mouse model of unilateral vestibular dysfunction that can be used for developing cell-based or gene therapy methods to treat vestibular disturbances caused by loss of vestibular hair cells and neurons. The following two different experimental conditions were generated using eight-week-old male C57BL/6N mice. (1) Vestibular hair cells were ablated by injecting 1 µl of gentamicin (360 mg/ml) via the posterior semicircular canal (modified Kawamoto et al., Hear Res 247: 17-26, 2008). (2) Vestibular neurons were ablated by injecting 1 ul of ouabain octahydrate (5 mM) via the posterior semicircular canal. Histology indicated that gentamicin and ouabain preferentially ablated either vestibular hair cells or neurons, respectively. Serendipitously we discovered Na+, K+-ATPase negative neurons within the vestibular ganglion, and ouabain ablated Na+, K+-ATPase positive neurons only. Functional analysis as determined by the vestibular ocular reflex (VOR) demonstrated that both models (i.e. hair cell loss, and neuronal loss) showed reduced VOR gain in the affected ear. Therefore, we have demonstrated the production of a model of unilateral vestibular neurosensory degeneration that can be a useful tool to investigate functional regeneration of the vestibular system.

# C62

**Ionic direct current stimulation results in spike-rate adaptation in vestibular afferents of the mouse crista in vitro**

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Direct current (DC) can be used to modulate (excite or inhibit) neuronal activity while maintaining a stochastic firing pattern. DC is commonly used to modulate the vestibular system with the aim to probe and understand vestibular function in normal function and pathophysiology (Fitzpatrick and Day, 2004; Schniepp et al., 2018). Typically, DC is applied externally through the mastoid processes of the head, called 'galvanic stimulation', to avoid any toxicity generated by electrochemical reactions occurring at the DC electrode. However, experiments have also attempted to increase the specificity DC by inserting the electrodes into or close to the inner ear. These experiments resulted in a generalized activation or inhibition of the vestibular end organs.

An evolution of this concept is the focused, safe delivery of ionic direct current (iDC) separately into each vestibular semicircular canal with the intent to restore functional vestibular sensation for patients suffering from bilateral vestibular dysfunctions. iDC steps evoke vestibular-ocular reflex (VOR) responses, with the direction of eye rotation dependent on the polarity of the iDC, but the physiological effect of iDC on vestibular afferent activity has not been reported.

In this study, afferent firing rates in response to iDC modulation were monitored in the excised vestibular mouse crista *in vitro* with loose-patch clamp recordings from afferent endings close to the hair cells. The results show that the position of the stimulation electrode can drastically influence the response of the afferent to cathodic and anodic iDC modulation. Moreover, cathodic and anodic iDC steps can

instantaneously reduce and increase afferent spike rate during brief stimulation durations. These spike rate responses match iDC/VOR relationships described in previous papers in the chinchilla (Aplin et al., 2019) and both datasets showed an increased sensitivity to stepped cathodic iDC over anodic iDC with a similar ratio (~3:1). However, a sustained constant anodic or cathodic current resulted in adaptation to the stimulus and a return to spontaneous spike rate with a time constant of adaptation ranging from ~0.1 to 10 s. Sinusoidal iDC modulation displayed high-pass characteristics including a higher sensitivity to stimulation at higher sinusoidal frequencies and a phase-lead at low frequencies. Post-adaptation spike rate responses to iDC steps were similar to pre-adaptation controls, but at high intensities spike rate response sensitivities were modified by the presence of an adaptation
step even though the baseline spike rate was not significantly different.

Our data show that iDC can modulate spike rate at the vestibular afferent, and that the relationship between iDC amplitude and spike rate change is time dependent. Differences in behavioral data across iDC baselines may be due to post-adaptation changes in sensitivity at the afferent. These results help to understand spike rate/amplitude relationships for both invasive and non-invasive DC stimulation of the vestibular system and inform the development of an iDC-based vestibular prosthesis.

**Keywords:** Direct current, Adaptation, Vestibular nerve fiber, Vestibular prosthesis

# C63

**Inefficient cranial venous outflow and increased CSF pulsatility in the Aqueduct of Sylvius in patients with Meniere's disease**

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**Introduction**
Meniere's disease (MD) is a multifactorial disease clinically characterized by intermittent severe bouts of vertigo, tinnitus and aural fullness. MD patients have a high incidence of abnormal neck venous vessels characterized by various degree of strictures in the internal jugular veins. This will cause obstruction to cerebral venous outflow, increased intracranial venous pressure, hampered CSF reabsorption, disturbed intracranial dynamics and intracranial pressure (ICP) resulting in disturbed inner ear circulation and possibly disturbed perilymphatic and endolymphatics fluid spaces. Such changes in overall intracranial fluid dynamics can cause a permanent increase in the volume of the endolymph, a condition also termed endolymphatic hydrops (EH). Since the endolymphatic space in the inner ear communicates directly with the subarachnoid space (SAS), an increase in ICP will be transmitted to the inner ear and cause MD. Inefficient cranial venous outflow is characterized by increased CSF pulsatility in the Aqueduct of Sylvius (AoS), a potential magnetic resonance (MR) biomarker for increased ICP. We report a phase contrast magnetic resonance imaging (PC-MRI) and magnetic resonance venography (MRV) findings to study the anatomy of the extracranial venous vessels and changes in AoS pulsatility.

**Materials and Methods**
T1 structural MRI data was used to segment the three tissue types in the brain: CSF, white matter (WM) and gray matter (GM) in 28 patients (17males, 60.6yrs: 40-77yrs) matched to similar data already available in literature. PC-MRI was performed at the level of the AoS to quantify CSF flow. CE-MRV was performed to characterize anatomical venous anomalies in the extracranial neck vessels. All data was collected on a 1.5T Siemens clinical scanner in a local hospital.

**Results**
**T**otal intracranial volume of 1438.35±109.8mL was within normal values (1367.3±147.4mL; p=0.11), however there was a significant increase in total CSF volume 302,41±61.98 mL (normal 151±54 mL; p<0.0001), a significant decrease in GM (582.20±55.52 mL; normal 675±53 mL; p=0.0001) and normal WM volume (563.02±46.53 mL; normal 583±124mL; p=0.47). Flow data could be calculated on 19 patients (9 were excluded due to the presence of artifacts). Anomalous venous anatomy characterized by stenosis, agenesis and atretic jugular vein segments were noted in all patients and various degree of dilated collateral veins

were noticed. There was a significant increase in CSF pulsatility at the AoS (-4.12±1.36cm/s; normal -2.99±1.40 cm/s; p=0.022).

**Discussion**

All our patients demonstrated increased anomalous venous anatomy and increased number of tortuous veins in the neck (Figure 2). This suggests hampered drainage towards the heart, increased intracranial venous volume and pressure and altered CSF reabsorption via the subarachnoid granulation. Elevated ICP is inferred by the presence of increased CSF pulsatility in the AoS. Our segmentation results suggest a very high total CSF volume in patients and a significantly reduced total GM volume which could suggest a compressive effect of the increased CSF in the SAS. We speculate that this could also cause reduced drainage of waste products from the GM towards the SAS, but this requires further investigation. Future work will include the study of MD patients after treatment.

# C64

**Biomarkers for the differential diagnosis of Vestibular Migraine and Meniere Disease**

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Vestibular Migraine (VM) and Meniere's Disease (MD) are episodic vestibular syndromes characterized by an association of symptoms such as hearing loss, tinnitus and/or migraine during attacks. These syndromes have a high symptom overlap, especially in the earlier stages of the disease, so at times based exclusively on medical history and symptomology they may be indistinguishable. Subgrouping of MD patients according to their IL-1β profile – high or low - has previously been reported. Therefore, considering the clinical resemblance of VM and MD, we aimed to characterize the cytokine profile of MD and VM to differentiate these patients. Firstly, we carried out gene expression microarrays and observed that gene expression profile in peripheral blood mononuclear cells (PBMC) showed significant differences in MD patients with high and low basal levels of IL- 1β, VM patients and healthy controls. Then, we measured the levels of 14 cytokines and 11 chemokines in 129 MD patients, 82 VM patients and 66 healthy controls. We observed that MD patients with high basal levels of IL- 1β (MDH) had higher levels of cytokines/chemokines when compared to the other subsets. CCL4 levels were significantly different between MDH, MD with low basal levels of IL- 1β (MDL), VM and controls. We determined by Logistic Regression that IL- 1β, CCL3, CCL22 and CXCL1 levels can be used to differentiate VM patients from MD patients (area under the curve=0.993), suggesting a high diagnostic value in patients with symptoms overlap.

**Keywords**: Vestibular Migraine; Meniere Disease; cytokines; differential diagnosis

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# C65

**Electrocochleography finding in meniere disease after active pressure treatment**

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**Objectives**

To identify a possible long-lasting effect of the low-pressure treatment on Ménière's Disease (MD) and to find eventual correlations between its active stage and endolymphatic hydrops (EH).

**Methods**
Twelve patients affected by definite MD and a severe degree of disability, received a ventilation tube and low-pressure treatment before considering a surgical procedure. All the subjects were assessed by electrocochleography (ECochG) for getting evidence of EH, and were asked to fill specific questionnaires at pre-determined timing, i.e. before starting the treatment, at the end of treatment and 3, 6 and 48 months later.

**Results**
All the selected subjects presented with an ECochG pattern indicative of EH before starting the treatment. At the end of one-month pressure treatment, 58.4% of the patients showed symptomatic improvement while the ECochG pattern remained hydropic-like in 75 % of them. At 3-month control stage, the hydropic pattern resulted normalized (< 0.5) in all the improved subjects (83.3 %). This result remained stable at 6 months of follow-up. After 2 years 80 % of the patients remained asymptomatic along with a normal ECochG pattern.

**Conclusions**
The normalization of the hydropic ECochG pattern after one-month low-pressure treatment, already observed at the six month follow-up assessment, showed to persist in the majority (80 %) of the study sample after two years. We can affirm from this study that this pressure treatment has a long term effect on EH. This would mean that EH is certainly accompanying the active phase of MD, while it subsides when the disease is quiescent as it was the case after the application of this therapy.

**Keywords**: endolymphatic hydrops; Ménière's disease; electrocochleography; pressure treatment

# C66

**Anatomical, biochemical and behavioural characterization of a mouse model for Meniere's Disease**

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Meniere's Disease (MD) is characterized as follows: recurrent episodes of vertigo, fluctuating and progressive sensorineural hearing loss (SNHL) and tinnitus. MD is a heterogeneous clinical syndrome. The prevalence of MD is about 0.5-1/1000 persons. The usual age of onset for MD ranges from 30-50 years. Both ears are affected, leading to severe hearing impairment and chronic imbalance, resulting in a huge burden for patients and a significant impact on health-related quality of life. Because of a lack of knowledge of the molecular mechanisms involved, it is difficult to generate treatments for these patients. We have been working with one mouse model, a Fam136a knockout (KO), using anatomical, biochemical and behavioural approaches, to understand how its inner ear might be degraded to generate the disease phenotype at an advanced age in these patients. Fam136a is a mitochondrially-localized protein that is highly conserved across species, and it is coded for by a gene located on Chromosome 2. Through whole exome sequencing, this gene was found in multiple generations of a human family with familial MD. We have found significant differences in both rotarod performance over time and in mitochondrial function between the WT and KO mice, supporting the hypothesis of a role for this gene in the dysfunction occurring in MD.

*This research was supported by the American Hearing Research Foundation*

# C67

**Metabolomics in Meniere's Disease: redox modulation by nutritional approaches with mushrooms**

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Metabolomics has been successfully applied to study neurological and neurodegenerative disorders for the identification of potential biomarkers of onset and disease progression, the identification of novel

mechanisms of disease progression and the assessment of treatment prognosis and outcome. Meniere's disease (MD) represents a clinical syndrome characterized by episodes of spontaneous vertigo, associated with fuctuating, low to medium frequencies sensorineural hearing loss (SNHL), tinnitus and aural fullness affecting one or both ears. To date, the cause of Meniere's disease remains substantially unknown, despite many study reports indicate idiopathic endolymphatic hydrops as the main pathophysiological event, and increasing evidence suggests that oxidative stress and neuroinflammation are central to the development of endolymphatic hydrops and consequent vestibular otholitic degeneration and crisis. Among the cellular pathways conferring protection against oxidative stress, a key role is played by vitagenes, which include heat shock proteins (Hsps), heme oxygenase-1 and Hsp70, as well as γ-GC liase, sirtuin and thioredoxin. Mushrooms, have been used in traditional medicine for thousands of years, reportedly endowed with various biological actions, including antitumor, immunomodulatory, antioxidant, antiviral, antibacterial, and hepatoprotective effects. Of the mushroom-derived therapeutics, polysaccharopeptides obtained from Coriolus versicolor are commercially the best established. In this study we tested the hypothesis that neurotoxicity is an important primary mediator of injury in Ménière's disease and may be reflected in measurable increases in markers of cellular stress response and oxidative stress in the peripheral blood of patients with Meniere's disease. We evaluated systemic oxidative stress and cellular stress response in MD patients in absence and in presence of treatment with a biomass preparation from Coriolus. Systemic oxidative stress was estimated by measuring in plasma protein carbonyls, HNE, ultraweak luminescence and F2- isoprostanes, as well as active biolipds such as lipoxin A4, whereas in lymphocytes we determined heat shock proteins 70 (Hsp72), heme oxygenase-1 (HO-1), thioredoxin (Trx), and γ-GC liase to evaluate the systemic cellular stress response. Increased levels of carbonyls, HNE and F2-isoprostanes were found in MD patients with respect to MD plus Coriolus treated group. This was paralleled by a significant induction in lymphocyte of HO-1, Hsp70, Trx, sirtuin-1 and γ-GC liase ($P<0.01$) and by a significant increase in the plasma ratio reduced glutathione GSH) vs oxidized glutathione (GSSG) ($P<0.05$). Lipidomics and Metabolomics approaches and results will be also discussed. In conclusion, patients affected by MD are under condition of systemic oxidative stress and the induction of vitagenes after mushroom supplementation indicates a maintained response to counteract intracellular pro-oxidant status. The search for novel and more potent inducers of vitagenes will facilitate the development of pharmacological strategies to increase the intrinsic capacity of vulnerable ganglion cells to maximize antidegenerative mechanisms, such as stress response and thus neuroprotection1.

# 56th Inner Ear Biology Workshop

**Sunday 8 September 2019**

# POSTER SESSION

## Imaging and Anatomy

## P1

**The innervation of the mammalian cochlea, an immunocytochemical study**

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The mammalian cochlea has a distinguished and unique innervation by the auditory neurons. These neurons have afferent peripheral processes with the ribbon synapses, which are specialized for the precise and fast processing of the hearing information. Spiral ganglion cells extend their central axons toward the auditory brainstem and form the auditory division of the eight cranial nerve. In addition, the olivocochlear bundle form the superior olivary complex and innervates the sensory cells through efferent fibers. This system of various nerve fibers and connections is needed to send the sound signal from the sensory hair cells to the auditory brainstem, but also to modulate the system.
The aim of the study was to investigate the nerve fibers, the synapses and the somata of the auditory neurons by new identified immunocytochemical markers. For this, postnatal day 21 mouses (P21) were fixed by paraformaldehyde, sectioned via cryostat and processed by immuncytochemical analysis. In addition, whole-mount preparations were performed. Digital photos were taken by confocal microscope and processed.
Two types of spiral ganglion neurons were characterized: bipolar type I neurons connecting the inner hair cell (IHC) to the cochlear nuclei of the hindbrain and are stained with Calretinin (Calb2). Pseudounipolar type II spiral ganglion neurons connecting the outer hair cells (OHC) of the organ of corti, which were labelled by Peripherin (PRPH).
The neurons of the olivocochlear bundle can be divided in the medial olivocochlear efferent neurons (MOC) and the lateral olivocochlear efferent neurons (LOC). MOC fibers innervate the OHC and are cholinergic neurons. They were labelled by choline acetyltransferase (ChAT) and the terminals by vesicular acetylcholine transporter (VAT). LOC fibers innervate afferent terminals of type I spiral ganglion neurons of the IHC. Predominantly, LOC fibers are cholinergic neurons, but a small amount is dopaminergic, which are labelled by tyrosine hydroxylase (TH). Calcium-activated potassium channels are also present, since they modulate the excitability of the neurons and the sensory hair cells. These channels can be divided into two groups; small conductance calcium-activated channels (SK) and large conductance, voltage-gated, calcium-sensitive channels (BK), which are distinguished by significant differences in voltage sensitivity, single-channel conductance, calcium affinity, and gating kinetics. In addition, presynapses contain inotrope nicotic acetylcholine receptor (nAChα9α10) as well as endocytosis proteins like Piccolo and Ribeye for the active zone and potassium voltage-gated channel subfamily KQT member 4 (KCNQ4).
In conclusion, the investigations display a comprehensive overview of the innervation of the mammalian cochlea. The results and the new identified antibodies will facilitate in future to investigate pathologies of the auditory pathway, for example in animal models of auditory neuropathies.

**Key words**: cochlea – innervation – afferent – efferent – neurons

# P2

**Abnormalities of the ear and far beyond: the long lasting Padova's experience on aural atresia**

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In Padova university hospital 190 patients affected by aural atresia are currently on follow-up. Aural atresia can be considered the slightest sign of a spectrum of pathologies called oculo-auriculo-vertebral spectrum (OAVS). This spectrum is characterized by the presence of malformations affecting at some degree the ear, face, eye and cervical spine.
In recent years our group published numerous papers describing unknown involvement of districts that are not strictly related to those responsible for the OAVS' phenotype.
This presentation will be a summary of our latest experience in this pathology highlighting what should be investigated by imaging in this craniofacial disorder. In particular, the presentation will take into account the peculiar imaging features of some previously unreported anatomical variations (affecting vascular, neural and bone structures) that should be remembered as these might be seen even in other pathologies.

# P3

**The vascularization of the labyrinth: an anatomical study**

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**Introduction**

The pathogenesis of diseases affecting the labyrinth is still somewhat unknown, especially when the metabolism and fluid homeostasis are involved.
A great effort was made in the second half of 20th century to study the vasculature of the labyrinth, through a meticulous dissection of injected temporal bones and stereoscopic images of the inner ear. A detailed description of vascular system was the result, which has not known in the years to come further investigations and still remain a cornerstone to understand the pathogenesis of those diseases affecting the blood supply, fluid homeostasis and metabolism.

**Methods**

The study, realized in 1990, was performed on 40 human temporal bones with vessel injection and 1mm serial sections.

**Results**

The main aspects of the vascular supply of the labyrinth are summarized as follow:
The internal auditory artery (IAa) derives from the anterior inferior cerebellar artery (AICa).
From IAa originates: the superior vestibular artery (SVa), the cochlear artery (Ca) and the vestibule-cochlear artery (VCa). The Ca reaches the cochlear turns. The VCa divides in a T pattern into a cochlear branch (basal cochlear artery – BCa) and a vestibular branch (inferior vestibular artery - IVa).
The utricular artery, branch of SVa, reaches the nerves of the superior and lateral canals and after the cristae and ampullar crura.
The first part of the IVa supplies the vestibular scala, the spiral lamina, the saccular artery, the artery of the vestibular caecum and a branch of the common crus. The second part of IVa bifurcate into two branches: the first supplies the posterior canal, the second supplies the utricle and ends in two terminal branches: the artery of the lateral canal and the artery of the scala media.
The venuos drainage of the vestibular labyrinth is made of three veins: the superior vestibular vein (SVv), the inferior vestibular vein (IVv) and the posterior vestibular vein (PVv). The SVv, the IVv and the common cochlear vein form the cochlea-vestibular vein (also called vein of the perylimphatic duct) that reaches the inferior petrosal sinus. The PVv (also called vein of the endolymphatic duct) enters in petrosal sinus or in the jugular bulb. The SVv receives veins from the utricle macula and from the membranous walls of the utricle and saccule. The IVv receives veins from the crista and ampullar crus of the posterior canal, from the utricle

and saccule walls, from the vestibular aspect of the scala media and the vein of vestibular caecum. The PVv receive blood from the ampullar crus and the simple crus of the lateral canal and from the common crus.
The cristae of the semicircular canals have a double way of drainage. The central part of the crista drains into the cochlea-vestibular vein, the periphery drains into the posterior vestibular vein. The latter is running parallel to the vestibular aqueduct and on its dural course lies close to the medial margin of the endolymphatic sac.

**Discussion and conclusion**
Operations on the endolymphatic sac to relieve symptoms of Meniere's disease have a long history of changing popularity in the otological practice and their effects on the labyrinth are still obscure. Surgery on the sac involves a high risk of hitting the posterior vestibular vein and thus causing an acute venous hiperhemia on the peripheral cristae in an area devoted to endolymph metabolism.

# P4

**Hearing and cognitive impairment: a functional evaluation of associative brain areas in patients affected by Alzheimer's disease**

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Auditory dysfunction observed in patients with cognitive diseases is probably due not only to mechanisms of aging or dysfunction of the peripheral auditory pathway, but also to the alteration of some brain networks that play an important role in sound stimulus processing. Central hearing is an elaborate process, which activates not only the auditory cortex, but also several associative areas where words are cognitively connected. In fact, associative brain areas (BAs) seem to play a fundamental role in the comprehension of the auditory message, allowing the subject not only to perceive the sound stimulus, but also to understand its semantic value. Recent studies have mapped the selectivity of various semantic regions located in different cortical areas by performing functional magnetic resonance imaging (MRI) in healthy subjects exposed to sound stimuli, however, the functioning of auditory areas in patients with cognitive diseases remains poorly known and difficult to assess. For these reasons, we recruited patients with Alzheimer's disease (AD), one of the most important forms of cognitive impairment, selecting those with a normal (i.e. for age and gender) audiometric evaluation and compared them with normal subjects. The aim of our study was to evaluate, through 2-deoxy-2- [18F]fluoroglucose (18F FDG) positron emission tomography/computed tomography (PET/CT) neuroimaging, the functional hearing networks in the primary auditory cortex and in other associative brain areas involved in the hearing process.
We recruited 131 patients with a new diagnosis of AD (diagnosed according to the NINCDS-ADRDA criteria). and a control group (CG) of 36 chemotherapy naïve subjects. A complete clinical investigation was performed in all the subjects, including medical history, Mini-Mental State Examination (MMSE), complete blood screening and neurological, otolaryngological, neuropsychological and neuropsychiatric examinations. All the patients underwent MRI. After the complete clinical investigation described, all subjects underwent a brain FDG PET/CT.
The comparison of glucose metabolism between the AD patients and the CG showed significant hypometabolism in the temporo-parietal lobes (with the higher differences being reported in right precuneus, angular gyrus, left inferior parietal lobule) and frontal lobe (right and left middle frontal gyrus, right superior frontal gyrus), corresponding to BA6, BA7, BA8, BA39, whereas we did not find differences in the primary auditory cortex metabolism. In particular, AD connectivity analyses showed a positive correlation of the primary auditory cortex with BA 6,8,21,31,39,40,42 and a negative correlation with BA 19, cerebellum and basal ganglia.
In conclusion, our findings seem to indicate a disruption of functional networks between the primary auditory cortex and other brain areas that play a fundamental role in the hearing process. These evidence may suggest the importance of exploring hearing function in patients with cognitive impairment for early diagnosis of cognitive impairments, while on the other hand, correction of auditory function through the appropriate hearing rehabilitation seems to be crucial to reduce the risk of cognitive decline.

**Keywords**: Alzheimer's disease, cognitive impairment, hearing loss, neuroimaging

# P5

**The complexity of multidisciplinary evaluation of children with sensorineural hearing loss: three case report**

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Through the universal hearing screening program it is possible to diagnose sensorineural hearing loss (SNHL) in the first months of life. Nevertheless an early and certain etiology of SNHL is not always possible. Most of scientific literature aims at a better understanding of the etiology of pediatric SNHL, but there is little information regarding the appropriate medical evaluation.
In our institute, all children affected by SNHL are subject to a multidisciplinary clinical evaluation. Each child receives an evaluation from otolaryngologist, audiologist, speech therapist, ophthalmologist, genetics, pediatric, neurologist and radiologist. This medical approach has enabled an accurate diagnosis in most cases with SNHL. It has also improved the time and the type of medical follow-up procedure.

We describe below three cases of children with SNHL.
The first case regards two sisters affected by different degrees of bilateral SNHL: one moderate and one profound. During pregnancy, intrauterine growth retardation was found in both cases. Clinical examination showed postnatal growth retardation, small head size, facial dysmorphism, cutaneous dyschromia and clinodactyly. An inner ear malformation was found after neuroradiology of the ear.
The genetic exam points to the mutation in DDX11 gene that resulted in Warsaw syndrome.
In the second case we describe a boy with profound SNHL in auditory neuropathy spectrum. Muscular weakness, peripheral sensorial deficit were observed during the neurological examination. The MRI showed no ear and brain alteration. Low visus and retinal alteration was found after ophthalmology evaluation. Genetic investigation are under way.
The last case regards a child affected by asymmetric SNHL. No alterations were found in opthalmological and neurological examination. Computed tomography showed right enlarged vestibular aqueduct and bilateral cochlear malformation whereas diffuse cerebral and cerebellar alterations were found in MRI exam.
Subjects performed a next-generation targeted re-sequencing to analyze 61 genes related to SNHL and different mutations in different gene. An accurate gene evaluation is in progress.

These three complex cases provide an understanding of the importance of considering thorough medical and specialistic evaluations among children whit hearing impairment.

**Keywords**: Infant sensorineural hearing loss, etiologic diagnosis, multidisciplinary evaluation

# P6

**Outcomes of Modified Canal Wall Down Mastoidectomy and Mastoid Obliteration Using Autologous Materials**

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**Objectives**
The traditional canal wall down mastoidectomy (CWDM) procedure commonly has potential problems of altering the anatomy and physiology of the middle ear and mastoid. This study evaluated outcomes in

patients who underwent modified canal wall down mastoidectomy (mCWDM) and mastoid obliteration using autologous materials.

**Methods**
Our study included 76 patients with chronic otitis media, cholesteatoma, and adhesive otitis who underwent mCWDM and mastoid obliteration using autologous materials between 2010 and 2015. Postoperative hearing airbone gap and complications were evaluated.

**Results**
During the average follow-up of 64 months (range, 20 to 89 months), there was no recurrent or residual cholesteatoma or chronic otitis media. No patient had a cavity problem and anatomic integrity of the posterior canal wall was obtained. There was a significant improvement in hearing with respect to the postoperative air-bone gap ($P<0.05$). A retroauricular skin depression was a common complication of this technique.

**Conclusion**
The present study suggests that our technique can prevent various complications of the classical CWDM technique using autologous tissues for mastoid cavity obliteration. It is also an appropriate method to obtain adequate volume for safe obliteration.

**Keywords:** Bone; Cartilage; Mastoid; Mastoidectomy

# P7

## Conformity between Magnetic Resonance Imaging and Surgery Outcome in Cholesteatomas

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**Background**
Cholesteatoma means that keratinization squamous epithelia is trapped in middle ear and/or mastoid. The degree and expanse of the cholesteatoma is often impossible to determine with an earmichroscopic examination. With computertomography bone destruction can be visualized, but cholesteatomas cannot be differentiated from other soft tissue changes in the middle ear or mastoid. In 2006 it was first reported about the possibility to differentiate between cholesteatomas and other soft tissue in middle ear and mastoid with diffusion weighed magnetic resonance image (DWMRI). Studies have shown high sensitivity and specificity for DWMRI regarding cholesteatoma tissues.

**Objectives**
To outline the usage of DW-MRI for cholesteatoma diagnostics and evaluate its accuracy in regular clinical use.

**Method**
A retrospective chart analysis. Patients examined with DW-MRI in area that covers a population of approximately 516,000 inhabitants between October 2010 and March 2019 was included. Primary outcome measure was diagnostic accuracy, calculated by comparing DW-MRI conclusions to surgical findings. A flow diagram was constructed by following the 2015 STARD (Standards for Reporting diagnostic accuracy studies) guidelines. The radiological conclusions from the MRI-examinations were coded as positive, negative or inconclusive. In cases where the patient had later undergone surgery, statements were compared to the surgical diagnosis. All middle ear/temporal bone operations in which a cholesteatoma would have been detected during surgery were included as reference standard. The surgical diagnosis was coded as positive or negative if a cholesteatoma was present respectively ruled out.
The sensitivity and specificity for the method was calculated having the DW-MRI as index test and diagnose from surgery considered as reference standard.
In the cases where radiological conclusion did not correspond to the surgical diagnosis (i.e. false positives and negatives) an experienced radiologist was consulted to reevaluate the MRI-examinations.

**Results**
110 patients with suspicion of uni- or bilateral cholesteatoma underwent one or more DW-MRI examination which resulted in 139 cases in total, of which 52 cases underwent surgery after examination. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of operated cases were 50.0, 75.0, 73.7, 51.7 and 60.4% respectively. The usage of DW-MRI has increased over time and the

diagnostic accuracy of DW-MRI seem to have increased over time. Accuracy was higher when including re-examined false results interpreted by an experienced head and neck radiologist and when adjusting for new classification criteria regarding the presence of retraction pockets.

**Conclusions**

The usage of DW-MRI as a diagnostic tool for primary and relapse cholesteatoma has increased over time. Overall, the sensitivity, specificity and accuracy do not reach acceptable levels to be reliable in everyday clinical use. The quality of the examination increases substantially over time, when interpreted by an experienced radiologist and when using the definition of cholesteatoma recommended by the new EAONO/JOS classification criteria.

**Keywords:** cholesteatoma; Diffusion Weighted Magnetic Resonance Imaging; Non-EPI DW-MRI

# P8

**Vascular network of the rat cochlear nuclei**

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Three-dimensional digital atlases for model organisms are becoming an essential tool in modern neuroscience. As the rat is an important model for hearing research, and the reference anatomy of its auditory system is less well characterized than in mouse, there is the necessity of producing maps of its structures. A segmentation of the rat auditory system regions has been recently added to the Waxholm Space Reference Atlas [Papp et al. 2019], yielding a starting point to add more details.

Cellular and subcellular details of the nervous system may be obtained using clarification protocols followed by fluorescent labeling [Vigouroux et al. 2017]. These protocols offer the advantage of imaging the whole brain in situ with light microscopy resolution, and selectively labeling cell populations. In our lab, we have developed a whole clarified rat brainstem/temporal bone preparation, in which the lower auditory system may be imaged without cutting artefacts from the cochlea to the inferior colliculus [Perin et al. 2019]. In the present work, we reconstructed the vascular network in cochlear nuclei (CN) by labeling capillaries (with collagen IV and IgG) and arteries (with SMA) in healthy adult rats.

Within the rat brain, the CN are among the regions with the highest blood perfusion at rest [Gharagouzloo et al 2017], but a detailed map of their vascular network is still lacking. Consistently with these functional data, in our collagen IV labeled brains, the capillary density of cochlear nuclei was clearly higher than in the surrounding brainstem, and also higher than in cerebellar lobules.

The CN are located at the lateral exit of the 4$^{th}$ ventricle; the DCN is intraventricular, whereas the VCN is mostly located outside the foramen of Luschka. This strongly affect their vascularization: as evident from SMA arterial labeling, VCN is irrorated by several small pial arteries surrounding the nuclear surface, and the cochlear nerve root is irrorated by an artery which bifurcates within the VCN, possibly following the course of cochlear nerve fibers.

On the other hand, the DCN has no superficial arteries, and is irrorated from its deep layers outward by collaterals of AICA (anteriorly) and medullary artery (posteriorly), which delimit the nucleus at its anterior and posterior edge. Deep DCN also receives collaterals from VCN arteries, and vessels delineating the fusiform layer are common. Similarly to the cerebellum, the DCN appears to receive separate irroration of the molecular and deep layers; however, whereas in the cerebellum arterioles (both superficial and deep) are mostly perpendicular to the surface, in the DCN they are not, and a clear direction is not discernible.

As regards veins, VCN and DCN drain into common vessels located on the lateral surface. The anterior vein also receives tributaries from the choroid plexus, and contains an evident muscular layer.

The present results show that the vascular organization of DCN is different from either VCN or cerebellum, and displays features which are comparable to those found in circumventricular organs [Duvernoy and Risold 2007].

## P9

**Relationship between the drainage patterns of the dural venous sinus and the affected side of sudden sensorineural hearing loss**

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**Objective**: To analyze whether there is a correlation between draining patterns of the dural venous sinuses and 1) affected side of sudden sensorineural hearing loss (SSNHL) and 2) hearing recovery.
**Study Design**: Retrospective study.
**Setting**: Tertiary referral hospital.
**Patients**: Adult patients with unilateral SSNHL.

**Interventions**
All 64 patients were treated with oral methylprednisolone. In 43 patients, intratympanic dexamethasone injection was performed along with oral steroid.

**Main outcome measures**
The dominance of the inferior petrosal sinus (IPS) on the affected side and the ipsilateral transverse-sigmoid sinus (TS/SS) as demonstrated by three-dimensional contrast-enhanced magnetization-prepared rapid gradient-echo sequence. 2) Recovery of hearing defined as average thresholds returning to better than 25 dB or within 10 dB of the unaffected ear.

**Results**
Asymmetrical venous drainage of the dural sinuses proved to be frequent (53.1% in IPS; 82.8% in TS/SS). The dominant side of the IPS or TS/SS alone was independent from the affected side. However, the relationship between the ipsilateral IPS and TS/SS showed a significant negative correlation. Interestingly, the recovery rate in patients with hypoplastic IPS and dominant TS/SS on the affected side was significantly higher than that in the other patients (60% versus 20.4% within 2 weeks; 77.8% versus 29.6% after 3 months).

**Conclusions**
Hypoplastic IPS combined with ipsilateral dominant TS/SS might be the anatomical background, which provides a predisposing factor for the development of cochlear venous insufficiency in SSNHL patients. This presumptive evidence of venous etiology also correlates with better hearing prognosis of SSNHL regardless of modality of treatment.

# Regeneration and Stem Cells

## P10

**The Gunn Rat - A Model for Cell Transplantation Therapy in Auditory Neuropathy?**

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The Gunn rat is a spontaneously-arising animal model for Crigler-Najjar type 1 syndrome, a recessive metabolic disorder in which an enyzmatic deficiency entails that bilirubin cannot be excreted as normal. Unconjugated bilirubin accumulates at toxic levels in the bloodstream, with subsequent deposition in the brain tissue, causing kernicterus. Homozygous Gunn rats carrying the jj genotype are phenotypically similar to affected human patients, displaying a pronounced jaundice and neurological deficits such as ataxia and cerebellar hypoplasia. However, of interest to our lab is the hearing loss reported in the literature, in the Gunn model, allegedly due in part to the loss of spiral ganglion neurons. This phenotype could relate to that found in children born with severe jaundice.
We have directed our attention to the use of the Gunn rat as a host for human otic neural progenitor transplants, as a parallel model to the ouabain paradigm we currently employ. However, despite our best efforts, the results in our hands were disappointing. We took two approaches for the development of a neuropathy model - namely, the investigation of the phenotype in older animals to see if auditory neuropathy

would arise from a lifetime of high bilirubin levels. In parallel, we administered sulfadimethoxine to young pups to exacerbate the leaching of bilirubin from the circulation into the tissues. In both paradigms, we assessed the resultant auditory phenotype and the results for both were unexpected. Firstly, an aged cohort of heterozygous Jj and homozygous jj rats between 6 and 18 months old were tested with our standard ABR schedule. There was no shift in ABR threshold for the click or pure tone protocols. There was no reduction in the amplitude of wave ii-iii in the click-evoked ABR wave complex at any of the measured sound intensities, nor any shift in the individual peak latencies for the jj animals compared with their Jj siblings, implying that neural conduction along the auditory pathway is occurring normally. When serum bilirubin levels were measured, the jj animals were found to have an average serum bilirubin level of 8.15mg/dl compared to 0.21mg/dl in their Jj relatives confirming a lifelong bilirubinaemia.
Meanwhile, injecting jj pups with sulfadimethoxine at early ages had no effect on the auditory system when ABRs were measured 4-6 weeks later - auditory thresholds were not significantly different in sulfa-treated animals compared with saline-treated sibs for both click and tone protocols, with no change in wave ii-iii amplitude or peak latencies. In neither the aged jj cohort nor the sulfa-injected jj animals did we see a significant reduction of spiral ganglion neurons - when compared to the rapid and catastrophic neural loss we achieve with ouabain, it becomes apparent that, currently, the Gunn rat is not an appropriate model for ONP transplantation.

**Keywords**: auditory neuropathy, cell transplantation, regeneration.

# P11

**Functional recovery of regenerating lateral line hair cells**

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The lateral line is a mechanosensory organ present in several aquatic vertebrates, which uses hair cells organised in rosetta-like structures called neuRomests to detect water motion. This organ is important for several behaviours, including avoiding predators, capturing preys, orienting in constant flow and shoaling. The zebrafish larva is an ideal model organism to study hair cell physiology *in vivo*, due to its transparency, genetic manipulation capabilities and superficial location of its neuRomests, which makes hair cells easily accessible for investigation and pharmacological manipulation.
In contrast to mammals, hair cells from the zebrafish lateral line can regenerate. However, the time course of functional regeneration of these hair cells is currently not known. Here, we used 3 days post fertilisation (dpf) transgenic zebrafish larvae to investigate the recovery of hair cell function during regeneration using electrophysiological recordings of afferent neuron activity and immunostaining of pre and post-synaptic machinery. Furthermore, we employed two-photon calcium imaging to investigate the recovery of post-synaptic responses in the afferent terminals. Following complete hair cell ablation with 10 µM copper sulphate, afferent fibre retracted despite the continuous presence of the post-synaptic marker (MAGUK). Afferent fibre re-emerged in the neuRomest around 3 hours post treatment (hpt), and hair cells were first detected after 6 hpt. After 12 hpt, pre-synaptic structures reappeared, which was followed by their co-localisation with the post-synaptic terminals. Around this time point, calcium responses started to be detected in hair cells and afferent fibres following cupula deflection with a fluid jet. Hair cell number, spontaneous afferent neuronal activity and mechanically-induced synaptic responses recovered to control levels after about 48 hpt. Interestingly, the number of functional synapses (CtBP2 and MAGUK colocalization) was increased at 48 hpt in copper-treated fish compared to control fish (5 dpf). Our results suggest that complete functional regeneration of hair cells may require further refinement after 48 hpt, in accordance with what is observed during the normal developmental programme.

**Keywords:** hair cells, zebrafish, regeneration, synaptic responses

# P12

**Engraftment of human induced pluripotent stem cells (iPS) and guinea pig bone marrow-derived stem cells (MSC) into the cochlea of guinea pig**

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This study aimed to evaluate the potential of human induced pluripotent stem cells (iPS) and guinea pig bone marrow-derived stem cells (MSC) for use as transplants for the replacement of the auditory primary neurons (SGN: spiral ganglion neurons).
The induction methods of iPS cells and MSCs into neural progenitor cells is as follows. We performed neural stem cell (NSC) induction from human iPS cells and preparation of guinea-pig MSCs according to our previous report (Ishikawa et al. 2017). NSCs were induced from human iPSCs expressing GFP by culture in the serum free medium containing GSK3β inhibitor, TGF-β inhibitor and human leukemia inhibitory factor (LIF) more than 7 days. For transplantation, human iPSC derived NSCs were prepared at a density of 1x107 cells/ml in serum free medium. Guinea-pig MSCs obtained from femoral bones were cultured in DMEM low-glucose containing 10% FBS and were labeled using PKH26 red fluorescent cell linker. Guinea-pig MSCs from two to four passages were used for transplantation at a density of 1x107 cells/ml in PBS.
Hartley guinea pigs were used as recipient animals. They were anesthetized with midazolam and xylazine. The bulla was exposed to visualize the basal turn of the cochlea. A small hole was made on the lateral wall of the scala tympani. A 35G needle was used for transplanting cells. The needle was penetrated the osseous spiral lamina to enter Rosenthal canal. Cells were transplanted slowly using a syringe pump. One to four weeks after transplantation, animals were sacrificed, and cochlea were collected.
Histological analysis demonstrated the survival and neural differentiation of transplants in the cochlear modiolus and in some cases active neurite outgrowth of transplants toward host peripheral or central auditory systems were found. Now we are investigating electrophysiological assessments whether functional recovery occur.
These findings support the hypothesis that transplantation of iPS cells and MSCs-derived neural progenitors can contribute to the functional restoration of spiral ganglion neurons.

**Key words**: iPS, MSC, SGN, transplant, regeneration, guinea pig

# P13

**Alteration in Atoh1 expression during the loss and regeneration of auditory hair cells in explant cultures of chick basilar papillae**

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Nowadays, the induction of hair cell (HC) regeneration is the major path for drug development for sensorineural hearing loss. Previous studies have demonstrated that HC regeneration can occur in the mammalian cochlea through direct conversion of supporting cells (SCs) to HCs, although its spontaneous capacity is very limited. In general, the manipulation of Notch signaling using chemicals or gene transfer is required for this process in mammals. Recent studies have revealed that some additional treatments can promote this process. However, at present, satisfactory recovery of hearing using these strategies has not been achieved. In contrast to mammals, in the chicken cochlea, namely the basilar papilla (BP), HC regeneration spontaneously occurs, which is enough for functional recovery. To explore the novel strategy to accelerate direct conversion of SCs to HCs in mammals, we aimed to investigate the mechanisms for direct conversion of SCs to HCs in chick BPs. First, we established the organ culture system of chick BPs, in which HC regeneration through direct conversion of SCs is the main path for HC regeneration. Thereafter, we examined time courses for HC loss and regeneration, and analyzed alterations in Atoh1 expression during this process.

**Methods**
We used post-hatch day-1 chicks as the experimental animals. BPs were dissected out from the temporal bones and provided for explant cultures. BP explants were exposed to streptomycin (SM) for 48 h followed additional 48-h culture without SM. Newly generated HC-like cells (trans-differentiating cells) were detected by the expression of both myosin VIIa and sox2 according previous publications. To elucidate the time course of HC loss and regeneration, BP samples were collected at various time points, and provided for immunohistochemistry or quantitative polymerase chain reaction. We counted numbers of SCs labeled with sox2, HCs labeled with myosin VIIa or double-labeled cells (trans-differentiating cells) in frozen sections of BP samples, respectively. *ATOH1* mRNA expression was estimated in BP samples before, during or after SM exposure.

**Results and Discussion**

HC loss was identified in specimens after 12-h SM exposure, and total HC loss achieved at 24-h SM exposure. SC numbers during 48-h exposure to SM were not changed. Trans-differentiating cells emerged from 18 h after the completion of SM exposure, and their numbers was increased overtime. *ATOH1* mRNA expression was up-regulated immediately after total HC loss, and was down-regulated 12 h earlier that the appearance of trans-differentiating cells. These results suggest that SCs or HC progenitors in chick BPs immediately initiated HC regeneration process in response to HC loss even in the presence of SM, and that the process for fate determination may also initiate in the early phase of the recovery processes.

**Keywords**: basilar papilla, hair cell, regeneration, trans-differentiation

# P14

**Examination of EYA4 gene mutation related hearing loss using the common marmoset (Callithrix jacchus) cochlea and patient-derived induced pluripotent stem cell (iPS cells)**

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The *eyes absent (EYA)*-like genes are essential for the formation of sensory organs among fly (*Drosophila melanogaster*) and mammals. *EYA4*, one of the vertebrate genes of *Eya* family, is reported to be causative for late-onset mid-frequency sensorineural hearing loss in humans. *EYA4* was mapped to chromosomes 6q22.3–q23.2 and it encodes a 639-amino-acid protein called the EYA4 protein. Mammalian EYA proteins translocate into the nucleus in association with members of the sine oculis homeobox (SIX) family of transcription factors. While *Eya4*-deficient mice exhibited congenital profound deafness and otitis media with effusion due to the eustachian tube dysmorphology. Because of the species difference in the phenotype, the pathophysiology of EYA4 in the human cochlea has yet to be elucidated.
To unravel the pathophysiology of *EYA4* gene mutation related hearing loss, we first examined the expression pattern of EYA4 in the primate cochlea by using common marmoset (*Callithrix jacchus*), a non-human primate. In the marmoset cochlea, EYA4 immunoreactivity was observed in spiral ganglion neurons, inner/outer hair cells, all supporting cells including the Deiters', Hensen's, and Claudius cells, inner/outer pillar cells, and inner/outer sulcus cells（Matsuzaki S. et al. Neuroscience Letters, 2018). No immunoreactivity was observed in the stria vascularis, lateral wall fibrocytes, and Reissner's membrane. Furthermore, our results revealed an EYA4-SIX1 co-localization in the cells of the organ of Corti including the inner/outer hair cells, supporting cells and the spiral ganglion neurons.
After confirming the expression pattern of EYA4 in the primate cochlea, we established iPS cells from the peripheral blood of 4 patients with *EYA4* gene mutation who are visiting Keio University Hospital. These cells were induced to inner ear progenitor cells *in vitro* and further to the matured supporting cells by using a previous published protocol (Hosoya M. et al. Cell Reports, 2017). We found expression of EYA4 protein in the differentiated cochlear cells. Interestingly, EYA4 was expressed in both nuclei and cytoplasm in *EYA4* gene mutation-derived cells, while it is exclusively distributed in the nuclei in healthy individual control. Furthermore, we investigated the cell vulnerability to stresses. Several stress agents were added to the culture medium for 24 hours.
The differences in the distribution of mutated EYA4 protein together with vulnerability profile strikingly correlated with the audiological history of individual patients, suggesting the pathology found in the iPS-derived cochlear cells can predict development of accelerated hearing loss in this genetic hearing loss patients. We are now further characterizing the pathophysiology of these patients' cells at the molecular and cellular level and, near in the future, discover drugs by drug screening.

**Key words**: EYA4, common marmoset, iPS cells, DFNA10

# P15

**The transplantation of sphere-forming stem cells from the inner ear into cochlea**

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Over 5% of the world's population has disabling hearing loss. Approximately one third of people over 65 years of age are affected by disabling hearing loss (WHO, 2019). Hearing loss is classified conductive or sensorineural hearing loss. Sensorineural hearing loss can be caused by the loss of hair cells or the loss of spiral ganglion neurons (SGNs). Cochlear implants (CIs) can functionally replace lost hair cells and stimulate the SGN electrically. But the benefit of CIs depends on the functions of SGNs.
So far various kinds of neural stem cells have been transplanted for restoring the function of SGNs. For example, embryonic stem cells (ESCs) derived neural stem cells, induced pluripotent stem cells (iPSCs) derived neural stem cells, bone marrow stRomel cells derived neural stem cells, adipose cells derive neural stem cells neural stem cells, etc., were recruited. In this study, we will present the results of transplantation of sphere-forming stem cells from the rat inner ear.

**Cell Culture**

Early postnatal (P0-5) SD-Tg(CAG-EGFP) rats of both sexes were dissected for primary cell harvesting for in vitro culture. The rats were rapidly decapitated, the skull was opened, the brain removed and the temporal bones was removed. Under the HBSS, bony wall of cochlea and basal membrane were removed. Then spiral ganglia was separated and transferred to ice-cold HBSS.
Spiral ganglia were pooled and digested in HBSS containing trypsin and DNase I. Trypsinization was stopped by withdrawal of the supernatant and addition of fetal calf serum (FCS). FCS, trypsin and DNase were washed out with serum-free culture medium and the SGC were mechanically dissociated.
Spiral ganglia were incubated with NeuroCult™ Proliferation Kit on non-adherent dishes. In a few days, cells were formed spheres. Medium were changed every 2 or 3 days. Sphere forming cells were collected after a few weeks culture.

**Transplantation**

Collected sphere forming cells were transplanted to guinea pig's cochlea. Hartley guinea pigs were anesthetized with midazolam and xylazine. A postauricular incision was made. The bulla was exposed and a small hole was opened on the bulla to visualize the basal turn of the cochlea. A small hole was made on the lateral wall of the scala tympani. A 35G needle was used for transplanting cells. The needle was penetrate osseous spiral lamina to enter Rosenthal canal. For transplanting cells into Rosenthal canal, we developed a new 6 axis manipulator. Cells were transplanted slowly using a syringe pump. After transplanting cells, wound was closed and animals were cultured for 1 to 4 weeks. One to four weeks after transplantation, animals were sacrificed and cochlea were collected. Cochleae were evaluated histologically to detect the transplanted cells in the cochlea.

# P16

**Improvement of Otic Induction from Human Induced Pluripotent Stem Cell**

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**Background**

Sensorineural hearing loss (SNHL) is one of the most common disabilities in humans, and effective drugs are not available at present. Based on such backgrounds, many researchers and pharmaceutical companies are going to develop novel therapeutics for SNHL. For research and development of SNHL therapeutics, the limitation in the availability of human tissues is included in the major obstacles. Recently human induced pluripotent stem (iPS) cells, which have the potential to differentiate into any types of cells within a whole body, have been gained considerable attentions in drug discovery. Previous studies have shown the capacity of human iPS cells for hair cell induction. We also reported a hair cell induction method from human iPS cells in 2D culture using stepwise induction (Ohnishi et al., 2015). However, the efficiency of otic induction step in this method was unsatisfactory. To improve our hair cell induction method, we modified otic

induction step according to a previous report (Ealy et al. in 2016) and validated the efficacy of otic induction of human iPS cells.

**Methods**
We focused on the conditions of differentiation method for otic placode from human iPS cell-derived preplacodal ectoderm. We modified the induction step for this process using several growth factors and small molecules. The efficacy of otic induction was evaluated by means of immunocytochemistry and RT-PCR analyses. We also examined the potential for hair cell induction using cultures in a serum-free medium containing Matrigel. The hair cell induction was estimated by immunocytochemistry and scanning electron microscopy.

**Results**
Five combinations of growth factors and small molecules were validated, and the best combination achieved over hundredfold improvement in the Pax2 expression ratios in comparison with our previous method. In addition, the expression of other otic placode marker genes was confirmed in the cells after otic induction. After additional cultures of these human iPS cell-derived otic cells, we obtained hair cell-like cells, which showed the expression of hair cell markers and the presence of stereocilia bundle-like structures on their apical surface.

**Conclusion**
Our modified method successfully improved the efficacy of otic induction of human iPS cells, and these human iPS cell-derived otic cells have the potential for differentiation into hair cell-like cells.

**Keywords**: human iPS cells, otic induction, hair cell

# P17

**Stimulation of spiral ganglion neurons cultured in vitro with a global electro-magnetic field**

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Electrical stimulation of neurons is a powerful investigative tool and has practical applications in the area of applied neural control, which seeks to restore functionality to impaired regions of the nervous system1,2. Profound sensorineural hearing loss is often caused by impaired hair cell function in the sensory organ of the cochlea. It results in an alteration in the auditory nerve stimulation and signaling to the brain. Application of external electric stimulation through a cochlear implant (CI) or an auditory brain stem implant can partly compensate for the sensory hearing loss in deaf patients. Although a CI with the intracochlear electrodes directly stimulates the auditory nerve fibers situated at a distance of a few micrometers away, optimal stimulation cannot be achieved due to the anatomical gap between the electrode and neurons3.
In our study, we assessed whether exposure to chronic global electromagnetic field (EMF) stimulated continuously for 3 and 6 days in vitro (DIV) may affect the outgrowth and morphological properties of neonatal spiral ganglion neurons. SGN extracted from cochleae of P5-P7 rat pups were cultured for 3 and 6 days in vitro (DIV) on glass coverslip in a Petri dish that was exposed to an electromagnetic field generated by a circular electromagnetic coil with 80 loops and a diameter of 4.1 cm, with sawtooth-shaped current pulses of peak-to-peak amplitude of 210 mA. Neuronal cultures that were not exposed to electro-magnetic field represent a control. After 3 DIV and 6 DIV, neuronal cultures were fixed with 4% paraformaldehyde and stained with mouse monoclonal anti-ßIII-tubuline (Tuj), and rabbit polyclonal anti-S100, and then analyzed for SGN presence, outgrowth, neuronal morphology, neurite orientation, neurite length, and neuronal branching.
Our results show that electromagnetic field induced neuronal growth with longer neurites compared to control and affect neurite orientation and neuronal branching. This results can contribute to a better understanding of spiral ganglion neurons' responses to different shapes of electrical stimulation.

**Keywords**: spiral ganglion neurons, electro-magnetic field, neuronal outgrowth, orientation

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# P18

**Selective induction of cochlear hair cells from human induced pluripotent stem cells**

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Loss of hair cells in mammalian cochlea is irreversible once injured and results in permanent sensorineural hearing loss. Induction of hair cells from human pluripotent stem cells (hiPSCs) may provide platforms for disease modeling, eventually to therapeutics by drug screenings. Although reliable and robust induction method is indispensable for the biomedical application, previously reported methods was unfortunately not perfect in their induction efficiency and maturity of the induced hair cells.
In this study, we developed a novel strategy for differentiation of hiPSCs into cochlear hair cell linage by using several candidate compounds (compound A-G).
Firstly, we differentiated hiPSCs to otic progenitor cells with high efficiency using a previously reported method (Hosoya et al., 2017). Immunocytochemistry and qRT-PCR showed that otic progenitor cells expressed early otic progenitor markers, including PAX2, PAX8 and SOX2. When we treated the induced otic progenitor cells with two liquid factors, we observed upregulation of otic progenitor markers, *PAX2, PAX8* and prosensory domain marker, *LGR5*. Next, we examined the effects of compound A-G whether these compounds can facilitate the induction of cochlear inner or outer hair cells from hiPSCs derived otic progenitors. Treatment of cells with compound A promoted upregulation of various hair cell markers including *ATOH1, MYO7A, MYO15A* by qRT-PCR analysis. Immunocytochemistry revealed that the induced hair cell-like cells expressed hair cell markers, MYO7A and BRN3C. Moreover, compound G significantly upregulated the expression levels of cochlear outer hair cell marker, *PRESTIN*. On the other hand, compound A-G treatment did not increase the expression levels of cochlear inner hair cell marker, *vGLUT3* by qRT-PCR.
We found novel drugs that promote differentiation of hiPSCs derived otic progenitor cells into cochlear hair cell linage. We will further refine induction protocol in its efficacy in hair cell differentiation and examine culture conditions to differentiate into mature cochlear hair cells.

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# P19

**Exogenous BDNF and NT-3 in mouse explant cultures: Only a neural survival factor or also promoting axonal outgrowth?**

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Cochlear implants (CI) are very well-established devices to replace hair cell function. Despite their success, only little progress in speech recognition, especially in monosyllabic words, could be achieved for decades. The main obstacle for further advancement is a lack in specificity of electrical stimulation. The gap between the CI's electrodes and the stimulation target, the spiral ganglion neurons (SGN), results in current spread and an unspecific stimulation of the tonotopically organized SGNs. This impedes frequency discrimination and deteriorates speech recognition. A proposed solution to overcome this gap could be directed regrowth of SGNs towards the electrodes' surface.

In this study, we tested the influence of two neurotrophins on regrowth and branching behavior in SGN explants from apical, medial and basal turns in vitro. Cochleae were extracted from p6-7 C57/B6N mice and dissected into two spiral ganglion pieces per turn and then cultured for four days. The culture medium was supplemented by different concentrations of BDNF and NT-3. Subsequently, the explants were fixed, immunohistochemically stained for beta-3-tubulin and analyzed in Matlab. In order to obtain more information about branching, axonal density and other properties, the resulting images were skeletonized and converted to graphs.
BDNF and NT-3 do not have an influence on the mean length of neurites. Compared to unsupplemented controls, no increase could be observed even at concentrations as high as 200 ng/ml. Regardless of the neurotrophin concentration, the mean length of neurites is more dependent on the number of outgrowing neurites indicating an influence of mutual support of neurites. However, total length and therefore the number of resprouting SGNs increased with higher concentrations of BDNF and NT-3. Also, the explant size had a small effect on the number of outgrown neurites. There were no differences in outgrowth performance within the three turns evident. Results considerably varied among single explants, which is probably caused by variances during dissection.
This study sheds more light on the effects of BDNF and NT-3 on murine SGN explants and suggests that their main beneficial effect is an increased number of surviving and resprouting SGNs rather than promoting elongation.

# P20

**The efficacy and safety of Wnt and Notch-signaling modulators in the cochlea**

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**Background**
Sensorineural hearing loss is usually caused by damage of cochlear hair cells, which is irreversible in mammals. *In vivo* regeneration of hair cells is a desired treatment option. Previous studies[1-3] described the feasibility to generate neonatal and adult mouse cochlear hair cells *in vitro* via manipulation of Wnt and Notch signaling pathways, leading to the differentiation of G-protein-coupled receptor 5 (LGR5) expressing supporting cells into hair cells.
The present study supplements these previous reports by targeting Wnt and Notch signaling pathways and extends these reports by ***(i)*** evaluating therapeutic efficacy of regeneration of functional hair cells by targeting the (trans-) differentiation of LGR5+ supporting cells, *in vivo*, in (partially-) deafened mice; and ***(ii)*** assessing and minimizing potential adverse effects related to the treatment molecules and/ or the procedure.

**Aims**
a) Characterize changes in status of mature hair cells and supporting cells (including expression of LGR5 and ligands) in the mouse cochlea after induction of (various levels of) drug- and noise-induced hearing loss
b) Study the effect of a selection of treatments with Wnt agonists and Notch inhibitors on the (trans-) differentiation of the supporting cells *in vivo* in (partially) deafened mice,
c) Optimize the treatment: the injection route, time interval of treatment after deafening, the optimal dose required with minimal or no side effects,
d) Evaluate the safety of the treatment molecules and procedure,
e) Understand the mechanism of action of the therapeutic compounds via characterization of the Wnt signaling pathway components before and after treatment,
f) Apply the treatment protocol *in vitro* on human-derived inner ear hair cells.

**Methods**
We use LGR5-EGFP-IRES-creERT2 mouse model to perform the deafening procedures (ototoxic drugs, acoustic trauma) and treatment delivery (selected Wnt agonists and Notch inhibitors). Surgical interventions for administration of treatments will be conducted and various routes of administration will be assessed. The outcome measurements to evaluate anatomical and functional effects of the treatments consist of histology, molecular analysis and electrophysiology. Electrophysiology includes auditory brainstem responses and electrocochleography to test hair cell function and their connections with the auditory nerve.

**Research outcome**
The overall goal is the development of a (pre-) clinical protocol for Wnt-signaling mediated regeneration of hair cells for the treatment of sensorineural hearing loss. Initial results will be presented.
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## Developmental Biology

# P21

**Identification of TMCC2 as novel hair cell marker and characterization of antibodies for phenotypic study of hair cell deficits**

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Inner ear sensory hair cells are responsible for auditory signal transduction as they respond to sound-induced vibrations and convert these mechanical signals into electrical impulses in a process known as mechanoelectrical transduction. Hair cells display remarkable apico-basal polarization and develop in a highly ordered architecture of the organ of Corti, surrounded by supporting cells and in contact with innervating neurons. The molecular mechanisms that control the development and maintenance of hair cells are only partially understood but it is clear that these cells express a large number of proteins that serve hair cell specific functions. Regulation of their expression as well as precise control of their intracellular localization and turnover are necessary for hair cell function and disturbances in these processes can lead to hearing deficits. Identification of novel protein markers expressed in hair cells has been one of the succesful approaches to deciphering the mechanisms of hearing and the causes of deafness. We present expression data supporting the identification of transmembrane and coiled-coil domains protein 2 (TMCC2) as a novel hair cell marker. TMCC2 is particularly interesting because it resides in intracellular membranes of the endoplasmic reticulum (ER) and the Golgi apparatus. Additionally, recent findings implicate its close paralog in intracellular protein sorting, raising the possibility that the loss of this protein might influence the development of hair cells. Based on the preliminary analysis of a newly generated knockout mouse model, we report that TMCC2 is dispensable for the generation of hair cells and the establishment of apico-basal polarity. We also discuss its potential cellular role in the regulation of transport and sorting of proteins or membrane components and present histological charaterization of several antibodies recognizing known hair cell expressed proteins that we have used to probe the molecular architecture of TMCC2 knockout hair cells.

# P22

**The inhibition of endogenous ceramide kinase alters the morphogenesis of the chicken inner ear primordium**

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In all vertebrates, the inner ear originates from the otic placode, an ectodermal patch in the head which invaginates and closes to form the otic vesicle (OV) or otocyst. The OV is considered the anlagen of the inner ear since their epithelial cells will differentiate generating most of the cell types building the complex structure of the adult inner ear. This transition is carried out by spatio-temporal restricted processes of proliferation, differentiation, migration, apoptosis, autophagy and senescence. A network of intracellular signals manages the precise process of development. The knowledge of the molecular bases that guide otic development is necessary for the design of novel treatments for the protection and repair of cells in hearing loss disorders.
One of the emerging family of compounds with critical roles in most cell biological processes are the bioactive sphingolipids. In fact the term “morphogenetic lipids” are coined for sphingolipids that regulate stem cells survival and differentiation during embryonic and postnatal development. Ceramide is the central

backbone precursor of all complex bioactive sphingolipids. We had previously shown that a synthetic short-chain ceramide analogue was a potent inductor of apoptosis in OV cultures. The action of ceramide is finished by its phosphorylation to ceramide-1-phosphate (C1P), a reaction catalysed by the ceramide kinase (CERK). The presence of CERK has not been studied in the OV.
In the chicken embryo, Insulin-like growth factor-1 (IGF-1) is required for the survival and differentiation of the epithelial auditory precursors. In addition, IGF-1 deficit causes syndromic deafness in mice and men. In this work we have studied whether the pro-survival role of IGF-1 are due to the generation of C1P. To tackle the study we have used a specific CERK inhibitor (NVP-231). We have addressed the study in *ex vivo* cultures of OVs described as a good model of inner ear development that mimics the *in vivo* program maintaining the morphogenetic spatiotemporal pattern.
Our results show that CERK is expressed during the developing inner ear in chicken. The inhibition of CERK reduced proliferation and increased cell cycle arrest followed by cell death. The inhibition of CERK also altered the neurogenesis in the acoustic vestibular ganglion (AVG). Taken together, these results would confirm the involvement of C1P in the morphogenesis of the OV and AVG. The inhibitor counteracted the effect of IGF-1 exogenously added, suggesting that a part of the IGF-1 protective role could be carried out by CERK stimulation and C1P production.

**Keywords**: Chicken otocyst, NVP-231, insulin-like growth factor 1, survival, neurogenesis.

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# P23

**Elucidating pathological mechanisms of hearing loss induced by hypothyroidism using Duox2 mutant mice**

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**Background**
Developmental thyroid disorders can lead to hearing loss. Previous studies showed that the tectorial membrane, middle ears, and otic capsule were defective in various mouse models of hypothyroidism. However, it is still unclear how the lack of thyroid hormones leads to such defects in the inner and middle ear structures, which may be responsible for the hypothyroidism-induced hearing loss. In this study, pathological mechanisms of hypothyroidism- induced hearing loss in Duox2 mutant mice was elucidated, and exogenous thyroid hormones were supplied to rescue hypothyroidism-induced hearing loss.
**Material and methods**
To investigate the pathological mechanisms of the hypothyroidism-induced hearing loss, we analyzed the inner ears of a spontaneous mutant from the Jackson Lab, which carries a mutation in dual oxidase 2 (Duox2). Duox2 is an essential enzyme in thyroid hormone synthesis, and mutations in the DUOX2 gene in humans have been shown to cause typical phenotypes of congenital hypothyroidism. We evaluated serum levels of T4, hearing function by ABR analysis, histological phenotypes by H&E staining, and gene expression patterns by in situ hybridization.

**Results**
Duox2 mutant mice suffered from hypothyroidism and growth retardation and severe hearing loss,and their tectorial membrane is severely thickened. To understand the molecular mechanisms of thickening of the tectorial membrane, we examined expression patterns of receptors for thyroid hormones. Size of round window is decreased and middle ear ossicles were enlarged. To rescue hearing loss of Duox2 mutants, we supplied exogenous thyroid hormones and growth was recovered and hearing ability was significantly improved. Tectorial membrane, middle ears and round window developed normally.
**Conclusion**
Hypothyroidism-induced hearing loss in Duox2 mutants is resulted from abnormally thickened tectorial membrane in the inner ear and enlarged middle ear ossicles.
Exogenous thyroid hormone treatment during neonatal stages can rescue structural defects of the inner and middle ears and significantly improve hearing ability in Duox2 mutant mice

**Keywords:** Duox2, hypothyroidism, hearing loss, growth retardation, thyroid hormone

# P24

**Analysis of auditory system of mice lacking brain-specific angiogenesis inhibitor 3 (Bai3)**

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**Introduction**

Hair cells in the organ of Corti send acoustic information to the brain through synapses. Inner hair cells, innervated by type I afferent neurons, are responsible for encoding sound stimuli. Outer hair cells, innervated by efferent neurons and type II afferent neurons, play roles in modulating cochlea mechanics thus regarded as “amplifiers.”

Brain-specific angiogenesis inhibitor 3 (Bai3) is an adhesion G-protein coupled receptor playing roles in synapse formation and/or maintenance with its ligands, C1q-like complement (C1QL) family proteins (Bolliger et al., 2011). Interaction of Bai3 and C1ql1 is required for pruning of climbing fibers in mouse cerebellum (Kakegawa et al., 2015; Sigoillot et al., 2015). Single nucleotide polymorphisms in *BAI3* is associated with schizophrenia in genome-wide association studies (DeRosse et al., 2008; Liao et al., 2012). Although it has been suggested that *Bai3* mRNA is expressed in mouse cochlea during development (SHIELD; https://shield.hms.harvard.edu), the role of Bai3 in auditory system is still unknown. In this study, we investigated the role of Bai3 in mouse auditory system using *Bai3* knockout mice.

**Methods**

To study functions of Bai3 in the mouse auditory system, we performed recordings of auditory brainstem responses (ABRs) of *Bai3*-/- mice at 5-6 weeks. We also performed immunohistochemisty using cryosections and surface preparations of cochleae to identify Bai3-expressing cells in detail and to identify ligands for Bai3 in mouse cochlea. Immunostaining with antibodies against synapse-related proteins were also carried out and compared between *Bai3*-/- and wild-type cochlea.

**Results**

*Bai3*-/- mice at 5 weeks showed elevated ABR thresholds at 8, 16 and 24 kHz compared to wild-type mice. Immunosignals of Bai3 was observed in outer hair cells at embryonic day 18.5 and in outer pillar cells in adult cochlea. While histological analysis showed no significant differences in the number of hair cells between wild-type and *Bai3*-/- cochlea, reduction of some immunosignals was observed in organ of Corti of *Bai3*-/- mice.

**Summary**

Bai3 is required for normal hearing in mice. Based on our histological analysis, in the session, we will further discuss functions of Bai3 in the mouse auditory system.

# P25

**Shh Signaling Pathway Role in the Differentiation of Mouse Pluripotent Stem Cells into Inner Ear Organoids**

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**Introduction**

Cochlear and vestibular hair cells of the inner ear are responsible for hearing and balance, respectively. Loss of cochlear hair cells in the inner ear is one of the most crucial causes of hearing loss, worldwide. Stem cell therapy is a promising approach for generating cochlear hair cells to be used in regenerative treatment of hearing loss. Several protocols are published for generating *in vitro* inner ear organoids consisting of vestibular hair cells from pluripotent stem cells. Generating adequate cochlear hair cells from pluripotent stem cells has remained challenging. Therefore, it is needed to study the developmental process and investigate the signaling pathways involved during the *in vivo* differentiation of cochlear hair cells from pluripotent stem cells to be applied in the generation of *in vitro* inner ear organoids.

**Methods**

In this regard, transcriptome profiles of the developing mouse vestibular and cochlear structures of the inner

ear in different time points from GEO database were collected in order to explore upregulated genes and involved signaling pathways in the cochlea development in comparison with vestibule. Afterwards, the main achieved signaling pathway was induced and inhibited on a specific day of inner ear organoids differentiation which was before the fate determination of prosensory cells in the organoids.

**Results**
Meta-analysis revealed Shh as the main signaling pathway in the cochlea development. Induction and inhibition of this pathway using SAG (Shh agonist), Sant1 (Shh antagonist) and existing a control group (no treatment) on day 8 of the inner ear organoids differentiation showed significant larger size and a higher number of otic vesicles in SAG treated organoids in comparison with the other two groups. It was seen that organoids in all three groups were expressing the markers of supporting cells (Sox2), vestibular hair cells (Calb2) and hair cells (Atoh1) but the expression of Calb2 in the control group and Atoh1 in the SAG treated group were significantly increased in comparison with the other two groups. In SAG treated group, co-expression and also lack of co-expression of Calb2 and Atoh1 on day 27 of differentiation were also observed.

**Conclusion**
As a result, on day 27 of differentiation in SAG treated group, expression of Calb2 and co-expression of Calb2 and Atoh1 showed the existence of vestibular hair cells, while the cells expressing Atoh1 individually are cochlear hair cells. This represents the existence of two kinds of hair cells in the SAG treated organoids.

**Key words:** inner ear organoids, vestibular hair cells, cochlear hair cells

# P26

**Expression of Carbonic Anhydrase 13 in the Developing Mouse Cochlea**

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Carbonic anhydrases (CAs) are zinc-containing enzymes that catalyze the reversible hydration of carbon dioxide to bicarbonate. CAs mainly contribute to stabilizing the concentration of carbon dioxide in the blood, but their roles differ depending on expression sites. It is known that mammalian CAs (α-CAs) has sixteen isozymes, and each shows the unique expression pattern. We analyzed the gene expression pattern in the developing inner ear based on the publically available data including the comprehensive gene expression patterns of whole mouse embryos (Cao et al., 2019) from embryonic day (E) 9.5 through E13.5. From the analysis of this data, we found that Carbonic anhydrase 13 (Car13) expression was limited to the cochlea more specifically than CA isozymes from E9.5 through E13.5. Then we focused on Car13, which is one of the α-CA cytosolic isozymes. The paper by Wu et al. (2013) has already indicated that it was expressed at a relatively higher level than other CA isozymes from E15.5 through early postnatal days. Here, we investigated the expression pattern of Car13 at earlier stages of developing inner ear, which is the important period for the inner ear formation and development. We firstly clarified the change of Car13 expression level in the inner ear from E9 through E15 by using quantitative polymerase chain reaction of Car13. Then we performed in situ hybridization of Car13 to make clear the expression pattern in the cochlea from E9 through E15. Car13 was expressed on the center and lateral side of sensory epithelial cells in the developmental cochlea from E10 through E15, but not expressed saliently at the other parts of inner ear, such as vestibule or neural tube. Its high expression region proceeded from the center to the lateral side of sensory epithelial cells with the passage of embryonic days. We could not see the remarkable expression pattern at E9. In conclusion, Car13 is highly expressed with the unique pattern in the cochlea during inner ear formation and development. These results suggest that Car13 contribute to developmental regulation or function of the inner ear, especially the lateral side of the cochlea. In the future, we are going to perform Car13 functional analysis of sensory epithelial cells in the cochlea and determine the role of Car13 in the inner ear development.

**Keywords**: carbonic anhydrase, development, in situ hybridization, cochlea

# P27

**Cochlear Implant-Based Electrical Stimulation Modulates Neural Stem Cell-Derived Neural Regeneration**

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Cochlear implantation is now the best therapeutic method for the profound sensorineural hearing loss. However, insufficient numbers of functional spiral ganglion neurons hinder the clinical effects of cochlear implantation. Stem cell transplantation is believed to provide a novel strategy for spiral ganglion neuron regeneration after injury. Some obstacles still need to be overcome, such as low survival, less migration into the site of the injury, uncontrolled differentiation and lack of functional regenerative neurons. It's promising to modulate neural stem cells behavior to address the mentioned issues by novel technologies. Here, a device capable of electrical stimulation was designed by combining cochlea implant and graphene substrate. Neural stem cells or primary spiral ganglion neurons were cultured on the graphene substrates and subjected to electrical stimulation transduced from sound by cochlear implant. Cell behaviors were further studied. It was found that this device was biocompatible for both neural stem cells and spiral ganglion neurons. More importantly, prolonged electrical stimulation could promote neurite outgrowth of spiral ganglion cells, which may be through enhanced development of growth cone located on the tips of neurites. Furthermore, the results show that prolonged electrical stimulation with complex frequency induced neural stem cell death and apoptosis. Interestingly, electrical stimulation could promote neural stem cells to proliferate and enhance the differentiation into neurons when high-frequency stimulation was removed. Current study provides experimental evidence for understanding the regulatory role of electrical stimulation in stem cells, and highlights the potentials of this above mentioned device in stem cell therapy for hearing loss treatment.

**Keywords**: Electrical stimulation; neural stem cells; neural regeneration; hearing loss

## Ear Physiology

# P28

**Otolin-1 as a possible Biomarker for Inner Ear Disease?**

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**Objective**
The expression of Otolin-1 mRNA is highly restricted to the inner ear. In particular, it is identified in the supporting cells of the maculae and cristae and also as a component of the tectorial membrane. A previous study showed that Otolin-1 is present in a significantly higher level in serum samples of patients with benign paroxysmal positional vertigo (BBPV) compared to healthy patients. Therefore we analyze the levels of the protein Otolin-1 in blood, urine or saliva in patients suffering from Menière's Disease (characterized by the rupture of the membranous labyrinth), sudden hearing loss, and neuritis vestibularis in comparison to healthy subjects.

**Material and Methods**
17 patients suffering from Ménière's Disease according to the AAO-HNS criteria, 10 patient with sudden hearing loss and 11 neuritis vestibularis were included in the present study. The control group consists of 17 patients without any history of otoneurological disease. Sampling of serum was performed in the morning after at least 12 hours of fasting. Urine and saliva was analyzed only in the first 10 Ménière's disease patients. All samples were stored at -20 C until analysis. Detection of Otolin-1 concentration was performed by the use of a highly sensitive ELISA-kit for human Otolin-1.

**Results**
Otolin-1 was detected in pg/ml range in all collected samples (i.e., urine, saliva and serum). Serum samples of patients suffering from sudden hearing loss and Menière's Disease showed significantly higher Otolin-1 values than control samples. There was no significant difference in the saliva or urine concentrations of

Otolin-1 between patients of the Ménière's Disease (n = 10) and of the control group (n=10) and no significant difference in serum samples of the patients with neuritis vestibularis compared to controls.

**Conclusion**
Our results indicate that Otolin-1 is present in serum and also in saliva, but rarely in urine of patients. The highly significant difference in serum samples of the Menière's Disease and also sudden hearing loss patients compared to healthy controls seems indicative for a biomarker function of Otolin-1 in the pathologies affecting the inner ear.

# P29

**Medial olivocochlear and middle-ear reflex: is it possible to differentiate?**

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The central nervous system can modulate the peripheral auditory system with feedback projections like medial olivocochlear (MOC) reflex and middle-ear muscle reflex (MEMR). The MOC decreases the gain of the outer hair cells and the MEMR increases the impedance of the middle ear. These two reflexes attract the attention because they can be affected in cases of auditory neuropathy spectrum disorders and synaptopathy. The ipsilateral or contralateral presentation of a sound could activate both efferent and middle-ear muscle loops. A contralateral acoustic stimulation (CAS) modulates the amplitude of distortion product otoacoustic emissions (DPOAE). Moreover, previous studies in chinchilla indicate that MEMR activation seems to occur primarily in the presence of low frequency CAS. Also, if the activation of MOC induces DPOAE amplitude reduction, the DPOAE phase shift is strongly correlated with stapes displacement and consequently MEMR activation. Few studies attempt to distinguish the involvement of these two mechanisms using non-invasive exploration methods (for translational applications).

The goal of this project is to evaluate the effect of CAS on DPOAE parameters (amplitude and phase) and to differentiate the MOC from the MEMR reflex.

First, in awake guinea pigs, our pilot study showed the CAS had maximal effect in amplitude and phase DPOAE shift with F2 frequency at 2, 4 and 8 kHz (in a range of frequencies between 2 and 24 kHz). The second stage of this project studies the shifts in DPOAE amplitude and phase for different types of CAS (white noise, narrow-band noise) in three conditions in addition to the already studied awake situation (ketamine, isoflurane, isoflurane and curare). The injection of curare and the consequent stapedius muscle blockage will single out the MOC contribution to the whole response to CAS, in order to assess whether it is possible to design a protocol for evaluating each reflex separately.

**Keywords:** olivocochlear reflex, middle-ear muscle reflex, guinea pig, DPOAE

# P30

**Dominant deafness due to a point mutation in TMC1**

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Cochlear hair cells transduce sound into electrical signals by activation of mechano-electrical transducer (MET) channels thought to be formed at least partly by TMC1. We generated mice possessing a single aspartate/asparagine substitution, D569N, in TMC1, an alteration at a homologous site to that in a dominant human genetic deafness, DFNA36. Neonatal mutant mice had MET currents of smaller amplitude, with a three-fold reduction in the calcium permeability of the MET channel, but no change in its single-channel conductance. The stereociliary bundles of the outer hair cells appeared normal until about postnatal day(P) 8, at which time the distinctive V-shape became rounded and there was a 35 percent reduction in bundle height. After postnatal day 14, there was loss of OHCs proceeding from the base to apex, and by four weeks, the mice were deaf as judged by ABR measurements. All phenotypic effects were seen in heterozygotes, as well as in homozygotes and must ultimately be controlled by the reduced MET channel

calcium permeability. The mouse mutant accounts for the dominant human deafness and implies that TMC1, besides forming the MET channel, can regulate its own expression.

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# P31

**Variation of intercellular K+ concentration at the mouse vestibular Type I hair cell-calyx synapse can contribute to afferent signalling**

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Vestibular sensory epithelia of Amniotes contain two Type of sensory cells, called Type I and Type II hair cells (HCs). Each Type II HC is contacted by several (10 to 20) small, bouton-like nerve terminals. The basolateral membrane of Type I HCs, at difference, is enclosed in a single giant chalice-shaped nerve terminal, whose function remains enigmatic. Deflection of the hair bundle opens mechano-sensitive ion channels producing an inflow of $K^+$. The resulting depolarization opens voltage-dependent $Ca^{2+}$ channels located at the HC basolateral membrane. The ensuing $Ca^{2+}$ influx triggers the release of glutamate, which binds to AMPA (alfa-Amino-3-Idrossi-5-Metil-4-isoxazolone propionate) receptors expressed at the opposite afferent terminal. However, a fraction of the afferent signal produced by Type I HCs appears to be resistant to AMPA-receptors blockers and might be non-quantal in nature, possibly mediated by $K^+$. To better investigate this possibility, we performed whole-cell patch-clamp recordings from mouse Type I HCs or their associated calyx ending. Since direct measurement of $K^+$ concentration in the cleft is not achievable, intercellular $K^+$ concentration was inferred by the shift in $K^+$ current reversal potential ($V_{rev}K^+$). We found that $K^+$ concentration in the calyceal cleft can decrease or increase depending upon hair cell membrane depolarization or hyperpolarization, respectively. $K^+$ flux involve a low-voltage gated $K^+$ conductance specifically expressed by Type I HCs, called $G_{K,L}$, and $K_V1$ plus $K_V7$ channels at the calyx inner membrane. Our data support a scenario where the calyx inner membrane effectively confines an intercellular compartment inside which $K^+$ can vary significantly relative to the interstitial medium, directly changing the postsynaptic membrane potential.

**Keywords:** Type I hair cell; calyx; $K^+$ channel; vestibular; synapse

# P32

**Biophysical Model of Synaptic Transmission at the Vestibular Hair Cell Calyx**

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Within the past decade, tremendous advances have been made identifying and characterizing the ion channels and pumps within the sensory epithelia of the vestibular inner ear. In amniotes, a striking feature of these epithelia is the presence of cup-shaped synaptic terminals (calyces) made by primary afferent neurons on specialized mechanosensory cells (type I hair cells). Recent work has shown that type I hair cells transmit to calyces by two mechanisms: (1) release of glutamate from vesicles ("quantal") and (2) unconventional flow of ions from the hair cell into the cleft and the postsynaptic calyx ("non-quantal"). Details of this complex transmission remain unknown, and the relevant compartments (cells and synaptic cleft) are hard to access, hindering the measurement of ion concentrations and potentials. **The objective of this study is to create a biophysical model of the vestibular hair cell-calyx (VHCC) synapse with the goal of understanding the role of both quantal and non-quantal transmission.** The VHCC model incorporates the specific location and surface density of ion channels (MET, HCN, $K_v$, $Ca_v$, $Na_v$) and pumps (Na-K ATPase, KCC) expressed in hair cells and calyces, along with measurements of cell and calyx

geometry from electron micrographs. The dynamic behavior of the system is determined from measured or estimated open probabilities, conductance, and activation time constants of the channels. The VHCC model is implemented in COMSOL and Matlab using a variant of the cable equation along with $K^+$ and $Na^+$ electrodiffusion in the cleft, simplified Hodgkin-Huxley-style ion currents, and stochastic vesicle release along an axisymmetric parametric surface. After establishing steady-state conditions, the input to the model is a step deflection of the hair bundle, and the outputs include the $K^+$, $Na^+$ and potential gradients within the cleft, the voltage changes on the inner surface of the calyx, and the pattern of spiking in the nerve fiber. The overall predictions are consistent with experimental recordings from the vestibular hair cell synapse, and the model is able to predict how increases in cleft $K^+$ can influence the extent of non-quantal transmission and alter time to spike. The model is also able to make quantitative predictions on how changes in the conductance of specific channels in both the hair cell and the calyx influence the ultimate pattern of firing in the vestibular nerve. For instance, an increase in conductance of the $K_v7.4$ channel located on the inner face of the calyx reduces the time to spike and increases the rate of non-quantal spiking. Overall, the VHCC model provides a quantitative framework for evaluating the relative contribution of quantal and non-quantal transmission to sensory processing in type I vestibular hair cells and elucidating how individual ion channels shape the signal encoded by vestibular afferents.

**Keywords**: Calyx, Hair Cell, Model, Vestibular, Ion Channels

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# P33

**Changes in intracellular pH, Na+, and Cl- induced by hydrogen sulfide in outer hair cells**

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**Objectives**

Hydrogen sulfide ($H_2S$) acts as an important class of gaseous signal transmitter similar to nitric oxide and carbon monoxide. $H_2S$ mediates a number of biological processes. Intracellular pH (pHi) is an important endogenous modulator of cellular function. Previous studies also suggested that $H_2S$ regulates pHi in vascular smooth muscle cells and glial cells. In the present study, we investigated whether $H_2S$ can affect pHi in cochlear outer hair cells (OHCs).

**Methods**

OHCs were enzymatically isolated from the guinea pig cochlea. Changes in pHi in OHCs induced by $H_2S$ were determined using the pH-sensitive dye BCECF. $H_2S$-induced changes in intracellular $Na^+$ and $Cl^-$ levels in OHCs were determined using the $Na^+$-sensitive dye SBFI and the $Cl^-$-sensitive dye MQAE, respectively.

**Results**

NaHS, an $H_2S$ donor produced sustained decreases in pHi in OHCs. NaHS increased intracellular $Na^+$ concentrations ($[Na^+]_i$) in OHCs. Cariporide, a potent NHE-1 (sodium-hydrogen exchanger isoform-1) inhibitor inhibited the NaHS-induced increase of $[Na^+]_i$ in OHCs. NaHS increased intracellular $Cl^-$ concentrations in OHCs. NaHS also induced cell shortening and swelling, which were accompanied by an increase of cell volume in OHCs.

**Conclusion**

Decrease of pHi induced by $H_2S$ may activate $Na^+/H^+$ exchanger in OHCs. The $Na^+$ influx was accompanied by the $Cl^-$ influx in OHCs. These $Na^+$ and $Cl^-$ influxes finally result in an increase of cell volume (cell swelling) in OHCs. It is concluded that two ion transporters, the NHE1 and $Na^+/Cl^-$ symporter may be important regulators of cell volume in OHCs.

**Key words**: hydrogen sulfide, sodium-hydrogen exchanger, cochlea, intracellular pH

# P34

**Piezoelectric Vibrator-Stimulated Potential and Heart Rate Accelerations Detected from the Fetus**

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**Objectives**

The fetus is well known to have a substantial capacity for sound recognition in the uterine environment. The aim of this study was to develop a sound stimulus system equipped with a piezoelectric vibrator (PV), record the PV-stimulated potential (PVSP) of the fetus and monitor changes of the fetal heart rate (FHR) under PV stimulation.

**Methods**

The relationship between the input voltage applied to a piezoelectric vibrator and the sound pressure generated in the uterus was calibrated based on a model of the maternal abdomen. Fourteen fetuses for the measurement of the PVSP and 22 fetuses for the measurement of the heart rate changes from low-risk pregnant women were recruited.

**Results**

The PVSP responses were obtained in 9 out of 14 fetuses. All the tested fetuses accelerated the FHR after the 2 kHz tone stimulation at 70 dB intensity generated by PV from 32 to 37 weeks gestational age.

**Conclusions**

Using a newly developed sound stimulus system equipped with PV, the electric responses of a fetus recorded from electrodes placed on the mother's abdomen may be closely related to the auditory evoked response. Significant accelerations of FHR were objectively, accurately and readily obtained after the sound stimulation.

**Key words:** Piezoelectric vibrator, Sound stimulus, Fetus, Heart rate acceleration

# P35

**Distribution of Na/K-ATPase Subunits and Voltage-Gated Ion Channels in the Human Cochlea and Auditory Nerve - A Study Using Super Resolution Microscopy with Special Reference to Cochlear Implantation**

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**Background**

The expression of the ion transport protein sodium/potassium-ATPase (Na/K-ATPase), its isoforms and voltage-gated ion channels were analyzed in the human cochlea and auditory nerve using confocal and super resolution structured illumination microscopy (SR-SIM). The purpose was to increase our understanding of the pump´s and ion channels´ roles in the propagation and processing of action potentials (AP) in the human cochlea. Moreover, it may bring information on how electric nerve responses are elicited from extra-cellular stimulation of auditory prostheses.

**Material and Methods**

Human cochlear sections were analyzed from archival material derived from trans-cochlear surgeries to remove petro-clival meningioma after ethical permission. Tissues were fixed with 4% PFA immediately in the operating room before being decalcified, frozen in Tissue-Tek OCT, and cryostat-sectioned. Antibodies against Nav1.6 and other voltage-gated channels (Nav, Kv, Cav, etc.), various isoforms of Na/K-ATPase and additional transporting proteins, were used for immunohistochemically localizing transporters/channels. Confocal laser scanning microscopy (CLSM) and super-resolution structured illumination microscopy (SR-

SIM) were used for investigation of immunofluorescent sections. SR-SIM is a Zeiss Elyra S.1 SIM system which is capable of achieving a lateral (X-Y) resolution of ≈100 nm and an axial (Z) resolution of ≈300 nm.

**Results**
The advantages of using surgically obtained cochlear specimen rather than postmortem cochlear tissue for immunohistochemistry study with regards to antigen preservation and super-resolution microscopy are shown. Na/K-ATPase α1 was expressed in satellite glial cells, and Na/K-ATPase α3 and β1 in the neurons. The neuronal Na/K-ATPase sub-types (α3 and β1) were expressed in nerve terminals, in the organ of Corti and in the plasma membrane of the neuronal cell bodies. The voltage-gated sodium channel 1.6 was expressed in SGN cell body, at the hemi-nodal region as well as in the node of Ranvier. Kv1.2 immunostaining was expressed in the node of Ranvier.

**Discussion**
Nanoscopy demonstrated unique molecular expressions in well-fixed human inner ears. The findings suggest that the human auditory nerve exhibits exclusive molecular features. Electric signals generated by hair cells may not go uninterrupted across the spiral ganglion in man, but are locally processed. This may be related to particular coding properties in the human acoustic pathway. The nature and distribution of voltage-gated ion channel along the human nerve needs further analyses.

**Keywords:** Na/K-ATPase, Voltage-gated ion channel, Human cochlea, Immunohistochemistry, Super-resolution microscopy

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# P36

**The Role of D1-like and D2-like Dopamine Receptors in Lateral Olivocochlear Efferent Function**

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The olivocochlear (OC) system provides a feedback system to modulate auditory nerve activity in the cochlear periphery. One subset of OC neurons, the lateral olivocochlear (LOC) neurons originate in and around the lateral superior olive (LSO) in the brainstem and send unmyelinated axons that synapse onto the dendrites of type I auditory nerve fibers (ANFs) underneath the inner hair cells (IHCs). These synapses are located before the spike initiation zone on the peripheral ANF axons, in order to directly modulate the hair cell induced postsynaptic activity in ANFs, affecting firing rates and neural coding in the auditory nerve. Dopamine (DA) is the one of the neurotransmitters released from LOC efferents. Among others, Maison *et al.* (2012) characterized expression patterns and knock-out phenotypes of specific dopamine receptor subtypes, using RT-PCR and genetic deletion of dopamine receptors. These data suggest that D1-like and D2-like DA receptors play an important role in lateral efferent function and are possibly involved in protection of the hair cell afferent synapses from noise exposure.

To further understand the effects of dopaminergic LOC input in the cochlea, we investigated the effects of D1-like and D2-like receptor agonists on ANF firing and IHCs, using whole-cell or extracellular loose patch recordings in acutely excised rat cochlear tissue. Secondly, utilizing several transgenic reporter mouse lines for D1-like and D2-like receptors, we analyzed the expression pattern of dopamine receptors in the cochlea.

Type I ANF recordings in 3-4 week old rats showed that DA reduces the spontaneous firing in a dose-dependent fashion. Both, the D2-like receptors agonist Quinpirole (20 □M) and D1-like receptor agonist SKF389393 (20 □M) reduced ANF firing. Interestingly, voltage-clamp recordings showed that DA reduced the rate of EPSCs, suggesting that transmitter release from the IHC was affected. In fact, whole-cell recordings from IHCs showed that dopamine and D2-like receptor agonists reduce the amplitude of calcium

currents, whereas the D2-like receptor antagonist Sulpiride (50 □M) increased it. The underlying mechanism is under investigation.

In *Drd1-TdTomato* mice, expression of D1-like receptors is found in most if not all type I ANFs. In contrast, D2 receptors are found to be expressed in the dopaminergic LOC efferents in *Drd2-Cre* mice, likely acting as auto-receptors. Finally, immunostaining against the vesicular monoamine transporter 2 (VMAT2) shows dense labeling in the inner spiral bundle region, further supporting the idea that dopamine is released from the lateral efferent terminals.

**Keywords:** Dopamine, auditory nerve fiber, inner hair cell, efferent neuron, lateral olivocochlear neuron

# P37

**Simultaneous detection of Ca2+ signaling and ATP release in the developing cochlea**

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During pre-hearing stages of development in mice, periodic transient elevations of cytosolic free $Ca^{2+}$ concentration occur spontaneously in the greater epithelial ridge (GER) and propagate as intercellular $Ca^{2+}$ waves invading variable portions of the GER. Prior work by our and other groups indicates that intercellular $Ca^{2+}$ waves in the GER rely on the interplay between $IP_3$, generated intracellularly, and ATP, released at the apical surface of cochlear non-sensory cells. A vast body of data supports the hypothesis that, in the developing cochlea, ATP is released through connexin hemichannels [1], however a direct proof is lacking.
To test this hypothesis, we designed and built a closed microfluidic chamber (10 µl max. volume) in which the transparent roof was covered with plated HEK23T cells (facing the fluid interior of the chamber), stably expressing $P2Y_2$ purinergic receptors and sensitive to ATP in the nM range. These biosensor cells sitted at <100 µm from the surface of an organotypic cochlear culture plated on the chamber bottom.
After loading both biosensor cells and cochlea with $Ca^{2+}$-selective dyes, or by using genetically encoded GCaMP6s $Ca^{2+}$ sensors, this architecture allowed us to monitor $Ca^{2+}$ responses in HEK293T biosensor during propagation of $Ca^{2+}$ waves in the GER of the cochlea underneath. To image $Ca^{2+}$ dynamics while discriminating optical signals originating from the two focal planes, we stepped up and down, rapidly and repeatedly, the objective of a custom-made multi-photon microscope. For these experiments, the saline solution trapped within the chamber contained an endolymphatic $Ca^{2+}$ concentration (20 µM) and ARL67156 (100 µM), an inhibitor of ectonucleotidases. $Ca^{2+}$ signals disappeared upon replacing ARL67156 with apyrase (40 U/ml, an enzyme that catalyzes the sequential hydrolysis of ATP) in the extracellular medium, confirming that ATP mediated both $Ca^{2+}$ wave propagation in the GER and the ensuing $Ca^{2+}$ responses in the HEK293T biosensors.
To determine the source of the released ATP, we tested cochlear organotypic cultures from mutant mice with global deletion of pannexin 1 (*Panx*$1^{-/-}$), and another strain with targeted deletion of connexin 26 in the sensory epithelium of the cochlea (*Gjb2*$^{-/-}$). Using the microfluidic chamber, we determined that $Ca^{2+}$ signals in the ATP biosensor cells were the same irrespective of whether they faced *Panx*$1^{-/-}$ or age-matched *Panx*$1^{+/+}$ or $Cx26^{+/+}$ cochlear cultures. In contrast, $Ca^{2+}$ signals strongly depressed in the presence of *Gjb2*$^{-/-}$ cultures. Furthermore, $Ca^{2+}$ waves in the GER were reversibly inhibited by flufenamic acid (50 µM) and the anti-connexin antibody abEC1.1 (952 nM) [2], both of which are not effective on pannexin 1 channels [3]. Together, these results validate our working hypothesis and confirm that pannexin 1 channels are not involved in the ATP release process that mediates $Ca^{2+}$ wave propagation in the GER.

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# P38

**Digital hearing: biophysical modeling of auditory processes**

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The experimental conformity base of the auditory receptors coordinates to the stimulated sound frequency was presented in the middle of the last century (1947). G. fon Bekesy developed it, but did not provide solid biophysical and mathematical rationale The analytical equation for this connection was developed at the Department of Biomedical Physics of Samara State Medical University at the beginning of this century. It is expressed according to the following equation: $\ell(f)=L_o 2^{lg/fmo}$ .
In this equation, $L_{o=}$32mm – is a standard cochlear duct length, $f_{mo}$=20kHz is the maximum frequency of perceived sound ranging, $\ell(f)$ is the auditory receptor coordinate that perceive test frequency f. This hearing sense mathematical model is based on sound waves properties and on the functions of the ear structures that are involved in sound conduction.
Among all sound effects manifested in the inner ear are the following: acoustic dispersion, its round window reflection, forward and reflected waves interference, standing wave formation in the perilymphatic canal (vestibular and tympanic) At the same time, the sound stimulus formation begins in the cochlear duct with vibrations of stapes and membrane oval window, generating a longitudinal wave in the perilymphatic chamber, exciting the vestibular membrane, which creates a wave in the endolymphatic duct, exciting the tinctorial memrane, which interacts with the basilar membrane, generating a membrane potential.
Another surprising fact of the model is manifested both on the vestibular membrane and on the basilar with the appearance of standing waves, these effects are set at equal distances from the apex at the same time $t=Lo/(2v_{mo})+n/(2\cdot f)$, where $v_{mo}$=1600m/s is the speed of sound in the perilymph of the maximum frequency, n=0, 1, 2 ... is the sequence number of the temporary maximum at the cochlear duct. It turns out to be equal to 0.04ms for a standard cochlear duct, reflecting the sound simultaneous relaxation time of any frequency.
This model keeps with G. fon Bekesy experiments, moreover the excitation maxima move with the increasing of frequency from the apex to the basal portion of the cochlear duct. An interesting feature of this model is not only upper frequency threshold limitation, which corresponds to the cochlear duct length, but also lower threshold limitation, which we associate with the presence of membranes apical ligament. We'll be handling histological confirmation for the time being.
Apical-basal paradox, first established in Russia, but found to be biophysicaly unsubstantiated, is the main application of this model, but this model has provided a strong rationale for this effect. The establishment of inter sensory distances of both external and internal cochlear hair cells is another application. The theory shows that, near the wide part of the cochlear apex, the intersensory distances are big enough, they reduce to the middle of the duct, and increase to the basal part of the duct again.
Thus, we presented a morpho-functional acousto-wave sound conduction model (hearing model), which is evidence-based and confirmed by numerous auditory effects.

## Aging

# P39

**Gender effect, quality of life and genetic biomarkers in a Portuguese sample with ARHL with or without tinnitus**

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It has being estimated that Presbycusis or age-related hearing loss (ARHL) will affect up to 1.5 billion people by 2025. In addition, tinnitus occurs in a large majority of cases with Presbycusis being associated with negative psychological and emotional effects leading to impaired quality of life. The aim of this study was to

explore gender effect on a presbycusis sample of older Portuguese with and without tinnitus according to their audiological and genetic profile concerning biomarkers for ARHL

Tonal and speech audiometry, tinnitus assessment, clinical interview, and DNA samples were obtained. The Brief Symptom Inventory (BSI) and the General Health Perceptions subscale (MOS-SF36 -Portuguese version) were also used respectively to evaluate psychological distress, quality of life, and emotional and social difficulties on a sub set of our sample. Tinnitus severity was evaluated through Tinnitus Handicap Inventory (THI). GRM7 analysis was performed by qPCR. Genotyping of SNPs in NAT2 was performed by PCR amplification followed by Sanger sequencing or by qPCR. All the volunteers signed an informed consent and ethical guidelines were followed.

We present epidemiological and psychological data from 475 elderly individuals. Our sample includes 366 women (77%), and nearly 51% of the individuals (n=241) present tinnitus, 69% (110) of the tinnitus subgroup are women. Results for comorbidities (high level of cholesterol, blood pressure, dizziness, smoking habits, infectious diseases and ototoxic medications) are discussed. Regarding emotional and social difficulties, the worst listeners present more difficulties and more depressive symptoms, being women more affected. Higher self-reported quality of life seems to be a protective factor towards tinnitus while psychological complaints and hearing loss, were both significant risk factors for tinnitus. T allele at GRM7 was the most observed and individuals with a T/T genotype have a different risk for ARHL and for tinnitus, compared to A/A and A/T genotype. Slow acetylator was the most common NAT2 phenotype, also with a gender effect distribution.

Tinnitus and hearing loss disorders can have a high negative impact on quality of life. The health-related perception and psychological factors appear to have a significant impact on the development of severe tinnitus. With our aging population, it is likely that the problems identified will be increasingly prevalent and add to the frailty of older adults.

# P40

**Neuro-otological treatment for patients with dementia and hearing loss in unique psychiatric hospital**

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**Introduction**

Japan is a hyperaging society. The number of patients with dementia are increasing and might be 4.62 million people in 2012. We reported the audiometric tests (AT) in patients with dementia and the usefulness of hearing aid (HA).

**Subjects**

The subjects were 152 patients with dementia (52 men, 100 women, age range, 61-95; mean age 80.3 years) and 43 patients with mild cognitive impairment (MCI) (16 men, 27 women). Dementia were classified as follows: Alzheimer's in 92 (60%), vascular in 12 (7.9%), Lewy Bodies (DLB) in 13 (8.3%), frontotemporal (FTD) in 22 (14.5%), mixed in 12 (7.9%), others in 1 (0.7%). We used revised Hasegawa's dementia scale (HDS-R) as a cognitive function test. The HDS-R results were classified as follows: *normal* and mild≧21; *moderate1*:: 16～20; *moderate2*: 11～15; *severe*: ≦10. AT results were classified as follows: *normal*: pure tone average (PTA)≦25 dB; *mild*: 26-40dB; *moderate*: 41～70dB; *severe*: ≧71dB.

**Results**

Out of 152 patients with dementia, in HDS-R, 53 (34.9%) cases gave normal and mild results, 40 (26.3%) showed moderate1, 32 (21.1%) showed moderate2 and 27 (17.8%) showed severe. Bilateral AT results showed normal in 11 (7.2%) case, right hearing loss (HL) in 3 (2%), left HL in 4 (2.6%) and bilateral HL in 133 (87.5%). In total therefore, 141 (92.7%) had HL. The rate of continuing to wear HA was get better as follows: 7/76 (9.2%) in 2015, 23.1% (22/95) in 2016, 24.7% (25/101) in 2017, 33.3% (44/132) in 2018 and 34.8% (49/141) in 2019. Out of 43 patients with MCI, 40 (90%) had HL and 14/40 (35%) continued to wear HA. We presented 13 cases of cognitive function improvement. Cognitive function tended to be maintained in the last 9 cases. Relationship with family seemed to be important.

**Discussion**
It is important for them to detect their HL and usefulness of HA. We have to be careful for the possibility of pseudo-dementia (cognitive dysfunctions caused by HL or earwax). Hearing level affects cognitive function, therefore hearing tests are very important for eldery persons to evaluate not only inner ear but also brain function. We need to keep their social happy life.

# P41

## Inhibition of DRP-1-dependent mitophagy promotes cochlea hair cell senescence and exacerbates AHL

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**Background**
DRP-1 plays a critical role in mitochondrial fissionand mitophagy. However, the role of DRP-1-dependent mitophagy altering mitochondrial function in senescent disease remains unclear. Here we examined whether the decline of DRP-1 altered mitophagy and mitochondrial dysfunction in cochlear cell senescence from cellular model to aged mice.
**Methods**
Cellular senescence induced by hydrogen peroxide (H2O2) and aged cochlea were evaluated by senescence associated β-galactosidase stain. Mitophagy levels were evaluated by fluorescence image and western blotting of LC3II and P62. Mitochondrial function was assessed by ATP assay, mtDNA assay and JC-1.
**Results**
We found that DRP-1 expression and mitophagy level decreased in senescent cells induced by H2O2 and senescent cochlea. DRP-1 overexpression cells initiated mitophagy and displayed better mitochondrial function exposed to H2O2, meanwhile silencing DRP-1 displayed otherwise. Moreover, inhibition of DRP-1 by mitochondrial division inhibitor-1(Mdivi-1) inhibited mitophagy and exacerbated hearing loss in aged C57BL/6 mice.
**Conclusions**
These results indicate that mitochondrial fission and mitophagy together eliminate dysfunction mitochondria and DRP-1 may be protective against oxidative stress-induced senescence by initiating mitochondrial fission and mitophagy to remove dysfunction mitochondria. These results may provide a potential therapeutic target for age-related hearing loss.

# P42

## Life-long-lasting functional damage of the inner ear cells induced by P-type Ca2+-ATPase mutations

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It remains elusive what precise mechanism underlies age-dependent hearing loss or presbyacusis onset and development. It also raises controversy that rodent model of age-dependent hearing loss can be applied to study of human's.
Using ENU-mutagenesis platform, we have isolated 4 mouse lines of Pmca2 mutant, each of which has a different missense mutation and shows quite diverse phenotype; there exists a large difference in age-dependent ascending rate of ABR thresholds or period of hair cells/SG cells disappearance. F1 genetic background of C57BL6/J and C3H/HeJ augmented these phenotypic differences.
The clear differences in phenotype among these mutants appear to result from the differences between Ca2+-pump activities of P-type Ca2+-ATPase, the product of mutated Pmca2 genes. Assuming a

biomolecular pathway towards the age-dependent emergence of phenotypes could be divided into two axes; functional impairment and survival odds, it may provide candidates of useful models for human progressive hearing loss to clarify these pathway axes. Besides Ca2+ ion as a second messenger in multiple signal pathways is generally thought to act in very short time range, it might also act in as-yet-unknown pathway for a life-long period.
To investigate these pathway axes in vitro, we try to establish cultured cell system in which the each mutated protein expression is properly controlled to give equal amount and localization. Molecular analysis of this system is expected to bring in latent bases of the mutant long-range effects.

# P43

**Deficiency of mitochondrial tRNA modification causes early spiral ligament damage**

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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 ($ms^2$) modifications of mt-tRNAs. Deficiency in $ms^2$ 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 $ms^2$ modifications of mt-tRNAs on age-related change in vivo and in vitro. Herein, we investigated the morphology of spiral ligament to determine the influence on age-related hearing loss.

**Materials and Methods**
Each stage cochlea of cdk5rap1KO and hetero mice was dissected, cryosectioned and stained with HE, and immunostained with anti-Cx26, anti-Na+/K+-ATPase, anti-Aqp1 antibody.

**Results**
HE staining showed that fibrocytes in spiral ligament of cdk5rap1 KO mice cochlea decreased from postnatal 1 months. While, fibrocytes in spiral ligament of hetero mice cochlea decreased from postnatal 5 months. Immunostaining showed the earlier decreased expression of Cx26 in KO mice compared to that in hetero mice
**Conclusions**
Our previous study and these results suggest that $ms^2$ modifications of mt-tRNAs may induce mitochondrial damage and oxidative stress in the spiral ligament and accelerated their aging, thereby causing aging-hearing loss.

**Key Words**: cdk5rap1, age-related hearing loss, spiral ligament

# P44

**Cx26 partial loss causes accelerated presbycusis by redox imbalance and dysregulation of Nfr2 pathway**

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Mutations in GJB2, the gene that encodes connexin 26 (Cx26), are the most common cause of sensorineural hearing impairment. Despite the undisputed correlation between GJB2 and hearing loss, the exact function of inner ear connexins and their role in etiopathogenesis of deafness remain largely undetermined. We generated and analyzed Gjb2+/− mice as a model of heterozygous human carriers of 35delG. Compared to control mice, auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs) worsened over time more rapidly in Gjb2+/− mice, indicating that they were affected by accelerated age-related hearing loss (ARHL). We linked causally the auditory phenotype of Gjb2+/− mice to apoptosis and oxidative damage in the cochlear duct, reduced release of glutathione from connexin hemichannels, decreased nutrient delivery to the sensory epithelium via cochlear gap junctions and deregulated expression of genes that are under transcriptional control of the nuclear factor erythroid 2-related factor 2 (Nrf2), a pivotal regulator of tolerance to redox stress. Moreover, a statistically significant genome-wide association with two genes (PRKCE and TGFB1) related to the Nrf2 pathway was detected in a very large cohort of 4091 individuals, originating from Europe, Caucasus and Central Asia, with hearing phenotypes (including 1076 presbycusis patients and 1290 healthy matched controls). Taken together, our results showed that (i) elements of the Nrf2 pathway are essential for hearing maintenance and (ii) their dysfunction may play an important role in the etiopathogenesis of human presbycusis.
In conclusion, our data suggest that presbycusis might arise from insufficient expression of the gap junction protein Cx26 and highlight interesting candidates to be further validated in larger cohorts and with potential powerful translational opportunities. Therefore, this work sheds new light on the etiopathogenesis of progressive hearing loss and also paves the way to its prevention in heterozygous carriers of 35deG, the prevalent GJB2 mutation in several populations.

**Keywords**: age-related hearing loss, connexins, oxidative stress.

# P45

**Age-related alterations in the hearing function of Fischer 344 rats**

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Age-related changes become very actual issue with the increasing life expectancy. Progressive decline in the hearing function with age is known as age-related hearing loss (ARHL) or presbycusis. Fischer 344 (F344) rats were previously described as an animal model of fast aging with a prevalence of the strial type of presbycusis. We examined hearing sensitivity (as thresholds of the auditory brainstem responses, ABRs), responsiveness to suprathreshold acoustic stimulation (as strength of the auditory startle responses, ASRs) and gap detection ability (as the efficiency of the prepulse inhibition of ASR induced by gaps of different durations, gap-PPI) in young adult (3–6 months old) and aged (24–27 months old) F344 rats. Aged animals showed considerable ABR threshold elevation (about 25 dB SPL) in the whole frequency range from 2-40 kHz compared to young adult rats. The weakening of the startle reactivity, characterized by slight increase of the ASR threshold (about 5 dB SPL) and pronounced decrease (about three-fold) of ASR amplitude in the range of high intensities, was found in the aged F344 rats. We observed reduction of gap-PPI in aged compared to young adult rats indicates worsening of the gap-detection threshold and decline in perceptual salience of suprathreshold gaps. Revealed changes of the animal responsiveness to gap reflected age-related deficit in the temporal resolution. Behavioral sings of tinnitus in the tested aged F344 rats were not found. F344 rats can serve as useful experimental model for investigation of mechanisms and manifestation of presbycusis. This work was supported by The Czech Science Foundation (GAČR) (grant number 18-09692S).

**Key words**: aging; age-related hearing loss; auditory brainstem responses; auditory startle responses; gap detection.

# P46

**Degradation of cochlear gap junction is a crucial pathogenesis in age related hearing loss**

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**Background**
AHL (Age-related hearing loss) is defined as a progressive, bilateral, high-frequency hearing loss in elderly people. Connexin (CX)26 is one of the major protein subunits to form gap junctions in the cochlea. Mutations in CX26 are one of the most common causes of inherited nonsyndromic deafness. The relationships between CX26 and AHL are not fully understood. Here, we investigated the CX26 quantitative change and molecular pathology of AHL.
**Methods**
C57BL/6J mice were used as a representative model of AHL. The hearing levels were evaluated by ABR (auditory brainstem responses). We investigated the formation of gap junction plaques in cochlear inner sulcus cells by confocal microscopy and the cochlear gap junction proteins such as CX26 and CX30 by western blotting and compared 4- and 32-week mice.
**Results**
ABR thresholds gradually increased to 32 weeks and rapidly elevated at 36 weeks. It was suggested that the pathological progression of hearing loss in early stage accelerated between 32weeks and 36weeks. Therefore, 32-week-old mice were investigated as initial stage model of AHL. In immunohistochemical analysis of the cochlea in 4-week-old mice, gap junctions showed linear plaques along the cell-cell junction sites with adjacent cells. In contrast, gap junction plaques in 32-week-old mice did not show the normal linear structure but instead formed small spots around the cell-cell junction sites and gap junction lengths were significantly shorter than in 4-week-old mice. Hair cell loss were also compared between 4- and 32-week-old mice. However, their differences were not significant. In western blotting, CX26 and CX30 protein level were significantly decreased in 32-week-old mice compared with 4-week-old mice.
**Conclusions**
These results indicated that the disruption of GJPs were crucial pathogenesis of AHL occurring before the hair cell degenerations. Moreover, disruption of the gap junction plaques and decreased gap junction proteins might contribute to the onset and progression of AHL. Furthermore, the treatment targeting CX26 such as GJB2 gene therapy may be effective for AHL.

**Keywords**: Presbycusis, Age-related hearing loss, Connexin26, Gap junction

# P47

**The potential effect of food intake in gaining or reducing an incidence of hearing impairment after middle age among Japanese community dwellers**

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**Objectives**
There is a paucity of data in human epidemiological studies to address the relationship between dietary intake and hearing impairment (HI). We aimed to obtain information from longitudinal analysis about the association between dietary intake and age-related HI.

**Methods**
Data were collected biennially between 2000 and 2012 in the Longitudinal Study of Aging. The participants with no HI in their forties at baseline and without any missing information necessary to analyze were 421 individuals. Hearing impairment criteria were taken as the better ear pure-tone average at frequencies of 0.5, 1, 2, and 4 kHz greater than 25 dB. Averages for 3-day food and nutrient intake (including alcohol intake) were calculated according to the Standard Tables of Foods Composition in Japan 2010 and other sources. The longitudinal analysis was performed in 1,845 samples from 421 participants aged 40-61 years old and

who participated in the survey at least once after baseline examination and were followed up - for up to 11.5 years. Cumulative data multivariable analyses were done using generalized estimating equations, in which odds ratios (ORs) per 1 standard deviation increase for 18 food groups with the risk of developing HI were calculated.

**Results**
The longitudinal observations indicate a significant association of mushrooms intake with risk reduction (OR = 0.362, 95% confidence interval [CI]: 0.164-0.802), and a significant association of beverages (OR = 1.560, CI: 1.069-2.278), and seasonings and spices intake (OR = 1.347, CI: 1.061-1.710) with risk increments for developing HI after adjustment for age at baseline, passed years from baseline, and sex.

**Conclusions**
The present observation implied that mushrooms, beverages, and seasonings and spices intake could be a potential modifier of risk for developing age-related HI in young middle individuals.

**Keywords**: age-related hearing impairment; dietary intake; longitudinal study

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## Cochlear Implant and Implantable Prosthesis

# P48

### Band-limited Chirp-evoked Compound Action Potentials in Guinea Pigs: Feasibility for Cochlear Implantation Monitoring

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**Background**
Patients with severely impaired high-frequency hearing and sufficient residual low-frequency hearing can be provided with cochlear implants (CIs), thereby facilitating ipsilateral electric and acoustic stimulation (EAS) with well-established advantages over electric stimulation alone. Still, residual hearing is often only partially preserved due to, inter alia, acute mechanical trauma during implantation. Possibilities of intraoperative monitoring using electrocochleography (ECochG) have been extensively studied in CI patients, primarily using the ongoing outer hair cell response to low-frequency tone bursts, i.e. cochlear microphonics. In contrast, neural phase-locking to the ongoing response as well as the neural transient response, i.e. compound action potential (CAP), were generally less prominent or sometimes absent, thus falling short of providing useful contribution to ECochG analysis. In this study, we investigate using band-limited chirps to better synchronize the neural response and provide more robust CAP in a guinea pig model of cochlear implantation.

**Methods**
In experiment 1, ECochG was measured in 9 normal-hearing guinea pigs (Dunkin Hartley) via gold-wire electrode placed at the round window niche. We compared input-output (IO) functions and derived-band responses using high-pass noise masking to infer neural contribution from the targeted frequency band to the CAP. Stimuli were constructed by adding a harmonic series either with zero phase delay (band-limited click) or by adjusting the phase delay according to a model of basilar membrane group delay (chirp) with 3 different parameters in order to examine level-specific changes in the delay model. The amplitude spectrum

was thus identical between stimuli with differences only in phase. In experiment 2, guinea pigs were implanted with MED-EL custom-built CI electrode carrier using a motorized micRomenipulator. ECochG was measured at each insertion step for the following stimuli: broadband click, band-limited click, chirp (3 parameters), and 10-ms tone bursts at frequencies 1, 2, 4, and 8 kHz. Insertion depths ranged between 4 and 5 mm.

**Results**
Chirps compared with equal-band click showed significantly lower thresholds and steeper slopes at inflection point of sigmoid-fitted IO functions. They also showed limited dynamic ranges and comparable or smaller CAP amplitudes at saturation. Derived-band analysis showed higher peak bandwidth of the Gaussian-fitted neural firing density with larger effective neural firing "mass", interpreted as more synchronous activation in the targeted frequency band. Finally, intraoperative monitoring "sensitivity", defined as relative amplitude change per unit distance, was comparable between chirp-evoked CAPs and tone burst-evoked ongoing responses, but it was smaller for other stimulus responses.

**Conclusion**
The chirp appears to be an effective stimulus to synchronize the neural response from a limited frequency band in guinea pigs. Therefore, it could be used to achieve a more holistic assessment of residual hearing in humans during cochlear implantation.

**Keywords**: chirp, electrocochleography, compound action potential, derived-band response

# P49

**Cochlear implants in elderly population: results and benefits**

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**Introduction**
The increasing of life expectation in elderly population has determined a rise of people with severe-profound sensorineural hearing loss requesting auditory rehabilitation and many studies have demonstrated that cochlear implantation in elderly is advantageous for their quality of life.This study aimed to analyse retrospectively functional results and benefits of quality of life in 39 patients with post verbal severe-profound bilateral hearing loss, aged more than 65 years, who underwent unilateral cochlear implantation in Carpi's hospital in the period between January 2000 and June 2014. All the patients have a follow up of at least 3 years.

**Methods**
In 27 cases patients underwent cochlear implantation in the "worst "ear, maintaining a hearing aid in the contralateral ear (bimodal stimulation). 4 subjects received the implant in the side of patent cochlea, 5 in the ear with lower length of hearing deprivation, 3 in the ear with more recent onset of hearing loss. Audiological evaluation included pure tone audiometry, speech audiometry, ABR, verbal perception tests. All the patients underwent speech therapy treatment and routine follow up for cochlear implant regulation. Finally, a self – assessment questionnaire to evaluate subjective benefits was administrated.

**Results**
no surgery related complications were observed, only in 1 case the array was inserted partially because of a post-meningitis cochlear ossification. 10 subjects left contralateral hearing aid as there was an aggravation in hearing loss. All the subjects reported benefits in speech perception and in quality of life, factors mainly conditioning the outcomes were the lenght of hearing loss and the possibility to use a bimodal stimulation. Frequency of speech therapy treatment resulted important as well.

**Conclusion**
Our data confirm that CI is an effective procedure also in elderly patients, conferring benefits in speech perception and quality of life. The association with bimodal stimulation and speech therapy is important and it allows to avoid social isolation and the possible consequence of depression which is more common among the elderly.

# P50

**Working memory function in children with unilateral hearing loss using a bone anchored hearing implant: a case control study**

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The importance of a good hearing function to preserve memory and cognitive abilities has been shown in the adult population, but studies on the pediatric population are currently lacking. This study aims at evaluating the effects of a bone-anchored hearing implant (BAHI) on speech perception, speech processing, and memory abilities in children with unilateral hearing loss (UHL). We enrolled n = 25 children with UHL and assessed them prior to BAHI implantation, and at 1-month and 3-month follow-ups after BAHI implantation using tests of perception in silence and perception in phonemic confusion, dictation in silence and noise, and working memory and short-term memory function in conditions of silence and noise. We also enrolled and evaluated n = 15 children with normal hearing. We found a statistically significant difference in performance between healthy children and children with UHL before BAHI implantation in the scores of all tests. After 3 months from BAHI implantation, the performance of children with UHL was comparable to that of healthy subjects as assessed by tests of speech perception, working memory, and short-term memory function in silence condition, while differences persisted in the scores of the dictation test (both in silence and noise conditions) and of the working memory function test in noise condition. Our data suggest that in children with UHL BAHI improves speech perception and memory. Speech rehabilitation may be necessary to further improve speech processing.

**Keywords**: Unilateral hearing loss, Bone anchored hearing implant, working memory

# P51

**VSB surgery for conductive and mixed hearing loss - the most sensitive predictable factor for speech perception with VSB -**

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**Educational Objective**
On the conclusion of this presentation, the authors demonstrate that the most sensitive predictable factor for the post-operative speech perception with VSB at six months after the surgery should be the pre-operative speech perception with as well as without best fitted hearing aids (HA).

**Objectives**
In our institute, 44 patients received VSB implantation for conductive and mixed hearing loss so far, and they were 19 males and 25 females. FMT was placed within RW area (RWV) in 23 cases, and within OW area (OWV) in 11 cases. VSB was implanted as VORP in 9 cases. RWC (Round window coupler) was used in 16/23 RWV cases.

**Study Design**
Retrospective simple and multiple regression analysis of the post-operative speech perception with VSB.

**Methods**
In 28 cases/ 44 cases who underwent the VSB surgery between 2012- 2018 and has been wearing his/her VSB for more than six months after its activation, regression analyses were conducted to find out which variable is the most sensitive predictable factor for the post-operative speech perception with VSB, among five independent variables include 1) the age at the surgery, 2) the pre-operative PTA (Pure tone audiogram), 3) the pre-speech perception (with and without best fitted HA), 4) the placement of the FMT (RW/OW/VORP), and 5) the use of RW-coupler in RWV, while the target (dependent variable) is“speech perception with VSB” at six months after its activation.

**Results**
With simple regression analysis, we found positive correlations between the post-speech perception with VSB and the pre-speech perception with as well as without best fitted HA, and found a negative correlation

between the post-speech perception with VSB and the pre-operative PTA. With multiple collinearity analysis, we found very close correlation between the pre-speech perception and the pre-operative PTA. No correlation was found between the pre-operative PTA and the age at the surgery, and between the pre-speech perception and the FMT placement. Multiple regression analysis indicated that the pre-operative speech perception with as well as without HA should be the best predictor for the post-speech perception with VSB.

**Conclusions**
The most sensitive predictable factor for the speech perception with VSB should be the "pre-operative speech perception with as well as without hearing aid".

## P52

**Restoring hearing can affect the degraded cognitive domains in older patients**

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**Background**
Hearing disability is an important issue in geriatric medicine because it is associated with numerous health issues, including accelerated cognitive decline, depression, increased risk of dementia, poorer balance, falls, hospitalizations, and early mortality. There are also social implications, such as reduced communication function, social isolation, loss of autonomy, impaired driving ability, and financial decline. Because Age-Related Hearing Loss (ARHL) is common and potentially treatable, it should be investigated as a modifiable risk factor for neurocognitive decline and dementia. The aims of the present study are: evaluate cognitive profile of patients with Cochlear Implant (CI) and analyse the relationship between audiological and neuropsychological performances of these patients during the follow-up.

**Material and Methods**
42 patients affected by ARHL (over 60 years old) with CI candidacy, without any neurologic disease or dementia underwent audiologic tests (tonal audiometry, impedentiometry, vocal audiometry, Speech-in-noise test, auditory brainstem response and transient evoked otoacoustic emission); neuropsychologic tests for global cognitive profile (through Mini Mental State Examination, MMSE), episodic memory, semanthic memory, working memory, attention, executive functions; and quality life questionnaires. These tests were performed at baseline (T0), at 6 months (T1) and in 32 patients having 12 months or more of follow-up after implantation (T2).

**Results**
There was a significant difference in audiological performances and overall neuropsychological tests at T1. Significantly better performances after rehabilitation were showed at T1 in MMSE and in memory tests, brief and long term. At T2 we reported the stability of mnesic functions and increasing of executive and attention functions. A significant improvement in quality life questionnaires was reported between T0 and T1, except for suffer pain and physical activity.

**Discussion**
Patients with CI showed an improvement in episodic memory tests, proving that mnesic disorders are due not only to sensorial deficit but also a weakened post-processing mechanisms useful for the consolidation of learnt information confirming the hypothesis of a link between ARHL and dementia. Furthermore, hearing impairment seems to reduce executive functions which are responsible for attention, planning, strategies' production and coordination between other cognitive processes. Hearing rehabilitation provide a recovery of superior cognitive domains through a re-allocation of cortical resources altered by hearing deprivation.

**Key words**: aging, age-related hearing loss, dementia, cochlear implant

# P53

**Defined BDNF and NT-3 Growth Factor Cocktail Enhanced Neurite Outgrowth in a Postnatal Day 7 (adult-like) Murine In Vitro Spiral Ganglion Explant Model**

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Cochlear implants (CI) are the method of choice for patients suffering severe to profound sensorineural hearing loss. A major obstacle is the spatial distance between the electrode array of the CI in scala tympani and the stimulated region of the auditory nerve. This anatomical gap restricts the number of information channels provided by the implant. To increase frequency selectivity and decrease energy consumption, a direct coupling between the CI electrodes and the cochlear nerve fibers should be achieved. Therefore, the neurite outgrowth from the spiral ganglia neurons (SGNs) towards and onto the electrode should be induced. The regulation of outgrowth, maintenance and survival of nerve fibers in the inner ear is crucially linked to neurotrophic factors like BDNF (brain-derived neurotrophic factor) and NT-3 (neurotrophin 3). In the cochlea, an opposing NT-3 and BDNF gradient from base to apex has been described that is reversed during development.
We tested SGN outgrowth *in vitro* by applying BDNF and NT-3 either individually or both in combination of various compositions. For better comparison to the adult situation and to account for the switch from embryonic to adult phenotype, we established a NMRI (Naval Medical Research Institute) mouse organotypic *in vitro* spiral ganglion explant model at postnatal day 7 (P7) utilizing 96-well plates. Effective stimulation of neurite outgrowth was analyzed using immunohistochemically stained fluorescent micrographs quantified with a custom adapted Sholl analysis procedure (ImageJ plugin).
In common early postnatal (P0-P4) organotypic culture models of spiral ganglion explants, outgrowth under control conditions is very limited, and the addition of individual neurotrophins increased neurite outgrowth in a dose-dependent manner.
In contrast, our later postnatal (P7) spiral ganglion explant model showed substantial neurite outgrowth, even under control conditions (no growth factor added). However, the addition of BDNF or NT-3 enhanced this outgrowth similarly to common models. Interestingly, the defined combination of both growth factors as a BDNF and NT-3 cocktail improved neurite outgrowth even over the extent of the individual compounds.
The presented new organotypic culture model of late NMRI mouse postnatal stage (P7) spiral ganglion explants, more resembling the adult situation, is suitable for the evaluation of compounds affecting the SGN neurite outgrowth *in vitro*.

# P54

**Cochlear duct length measurements in congenital single side deafness**

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Today, 43% of children with unilateral hearing loss (UHL) are identified prior to 6 months of age by means of the newborn hearing screening testing. Currently, different treatment options have been proposed for UHL, with increasing prescription of cochlear implantation (CI) in case of UHL of severe to profound degree (Single Side Deafness-SSD). Nevertheless, little is known about the possibility and difficulties in obtaining an auditory balance between affected and normal side with CI. In this regard, the different length of the cochlear duct between affected and unaffected side could be an important factor in CI fitting and prospective balanced auditory discrimination outcomes.
The objective of this study was to identify the anatomic factors that can be relevant to CI surgery and fitting in SSD. Specifically, the length of the cochlear duct in affected compared with the contralateral side could influence the electrode choice and modality of frequency stimulation and matching.
We analysed CT (computed tomography) scans of 39 children with congenital isolated sensorineural UHL using OTOPLAN software (MED-EL, Innsbruck, Austria), a specific radiological viewer used to select the most suitable implant and surgical approach.
This software uses CT images to obtain anatomic reconstructions and accurate measure the cochlear dimensions.

The results of the study can be of help in developing recommendations and guidelines for selecting the CI as a treatment in case of congenital SSD, and in suggesting the most appropriate electrode and stimulation strategy.

**Keywords**: unilateral hearing loss; cochlear implant; cochlear duct length; Otoplan.

# P55

**Does proteome analysis of human perilymph give information about hearing performance after cochlear implantation?**

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**Objective**

The molecular pathophysiology of the inner ear leading to sensorineural hearing loss is not elucidated in detail at all. Also it is unknown if the composition of perilymph can give information about patients individual hearing performance after cochlear implantation. We hypothesize that proteome analysis of perilymph offers an opportunity to predict on speech understanding of cochlear implant patients postoperatively.

**Methods**

Established perilymph liquid biopsy and proteome analysis was performed in a variety of cochlear implantations (n = 75). The perilymph samples derive from adults and children with different etiology undergoing cochlear implantation or vestibular schwannoma surgeries. The proteins were identified by a shot-gun proteomics approach and data-dependent analysis using orbitrap mass spectrometry (Thermo Fisher Scientific) and Max Quant software for identification and Perseus software for data analysis. For the postoperative hearing performance the data of audiologic tests (HSM sentence test, Freiburg monosyllable word test) one year after cochlear implantation were analyzed.

**Results**

In the stringent proteomics analysis of 75 perilymph samples from 68 patients 935 proteins were detected. From the 68 patients, the majority of the patients participated in the two audiologic tests (HSM sentence test in noise 10 dB, n = 45; Freiburg monosyllable word test, n = 47) used for the classification of hearing performance one year postoperatively. For children, these types of tests were not applicable. The patients could be categorized by the audiologic tests into two groups depicting good or bad hearing performance after cochlear implantation. In the group good hearing performance 5 proteins were significantly upregulated. In the group bad hearing performance 6 proteins were significantly upregulated, for example different immunoglobulin heavy and light chains, showing a different immune response in the two groups.

**Conclusions**

The statistical analysis of these two groups by the proteomics data shows significantly up- and downregulated proteins and may give new predictive information about the individual cochlear performance of patients undergoing a cochlear implantation.

**Keywords**: Hearing performance, Proteomics, Perilymph diagnostics, Cochlear implantation

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# P56

**Effect of alginate-cell based drug-delivery systems on the insertion forces of CI-electrodes**

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**Background**

The outcome of the cochlear implant (CI) can possibly be improved by the treatment with neurotrophic growth factors (NTF) to support the survival of the stimulated spiral ganglion neurons. For a longer lasting effect, a chronic application of NTF is a prerequisite. This could be achieved by the implantation of drug-producing cells into the inner ear. To improve biosafety, cells can be encapsulated into a bioinert alginate-matrix to avoid their uncontrolled migration and release and to protect them against the host immune system. An application of alginate encapsulated NTF-producing cells as a viscous solution or electrode coating are possible delivery methods but may have an impact on the implantability of the CI.

**Methods**

To detect the feasibility of the two drug-delivery strategies viscose solution or electrode coating, insertion forces and coating stability were analyzed. NTF-producing cells were encapsulated in ultra-high viscous alginate and injected into a saline filled artificial human cochlea model (aCM) or applied as coating to human-sized custom-made electrode arrays before insertion into the aCM. The insertion of uncoated arrays into the saline filled aCM served as control. Dip coating was performed manually after poly-L-lysine pre-coating and consisted of inner cell-containing and outer, shielding cell-free alginate layers. Insertion force measurements were performed in an automated insertion test bench with the aCM mounted on a force sensor. Each array was inserted three times. Microscopic pictures were taken before and after first and final third insertion to evaluate the coating stability by detecting the degree of abrasion.

**Results**

The injection of the viscous alginate-cell solution into the aCM as well as the manual dip coating of the array before insertion into the aCM was feasible. Compared to the insertion of uncoated control arrays, the tested coating reduced the insertion forces by 75 % and showed no buckling or tip fold over. In contrast, the filling of the aCM with the viscous alginate-cell solution slightly increased the insertion forces. The coating stability was good after first insertion with 85 % coated arrays graded to no or minimal abrasion. Repeated insertions had a negative effect on the coating stability, so that only half of the arrays showed no or minimal signs of coating abrasion.

**Conclusions**

Both tested application strategies are promising options for a cell-based drug-delivery to the inner ear. However the alginate-cell coating offers the great advantage of combining cell-based drug-delivery with a tremendous reduction of insertion forces and therefore bares a huge potential for patients with residual hearing undergoing a CI-implantation.

**Keywords**: chronic drug delivery, alginate-cell solution and coating, insertion force reduction

*The project was supported by the German Research Foundation.*

# P57

**GWAS meta-analysis points to new loci for normal hearing function and age-related hearing loss**

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Normal human hearing function (NHF) and its decline with age (Age-Related Hearing Loss, or ARHL) are defined by both environmental and genetic factors, but which exactly the involved genes are is still an open question. Because of the social and economic impact of ARHL, and with the general ageing of world population, the importance of an in-depth knowledge of the genetic inner workings behind these traits is rising. With this study we aim at taking a step further in their understanding.
Eight different cohorts belonging to the G-EAR consortium and coming from Italy, Northern Europe, Caucasus and Central Asia, for an overall number of 4789 subjects aged>=18, were considered for genome-wide (meta)-analyses on NHF and ARHL. For all subjects, audiometric data (hearing thresholds at 250Hz, 500Hz, 1 KHz, 2 KHz, 4 KHz, 8KHz), clinical characterization, work and lifestyle data were available.
NHF. Quantitative GWAS analyses were performed using single thresholds or their average value across specific frequencies in the subject [Pure-Tone Averages (PTA-Low: 250,500,1KHz; PTA-Medium: 500,1K,2KHz; PTA-High: 4K,8KHz)].

ARHL. Case-control GWAS analyses were carried out using a phenotype definition based on PTAH value (described in DOI:10.3109/21695717.2014.911472). 2663 individuals aged>=50 from all eight cohorts were suitable for the task.

Genotype data was imputed with 1000Gph3 reference panel; GWA studies were performed with GenABEL, adjusting for sex, age and relatedness. Results were pooled together using METAL.
REPLICA. The subset of UkBiobank project with digit-triplet test data was selected as a replica cohort, using the SRT (Speech-Reception-Threshold) phenotype.
Analyses on NHF have highlighted several putatively associated loci on chromosomes 3, 5, 6, 8, 12 and 16.

In particular, on chromosomes 3, 6 and 12, a strong association (p-values $<10^{-8}$ and concordant direction of effect across all cohorts) with PTAH, 8KHz and PTAM respectively has been found. On chromosome 3, a gene encoding for a protein that acts as a calcium channel, whose expression has already been related to the hearing phenotype in mice, seems to have an important role. Concerning chromosome 6, the signal falls within a gene belonging to the succinylCoA ligase family, members of which have been reported as involved in the hearing phenotype. Moreover, on chromosome 12, a new candidate belonging to the glycosyltransferase family could be a relevant finding.

ARHL analyses detected a highly significant signal on chromosome 5, on a locus referring to a gene/protein catalysing the conversion of homocysteine to other amino-acids. Interestingly, literature showed a relationship between hyperhomocysteinemia induced by folate deficiency and hearing loss. Association signal was mainly driven by Northern European populations, in which a signal of natural selection (|iHS|score>2) is also reported.
Further analyses on enlarged cohorts and in vitro/in vivo studies will clarify the role of these candidate genes in the hearing phenotype. As specifically regards ARHL, results suggest that a possible population-specific haplotype involved in folate metabolism may also be involved in ARHL.

# P58

**Genetic mutations in Non Syndromic Deafness patients, clinical cases of Varese.**

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The genetic sensorineural hearing loss represent about 50% of all forms of hearing loss and is a heterogeneous group of pathologies. We performed ( period 2004-2017) molecular investigations on 902 patients with congenital profound sensorineural hearing loss; we researched alterations in the genes *GJB2 and his promoter and 5'-3'UTR, GJB6 (D13S1830 e D13S1854), GJB3 (exon2), GJA1 (exon2 and pseudogene GJA1P1)*, alterations in the *MIR96* gene encoding a miRNA involved in the post-transcriptional regulation of genes implicated in auditory function; specific mutations of the mitochondrial *MT-RNR1 and MT-TS1* genes ( m.1555A>G and m.7445A>G) that cause non-syndromic hearing loss with matrilineal inheritance. In 23.6% of patients we have identified the molecular cause. *GJB2* is the gene mainly involved in hearing loss and c.35delG is the most frequently detected mutation (34% of the GJB2 mutations). Homozygous 35del G mutation is present in 14% of GJB2 alterations. Compound heterozygosis 35delG / other GJB2 mutations is present in 51 patients and p.L90P is the second most frequent mutation (11 patients). These results reflect the literature data and confirm the important role of *GJB2* in the cochlear functions. We also identified 8 patients (0.1%) double heterozygous for D13S1830 deletion of the *GJB6* gene and c.35delG mutation. No patients have the D13S1854 deletion, and it confirms that the frequency of this alteration is variable in different geographical regions and very rare in Italy. Two dominant GJB2 mutations described in literature associated with dermathological anomalies ( pR75W and p.G130V) where found in two dominant families without dermatologic pathologies and confirm a very variable expression of cutaneous manifestation due to these mutations. Heterozygous GJB2 mutations were found in 35 Deafness patients, making the hypothesis plausible of the involvement of a second not investigated gene. Different detected mutations in GJB2 promotor region or UTR 5' and UTR 3' were classified as doubtful, but two of these mutations ( IVS1+1G>A, c.-22-6T>C) may have pathogenic relevance if associated with 35delG. No causative mutations were detected in *GJB3* (10 mutations found in 46 patients) and *GJA1* genes (2 mutations found in 3 patients), genes suggested to be involved in the pathogenesis of deafness; therefore, we definitely support that there isn't genetic relevance of these genes to the development of hearing loss.In 12 families, we have observed mitochondrial mutations m.1555A> G or m.7445A>G related to incomplete penetrance and variable expression of deafness. We have not identified any causative mutation in *MIR96* gene; we have supposed a role of this mutated gene in 2 families (miR96(+51C>G and miR96(+57T>C) with dominant pedigree, but without a clear segregation of the mutation with deafness.In our series of patients, genetic counseling is performed to all families and it is very important for diagnosis and an appropriate recurrence risks definition.

# P59

**Audiological phenotype of GJB2 gene variants in Slovakia**

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**Introduction**

Mutations in the Gap Junction Protein Beta 2 (*GJB2*) gene, encoding the connexin 26 protein, are responsible for approximately 50% of autosomal recessive non-syndromic hearing loss (HL). To date, more than 150 different variants have been reported in *this* gene. The spectrum and prevalence of *GJB2* mutations vary significantly among different ethnic groups. Besides the genotype, we also examined HL phenotype by collecting audiological data from investigated subjects in our cohort to characterize the clinical impact of particular *GJB2* variants.

**Materials and methods**

We included 295 subjects (243 probands plus their family members) diagnosed with hereditary HL due to *GJB2* mutations analyzed by Sanger sequencing. Apart from audiological investigation (otoacoustic emissions, pure tone thresholds, auditory steady-state response, ABR), every subject answered a questionnaire focused on clinical history of HL and associated symptoms.

**Results**
We identified 33 pathogenic *GJB2* variants. Regarding the severity of HL, the most frequent was profound HL (half of the cohort), second most frequent was severe HL in 21%. Majority had congenital or prelingual onset. The most common genotype was c.35delG/c.35delG, found in 55% of subjects in our cohort. About 60% of subjects with this genotype had profound HL. The second most frequent genotype was c.35delG/c.71G>A, found in 12% of the cohort, in most cases connected with moderate to severe HL (in 49%). Third most common variant was c.35delG/c.313_326del14 found in 7% of cohort, related to deafness in 70% of patients with this pathogenic variant.

**Conclusion**
Genotype-phenotype studies provide essential data on natural history of hereditary hearing loss. Research on individuals with extreme or atypical phenotype may reveal yet unknown molecular mechanisms involved in pathogenesis of hearing impairment and contribute to future therapeutic strategies.
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**Key words**: hereditary hearing loss, phenotype, audiological testing.

# P60

**Genetic analysis of hearing loss: some relevant cases from Campania Region (Southern Italy)**
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Hearing loss is the most common sensory deficit in humans. Its incidence is estimated between 1-3 children per 1,000 newborns. In our region (Campania) universal newborn hearing screening (UNHS) has been in place since 2006. Since then, we are able to detect and treat permanent hearing impairment (PHI) as soon as possible. Although in developed countries causality is frequently multifactorial, genetic factors contribute to about half of the cases. For non syndromic forms molecular analysis starts with *GJB2*/*GJB6* and when negative, according to the medical history of the patient, we extend molecular analysis to different genes. The carrying out of molecular investigations, necessary for the identification of a possible genetic etiology, has allowed us to identify several particularly interesting cases.
Here we are presenting some notable cases detected during our practice.

We studied the case of a child with profound sensorineural hearing loss, diagnosed through UNHS, in which we have highlighted the presence, in the heterozygous state, of a new sequence variant in exon 2 of the *GJB2* gene. This variant has a possible pathogenetic role, as suggested by available bioinformatics sources. The molecular study performed in the parents established that the variant is of maternal derivation, suggesting an autosomal dominant transmission.
In another case, it has been identified a new pathogenic variant of *MYO7A* present in compound heterozygosis with another mutation that is involved in the Usher syndrome. The subject is therefore now under observation from the eye point of view too.
In subjects presenting enlarged vestibular aqueduct (EVA) or other signs of the Pendred syndrome, we analyse the *SLC26A4* gene, another relevant cause of recessive hereditary hearing loss: its pathogenic variants account for about 10% of hearing loss. Recently, in the contest of our researches, we found a new *SLC26A4* mutation, expressed in homozygous form, in a girl affected by sensorineural hearing loss and EVA. This mutation had never been described in the past. This variant, never described in literature until now, introduces a STOP codon causing the formation of a truncated protein. The presence of the same mutation in heterozygosis was confirmed in her parents.
Finally, in the most peculiar syndromic forms, we perform array-CGH, to find out chromosomal abnormalities. Recently through this analysis we highlighted the presence of a duplication (q.21.2) in chromosome 1, in a little patient with conductive hearing loss and stenosis of the external auditory canals. The comparison of the literature with our data indicates the association of some genes present in this chromosomal area with the patient's symptoms. Concluding, thanks to the genetic protocol we applied, we are able to provide an etiopathogenetic description of the hearing losses detected through our UNHS for a lot of cases. Genetic diagnosis of deafness provides prognostic information on possible progression of hearing loss, permits meaningful genetic counselling, and impacts treatment decisions.

**Key words**: genetic, hearing loss, pathogenetic variant

# P61

**AAV mediated whirlin gene therapy for the treatment of DFNB31 and USH2D**

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Whirlin (WHRN), a protein essential for hearing and vision, plays a role in the stabilization, elongation and maintenance of the stereoliciary bundle in inner ear hair cells. Distinct disruptions of long and/or short whirlin variants (FL-WHRN and C-WHRN) lead to deaf-blindness (Usher syndrome type IID, or USH2D) or congenital, bilateral sensorineural hearing loss (DFNB31). To explore improved gene therapy treatment for these two disorders, we analyzed the benefits of different neonatal injection sites along with supplementation with specific variants. Efficacy of the treatments was assessed at the cellular and system level with analysis of mechanosensitive transduction currents, protein expression, hair cell survival and evaluation of the auditory and vestibular phenotype. This work shows that while neonatal supplementation of FL-WHRN alone lead to significant phenotypic recovery in mice models of USH2D and DFNB31, dual injection of C- and FL-WHRN in mice models of USH2D did not improve the outcome. No differential benefit was observed with injection either directly into the perilymphatic or the endolymphatic fluid. This work validates the use of novel synthetic vectors for the treatment of USH2D and DFNB31 and undermine the need for dual vector therapy for the treatment of USH2D.

# P62

**Gene therapy in a mouse model of genetic hearing loss**

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**Objectives**

Hearing loss is a highly prevalent sensory defect in humans. Nevertheless, there is no fundamental therapy to treat this disease. Gene therapy is a promising approach to restore hearing.

**Methods**

We achieved gene delivery by introducing a recombinant adeno-associated virus (rAAV) expressing the MsrB3 gene directly into the otocyst. we analysed the functional and histological changes of ears of DFNB 74 and DFNB2 mouse model

**Results**

We observed hearing recovery in the treated ear of the DFNB 74 and DFNB2 mouse model. We confirmed mRNA and protein expression in the ear, and rAAV-rescued ears exhibited normally shaped hair cells, similar to those of control mice.

**Conclusions**

To our knowledge, this is the first study to demonstrate restoration of hearing in a mouse model of hearing loss using an in utero, virus-mediated gene therapy. Our results provide a significant advancement in the treatment of hearing loss.

# P63

**Identification of a potential founder effect of a novel PDZD7 variant involved in moderate-to-severe sensorineural hearing loss in Koreans**

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A PDZ domain-containing scaffold protein is critical for organizing the Usher syndrome type 2 (USH2) interactome and is considered a USH modifier and digenic USH contributor. However, biallelic *PDZD7* variants potentially cause autosomal-recessive, non-syndromic hearing loss (ARNSHL) in humans. Here, we identified novel *PDZD7* variants from four unrelated families manifesting putative moderate-to-severe prelingual ARNSHL: (i) a homozygous missense mutation (c.490C>T: p.R164W), (ii) compound heterozygosity for a frameshift (c.1669delC: p.A557fs) and a missense mutation (c.490C>T: p.R164W), and (iii) compound heterozygosity for two missense mutations (c.1526G>A: p.G509E and c.490C>T: p.R164W). These novel *PDZD7* variants were found responsible for ARNSHL, based on the segregation study and *in silico* prediction analysis. Given a recurring missense variant (p.R164W) from our cohort, we compared genotyping data using six short tandem-repeat (STR) markers within or flanking *PDZD7* between four probands carrying p.R164W and 81 normal-hearing controls to identify whether p.R164W is associated with a specific single haplotype. Although we could not identify a definite single common haplotype shared by all four probands, a partially identical haplotype across three out of six STR genotyping markers exclusively shared by two unrelated probands but not by any of the 81 normal hearing controls were observed. The presence of biallelic variants of *PDZD7* is expected to induce ARNSHL, although longitudinal follow-up is necessary to exclude the retinal phenotype. Importantly, the p.R164W allele could exert a potential founder effect as well as a mutational hotspot in Korean pediatric moderate-to-severe hearing loss.

**Keywords:** haplotype; hot spot; *PDZD7*; p.R164W

# P64

**1Rare KCNQ4 variants found in public databases underlie impaired channel activity that may contribute to hearing impairment**

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*KCNQ4* is a frequently mutated gene in autosomal dominant non-syndromic hearing loss (NSHL), a typically late-onset, initially high frequency loss that progresses over time (DFNA2). Most *KCNQ4* mutations linked to hearing loss are clustered around the pore region of the protein and lead to loss of KCNQ4-mediated potassium currents. To understand the contribution of *KCNQ4* variants to NSHL, we surveyed public databases and found 17 loss-of-function and six missense *KCNQ4* variants affecting amino acids around the pore region. The missense variants have not been reported as pathogenic and are present at a low frequency (minor allele frequency < 0.0005) in the population. We examined the functional impact of these variants, which, interestingly, showed a reduction in potassium channel activity without altering expression or trafficking, being functionally similar to DFNA2-associated *KCNQ4* mutations. Therefore, these variants may act as risk factors for late-onset hearing loss and individuals with one of these variants may develop hearing loss during adulthood. In addition, reduced channel activity could be rescued by KCNQ activators, suggesting the possibility of medical intervention. These findings indicate that *KCNQ4* variants may contribute more to late-onset NSHL than expected, and therefore, genetic screening for this gene is important for prevention and treatment of this condition.

**Keywords:** Non-syndromic hearing loss, Deafness non-syndromic autosomal dominant 2, KCNQ4, Voltage-gated potassium channel

# P65

**Cochlear Nerve Hypotrophy and Degeneration in a Mouse Model of Charcot-Marie-Tooth Disease Type 2E**

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Charcot-Marie-Tooth disease (CMT) is the most common inherited peripheral neuropathy. Mutations in the neurofilament light polypeptide gene (*NEFL*) have been reported to cause CMT type 2E (CMT2E). Neurofilaments (NFs) are heteropolymers made of three different subunits: light, medium, and heavy (NFL, NFM and NFH, respectively). These subunits assemble into 10 nm filaments that compose the main part of the axonal cytoskeleton. Mutations in NFL are thought to disrupt NF assembly and axonal transport, resulting in the accumulations of NFs and leading to axonal degeneration. CMT2E patients with one of the *NEFL* mutations (N98S) have been reported to have hearing loss. Recently, a knock-in mouse model bearing the N98S mutation has been generated, and its behavioral phenotypes and histological results reported. In this study, we confirmed the presence of hearing loss in this mouse model and histologically evaluated the cause. *Nefl*$^{N98S/+}$ mice were compared with *Nefl*$^{+/+}$ mice to evaluate hearing thresholds according to the auditory brainstem response (ABR) at 1, 2, 4, and 6 months of age. The cochleae were extracted at 4 or 6 months of age for histologic evaluation of hair cells, peripheral processes, and cochlear nerves. *Nefl*$^{N98S/+}$ mice showed a significantly higher hearing threshold and more prolonged latency than *Nefl*$^{+/+}$ mice from 1 month of age, and their hearing became worse with age. Immunohistochemistry of cochlear whole mounts revealed that hair cells in both genotypes were intact, except in the basal turn. However, severe accumulation of NFs based on NF (NFL, NFH) staining was observed at the distal peripheral processes under inner hair cells in every turn of the cochleae of *Nefl*$^{N98S/+}$ mice, but not in those of *Nefl*$^{+/+}$ mice. The efferent fibers to outer hair cells were fewer and thicker in *Nefl*$^{N98S/+}$ mice than those in *Nefl*$^{+/+}$ mice. Immunostaining of transverse-sectioned cochlear nerves showed that NFL and NFH were significantly reduced in *Nefl*$^{N98S/+}$ mice; however, no differences were observed in $\beta$-tubulin staining. Furthermore, the diameter of the cochlear nerves was significantly smaller in *Nefl*$^{N98S/+}$ mice. These findings indicate hearing loss similar to the clinical results of auditory neuropathy observed in CMT2E patients. The hearing loss is likely caused by cochlear neuropathy following the pathological accumulation of NFs in peripheral processes. The *Nefl*$^{N98S/+}$ mouse model could be useful for testing potential treatments for hearing loss caused by the disruption of NF assembly.

**Keywords:** Neurofilament, Charcot-Marie-Tooth disease, hearing loss, auditory neuropathy

# P66

**Functions of NEDD4-2 in the auditory portion of mouse inner ear**

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Hearing loss is the most common neurosensory disorder with more than 466 million people affected worldwide. In most cases, hearing loss is caused by the dysfunction or the loss of the sensory cells (the hair cells) and/or their afferent neurons (spiral ganglion neurons) within the ventral portion of the inner ear, called the cochlea. To date, many genetic mutations have been associated with congenital deafness or early-onset hearing loss, allowing for the discovery of genes involved in the development or maintenance of the cochlea. Given the importance of protein ubiquitination in controlling a variety of important biological processes during development, together with a recent report identifying deafness-associated mutations in the human gene encoding the E3 Ubiquitin-ligase NEDD4L (Neurally Expressed and Developmentally Downregulated 4-like), we plan to uncover its implication in cochlear development and function.
We first characterized the spatio-temporal expression profile of Nedd4-2, the mouse ortholog of Nedd4L, together with the closely related Nedd4-1 gene. In situ hybridization experiments allowed us to confirm the presence of Nedd4-1 and Nedd4-2 transcripts during the embryonic stages of cochlear development. More specifically, both genes are expressed in the sensory epithelium, comprising the hair cells, and in the spiral ganglion. After birth, Nedd4-1 expression persists in these tissues while Nedd4-2 transcripts were undetectable. Next, we generated conditional knockout animals (cKO), in which Nedd4-2 gene is specifically invalidated in the otic vesicle at embryonic day 8.5 (E8.5), in order to decipher its role during mouse cochlear

development. Histological analyses, Scanning Electron Microscopy and specific immunostainings indicate that Nedd4-2 cKO cochleae develop normally during embryonic and early postnatal stages. However, similar analyses performed at postnatal days 30, 45, 60 and 90 (P30, P45, P60 and P90) suggest an early degeneration of the hair cells and their innervating spiral ganglion neurons. Interestingly, cochlear cell loss in Nedd4-2 cKO animals follows a gradient from the basal to the apical turn of the spiraled cochlea, similarly to what occurs in age-related or sound-induced hearing loss. At P90, Nedd4-2 cKO mice are likely to be profoundly deaf as we do not observe any hair cells remaining in the cochlear epithelium. Altogether, our results suggest a major contribution of the ubiquitin ligase NEDD4-2 in the maintenance of the cochlea integrity. In future experiments, we will combine the use of transgenic mouse models, gain- and loss-of function experiments in tissue cultures and biochemical assays to uncover the specific substrates of Nedd4-2 involved in cochlea preservation. These findings may provide future therapeutic perspectives against hearing loss.

# P67

**The creation of LOXHD1b gene knockout zebrafish**

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**Background**

Deafness caused by LOXHD1 gene mutation is clinically autosomal recessive inheritance and progressive. Hereditary families caused by LOXHD1 gene mutation has been also reported in Japan. Reported by Grillet et al.as a causative gene of hereditary hearing loss, the LOXHD1 gene is considered to be the causative gene of DFNB77 and expressed in hair cells in mouse. We used zebrafish as an experimental animal. In zebrafish, hundreds of eggs are produced at once, and eggs and embryos are transparent and can be easily observed after spawning. Genetic modification technology can be easily introduced. zebrafish was selected as an experimental animal because zebrafish is useful in conducting screening of medicines such as therapeutic drugs in the future. We verified that the LOXHD1b gene was expressed in hair cell bundles of zebrafish by in situ hybridization. We report that we created the LOXHD1b gene knockout zebrafish by using the CRISPR-Cas9 system and studied the morphology and physiological function of stereocilia of hair cells of LOXHD1b gene knockout zebrafish.

**Method**

We injected Cas9 mRNA and LOXHD1b mRNA into the zebrafish eggs of the first cell phase and the second cell phase using the CRISPR - Cas9 system. We crossed the injected F0 generation zebrafish and wild type zebrafish, and grew the F1 generation zebrafish. We cut the tail of the F1 generation zebrafish, recovered the genomic DNA, and performed genotyping with Heteroduplex Mobility Assay (HMA). A hetero mutant pattern was observed by electrophoresis. In the nucleotide sequence, deletion of the base on the N terminal side of the PAM sequence was observed. As a result of mating heterozygous mutants, electrophoretic patterns of homozygous mutants were obtained.

**Result**

Immunohistochemistry for anti paralbumin antibody was performed on zebrafish at 1 day post fertilization. In the wild type, the stereocilia of hair cells of the lateral line are normal, whereas in the homozygote the stereocilia of hair cells of the lateral line are shortened and decreased. For behavior, when we looked at the water tank from the side, the individuals rotating in part of the homozygote zebrafishes and those sinking from the head were observed.

**Prospect**

Detailed morphological observation of stereocilia of hair cells in LOXHD1b homozygote will be carried out using electron microscope. In addition to the LOXHD1b gene, Knockout of LOXHD1a gene is also being prepared, and these gene mutation zebrafish Establishment of the human hearing disorder model used is expected to be useful for screening future therapeutic agents.

# P68

**Association of polymorphisms in genes encoding uncoupling protein 1, 2 and protein kinase C-eta with the risk of tinnitus in community-dwelling middle-aged to elderly Japanese**

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Tinnitus is the perception of sound which has no external source. Risk factors of tinnitus include hearing loss, aging, sound exposure, smoking, anxiety, depression and ischemic cardiac disease. Although the etiologies of tinnitus remain unclear, genetic factors could contribute, at least in part. Thus, we investigated the association between genetic polymorphisms and tinnitus in the present study.

The subjects in the present analyses were derived from the National Institute for Longevity Sciences, Longitudinal Study of Aging (NILS-LSA), a population-based biennial survey between 1997 and 2012. Cumulative data from 1st to 7th wave (1997-2012) collected from 1964 participants (996 men, 968 women) aged 40-79 years old (average 59.1 years old) whose blood samples were taken to examine genetic polymorphisms in 1st wave from November 1997 to April 2000, were used for analysis. Among the 1964 participants, 676 had tinnitus and 1288 had no tinnitus in the baseline (1st wave). Genetic polymorphisms described below were investigated: interleukin 6 (*IL 6)* C-572G, *IL1A* -889C/T, *IL1B* -511C/T, uncoupling protein 1 *(UCP1)* A-3826G, *UCP2* Ala55Val, protein kinase C-eta *(PRKCH)* 1425G/A, endothelin 1 *(EDN1)* Lys198Asn (G/T), nitric oxide 3 *(NOS3)* G894T and *NOS3* 4a/4b. Generalized estimating equations were performed to evaluate the effect of each polymorphism on tinnitus after adjustment for age, sex, hearing loss, history of noise exposure, depression and smoking habits.

The odds ratio (OR) of *UCP1* A-3826G for the risk of tinnitus was 0.78 (CI: 0.65-0.94, p=0.01) in the recessive model. The OR of *UCP2* Ala55Val for the risk of tinnitus was 1.23 (CI: 1.03-1.47, p=0.02) in the dominant model. The OR of *PRKCH* 1425G/A for the risk of tinnitus was 1.56 (CI: 1.08-2.26, p=0.02) in the recessive model. No other polymorphisms showed a significant association. Our results imply that UCP1 A-3826G, UCP2 Ala55Val, PRKCH 1425G/A polymorphisms are associated with the risk of tinnitus in the middle-aged to elderly Japanese.

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# P69

**A novel splicing variant confirms COL11A1 as a cause of autosomal dominant non-syndromic hearing loss in the DFNA37 locus**

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Hereditary hearing loss is a clinically and genetically heterogeneous disorder. Over half of hearing loss has a genetic background and is inherited in an autosomal recessive pattern. Autosomal dominant non-syndromic hearing loss is the second most commonly inherited form and is present in roughly 20% of hearing impaired individuals. Many genes exhibiting either non-syndromic or syndromic hearing loss have also been identified as causing both forms of hearing loss, owing to the extensive phenotypic manifestations in the form of pleiotropy.
The gene *COL11A1* (collagen type XI alpha 1 chain) has long been associated with Marshall syndrome and Stickler syndrome type II, which are both inherited in an autosomal dominant pattern, as well as autosomal recessive fibrochondrogenesis. Each of these syndromes has phenotypic overlap that includes skeletal abnormalities and dysmorphic features, as well as variable cleft palate, ocular and auditory phenotypes that can include mild to moderate hearing loss and outer ear malformations. Recently, the gene *COL11A1* has

been associated with autosomal dominant non-syndromic hearing loss (DFNA37) through the genetic analysis of a large European-American family presenting a novel splice-site altering variant.
We ascertained a proband of a four generation family with non-syndromic hearing loss. The patient was diagnosed with moderate sensorineural hearing loss. Additional syndromic features were absent. The genomic DNA the proband was subjected to a custom-designed high-throughput sequencing panel consisting of 160 hearing loss-associated genes. Bioinformatics analysis disclosed a heterozygous variant (c.4338+2T>C, p.?, NM_080629.2) in the gene *COL11A1* that is predicted to abolish the 5' splice site in exon 58 out of the 68 exons comprising *COL11A1*. Furthermore, this novel variant affects a highly conserved nucleotide in the alpha chain domain. Functional testing of the variant using an in vitro splice assay confirmed abnormal splicing.
We report on the second DFNA37-associated splice-altering variant, providing confirmatory evidence of *COL11A1* as a *bona fide* autosomal dominant non-syndromic hearing loss gene.

**Keywords**: Autosomal dominant hearing loss, non-syndromic hearing loss, *COL11A1*, DFNA37

# P70

**Cyclin-dependent kinase 5 is essential for auditory function in mice**

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Cyclin-dependent kinase 5 (CDK5) is abundantly expressed in post-mitotic cells including neurons. It is involved in multiple cellular events, such as cytoskeletal dynamics, signaling cascades, gene expression, and cell survival, et al. Dysfunction of CDK5 has been associated with a number of neurological disorders. Here we show that CDK5 is expressed in mouse cochlea, and CDK5 inactivation in hair cells or spiral ganglion cells causes hearing loss in mice. In the cochlear hair cells, CDK5 inactivation has no effect on stereocilia development. However, it affects stereocilia maintenance, resulting in stereocilia disorganization and eventually stereocilia loss. Consistently, hair cell loss was significantly elevated by CDK5 inactivation. Despite that CDK5 has been shown to play important roles in synapse development and/or function, CDK5 inactivation does not affect the formation of ribbon synapses of cochlear hair cells. Further investigation showed that CDK5 inactivation causes reduced phosphorylation of ERM (ezrin, radixin, and moesin) proteins, which might contribute to the stereocilia deficits. Taken together, our present data suggest that CDK5 plays pivotal roles in auditory function through regulating the phosphorylation status of its target proteins in the cochlea..

**Key words:** inner ear, hair cells, spiral ganglion cells, hearing loss, CDK5

# P71

**THOC1 deficiency leads to late-onset nonsyndromic hearing loss through p53-mediated hair cell apoptosis**

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Apoptosis of cochlear hair cells is a key step towards age-related hearing loss. Although numerous genes have been implicated in the genetic causes of late-onset, progressive hearing loss, few show direct links to the proapoptotic process. By genome-wide linkage analysis and whole exome sequencing, we identified a heterozygous p.L183V variant in *THOC1* as the probable cause of the late-onset, progressive, non-syndromic hearing loss in a large dominant family. Thoc1, a member of the conserved multisubunit THO/TREX ribonucleoprotein complex, is highly expressed in mouse and zebrafish hair cells. The *Thoc1* mutant zebrafish generated by gRNA-Cas9 system lacks the C-startle response, indicative of the hearing dysfunction. Both *Thoc1* mutant and knockdown zebrafish have greatly reduced hair cell numbers, while the latter can be rescued by embryonic microinjection of human wild-type *THOC1* mRNA but to significantly lesser degree by the p.L183V mutant mRNA. The *Thoc1* deficiency resulted in marked apoptosis in zebrafish hair cells. Consistently, transcriptome sequencing of the mutants showed significantly increased gene expression in the p53-associated signaling pathway. Depletion of p53 or applying the p53 inhibitor Pifithrin-α

significantly rescued the hair cell loss in the *Thoc1* knockdown zebrafish. Our results suggested that *THOC1* deficiency lead to late-onset, progressive hearing loss through p53-mediated hair cell apoptosis. This is to our knowledge the first human disease associated with *THOC1* mutations and may shed light on the molecular mechanism underlying the age-related hearing loss.

# P72

**Epidemiologic and Genetic aspects of hearing loss in Algeria**

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**Introduction**
In Algeria, the prevalence of deafness in neonates is estimated to 3.2‰ in maternity, which makes this sensory deficiency a major public health.

**Aims**
Study of the epidemiologic, genetic and molecular aspects of cochlear implanted patients.

**Materials and methods**
It is a prospective study on 73 patients diagnosed with severe to profound hearing loss and fitted with a cochlear implant. They were, also, screened for the presence of *GJB2* mutations by direct sequencing of a single coding exon of *GJB2*. Patients without mutations were then screened for mutations in known HI genes.

**Results**
Over a period of three years, 73 patients aged between 2 to 7 years, originating from different enclaved regions were diagnosed with isolated deafness. All had severe to profound bilateral deafness and fitted with cochlear implant. During their follow up, they were screened for the presence of *GJB2* mutations. 66 patients had profound deafness and 7 had severe one.
*GJB2* mutations were found in 21 patients. The c.35delG, p.Gly12Valfs*2 mutation remains the most important mutation found in 17 patients. All detected patients were homozygous for this mutation. In addition, two other mutations (c.139G > T, p.Glu47* and c.167delT, p.Leu56Argfs*26) were found homozygous and two patients were compound heterozygotes for (c.35delG p.Gly12Valfs*2/c.139G > T, p.Glu47*). No deletion of GJB6 was detected.

**Conclusion**
The results of the study substantiate the genetic heterogeneity of deafness. Clinical and molecular screening of hearing impairment in newborns should be implemented to prevent such a disability in newborn with a progressive hearing loss, especially those affected by Usher syndrome, who will later suffer from sight loss.

**Keywords:** Genetic etiology, hearing impairment, cochlear implant

# P73

**From hearing screening into a systematic etiologic approach and follow-up of Paediatric Permanent Hearing Loss in Friuli-Venezia Giulia Region**

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Permanent hearing loss (PHL) of uncertain etiology ranges from 30% to 60% of identified cases. Most universal newborn hearing screening and surveillance programs lack a systematic etiologic focus.
The aim of this study was to obtain data on the diagnostic yield of a systematic etiologic approach within the activity of the newborn hearing screening and childhood hearing surveillance program in Friuli-Venezia Giulia Region, Italy.
During the period June 2012-July 2014, 18 656 newborns and 1139 at risk children aged 0-3 have been screened and evaluated within UNHS and CHS program of Friuli-Venezia Giulia region: 48 PHL (7 unilateral, 41 bilateral) have been identified and underwent a three-phases path of audiological and etiological investigation comprising: 1) complete audiological evaluation; 2) etiological and multidisciplinary diagnosis (first level investigation: ears, nose and throat examination, ophtalmology, TORCH sierology, echocardiography, GJB2/GJB6 mutation analysis and 2. level phenotype driven etiologic assessment (ie. imaging in case or asymmetric, progressive or severe to profound PHL; clinical genetic evaluation for non isolated PHL; other specialistic evaluations); 3) audiological revisitation after 3 years.
Results have been discussed by a multidisciplinary team and cases further classified into one of the following etiologic groups: PHL of exogenous origin (11); syndrome including PHL (12); GJB2/GJB6 biallelic PHL (5); putative non-syndromic genetic PHL (16); unilateral PHL of undefined origin (4). 12 of the 16 candidated subjects performed a next-generation targeted re-sequencing by Ion Torrent PGM (Life Technologies) to analyze 96 genes related to PHL and hearing function. In 30/48 cases hearing characteristics have been re-evaluated at the 3-years follow up to define audiometric characteristics in each etiological group.
Sistematic, multidisciplinary evaluations achieved to identify an etiologic diagnosis in 92% of examined bilateral PHL cases. A precise pathogenetic origin have been detected in 75% of them. The cross-match of audiologic, genetic and specialistic information provides the knowledge to plan a child-oriented treatment and rehabilitation. New strategies are required to guarantee ongoing follow-up in the complex and articulated path of audiological and etiological investigation of a neonate or an identified child.

**Keywords**: Infant permanent hearing loss, universal newborn hearing screening, etiologic diagnosis, next-generation targeted re-sequencing

# P74

## DFNB1: the experience of the Audiology Unit of the University Hospital of Padova

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### Introduction

Autosomal recessive deafness-1 (DFNB1) is caused by biallelic pathogenic variants in GJB2 and/or in its upstream cis-regulatory elements (usually deletions involving also GJB6). The aim of this study was to describe the phenotypic features of a cohort of patients with DFNB1 followed up in the Otolaryngology Unit of the University Hospital of Padova and to evaluate genotype-phenotype correlations.

### Material and methods

The study sample consisted of 201 pediatric patients with biallelic DFNB1-causing variants (108 males and 93 females, mean age of 11.6 ± 7.4 years at the last follow up evaluation). The sample was divided into 3 subgroups according to the presence of truncating (T) and non-truncating (NT) variants: Genotype T/T (78.6%), Genotype T/NT (18.4%), Genotype NT/NT (3.0%). For a better genotype-phenotype correlation, the recurrent truncating mutation 35delG was analyzed separately from the other truncating mutations (other-T), obtaining 5 different genotypes: 35delG/35delG (58.2%); 35delG/other-T (17.4%); other-T/other-T (3.0%); 35delG/NT (15.4%); other-T/NT (3.0%); NT/NT (3.0%).
The degree of hearing loss (HL) was considered mild if the $PTA_2$ in the better ear (average tonal threshold for the frequencies from 500 Hz to 4000 Hz) was between 21 and 40 dBnHL; moderate between 41 and 70 dBnHL; severe between 71 and 90 dBnHL and profound when > 90 dBnHL.

### Results

The hearing loss was stable (97.5%) and symmetric (92.5%) in the majority of cases. The distributions of patients according to the degree of hearing loss and the genotype (T/T, NT/NT and T/NT) were different ($p < 0.001$), with T/T patients showing the most severe phenotype. There was a decreasing trend in the rate of severe-profound HL from patients with T/T (93%) to those with T/NT (54%) to those with NT/NT (33.4%).

Among T/T patients, no cases with mild HL were observed and the 35delG/35delG genotype was associated with a more severe HL (87.2% profound HL and 10.3% severe HL) compared to the 35delG/other-T and the other-T/other-T genotypes (48.8% profound HL and 31.7% severe HL) ($p < 0.001$). The phenotype of patients carrying one or two NT variants depended on the specific type of genetic variation.

**Discussion**
There was a great phenotypic variability, not only between different families, but also within the same family; therefore, environmental factors and/or other genetic factors are likely involved in the expression of the disease.

**Key words**: DFNB1, GJB2, Genotype- Phenotype correlation

# P75

**Phenotype of 41 children affected by non-syndromic hearing loss with gene mutations detected by NGS analysis**

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**Introduction**
Non-syndromic hereditary hearing loss is characterized by extreme genetic heterogeneity and pathogenic variants in more than 100 genes have been described to date. The introduction of the next generation sequencing (NGS) technology in clinical practice has revolutionized the clinical/molecular diagnosis of HL, allowing the contemporary analysis of a large number of genes. The aim of this study was to characterize from the molecular point of view a sample of pediatric patients affected by non-DFNB1 non-syndromic hearing loss, to describe their phenotypic features, and to identify possible genotype-phenotype correlations.

**Material and methods**
The study sample consisted of 41 pediatric patients followed up in the Pediatric Audiology Clinic of the Otolaryngology Unit of the University Hospital of Padova, from January 2014 to May 2019. All the patients carried pathogenic or likely pathogenic variants in HL-associated genes detected by NGS analysis: the coding sequences of 62 genes associated with HL were captured using the HaloPlex Target Enrichment system (Agilent Technologies, Santa Clara, CA, USA) and sequenced on a MiSeq Dx instrument (Illumina, San Diego, CA, USA). Cases with biallelic DFNB1-causing variants were excluded.
The degree of hearing loss (HL) was considered mild if the $PTA_2$ in the better ear (average tonal threshold for the frequencies from 500 Hz to 4000 Hz) was between 21 and 40 dBnHL; moderate between 41 and 70 dBnHL; severe between 71 and 90 dBnHL and profound when > 90 dBnHL.

**Results**
In order of frequency, pathogenetic variants were found in: SLC26A4 (24.4%), STRC (9.8%), TECTA (9.8%), CDH23 and PCDH15 (9.8%), OTOG (7.3%), OTOA (7.3%), MYO15A (7.3%), genes on X chromosome (7.3%), LOXHD1 (4.9%), TMC1 (4.9%), TMPRSS3 (2.4%), COL11A2 (2.4%), MYH14 (2.4%). The phenotypic features were very variable and not always in agreement with the literature. Unlike previously published data, the hearing loss in our patients with OTOA variants was always progressive; the characteristic ski-sloping audiometric configuration associated with variants in STRC gene was evident only in one patient and we found two new mutation in TECTA associated with a hearing loss most pronounced in mid-high frequencies.

**Discussion**
The genetic heterogeneity of hearing loss underlies the importance of the analysis of multigene panels. The data in the literature about rare variants in genes associated with non-syndromic hearing loss vary among the studies, and further research with large samples is needed to detect genotype-phenotype correlations.

**Key words**: NGS, Deafness, Phenotype, Genetic heterogeneity

# P76

## Noise-Induced Hearing Loss among shipyard workers: Review of the literature

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**Objective**
Shipyard employees perform painting, electrical work, welding, plumbing, insulating, repairing and general contracting at ships. Regardless of the specialty, these laborers usually expose to metals, solvents, vibration and noise.

**Method**
A retrospective review of the scientific literature was performed in PubMed database, using MeSH terms such as [hearing loss]; [shipyard]; [noise-induced]; [employee]. The articles are identified by the present of the abstract, the full-text reading, the English language, and from the publication from January 1, 1988 to December 31, 2018.

**Results**
During 1988-2018 five follow-up surveys were identified. The sample size was between 78 to 757 workers while the follow-up period was from 1 to 12 years. The researchers studied whether impulse noise, simultaneous exposure to noise and vibration can cause or amplify sensory neural hearing loss (SNHL) among shipyard employees. The hearing threshold was estimated by audiometry and the noise level was measured by a miniature microphone outside and inside the earmuffs.
The impulsiveness of the noise was significantly high both outside and inside the earmuffs. The average SNHL was higher than predicted for the shipyard workers. The total exposure level inside the earmuffs was influenced by the total wearing time. The mean hearing level increased between 12 to 21.5 dB HL at 4 kHz. Specifically, among employees with past medical history of SNHL, the threshold change at 4 kHz was 3.2 dB HL statistically significant higher in SNHL ears. However, when workers were exposed to noise levels of 85 dBA and above, threshold change at 4 kHz was 5.6 dB HL statistically significant higher in SNHL ears. Among employees aged below 50, the threshold change values were lower in low-frequency (0.5-2 kHz) in SNHL ears, with a small range of changes, whereas in high-frequency (3-6 kHz), the range of changes was greater SNHL ears. Among workers aged 50 and above, SNHL ears showed a wider range of changes in both high- and low-frequency areas. Moreover, hearing handicap (HH) was 27.1%, with an 11.8% being over 5% according to Guide for the evaluation of HH by American Academy of Otolaryngology.

**Conclusions**
Noise-induced hearing loss typically affects the outer hair cells of the cochlea, in the basal portion of the basilar membrane, and decreases hearing between 2000-4000 Hz. The results of this study emphasize the burden of disease in the shipyard industry, and the need for continuous monitoring, implementation of preventive measures and hearing conservation programs.

**Keywords:** hearing loss; shipyard; noise-induced

# P77

**Peroxysome Proliferator Activated Receptors (PPARs): Effective Modulator of Cochlear Inflammatory Damages induced by Noise Induced Hearing Loss**

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Noise Induced Hearing Loss (NIHL) is the second most common cause of hearing loss after presbyacusis and there is still no curative treatment. NIHL leads to irreversible damage of both Hair Cells and Spiral Ganglion Neurons in the cochlea. Previous researches have provided the evidence of oxidative stress and more recently of inflammation as predominant mechanisms of cellular damages. Peroxysome Proliferator-Activated Receptors (PPARs) are a group of nuclear receptor proteins acting as transcription factors involved in the regulation of numerous biological processes including lipid and glucose metabolism, organ protection and inflammation. This work aims to investigate the potential role of PPARs in the cochlea during the inflammatory response following noise trauma. To proceed, we used a model of noise-induced hearing loss in Wistar rats. The animals were exposed to a pure tone noise (120dB, 10kHz, 1h). Following noise exposure, the inflammatory reaction were explored in the cochlea at different time schedules: 1,3,7,14 days after noise exposure. Moreover, Auditory Brain Responses (ABRs) were assessed for each time point to confirm the hearing loss model effectiveness. We found that the inflammatory response with rising of pro-inflammatory factors (IL1-□, TNF-□) and decreasing of anti-inflammatory cytokine IL-10 start day 1 after noise trauma with a peak of inflammation between day 3 and day 7 and a return to physiological condition at day 14 after noise trauma. Interestingly, as shown by immunohistochemical and Western blot analyses, the inflammatory reaction was associated to a decreased expression of both PPAR□ and PPAR□. This confirms the modulatory role of PPAR in cochlear inflammation after noise exposure possibly exerted by inhibition of NF□-B pathway. These findings contribute to better understand the profile of the inflammatory response following acoustic trauma and suggest the importance to maintain a physiological level of PPARs in cochlear cells to allow the modulation of inflammation and preventing damage of Hair Cells and Spiral Ganglion Neurons during NIHL.

**Key words**: Noise Induced Hearing Loss, Inflammation, Cochlea, Peroxysome Proliferator Activated Receptors

# P78

**Noise-induced hearing loss accelerates cognitive decline in a mouse model of Alzheimer's disease**

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Dementia and hearing loss are both highly prevalent neurologic conditions in older adults, each having considerable impact on the quality of life. A growing body of literature suggests that these two conditions are interrelated and that hearing loss may be a risk factor for the development of dementia in older adults. Though several epidemiological studies have demonstrated this association, the causal link of how hearing loss increases the risk of developing dementia is not well understood. Better understanding the etiology behind the connection between hearing loss and dementia could lead to interventions that preserve cognitive function in hearing loss patients. In this way, hearing loss could serve as a potential modifiable risk factor. Here we investigated the effects of noise exposure (pure tone, 100 dB, 10 kHz, 10 consecutive days) on memory performances and synaptic plasticity in the auditory cortex (AC) and hippocampus (HP) in a mouse model of Alzheimer's disease (AD) in order to evaluate if hearing loss may accelerate cognitive decline in AD. Transgenic mice developing a time-dependent AD-like phenotype (3×Tg-AD) were exposed to noise (100 dB, 60 min/day, 10 days) starting from 2 months of age and they were studied at 3, 6 and 9 months. Auditory brainstem response (ABR) recordings were used to evaluate auditory function. To analyze structural plasticity, we evaluated spine density in AC and HP neurons by using Golgi-Cox staining. Moreover, to investigate basal synaptic transmission, electrophysiological recordings were performed at layer 2/3 horizontal connections of AC brain slices. Novel object recognition (NOR) and open field (OF) tests were performed to evaluate recognition memory and locomotor activity, respectively. Noise exposure

induced a significant auditory threshold elevation in 3×Tg-AD mice at each time point studied thus negatively contributing to age-related hearing loss. Spine density was significantly altered both in AC and HP neurons, up to 2 months of age. At electrophysiological level, basal synaptic transmission at layer 2/3 horizontal connections was impaired in 3×Tg-AD mice exposed to noise compared to normal-hearing mice at all time points analyzed. At the behavioral level, cognitive decline was clearly manifested in 3×Tg-AD mice at 9 months of age, as revealed by NOR tests assessing short-term and long-term memory. Of note, noise-induced hearing loss anticipated cognitive deficits inducing memory alterations at 6 months of age. No changes of locomotor activity were observed in noise-exposed 3×Tg-AD mice compared to controls at any studied ages. Our findings provide novel evidence that hearing loss may accelerate cognitive decline in Alzheimer's disease and pave the way to further studies investigating the cross-talk between auditory cortex and brain areas directly involved in cognitive functions.

**Keywords**: aging, auditory cortex, cognition

# P79

**Acoustic Trauma Modulates the Cochlear Blood Flow and Vasoactive Factors in a Rodent Model of Noise-induced Hearing Loss**

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The inner ear vasculature is responsible for supplying substrates for metabolic functions, transporting systemic hormones for ion homeostasis and maintaining the endocochlear potential. Disrupted cochlear microcirculation has been considered an etiologic factor in noise-induced hearing loss (NIHL). Very few studies have identified how noise exposure alters microcirculation in the cochlea, to our knowledge, none have examined these alterations in different severity of noise trauma. In the present study, male 7-week-old C57BL/6 mice were exposed to noise trauma to induce the transient (TTS) or permanent hearing threshold shift (PTS). PTS group showed that significantly reduced the cochlear blood flow, decreased vessel diameter of the stria vascularis, and type IV fibrocytes in the spiral ligament as compared to TTS group, indicating that the cochlear blood flow and fibrocytes are differentially modulated depending on magnitude of acoustic trauma. PTS group showed a significant decrease in genes involved in vasodilation and an increase in gene involved in vasoconstriction as compared to TTS group. Moreover, PTS group also exerted higher expression levels of pro-inflammatory cytokines compared to TTS group. Collectively, these data suggest that cochlear blood flow is differently affected by the severity of noise exposure and these could be associated with the changes of vasoactive factors and inflammatory responses in the cochlea.

# P80

**Cochlear Glucocorticoid Receptor and Serum Corticosterone Expression in a Rodent Model of Noise-induced Hearing Loss: Comparison of Timing of Dexamethasone Administration**

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Glucocorticoid (GC) is a steroid hormone secreted from the adrenal cortex in response to stress, which acts by binding to cytoplasmic glucocorticoid receptors (GRs). Dexamethasone (DEX) is a synthetic GC exhibiting immunosuppressive effects in both human and rodent models of hearing loss. While clinical evidence has shown the effectiveness of DEX for treatment of various inner ear diseases, its mechanisms of action and the optimal timing of treatment are not well understood. In the present study, intergroup comparisons were conducted based on the time point of treatment with DEX: 1) pretreatment (pre-noise, −1 day and immediately before noise exposure); 2) posttreatment (post-noise, immediately after noise exposure and +1 day); and 3) pre&post-noise (−1 day and +1 day). The pre&post DEX treatment group showed a significant improvement in threshold shift compared to the pre and post groups. Both TTS and PTS significantly reduced cochlear GR mRNA expression and increased serum corticosterone and inflammatory cytokine levels in the cochlea. While TTS-treated animals showed preservation of inner and outer hair cells (OHCs), PTS induced significant loss of o OHCs. These data suggest that the timing of DEX administration may play a critical role in noise exposure/trauma treatment by decreasing GR and cytokine expression.

## P81

**Mesechnymal stem cell effects on the noise damaged inner ear: an RNAseq analysis of protective pathways**

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Mesenchymal stem cells (MSCs) are an adult derived stem cell population that have been shown to produce growth factors, modulate the immune system and can differentiate into a wide variety of tissue types. Within the inner ear, MSCs have been shown to modulate tissue damage induced by sound and a variety of ototoxins. To better understand the mechanism of action of these cells, mice were exposed to narrow band noise. After exposure MSCs derived from human umbilical cord were injected into the perilymph. Controls consisted of sound trauma only treated mice. Forty-eight hours post cell therapy total RNA was extracted and RNAseq performed to evaluated, the gene expression induced by cell therapy. A separate cohort of animals was treated in a similar fashion and allowed to survive for 2 weeks post cell therapy and hearing outcomes determined. Treatment with MSCs after sound trauma induced a moderate hearing protective effect. Activation of multiple protective pathways was identified in animals that received the cell therapy. This may aid in the identification of novel strategies to protect hearing.

# Ototoxicity

## P82

**Protective effect of ferulic acid against cisplatin-induced ototoxicity**

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**Objectives**
While cisplatin is an effective chemotherapeutic agent, it can cause irreversible hearing loss. Ototoxicity leads to dose reduction during the cisplatin chemotherapy and results in inadequate treatment of malignant tumors. This study aimed to investigate the protective effects of ferulic acid on cisplatin-induced ototoxicity.

**Methods**
House Ear Institute-Organ of Corti 1 (HEI-OC1) cells were exposed to 30 μM of cisplatin for 24 h with or without pretreatment with ferulic acid. Cell viability was determined using the WST assay. Apoptotic cells were identified using TUNEL assay. Western blot analysis was performed to examine the change in expression of cleaved caspase, cleaved poly-ADP-ribose polymerase (PARP), nuclear factor erythroid 2-related factor 2 (Nrf2), and catalase. Intracellular reactive oxygen species (ROS) were determined by flow cytometry. Real-time PCR analyses were performed to examine the mRNA levels of antioxidant enzymes including glutamate-cysteine ligase catalytic subunit (Gclc), glutathione peroxidase 2 (Gpx2), catalase, and superoxide dismutase 2 (SOD2). Phalloidin staining of the organ of Corti was performed to determine hair cell survival or degeneration.
**Results**
Pretreatment with ferulic acid before cisplatin exposure significantly increased cell viability, levels of antioxidant enzymes, and hair cell survival. In addition, pretreatment with ferulic acid significantly reduced apoptotic cells, levels of cleaved caspase, levels of cleaved PARP, and intracellular ROS production.
**Conclusion**
Our results demonstrated that ferulic acid inhibited cisplatin-induced cytotoxicity by preventing ROS formation and inducing the production of endogenous antioxidants and indicated that ferulic acid might be used as a protective agent against cisplatin-induced ototoxicity.

**Keywords**: Cisplatin; Ototoxicity; Cell death; Ferulic acid; Apoptosis

# P83

**Cisplatin-induced ototoxicity in rats is driven by RIP3-dependent necroptosis**

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Cisplatin-induced early-onset ototoxicity is linked to hearing loss. The mechanism by which cisplatin causes ototoxicity remains unclear. The purpose of this study was to identify the involvement of receptor-interacting protein kinase (RIP)3-dependent necroptosis in cisplatin-induced ototoxicity in vitro and in vivo. Sprague–Dawley rats (SD, 8 week) were treated via intraperitoneal (i.p) injection with cisplatin (16 mg/kg for 1 day), and their hearing thresholds were was measured by the auditory brainstem response (ABR) method. Hematoxylin and eosin (H&E) staining, immunohistochemistry, and western blots were performed to determine the effect of cisplatin-induced ototoxicity on cochlear morphology. Inhibitor experiments with necrostatin 1 (Nec-1) and Z-VAD were also performed in HEI-OC1 cell line. H&E stains revealed that the necroptotic changes were increased in the organ of Cortis (OCs) and spiral ganglion neurons (SGNs). Moreover, immunohistochemistry and western blot analysis showed that cisplatin treatment increased the protein levels of RIP3 in both OCs and SGNs. The treatment of Nec-1, a selective RIP1 inhibitor, resulted in markedly suppression of cisplatin-induced cell death in HEI-OC1 cells, whereas Z-VAD treatment did not change the cisplatin-induced cell death. Our results suggest that RIP3-dependent necroptosis was substantial in cisplatin-induced ototoxicity; inner cochlear regions, the OCs, and SGNs were especially sensitive to necroptosis.

**Keywords**: Necroptosis; Cisplatin; Ototoxicity; Organ of Corti; Spiral ganglion neuron

# P84

**Strong dose-dependence of hearing loss and general toxicity in a rat model of cisplatin-induced ototoxicity**

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Cisplatin is used to treat both adult and pediatric tumors and for children, cisplatin routinely forms the backbone of chemotherapy regimens. The incidence of cisplatin-induced hearing loss in the pediatric population varies substantially between reports, ranging from 2 to 90% depending on age, dose, evaluation scales, cohort size, and tumor type (van As JW et al, Cochrane Database Syst Rev, 2016), impacting language acquisition, general learning, cognitive dysfunction social-emotional development (Gurneyet al Pediatrics, 2007). Several cisplatin-induced ototoxicity paradigms have been developed in animal models using different dosing schedules and routes of administration. The use of a single administration of cisplatin offers ethical and logistical advantages, supported by data demonstrating that a single bolus administration was equally effective for inducing cochlear injuries as repeat infusions at lower doses (Harrison et al, Anticancer Drugs, 2016). We here explore the sensitivity of such a single dose regimen to the precise dose of cisplatin used, in terms of hearing loss and overall survival in a rat model.
Following baseline ABR (8/16/24/32 kHz) and DPOAE (4/8/16/24/32 kHz) recordings, groups of n=10 female 7-weeks old Wistar rats received a single intravenous infusion of cisplatin (8/9/10 mg in 20 mL/kg 0.9% NaCl) during 30 minutes under isoflurane anesthesia. Twice daily oral rehydration (10 mL/kg 0.9% NaCl) was performed for 14 days before final audiometry was performed for characterization of final hearing loss. Survival rate was characterized as a percentage of the number of originally included animals per group.
Survival rate of cisplatin infused animals showed a negative correlation with administered dose, with 100% survival on D15 for animals receiving the 8 mg/kg dose, 70% for the group receiving 9 mg/kg and 60% for the highest dose of 10 mg/kg. D15 ABR threshold shift showed a mean of 17 dB shift across all frequencies tested for the 8 mg/kg cisplatin group, with 8 dB shift at 8 kHz reaching a plateau around 21 dB from 16 kHz to 32 kHz. Animals receiving 9 mg/kg CDDP cisplatin infusion showed mean ABR threshold shifts of 21 dB across all frequencies, with a frequency dependent increase from 8 kHz to 32 kHz from respectively 9 to 34 dB. After 10 mg/kg cisplatin, ABR threshold shifts showed a general increase with a mean shift of 37 dB across frequencies with a loss of clear frequency dependence and a plateau from 24 kHz to 32 kHz with an average ABR threshold shift of 41 dB SPL, compared to respectively 31 and 35 dB SPL for 8 kHz and 16

kHz. DPOAE amplitude losses were consistent with the dose- and frequency dependence of the described ABR threshold shifts.
These data demonstrate a strong dose-dependence of both hearing loss and survival after cisplatin administration in rats, and reinforces the need for a highly controlled preparation and administration to maintain robust and reliable induction of ototoxicity, balanced with ethically acceptable survival rates and a clinically relevant hearing loss profile.

# P85

**Non-linear dose-dependence of tobramycin induced hearing loss in a rat model of aminoglycoside ototoxicity**

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Aminoglycoside antibiotics are used to treat life-threatening bacterial infections but can cause hearing loss due to cochlear hair cell death. While many efforts have been made to develop strategies for aminoglycoside ototoxicity prevention, no pharmaceutical treatment is currently approved. Recent clinical data suggest that a single course of intravenous aminoglycosides already induces hearing threshold shifts ~5 dB detectable using extended high-frequency audiometry in cystic fibrosis patients (Gleser & Zettner, Int J Audiol, 2018). With cumulative courses of aminoglycosides, hearing threshold shifts reach ~20 dB at higher frequencies and also extend into speech frequencies (Zettner & Gleser, Otolaryngology–Head and Neck Surgery, 2018). To facilitate implementation of robust and representative in vivo preclinical models of aminoglycoside induced ototoxicity, we here investigated the dose-dependence of hearing loss following tobramycin administration based on a previously published single-course paradigm in rats reported to yield ABR threshold shifts in the ~25-35 dB range at higher frequencies (Asplund et al, Acta Oto-Laryngologica, 2009).
Groups of n=10 male 6-weeks old Sprague-Dawley rats received once daily subcutaneous injections of either tobramycin sulfate (160/80/40 mg/kg, 3.2/1.6/0.8 mL/kg of Nebcine 50 mg/mL) or vehicle control (NaCl 0.9%) plus rehydration (0.3 mL NaCl 0.9%, 5% glucose i.p.) for 10 days after baseline ABR (8/16/24/32 kHz) and DPOAE (4/8/16/24/32 kHz) recordings. On day 28, audiometry was repeated for characterization of final hearing loss.
Over the course of the experiment, mean ABR threshold shift in the vehicle control group was 3.2 dB across frequencies (range of 1.9-4.7 dB) while mean DPOAE amplitudes increased by 1.9 dB across frequencies (range -1.2 to 5.4 dB). Groups receiving 10 daily administrations of 80 and 40 mg/kg of tobramycin sulfate showed no signs of ototoxicity compared to vehicle control: Mean 2.2 dB ABR threshold shifts (range -1.9 to 4.4 dB) and 0.9-1.1 dB DPOAE amplitude loss (range -3.0 to 3.8 dB). Conversely, the group receiving 160 mg/kg tobramycin sulfate for 10 days showed ABR threshold shifts ranging from 9.0-25.3 dB (mean of 16.8 dB), along with DPOAE amplitude changes from -15.9 to 2.7 dB (mean of -7.1 dB). Maximal effects on both ABR thresholds and DPOAE amplitudes were seen at 32 kHz, while ABR threshold shifts and DPOAE amplitude losses > 10 dB were already evident from 16 kHz.
Overall, this demonstrates tobramycin induced ototoxicity and resulting hearing loss effects on audiometric measures with non-linear dose-dependence in male Sprague-Dawley rats following 10 days of subcutaneous administration. Notably, for the tested doses, none resulted in hearing loss comparable with those reported for a single course of intravenous aminoglycosides in the clinical setting (Gleser & Zettner, Int J Audiol, 2018). At the highest dose tested, ABR threshold shifts exceeded those reported for 10+ courses of aminoglycosides for cystic fibrosis patients (Zettner & Gleser, Otolaryngology–Head and Neck Surgery, 2018). Further refinement of animal models will be required to reliably reproduce the clinical situation and provide a realistic test system for characterization of otoprotective drug candidates.

# P86

**Validation of a mouse model for otovestibular loss induced by allylnitrile**

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**Background**
Growing evidence indicates that loss of auditory and vestibular function negatively impacts on cognition. Dependable models for otovestibular loss in mice are a long-standing need to further correlate otovestibular status to cognition. One strategy to establish such models is through ototoxic agents, since they primarily

target the inner ear, causing permanent disability due to degeneration of sensory hair cells (HCs). Allylnitrile was long considered as an unsuitable ototoxic tool in mice due to its high associated mortality. However, recently, a viable allylnitrile intoxication mouse model has been established, paving the way for allylnitrile as specific ototoxic tool for induction of otovestibular loss. For hearing evaluation, it is imperative for animals to remain still. Temporary immobilization is often accomplished using the general anesthetics isoflurane or ketamine/xylazine. However, so far, only few studies have directly compared their effects on auditory sensitivity in mice.

**Objectives**
To validate a mouse model for otovestibular loss by functional and histological examination of cochleovestibular allylnitrile ototoxicity, and to directly compare anesthesia effects of isoflurane and ketamine/xylazine on hearing function in mice.

**Materials and Methods**
Adult male CBACa mice were randomly allocated into an allylnitrile (n=8) and a control group (n=6). After baseline audiovestibular measurements, mice were co-administered with a single-dose of allylnitrile peroral at 0 or 1.0 mmol/kg and, to reduce systemic toxicity, three injections of *trans*-1,2-dichloroethylene (TDCE) intraperitoneally at 0 or 100 mg/kg, 30 min before and 6 and 24 h after allylnitrile. Hearing loss was evaluated by auditory brainstem responses (ABRs), distortion product otoacoustic emissions (DPOAEs), and prepulse inhibition (PPI). Behavioural test batteries were used to assess vestibular dysfunction. After testing, temporal bones were dissected for evaluation of HC loss using confocal microscopy. For direct comparison of anesthesia effects on hearing function, baseline ABR and DPOAE measurements were performed in all mice (n=14) under isoflurane and ketamine/xylazine.

**Results**
Allylnitrile-treated mice lacked detectable ABR thresholds at each frequency tested (2 to 32 kHz), while DPOAE thresholds were significantly elevated in the low-frequency region of the cochlea (5 to 11 kHz) and completely lacking in the mid-to high frequency region (12 to 32 kHz). Behavioural evidence of allylnitrile-induced hearing loss was provided via PPI measurements, revealing significantly reduced PPI percentages at all prestimulus intensities. One-week post-dosing, increased vestibular dysfunction rating (VDR) scores to intermediate values (i.e. 11 to 15) were observed in three out of six allylnitrile-treated mice. Over time, however, VDRs of affected subjects progressively declined to values in the control range (<3). Gait analysis and stationary beam test performance confirmed the preserved vestibular function 11 weeks post-dosing. Hearing function of mice, as determined by ABRs and DPOAEs, was significantly poorer under isoflurane anesthesia relative to ketamine/xylazine anesthesia.
**Conclusion**
We present the allylnitrile intoxication CBACa mouse model as a new, reliable and nonsurgical approach for induction of isolated hearing loss. Despite all the advantages that make isoflurane an attractive option as general anesthetic in auditory research, caution should be taken due to its confounding influence on the auditory system.

**Keywords:** Allylnitrile – Ototoxicity – Anesthetics – Mouse model

# P87

**The prevalence of hearing loss among adult patients admitted to emergency intensive care unit**
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**Background**
Recent improvement of critical care decrease mortality rate, and it is important to prevent health problems that remain after critical illness. In intensive care unit (ICU), many drugs are used for treating the patients and some of the drugs have a potential to lead hearing loss. In neonatal intensive care unit (NICU), post NICU hearing impairments were well evaluated, but the change of hearing function after admission of ICU was not evaluated enough.

**Methods**
We recruited adult patients (20 years or older) who met following inclusion criteria;
- Admit to emergency ICU in Kurashiki Central Hospital during 2017 January to 2019 May
- Patients who had pure-tone audiogram within 5 years before the admission

- Patients who can have pure-tone audiogram after discharge of emergency ICU
- Patients who want to participate this research

Patients who met the inclusion criteria had pure-tone audiogram after discharge of emergency ICU. We compared pre- and post- emergency EICU audiogram. Pure-tone averages (PTAs) were calculated using 0.5-, 1-, 2-, and 4-kHz.

**Result**

During the study periods, we enrolled 17 patients (male 7, female = 10, mean age = 69.3 [range 41-85]). The reasons of admission were infection (n = 4), metabolic disease (n = 5), bleeding (n = 2), overdose (n = 1), trauma (n = 1), and others (n= 4). The mean days of ICU stay was 5.6 days (median 4; range 2-15).
The reasons of audiometry before ICU admission were mainly are-related hearing loss and tinnitus. We excluded one ear because the patient had functional hearing loss. We exclude one ear because the patients had audiometry before removal of earplug. Therefore we included 32 ears from 17 patients for analysis.
The change of pre- and post-PTAs was 7.8 dB. Of the 32 ears, 10 ears had hearing loss (an elevation ≥ 10 dB in air cnduction). No patients had new tinnutis after emergency ICU admission. Of the 10 ears withhearing los, the reason of one ear was conductive hearing loss (otitis media effusion), and others was sensorineural hearing loss.

**Conclusions**

The preliminary study revealed about 30 % of ears had hearing loss (an elevation ≥ 10 dB in air cnduction) after ICU admission. However, we recruit patients who admitted to emergency ICU and we can not include patients who admitted to cardiac critical care unit. Diuretic drugs are well known as ototoxic drugs and these drugs are often used for patients with cardiac disease. The incidence of exacerbation of hearing function would be higher in patients admitted to cardiac critical care unit.
It is important to recognize the ototoxicity of ICU admission and to find the risk factor of hearing loss.Updated data will be presented and discussed.

**Key words**: Hearing loss; Intensice care unit; Adults

# P88

## Alcohol-induced glycolysis inhibition contributes to cell death in mouse auditory cells

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Cellular metabolism is important for growth and survival of cells. Abusive drinking can cause metabolic dysfunction related to human diseases. Metabolic dysfunction has been linked to cell death in various cells. While metabolic dysfunction has been linked to cell death, the mechanisms by which alcohol-induced metabolic dysfunction regulates hearing loss remains unclear. Our results demonstrate that the inhibition of glucose metabolism contributes to alcohol-induced cell death in mouse auditory cells. Treatment of alcohol increased cell death in house ear institute-organ of corti-1 (HEI-OC1) cells. Moreover, treatment of alcohol suppressed the glycolysis, an important pathway in glucose metabolism, in HEI-OC1 cells. Furthermore, inhibition of glycolysis by 2-Deoxy-D-glucose (2DG), a specific inhibitor of glycolysis, significantly increased cell death in HEI-OC1 cells. These results suggest that alcohol-induced glycolysis inhibition contributes to cell death in mouse auditory cells.

**Key words**: Alcohol, Glycolysis, Cell death, Hearing loss

# P89

**Efficacy and Safety of Intratympanic Botulinum Toxin: A Rat Study**

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**Background**
Middle ear myoclonic tinnitus (MEMT) is an unusual condition caused by repetitive contractions of middle ear muscles (MEMs), i.e. tensor tympani and stapedius muscles. To date, management of MEMT is largely limited to supportive therapy and/or few pharmacological agents with uncertain efficacy. Tenotomy of MEMs could be a treatment option for intractable MEMT, but it is an invasive procedure under operating room setting. Therefore, intratympanic injection of botulinum toxin that may induce paralysis of MEMs could be an alternative treatment modality. This animal study was conducted to investigate safety of botulinum toxin to inner ear structures as well as to examine its effect on MEMs prior to clinical studies.

**Methods**
Male Sprague-Dawley rats aged 1 month were divided into 3 subgroups according to the sacrificing day after botulinum toxin injection: 3 days (3D), 1$^{st}$ week (1W), and 3$^{rd}$ week (3W). After initial hearing tests using ABR and DPOAE, one ear was randomly assigned as the experimental ear, the other, as the control ear. Experimental ears were intratympanically injected with 0.1 ml of botulinum toxin A, whereas control ears were injected with saline. Final hearing tests and collection of MEMs with cochleas were done according to the subgroup for analysis. Cochleas were evaluated for morphologic changes, and degenerations of MEMs were evaluated through electron microscopic study.

**Results**
There were no significant changes in hearing thresholds of all experimental ears in all 3 subgroups ($P$>0.05). Cochlear morphologic studies also did not show significant changes in the organ of Corti, spiral ganglion, or stria vasularis, indicating safety of botulinum toxin to inner ear structures. MEM sections obtained from the experimental ears showed some significant alterations in muscle ultrastructures compared to the control ears, such as loss of myofibrils and crystalysis in mitochondrias.

**Conclusion**
Intratympanic injection of botulinum toxin do not seems to have ototoxic effect on inner ear function. Muscular degenerations of tensor tympani and stapedius muscles were observed in electron microscopic analysis after intratympanic botulinum injection, suggesting its possible use as a MEM-inactivating substance. Further transitional clinical study may support the therapeutic use of intratympanic botulinum toxin for the management of MEMT.

# P90

**Interactions between the ribbon-synapses and a combination of electrical stimulation and dexamethasone**

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More and more patients with residual hearing are candidates for cochlear implantation. However, some of them experience late hearing loss post-implantation and the etiology is not well understood yet. The surgical trauma occurring during implantation is unlikely the reason for the observed hearing loss. In own studies, the influence of electrical stimulation on isolated spiral ganglion neurons and the ribbon synapses in organ of Corti preparations were investigated and both safe and unsafe charge densities were determined. Dexamethasone is known as an anti-inflammatory otoprotective substance as well as radical scavenger and thus has the potential for the protection of the residual hearing. The aim of the current study was to investigate possible changes in the organ of Corti induced by application of electrical stimulation and dexamethasone.
Explanted organs of Corti from rats (p2-4) were cultured for 24 h with two different concentrations of dexamethasone. Thereafter, some of the organs of Corti were subjected to a biphasic pulsed electrical stimulation (pulse amplitude 0.44 and 2.0 mA, pulse width 400 µs, interpulse delay 120 µs, repetition rate 1 kHz) for a further 24 h. Finally, the cytoskeleton, ribbon synapses and possible free reactive oxygen species were visualized by immunocytochemistry. For the assessment of an interaction with the current, the ribbon

synapses and the fluorescence signal of possible free reactive oxygen species (ROX) were quantified. In addition, a subjective assessment of the ciliary structure of the hair bundles was made. Organs of Corti without electrical stimulation served as control.
Compared with the non-stimulated and dexamethasone-treated references, the apical turn of the organ of Corti appeared to be more responsive to dexamethasone treatment than the basal one. It has also been shown that at the lower pulse intensity, the treatment may have a protective effect on the apical turn of the organ of Corti. Such screening studies may be of great relevance for future synaptic studies on the organ of Corti to identify other protective substances for the protection of cochlear cells under electrical stimulation.

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# P91

**Studying of the stria vascularis of the inner ear under exposed to ototoxic drugs**

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**Introduction**
Currently, there are many theories of the development of sensorineural hearing loss (SNHL). A number of authors believe that changes in the vascular strip play an essential role in the development of hearing loss. Ultrastructural changes in the vascular stripe of the inner ear with experimental hearing loss are little studied.

**Objective**
To study the ultrastructure of the stria vascularis of the guinea pig inner ear when exposed to kanamycin and furosemide.

**Materials and methods**
The study was conducted on 12 guinea pigs, which were divided into 2 groups (6 animals each): experimental group - in which animals received kanamycin and furosemide in an ototoxic dose and intact group, which did not receive any drugs. A dose of 900 mg/kg body weight kanamycin was given for 14 days by intra-peritoneal injection. Intra-peritoneal injections of furosemide at 50 mg/kg 20 min following the injection of kanamycin for 14 days. The auditory function was assessed using the Preyer reflex. After simulating SNHL using kanamycin and furosemide, in the ototoxic dose of animals, the cochlea of the inner ear was collected and a stria vascularis was isolated. Ultra-thin sections were made on an ultratome Reichert Supernova (Austria) and
analyzed in a JEM-100S transmission electron microscope (Japan).

**Results**
In animals treated with kanamycin and furosemide in the ototoxic dose in the marginal cells of the stria vascularis of the basal part of the cochlea were revealed a number of significant violations of the ultrastructure. The vast majority of cells contain single endoplasmic reticulum cisterns, a reduced number of ribosomes and polyribosomes, vacuolation mitochondria, expansion of the Golgi apparatus cisterns, and significant discharging of the cytoplasmic matrix of cells. Mitochondria with expansion of the lumen between the membranes of the cristae, which have the form of small bubbles, are found. In addition, a significant number of large vacuoles are found in the cytoplasm of many cells. There are single marginal cells with a violation of the integrity of the outer cytoplasmic membrane. In the marginal cells of the apical part of the cochlea, similar disorders with less pronounced nature were found. In the interstitial cells of the basal part of the cochlea, a significant depression of the cytoplasmic matrix and a decrease in the number of organoids were detected. In some processes, the matrix is completely lost. Ultrastructure abnormalities of the interstitial cells of the apical
part of the cochlea are similar to those found in the interstitial cells of the basal part.

**Conclusion**
Thus, in experimental hearing loss caused by kanamycin and furosemide, changes in the stria vascularis were detected, which were characterized by structural disorders in the marginal and interstitial cells of the stria vascularis. Ultrastructure abnormalities are most pronounced in the basal part of the cochlea.

# P92

**Ototoxic and neurotoxic effects of styrene exposure: implication of inflammatory processes**

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Styrene is an organic solvent used as a precursor for polystyrene plastics in many industries making plastics, resins, synthetic rubbers and fiberglass reinforced products. Our previous studies (Fetoni et al., 2016) demonstrated that the mechanism of styrene ototoxicity is based on a redox imbalance due to an increase of oxidative stress. Functional and morphological analysis demonstrated that styrene exposure lead to hearing loss due to oxidative stress and that the primary vulnerable target of solvent toxicity seems to be the supporting cells (Deiters' cells). The nonsensory supporting cells provide trophic and structural support for sensorineural cells and spiral ganglion neurons. For this reason, the damage of Deiters' cells by styrene may represent the hallmark of OHCs (outer hair cells) dysfunction and OHCs death may result from ototoxic effect of styrene on Deiters' cells, possibly by DNA adducts formation.
Based on these considerations, the aims of our study were to evaluate whether the oxidative stress induced by the exposure to styrene could trigger an inflammatory response in the organ of Corti and, moreover, to analyze the toxic effect of styrene administration on the auditory cortex, in order to establish whether the neurotoxic effect could be considered a direct effect or a consequence of cochlear deafferentation.
We performed functional analysis by measuring the Auditory Brainstem Responses (ABR) in order to assess auditory function. F-actin and H&E staining were performed to evaluate OHCs and spiral ganglion neurons survival, respectively. Analysis of inflammatory mediators (TNFα, IL-1β and NF-κB) were performed in the cochlea and auditory cortex by using Western Blot and immunofluorescence analysis. Moreover, morphological evaluations in auditory cortex and hippocampus were performed by evaluating spine density in pyramidal neurons of layer II/III and in the dentate gyrus, respectively.
Immunofluorescence and Western Blotting analysis demonstrated that styrene administration enhances the expression of TNFα, IL-1β and NFκB, as well as of the pro-apoptotic factor p53 in the cochlea.
Altogether, our results demonstrated that styrene exposure causes an inflammatory response on auditory system, involving both the cochlea and pyramidal neurons of auditory cortex, inducing structural changes as a decrease of spine density. We conclude that the effect of styrene is a direct effect rather than a consequence of cochlear deafferentation.

**Keywords**: ototoxicity, cochlear damage, inflammation

# P93

**Potential Gadolinium-induced Sensory Hair Cell Toxicity: In vivo assay using zebrafish**

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**Objectives**
To assess Gd (gadolinium)-induced sensory hair cell toxicity using zebrafish animal model and to evaluate potential underlying mechanisms.

**Materials and Methods**
Zebrafish embryos (AB type) were exposed to various concentrations of Gd (0.625, 1.25, 2.5, 10, and 20%) for 24 hours (6 to 8dpf). The concentration and duration of Gd administration were calculated based on the realistic range after intratympanic injection of frequently used Gd-based MRI contrast agent (Gd-DO3A-butrol, Gadovist™, Bayer AG, Leverkusen, Germany). The average number of hair cells on four neuRomests (SO1, SO2, O1 and OC1) were counted and analyzed (n=36-41 for each groups). A TUNEL assay was carried out to see the hair cell apoptosis (n=31 for normal control, 2.5, 10, 20% Gd concentrations; using Brn3c:GFP transgenic zebrafish). To see the potential neurotoxicity induced by Gd, HuC-GFP transgenic zebrafish was employed for normal control,2.5, 10, 20% Gd concentrations. The sensory neurons and its synapse with hair cells were assessed for SO1, SO2, O1 and OC1 neuRomests by fluorescent microscope (n=21-24) and the number of nerve degeneration was counted per zebrafish.

**Results**
The control group had a total of 47.18±2.30 hair cells and a decrease in the hair cell count was observed after Gd administration reaching 36.68±7.33 hair cells in 20% Gd-exposed group. A sudden decrease of the

hair cell counts was obvious in 20% Gd-exposed group (15.09±10.82, $p<0.01$, Turkey's HSD test). TUNEL assay revealed a prominent increase of apoptosis in 20% group (2.17±1.53, $p<0.01$, Turkey's HSD test) compared with normal control (0.06±0.17) or 10% group (1.07±0.85). Significant sensory nerve degeneration was confirmed in 20% administration group (11.5±8.3) with disconnected synapses (7.1±6.8) compared with intact neural connections in normal control group. ($p=0.04$; $p<0.02$, chi-squared test). The 2.5 and 10% Gd-exposed groups also exhibited nearly normal neural connections.

**Conclusion**
The Gd had a significant toxicity for sensory hair cells with increased apoptosis and neural degeneration in high concentration. However, there seems no significant damage for relatively low Gd concentrations, which corresponds to estimated inner ear concentrations after intratympanic Gd injection.

**Key Words:** Gadolinium, Hair Cell, Toxicity, Zebrafish

# P94

**Expression of advanced glycation end-product (AGEs) in the cultured utricles**

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**Introduction**
It is known that the prevalence of hearing loss is high in the patients with diabetes. In the last meeting, we presented that the model mice of metabolic syndrome showed the progressive hearing loss with an obesity and hyperlipidemia, high blood pressure. Advanced glycation end products (AGEs) are proteins or lipids that become glycated after exposure to sugars. AGEs are prevalent in the diabetic vasculature and contribute to the development of atherosclerosis. We have shown that the formation of AGEs in the inner ear plays an important role in the progressive hearing loss with diabetes. In the present study, we tried the formation of AGEs in the inner ear tissue with in vitro model.

**Materials and Methods**
Cultured utricles of CBA/N mice were used. The utricles were divided to 2 groups (Control group, High glucose group). In the high glucose group, utricles were cultured with glucose (60 mM). Five days after exposure to glucose, the cultured tissues were fixed with 4% paraformaldehyde. To evaluate the expression of AGEs, immunohistochemistry was performed using anti-AGEs antibody. In addition, the immunohistochemistry with anti-calmodulin antibody could label the hair cells. The signal intensity was evaluated with the fluorescence microscope.

**Results**
The signal intensity of immunohistochemistry against AGEs were stronger in the high glucose group than in the control group. The formation of AGEs was observed in the whole cultured utricles, not hair cell specific. The hair cell degeneration was not observed in both groups.

**Discussion**
The results suggested that the inner ear tissues exposed to high concentration glucose accumulate AGEs. This phenomenon has not been reported in the past. AGE is closely related to the tissue damage in the patients with diabetes. The relationship between inner ear damage and AGE formation have been unknown. However, I would like to clarify the role of AGE in inner ear disorders.

## P95

**Effects of the piezoelectric nanomaterial litium niobate on neuronal and inner ear cell lines**

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Nanoparticles and molecular aggregates with a diameter in the nanometer range, over the past few years, are object of interest and investigation in many research fields. Among which there is the bioengineering of the ear that exploits the nanoparticle properties for the development of less invasive and more efficient cochlear implants.

For this reason in the present work we tested the biocompatibility of lithium niobate, a piezoelectric nanomaterial, on two different cell lines: OC-k3 and PC12. The OC-k3 are sensorial cells deriving from the organ of Corti, while the PC12 are neuronal cells.

The results showed that lithium niobate does not influence OC-k3 cell viability up to 48h, while significantly increases cell proliferation after 72h. Further investigation showed no changes on cell morphology, cytochrome C cell distribution and ROS production. On the other hand it caused a significant decrease of cell viability and neurite outgrowth on PC12 cells, without influencing cell morphology.
These data showed that lithium niobate has a good biocompatibility on sensorial cells, but slightly affects neuronal viability and neurite lengthening.

Future studies should be directed to better investigate the significance of the results obtained on PC12 cells, since the alterations of cell viability and neurite development may be temporary or may be not mirror toxic effects. Therefore, up to now, we cannot exclude the hypothesis that lithium niobate nanoparticles may be used to develop a cochlear implant based on this piezoelectric nanomaterial.

This study was performed as part of the Italyn Health Ministry project “New self-powered devices for cochlear stimulation based on piezoelectric nanomaterials” (RF-2011-02350464).

# Otoprotection

## P96

**Screening Hair Cell Protection in Autophagy Library using the zebrafish lateral line**

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Zebrafish lateral line hair cells are physiologically and morphologically similar to inner ear hair cells. As the zebrafish lateral line is located on the body surface, damage to the hair cells can be rapidly assessed. Therefore, the zebrafish lateral line is an effective system for the evaluation of drugs that damage or protect hair cells. We have been used to screen protective effect of supplement and herbal medicines drugs, and damage effect of anti-cancer drugs.

Autophagy is one of the mechanisms of cells to break down intracellular proteins. It is a mechanism found in eukaryotic organisms from yeast to humans, preventing the accumulation of abnormal protein in the cell, recycling proteins when protein synthesis is excessive or when the nutrient environment deteriorates. It is involved in maintenance of homeostasis of living organisms by eliminating pathogenic microorganisms invaded into the cytoplasm. In addition, it is known to be involved in programmed cell death in the process of ontogeny, occurrence of diseases such as Huntington's disease, and inhibition of canceration of cells.

There will be a possibility that autophagy plays an important role in the inner ear. In this research, we have used the zebrafish lateral line to screen a library of 94 Autophagy drugs (Cancer Research Institute of Kanazawa University Drugs Set) that induce or inhibit autophagy for hair cell protection.

5 dpf Zebrafish embryos of the AB wild-type strain were produced by paired mating of adult fish. Zebrafish larvae were exposed to the drug library at concentrations of 0, 1, 10, 100, and 1000 mM for 1 h prior to neomycin exposure. After fixation in 4% paraformaldehyde, zebrafish were incubated with anti- parvalbumin antibody at 4℃ overnight. Hair cells from the SO1, SO2, O1, and OC1 neuRomests were counted. Ten fish per dose were counted. Results were calculated as the mean hair cell survival as a percentage of the control.

Screening identified some protective drugs against neomycin. Especially, Rapamycin, Timosaponin A-Ⅲ showed a strong protection, and both related to mTOR inhabitation.
This study suggested that autophagy play an important role in hair cells protection.

# P97

**A Role of the Guanylyl Cyclase GC-A in Auditory Function**

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**Background**
In the inner ear, the cGMP signaling pathway has been described to facilitate both protective and harmful processes in response to traumatic events. The aim of this study was to investigate the otoprotective role of the particulate cGMP generator guanylyl cyclase type A (GC-A) and its ligands atrial and B-type natriuretic peptides (ANP, BNP) for auditory function.

**Methods**
Transgenic mice knockout (KO) for GC-A were tested for hearing function and for auditory recovery from noise injury. We studied GC-A, ANP and BNP expression in the hearing organ, the inner ear (cochlea) with the organ of corti containing hair cells for mechano-electrical transduction of sound information. We determined the hearing function of the mice from auditory brainstem responses (ABRs) and distortion-product otoacoustic emissions (DPOAEs).

**Results**
Our results show that GC-A is expressed in outer hair cells (OHCs), while ANP and BNP are expressed in OHCs and sensory inner hair cells (IHCs). We analyzed the hearing function of GC-A KO animals before and after auditory trauma from loud sound exposure. In the mouse, GC-A deficiency leads to age related, progressive hearing impairment. Already at young age, GC-A deficiency increases the vulnerability of our hearing organ to loud sound exposure

**Conclusion**
A role of cGMP within the ANP and BNP/GC-A/cGMP signaling pathway in the inner ear is considered for age and noise induced hearing loss.

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# P98

**The PPARγ agonist pioglitazone ameliorates gentamicin ototoxicity by downregulating interferon and STAT pathways in cultured Organ of Corti explants**

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Pioglitazone is an oral antidiabetic drug that functions via activation of peroxisome proliferator-activated receptor gamma (PPAR-γ). Beyond controlling genes in lipid and glucose metabolism, PPAR-・agonists agonists have shown pleiotropic effects to reduce oxidative stress and inflammation in many tissues. We previously demonstrated that PPAR-γ is expressed in cochlear structures including inner and outer hair cells, supporting cells, the stria vascularis, spiral ganglion and cochlear nerve. We also showed that pioglitazone

was able to protect cochlear hair cells (HCs) from gentamicin ototoxicity by reducing ROS-induced HC death *in vitro* (Sekulic-Jablanovic M et al., 2017). Another recent report confirmed the relevance of these effects in an animal model of noise-induced hearing loss (Paciello F et al., 2018). In this study, investigators demonstrated that intratympanic pioglitazone promoted significant hearing recovery after noise exposure in rats. *In vivo*, pioglitazone reduced hair cell loss by blocking both the immediate increase in cochlear ROS as well as the intermediate activation of cochlear NF-KB.

In the current work, we explored the mechanisms of pioglitazone by performing RNASeq complemented by qPCR analysis in cultured mouse organ of Corti (OC). Organ of Corti explants were cultured in the presence of 10 µM pioglitazone, 50 µM gentamicin, or both, and total RNA was isolated after 6h and 24h of treatment. Pathway analysis by bioconductor package QuasR revealed that gentamicin treatment resulted in significant induction of the IFN-alpha, -gamma, and inflammatory pathways. Addition of pioglitazone was able to significantly reduce the gentamicin response, reflected by downregulation of multiple pro-inflammatory and stress response genes including *Ccl2, -9, -12, Cxcl10, Cxcl16, Gbp2* and *Il-16*. Multiple interferon-induced genes including *Isg15, Irf7, Irf3, Usp18, Ifi44* were also downregulated by pioglitazone. Irf7 protein undergoes nuclear translocation in order to mediate activation of interleukin-stimulated genes. By immunofluorescent staining of OC explants, we found that IRF7 was translocated to hair cell nuclei in gentamicin treated explants, but not in the combined treatment with pioglitazone, suggesting that pioglitazone prevents Irf7 activation. We also found protein inhibitor of activated STAT1 (PIAS1) to be significantly upregulated by pioglitazone treatment. PIAS1 regulates innate immune responses by controlling transcriptional induction by the Toll-like receptor (TLR) and JAK/STAT pathways. We found that *Tlr4* and *Tlr2* as well as the *MyD88* and *Mal* components of those pathways were modestly downregulated in the presence of pioglitazone.

These data show for the first time in cultured OCs that cytokine pathways induced by gentamicin are downregulated by the PPAR-gamma agonist pioglitazone. These results indicate that pioglitazone, aside from reducing oxidative stress levels, modulates cochlear immune/inflammatory pathways, revealing new aspect of its protective role against hearing damage.

**Keywords**: PPAR- agonist, Pioglitazone, Interferon pathway, Inflammatory pathway, Organ of Corti

# P99

**Preservation of auditory nerve peripheral processes by neurotrophic treatment in deafened guinea pigs**

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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. Over the last two decades the protective effect of neurotrophic treatment on the nerve has been well demonstrated. Clinically applicable methods have been developed to optimize possible treatment for CI patients and also for patients with synaptopathy. In particular for the latter group, preservation of peripheral processes is crucial. The largest focus in this field has been on the spiral ganglion cell soma (SGC). Here, we examine the survival of peripheral processes (PPs) relative to the survival of SGCs comparing various neurotrophic treatments in deafened guinea pigs.
Guinea pigs were ototoxically deafened two weeks prior to neurotrophic treatment. In one study, brain-derived neurotrophic factor (BDNF) was delivered to the cochlea by means of a mini-osmotic pump; delivery lasted four weeks, and the animals were sacrificed immediately, four weeks, or eight weeks after treatment cessation (Ramekers et al., J. Neurosci., 2015). In the second study, the animals received BDNF, neurotrophin-3 or a combination of these two by means of gelfoam placed on the round window, which is a clinically applicable method (Havenith et al., Otol. Neurotol., 2015). These animals were sacrificed four weeks after treatment onset. The cochleas of each animal were harvested and, following histological preparation, the PPs and SGCs were quantified and normalized to the normal hearing situation.
The different neurotrophic compounds delivered through gelfoam showed similar outcomes. Preservation of SGCs and PPs was observed in the basal cochlear turn, and the PP/SGC ratio was comparable to that found in normal-hearing animals. In contrast, delivery by osmotic pump yielded complete preservation of SGCs not only in the basal but also in the middle turn, and the PP/SGC ratio was lower than in normal-hearing controls.

Our data indicate that neurotrophins delivered by means of gelfoam provide preservation of SGCs in the basal turn with each SGC being intact with its PP. Therefore, we conclude that gelfoam-mediated delivery not only has the advantage of being a clinically attractive delivery method but it can also preserve the PPs to a higher ratio than the pump-mediated delivery.

**Keywords**: Spiral ganglion cell, Sensorineural hearing loss, Neurotrophic factor, Neuroprotection, Guinea pig

# P100

**Anti-inflammatory and oto-protective effect of the small heat shock protein alpha B-crystallin (HspB5) in experimental pneumococcal meningitis**

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** S.L.L. and M.R. contributed equally to this work.*

Sensorineural hearing loss is the most common long-term deficit after pneumococcal meningitis (PM), occurring in up to 30% of surviving patients. The infection and the following overshooting inflammatory host response damage the vulnerable sensory cells of the inner ear, resulting in loss of hair cells and spiral ganglion neurons, ultimately leading to elevated hearing thresholds.

Here, we tested the oto-protective properties of the small heat shock protein alpha B-crystallin (HspB5) with previously reported anti-inflammatory, anti-apoptotic and neuroprotective functions, in an experimental model of PM-induced hearing loss.

We analyzed the effect of local and systemic delivery of HspB5 in an infant rat model of PM where 11 day old Wistar rats were inoculated intracisternally with *S.pneumoniae* and treated with antibiotic therapy. Cytokine secretion profile, hearing thresholds and inner ear damage were assessed at predefined stages of the disease up to one month after infection. Further, we tested the HspB5 administration *in vitro*, using whole mount cultures of the organ of Corti.

Endogenous HspB5 was found to be expressed in numerous supporting cells of the organ of Corti and in Schwann cells of the spiral ganglion. Exogenous HspB5 protected outer hair cells from bacteria-induced cell death *in vitro. In vivo* PM was accompanied by elevated pro-inflammatory cytokine concentrations in the cerebrospinal fluid, leukocyte and neutrophil infiltration in the perilymphatic spaces of the cochlea with neutrophils extracellular trap formation during the acute phase of the disease. Elevated hearing thresholds were measured after recovery from meningitis. Intracisternal but not intraperitoneal administration of HspB5 significantly reduced the levels of TNF-α, IL-6 IFN-γ and IL-10 in the cerebrospinal fluid 24 hours post infection. This resulted in a greater outer hair cell survival, as well as improved hearing thresholds at later stages.

These results suggest that high local concentrations of HspB5 are needed to prevent inner ear damage in acute PM. HspB5 represents a promising therapeutic option to improve the auditory outcome and counteract hearing loss after PM.

**Keywords:** sensorineural hearing loss, *S.pneumoniae*, HspB5, inflammation, oto-protection

# P101

**Human mesenchymal adipose stem cell locally applied to prevent cochlear damages in rats treated with cisplatin**

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Chemotherapy based on cisplatin (cis-diaminedichloroplatinum II, CDDP) is the major therapy applied for many common solid tumors such as breast, lung, testis and ovarian cancer in adults, and neuroblastoma in pediatric patients. However, it is well known that ototoxicity is one of the main adverse effects caused by CDDP treatments. The hearing loss is due to damages to the cochlear sensorineural tissues triggered by oxidative and inflammatory stress leading to apoptosis. Several studies show the preventive effects against CDDP ototoxicity of different compounds applied systemically or locally in *in vitro* and *in vivo* models.
In a recent work, we demonstrated for the first time the preventive effect obtained by the local injection of mesenchymal stem cells derived from human adipose tissue (HMSC) applied near the round window, in rats treated with a cumulative CDDP dose of 14 mg/kg: the pretreatment with HMSC before exposure to CDDP significantly reduced hearing loss caused by the ototoxic compound, showing a lower histological damage of hair cells. A further investigation of the previous described preventive effects revealed that the injected stem cells localize nearby the round window, and the changes to the expression of proteins involved in the oxidative and inflammatory pathways.

## Pharmacology of Inner Ear

# P102

**Effect of Immunophilin Inhibitors on Fibroblasts and Spiral Ganglion Cells**

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Hearing disorders significantly affect the communication between people and often cause those affected to withdraw from society. In order to ensure the best possible care of patients with electrical inner ear prosthesis, a large number of vital spiral ganglion neurons (SGN) and a close nerve-electrode contact are necessary. Due to the loss of hearing also the SGN degenerate. In addition, after implantation of the electrode into the cochlea, connective tissue forms around the electrode which impairs signal transmission to the nerve cells of the auditory nerve. Involved in these inflammatory processes are cyclophilins, which can be inhibited by cyclosporin A (CsA). As CsA is also immunosuppressive the focus was put on immunophilin inhibitors, some CsA derivatives. These still contribute to the reduction of inflammatory processes but shall not suppress the immune response.

To investigate the effect of the immunophilin inhibitors MM284 and compound V20, these substances were tested for cytotoxicity on standard cell lines (NIH/3T3 fibroblasts) using MTT-Test and on freshly isolated ear-specific neurons (spiral ganglion neurons, SGN) and fibroblasts from neonatal rat (P3-5) cochleae. Based on the solubility in water up to 20 µM (Franke, 2018), the concentration range of the immunophilin inhibitors was selected from $10^{-4}$ M up to $10^{-12}$ M and the respective concentration added to the seeded SGN for 48 hours incubation at 37 °C, 5 % $CO_2$. The 3T3 fibroblast cell line and the freshly isolated primary fibroblasts were incubated 24 hours before adding the substances for 48 hours and performing MTT tests. SGN were cultured for 48 hours before immunocytochemical staining and evaluation of cell survival and neurite length. All experiments were repeated 6 times with n=3 within each experiment.

The metabolic cell activity of the 3T3 fibroblasts as well as of the primary fibroblasts is comparable to untreated controls for concentrations of $10^{-5}$ M and $10^{-6}$ M, respectively, and lower. The investigated immunophilin inhibitors can be considered as biocompatible with both cell lines within these concentration

ranges. The SGN show on average cell survival and neurite length of about 100% compared to controls at concentrations of $10^{-6}$ M and below with both immunophilin inhibitors. Therefore immunophilin inhibitors are considered suitable candidates for further studies on reduction of inflammatory reaction after cochlear implantation.

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# P103

**Hyperbaric oxygen with steroid and prostaglandin E1 for idiopathic sudden sensorineural hearing loss**

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**Objective**
To investigate the efficacy of the addition of hyperbaric oxygen therapy (HBOT) to systemic corticosteroid therapy and prostaglandin $E_1$ ($PGE_1$) therapy in patients with severe idiopathic sudden sensorineural hearing loss (ISSNHL).

**Methods**
We recruited 35 patients with ISSNHL who had been treated with systemic corticosteroid and $PGE_1$, either with HBOT (n=24) or without HBOT (n=11). Inclusion criteria were ISSNHL diagnosed within 14 days of onset, with severe hearing loss (arithmetic mean hearing of 250, 500, 1000, 2000, and 4000 Hz ≧ 60 dB), and clinical follow-up of at least 1 month. Patients' hearing level was evaluated 1 month after hearing loss onset. The main outcome was hearing improvement, assessed with pure tone audiometry. We also evaluated patients' sex, age, date of birth, affected side, the interval between onset and treatment, incidence of vertigo, presence of diabetes mellitus, and presence of hypertension.

**Results**
The hearing improvement for patients treated with systemic corticosteroid therapy and $PGE_1$ therapy combined with HBOT was significantly greater than for those without HBOT, especially at the low frequencies (125 Hz and 250 Hz).

**Conclusion**
These findings suggest that HBOT conferred a significant additional therapeutic benefit for severe ISSNHL when combined with systemic corticosteroid therapy and $PGE_1$ therapy, especially at the low frequencies.

**Keywords**: Idiopathic sudden sensorineural hearing loss, Hyperbaric oxygen therapy, Corticosteroid, Prostaglandin $E_1$

# P104

**Design and characterization of a human monoclonal antibody that selectively modulates Connexin hemichannels implicated in inherited hearing loss and skin disorders**

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Connexins (Cx) are a large family of membrane-spanning proteins, which can either form hexameric plasma membrane channels (named connexons or hemichannels), or form gap-junction intercellular channels by docking head-to-head with another connexon from an opposing cell. Mutations in the *GJB2* gene, which encodes for Connexin 26 (Cx26), have been identified as the major cause of inherited autosomal recessive sensorineural hearing impairment. A number of single point pathological mutations translate into gain-of-function hemichannels imparting them a 'leaky' character that causes syndromic deafness associated with devastating and even fatal skin disorders. Lack of non-toxic inhibitors with defined mechanisms of action and

specific selectivity for hemichannels poses a serious obstacle to therapeutic interventions for diseases caused by these mutant aberrant connexins. In this context, monoclonal antibodies developed in the last three decades, have become the most important class of therapeutic biological and a key tool in drug discovery. By screening combinatorial antibodies phage libraries using a bait peptide corresponding to 15 amino acids of Cx26 extrcellular loop 1 (EC1), we identified a human-derived monoclonal antibody, that we named abEC1.1 [1]. We characterized this antibody by means of a variety of biochemical and biophysical assays in HeLa DH cells, organotypic cultures of mouse cochlea, and human-derived skin cells. We found that abEC1.1 is able to inhibit both wild type and hyperactive hemichannels composed of human Cx26 subunits. We determined that abEC1.1 antibody is remarkably efficient, non-toxic, completely reversible inhibitor and does not affect direct cell-cell communications via gap junctions. Importantly, molecular modelling and dynamic simulation experiments allowed us to identify residues located on the extracellular domain of Cx26 hemichannels that are critical for binding, and are conserved also in Cx30 and Cx32. Patch clamp experiment performed in HeLa DH transfectants validated model predictions, confirming that the reversible inhibition efficiency of abEC1.1 towards Cx30 and Cx32 hemichannels was comparable to the one observed for Cx26. Of note, we further characterized abEC1.1 specificity, demonstrating that even a single amino acid difference in the putative binding region reduced drastically the inhibitory effect of the antibody on all the other tested hemichannels (as well as on another plasma membrane channels composed of Pannexin1 protein). Finally, we assessed abEC1.1 *in vitro* stability providing evidence that this antibody does not aggregate appreciably [2]. Although further studies will be necessary to validate the effects of the antibody *in vivo*, taken altogether these results highlight the potential of abEC1.1 antibody as an effective drug candidate to treat disorders caused by augmented connexin hemichannels activity. *In vivo* experiments with relevant animal models will be the key to test the therapeutic potential of this antibody.

**Key words**: Connexin hemichannels, hearing impairment, therapeutic monoclonal antibodies, binding specificity.

[1] Xu L, *et al*., Front Mol Neurosci, 10 (2017) 298.
[2] Ziraldo G, *et al*., Frontiers in Physiology, 10 (2019) 392.

## 14. Physiopathology of Auditory Pathways

# P105

**"Wave I amplitude": an invaluable index for the assessment of hidden hearing loss**

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**Background**
Common causes of sensorineural hearing loss are often associated with hair cell damage or loss. Recent studies in animal models suggest a much more insidious process. Small rodents exposed to even a moderate level of noise have normal cochlear outer hair cells but damaged cochlear synapses. This so-called synapthopathy or “hidden hearing loss”. The electrocochleography (Ecog) has been proven an effective diagnostic method for detecting cochlear synaptopathy. Indeed, the decreases in SP/AP or wave-I amplitude have been attributed to the loss of the cochlear synapses. Thus, it seem unavoidable to be efforts to develop clinically available measures and also to normalize SP/AP or wave-I amplitude. Indeed, the usual method involves an invasive transtympanic needle insertion, actually it is possible to record non invasive Ecog with an electrode placed closed to the tympanic membrane or with a gold foil Tiptrode electrode placed in the ear canal.

**Material and methods**
To get quality Ecog recordings in patients, the goal of this study is to evaluated in normal-hearing adult volunteers (both sex, aged between 18 to 25 years) the impact (on SP/AP or wave-I morphology) (1) type of electrode (cup or Tiptrode electrode) (2) electrode position (vertical or horizontal montage) (3) sound pressure level of the acoustic stimulation in the ear canal (4) type of acoustic stimulation (clic vs tone burst).

**Conclusion**
After this first stage of normalization, our research project will be to assess -with this protocol- Ecog recordings among people with normal hearing but who complain difficulty understanding speech in noisy environments.

# P106

**In silico examination of hidden hearing loss**

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**Background**
In experimental and clinical settings, sound-evoked compound action potentials (CAP) of the auditory nerve are commonly used to detect a neuropathy (a loss of auditory nerve fibers – ANFs or their function). The contribution of ANFs to the CAP waveform is, however, difficult to directly address because the CAP reflects an ensemble of fibers spiking in synchrony.
**Methods**
In this study, we developed a computational model of the mouse cochlea to examine the relationship between ANF spiking (neurogram) and the CAP waveform. The model considers the main biophysical properties of the mouse cochlea, including the place frequency map, the fiber tuning curves, the number of ANFs per inner hair cell (IHC) all along the tonotopic axis, and the proportion of low-, medium- and high-spontaneous rate (SR) fibers per IHC.
**Results**
This *in silico* model allows us to simulate different scenarios of neuropathy (including noise exposure and aging) that are difficult to probe *in vivo*. Simulations show that the fiber loss can reach 80% of the total number of ANFs without any significant effect on the CAP threshold (less than 10 dB threshold shift). Although the CAP amplitude appears to be a more reliable index, the CAP amplitude fails to predict linearly the fiber loss especially in case of a specific deletion of high- or low-SR fibers.
**Conclusion**
In complement to experimental studies, the systematic use of *in silico* models will provide a better understanding of the neuronal disorders underlying auditory neuropathy phenotype with normal audiogram (hidden hearing loss).

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# P107

**Objective measures of listening effort in young children with cochlear implants**

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Hearing and language acquisition are some of the most important landmarks for the development of cortical organization. In congenitally impaired children, by enabling auditory input early in life, cochlear implants are thought to reduce significantly the overall effort to learn spoken language and offer an improvement of the quality of life (Sarant et al., 2014). Nonetheless, a substantial variability in their auditory and communicative outcomes is observed in children with cochlear implants (Litovsky & Gordon, 2016). Even in patients with very successful recovery, complex auditory environment provoke more listening effort, and consequently fatigue, compared to normally hearing population (Bess et al., 2014; see Ohlenforst et al., 2017 for a review). Consequently is possible a decrease in attention to speech (Houston & Bergeson, 2014). One possible way of approaching this issue is to establish auditory conditions that would be beneficial for children with hearing impairment. To achieve this, it is necessary to understand the scale and the types of listening effort, but also the effect of different types of hearing amplification on listening effort. To our knowledge, the present study is

the first to address both questions by measuring listening effort in young children with bilateral cochlear implants using an objective measure of pupil dilation. Children's pupil dilation is measured while they listen to music or speech stimuli in various auditory environments: no noise, signal to noise ratio (SNR) +10 dB, SNR 0 dB. To address the question of the impact of different types of hearing amplification on listening effort we also measure the levels of listening effort with only one implant active compared to both. During the presentation, we will present preliminary results obtained with 13 1.5 to 3-year-old children implanted bilaterally. Because speech in humans tends to elicit unintentional attention (Vouloumanos et al., 2001), we hypothesize that children with cochlear implants will experience the highest listening effort while listening to speech stimuli in highly noisy environment, possibly even more so if only one of the two implants is active. In any case, the results will shed additional light on the auditory environments experienced by children that benefit most from the recent technological advancement, and the possible effects it has on listening effort and subsequent fatigue.

# P108

**Normalization of the hearing threshold in a newborn with a congenital CMV infection: preterm birth or an effect of the therapy?**

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Cytomegalovirus (CMV) is the most frequent cause of congenital infection, affecting between 0.2% and 2.2 % of live births that is responsible for sequelae of varying severity. It is a major cause of hearing loss that may be present at birth or later. Given that CMV infection can have serious consequences for the unborn child, prenatal diagnosis is essential, as are the appropriate primary prevention methods. Our case study regards a patient with congenital CMV infection diagnosed at 22 w gestation with a nett improvement in auditory function after an antiviral treatment, to the point of reaching a normal level.

**Key Words**: congenital cytomegalovirus, hearing loss, prematurity, antiviral treatment

# P109

**Losartan prevents axonal sprouting and the decrease of inhibitory neurotransmitters in inferior colliculus after unilateral hearing loss**

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**Objective**

Several studies reported that the neuronal activity and neurotransmission in the inferior colliculus (IC) are altered following hearing loss. We hypothesized that axonal sprouting, which is promoted by TGF-β signaling, might be associated with the alteration of the excitatory and inhibitory neurotransmission in IC, and investigated whether the neurotransmission and axonal sprouting in IC are changed with losartan which blocks TGF-β signaling.

**Methods**

Rats were randomly divided into three groups: control group that underwent a sham operation, deaf group that underwent cochlea ablation on the left side and losartan group that underwent cochlear ablation on the left side and received losartan for 2 weeks. IC of the right side were harvested 1 or 2 weeks after surgery. Hearing level was estimated with auditory brainstem responses (ABRs). Western blotting was performed for NR2A, Calb, GaBAAa1, GAD67, GAP43, Synaptophysin, PDS95, ATRX, GDF10, Lingo1, IGF1, pSmad2/3.

**Results**
Baseline ABR thresholds before surgery ranged from 20 to 35 dB SPL. After cochlear ablation, ABR thresholds were higher than 80dB. Comparing control, deaf and losartan group at 2 weeks after surgery, deaf and losartan group had significantly lower levels of NR2A than control group. There was no significant difference between deaf and losartan groups. The expression of GABAAa1 and GAD67 showed significantly lower level in deaf group than control group. However, losartan group showed significantly higher level than deaf group. Additionally, GAP43 and p-Smad2/3 were significantly higher in deaf group than control group, and significantly decreased in losartan group than deaf group.

**Conclusion**
Losartan might prevent axonal sprouting in IC, following unilateral hearing loss by blocking TGF-β signaling. Axonal sprouting might be involved in the change of inhibitory neurotransmission after hearing loss.

# P110

**Zika Virus Effects on the Auditory System; The Puerto Rican Experience**
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ZIKA Virus altered the pediatric health panorama in Puerto Rico. From January 1,2016 up to July 2018 the PR Department of Health reported 40,460 cases of ZIKA. approximately 3,923 were pregnant women with laboratory evidence of ZIKA virus infection. Statistics also reported that some of the neonates born to the infected females have smaller head circumference even when they were not categorized clinically as microcephalic. ZIKA attacks "neural progenitor cells" which are present in the fetal brain prior to brain specialization. Babies exposed to ZIKA in utero are also at risk of birth defects including sensorineural hearing loss. Evidence points to brain calcifications, insufficient neurons or eight nerve damage. The American Academy of Audiology warns that infants who contracted the virus through infected mothers might have absent or poorly functional hearing at birth or that hearing loss could have a later onset. The PR Department of Health implemented a protocol following US CDC guidelines that comprise periodic re-evaluations during the first three years of age. The infants were assessed in other sensory as well as cognitive areas. The PR protocol and auditory findings in Puerto Rican infants born to ZIKA infected mothers will be described. We will also discuss our position regarding the need to follow not only the ones that were diagnosed with hearing loss but also babies without hearing loss to assess auditory development and rule-out auditory processing deficits.

# P111

**Distortion product otoacoustic emissions and cochlear impairment in Parkinson's disease**
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**Background**
Several studies have hypothesized a connection between Parkinson's disease (PD) and impairment in the auditory pathway. When studying pure tone audiometry (PTA) in cohorts of PD subjects, uncertainties emerged about different frequency range impairment and relation with disease severity and progress. Auditory impairment in PD has been linked to the degeneration of dopaminergic and nondopaminergic neurons located outside of the substantia nigra. Animal studies have demonstrated the presence of dopaminergic signaling in the auditory pathway, mainly in the lateral olivocochlear efferent system (LOC), related to suppression of cochlear nerve activity and implicated in noise-induced excitotoxicity. Moreover, previous studies have demonstrated alterations in distortion-product otoacoustic emissions (DPOAE) in PD patients, postulating outer hair cell (OHC) impairment, possibly due to medial olivocochlear efferent (MOC) involvement.

**Materials and Methods**
Hearing levels and OHC function were evaluated, by means of PTA and DPOAE, in a cohort of 86 PD patients (45 M; 41 F, mean age 65±18) compared to age- and gender-matched healthy controls (n=49, 26 M; 23 F, mean age 63±17). DPOAE spectra were recorded, with high frequency resolution and time-frequency analysis to unmix the distortion and reflection components. Ear-canal calibration of the forward pressure and of the OAE signal was performed. Statistical analysis using a multivariate linear model was performed: continuous variables (DPOAE amplitude and audiometric threshold) were treated as outcome, while clinical status (patient vs control) and DPOAE and PTA spectral frequency distribution were treated as predictors.

**Results**
PTA and DPOAE testing showed an impairment in auditory function in PD patients, with respect to healthy subjects.

**Discussion**
In our study cohort, PTA impairment encompassed frequency bands ranging from 1 to 8 kHz, indicating widespread hearing dysfunction, while previous works showed variability in the extent of frequency involvement, possibly due to smaller sample sizes. It has been speculated that LOC function is to modulate auditory nerve discharge, by facilitating or decreasing sound transmission according to the presence of harmful inputs (e.g. noise); in this scenario, dopaminergic activation seems to have a net inhibitory effect, as a depletion in dopamine levels has shown to decrease auditory function, possibly due to an increase in excitotoxicity. It is unclear whether such alterations may influence OHC function, as previous animal experiments involving DPOAE measurements showed conflicting results: several studies have hypothesized a mutual influence between the two efferent systems, either due to the presence of MOC-LOC synapses or to possible paracrine effects of dopamine on OHC. In this light, our findings are in accordance with previous works indicating DPOAE to be impaired in PD subjects.

**Conclusion**
Taken together, our findings further confirm evidences pointing at hearing loss being a non-motor feature of PD, with important implications, as non-motor symptoms appear to be pivotal in quality of life impairment. From a technical standpoint, the consistent correlation between audiological measurements in our dataset confirms DPOAE to represent a reliable, objective diagnostic tool for auditory dysfunctions.

**Keywords:** Parkinson's disease, DPOAE, Olivocochlear efferents, Dopamine

# P112

**Investigating OAEs and audiogram fine structure in the extended high-frequency region**
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**Introduction**
Several studies suggest that both noise induced hearing loss (NIHL) and age related hearing loss (ARHL) lead to more rapid hearing threshold level (HTL) shifts in the extended-high frequency (EHF) 10-18 kHz region than in the conventional frequency region (0.25 – 8 kHz). It has therefore been hypothesised that the EHF region can provide early signs of hearing damage, before the conventional frequencies.
Previous studies at conventional audiometric frequencies (≤8 kHz) have found that the presence of audiogram fine structure (AFS) is associated with normal HTL and normal transient evoked otoacoustic emissions (TEOAEs). The main aim of the present study is to investigate whether the relationships between HTL, otoacoustic emissions (OAEs) (TEOAEs and distortion product otoacoustic emissions (DPOAEs)), and audiogram fine-structure obtained in the EHF region are similar to those reported at conventional frequencies.

**Research Questions**
1. Do we see a reduction in the depth of the EHF audiogram fine structure with increasing EHF-HTLs and with a reduction in amplitude of EHF-OAEs?
2. Do we see an average spectral periodicity in the EHF audiogram fine structure that is consistent with predictions from cochlear mechanical theory?

**Method**
Hearing will be assessed in normal otological adults with measurable EHF-HTL. Hearing sensitivity will be assessed using EHF audiometry (10-18 kHz) and EHF-AFS (8-16 kHz), while cochlear function will be evaluated with EHF-TEOAEs, EHF-DPOAEs and spontaneous OAEs.

**Results**
Within subject design will be conducted to assess the correlation between EHF- measurements.
Results will be present the correlation between the EHF-HTL, TEOAEs, DPOAEs, and AFS at EHF hearing level. The average spectral periodicity of the EHF-AFS will also be presented.

# P113

**Type 2 diabetes and hearing loss, but also possible auditory neuropathy! A French study**

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**Background**
Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. T2DM is the most common form of DM, which accounts for 90% to 95% of all diabetic patients. T2DM is a serious and common chronic disease resulting from a complex inheritance-environment interaction along with other risk factors such as obesity and sedentary lifestyle. T2DM and its complications constitute a major worldwide public health problem. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Many aspects in overall health are affected by DM and the disease is also an important risk factor for the development of hearing loss.

**Material and methods**
Auditory function was measured through transient evoked otoacoustic emissions (TEOAE), distortion product otoacoustic emissions (DPOAE), tympanometry and acoustic reflexes, pure tone audiometry. The hearing complaints or difficulties was assessed with a simple questionnaire Results were compared to normative data and correlated regarding age, presence of nephropathy, retinopathy, peripheral neuropathy scores, number of hypoglicemies and glycosylated hemoglobin.

**Results**
A total of 166 patients with T2DM were included in the study (66 women vs 97 men, mean age 60 ±12 years (23-79y), disease duration 12 ± 10 years (0-42y)). 116 out of 166 patients (70%) report to have a hearing complaints and 58 patients (35%) have a subjective tinnitus. 57 out of 166 patients (35%) report to have been exposed to noise exposure during the work and leisure activities. The otoacoustic emissions responses were considered abnormal in 60% patients. The acoustic reflex was present in 57%.The hearing threshold were compared to the statistical distribution of hearing thresholds related to age and gender (ISO 7029:2017)

**Conclusion**
Audiometric analysis showed that type 2 diabetic patients present an increased prevalence of hearing loss in relation to normative values according to age. Results indicate that probable auditory neuropathy (abolition of the stapedial reflex) and cochlear pathology (reduced/absent OAE) may coexist in this population.

**Keywords,** type 2 diabetes mellitus, audiological evaluation, cochleopathy, auditory neuropathy

# P114

**Type 1 diabetes and the risk of incident hearing loss : A French study**

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**Background**

Diabetes mellitus (DM) is characterized by a metabolic disorder in which there is a state of hyperglycemia. While type 2 DM (T2DM) is a result of insulin resistance, type 1 DM (T1DM) is mainly due to a lack of insulin caused by the destruction of beta cells in the pancreas by an autoimmune process. T1DM manifests at earlier ages than T2DM and therefore its complications tend to present at young adulthood.

**Material and methods**

Auditory function was measured through transient evoked otoacoustic emissions (TEOAE), distortion product otoacoustic emissions (DPOAE), tympanometry and acoustic reflexes, pure tone audiometry. The hearing complaints or difficulties was assessed with a simple questionnaire Results were compared to normative data and correlated regarding age, presence of nephropathy, retinopathy, peripheral neuropathy scores, number of hypoglicemies and glycosylated hemoglobin.

**Results**

A total of 114 patients with T1DM were included in the study (73 women vs 41 men, mean age 46 ±16 years (18-79y), disease duration 18 ±13 years (0-60y)). 68 out of 114 patients (60%) report to have a hearing complaints and 17 patients (15%) have a subjective tinnitus. 43 out of 114 patients (38%) report to have been exposed to noise exposure during the work and leisure activities. The otoacoustic emissions responses were considered abnormal in 35/114 (31%) patients. The acoustic reflex was present in 84%.The hearing threshold were compared to the statistical distribution of hearing thresholds related to age and gender (ISO 7029:2017).

**Conclusion**

The results indicate a reduced cochlear function in T1DM. The underestimation of hearing loss, while patients report hearing complaints, can be revealed using otoacoustic emissions. The recurrent hypoglycemia could be one of the hypothetical and observable consequences of diabetes on the hearing function.

**Keywords,** type 1 diabetes mellitus, audiological evaluation, cochleopathy,

# P115

**Is CD105-based assessment of neoangiogenesis in neurofibRometosis type 2 schwannoma predictor of tumor growth?**

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**Background**

NeurofibRometosis type 2 (NF2) is an autosomal dominant, multiple neoplasia syndrome.
Pathognomonic is the diagnoses of bilateral vestibular schwannomas (VSs), other neoplastic lesions include optic gliomas, meningiomas, and other central nervous system tumors such as ependymomas. Worldwide, NF2 incidence is 1 in 25000 population. Although benign, NF2-associated VSs might reveal rapid growth and affect hearing or facial nerve function. Deciding between conservative (observation) or proactive treatments in order to preserve the facial nerve or hearing function, or to rehabilitate deafness, and the exact timing for surgery are thus crucial issues in NF2-associated VSs. Endoglin is a proliferation-associated protein expressed in angiogenic endothelial cells.
The present study aims at investigating endoglin expression in a series of NF2-associated VSs, as compared with a group of sporadic VSs.

**Methods**
Through image analysis application, vessel cross-sectional area (AA) and density (VD) were calculated from CD105 expression in 7 NF2-associated VSs and 14 size-matched sporadic VSs (ratio 1:2).
Tumor size was calculated as the largest diameter of the extrameatal lesion on T1-weighted contrast-enhanced MRI (ceMRI) scan. When more than one ceMRI scan was available, tumor growth rate was calculated in mm/months with (tumor size at latest ceMRI - tumor size at first ceMRI)/time elapsing between the two images. All the lesions were diagnosed as schwannomas at final pathological examination.

**Results**
The NF2-associated VS group encountered four men and three women (mean age 23.3 ± 19.5 years). Three patients underwent VS removal through a retrosigmoid approach in attempt to preserve hearing; four patients were treated via a translabyrinthine approach. The sporadic VS group included 10 men and 4 women, (mean age 48.7 ± 13.3 years). They all underwent surgery via a translabyrinthine approach.
No significant differences were observed between NF2-associated VSs and sporadic cases in terms of AA ($p = .28$), or VD ($p = .39$). A positive correlation emerged between tumor growth rate (measured on ceMRI) and VD in the cohort of NF2-associated VSs ($rho = 0.95$, $p = .05$).

**Conclusions**
To the best of our knowledge, the present study is the first to have investigated CD105 expression in NF2-associated VSs. In the NF2-associated VSs cohort, a positive correlation was evidenced between tumor growth rate and vessel density ($p = .05$), meaning that endothelial CD105 expression probably relate to progression in NF2-associated VSs.
These preliminary results need further confirmation in larger series of NF2-associated VSs, preferably in multicenter studies, focusing on CD105-related issues. Further investigations are also advocated to ascertain the feasibility of both measuring circulating endoglin levels to monitor tumor growth rate and targeting tumor neoangiogenesis with anti-endoglin approaches in NF2-associated VS.

**Keywords**: vestibular schwannoma; neurofibRometosis type 2; CD105; endoglin; schwannoma growth rate

# P116

**A historical review of the terminology of Auditory Neuropathy Spectrum Disorders**

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**Objective**
Otoacoustic Emissions (OAEs) and Auditory Brainstem Responses (ABRs) reflect the cochlear outer hair cells function, and the neural response, retrospectively. Patients with an auditory pathway dysfunction may have normal OAEs, absent or abnormal ABRs and a paradoxical audiogram according to these tests.

**Method**
A historical retrospective review of the scientific literature was performed in PubMed database, using MeSH terms such as [auditory neuropathy]; [auditory dys-synchrony]; [auditory neuropathy spectrum disorders]. The articles are identified by the present of the abstract, the full-text reading, the English language, and from the publication date up to February 1, 2019.

**Results**
During 1981-1996, interesting cases with hearing impairment were reported by Worthington (4 cases), Krause (7 cases), Starr (1 case) and Rui (14 cases). These patients characterized by unmatched speech discrimination relative to audiogram thresholds and absent or abnormal ABR waves. In 1996, Starr et al. used the term “auditory neuropathy” (AN) to describe this pathology in a study of 8 patients. They identified as main cause of the disease the damage of afferent nerve fibers and total or partial demyelination in surviving nerve fibers. Moreover, between 1999 to 2000, Yasunaga et al. marked the mutation of the otoferlin (OTOF) gene, which results in synaptic dysfunction relating to the inner hair cell and the auditory nerve.
In 2001, Berlin et al. proposed the term “auditory dys-synchrony” (AD). They confirmed a timing deficit in neural synchrony that may result to a partial dys-synchrony of the auditory nerve fibers to an auditory stimuli. Furthermore, during 2003-2006, Rapin and Gravel recommend the term "nerve conduction disease" (NCD)

and supported that the afferent neural conduction in the auditory pathway was disordered. They identified as main causes the lack of temporal consistency in auditory brainstem response to series' of audible stimuli, the myelin disorders and other mechanisms which may not involve dys-synchrony.
In 2004, Starr et al. recommended segmenting the term AN into Type I or Pre-synaptic and Type II or Postsynaptic. In 2008, Starr et al. suggested refining the terminology by the site of disorder. Therefore, the appropriate term was "auditory nerve disorder" if the auditory nerve was affected while the inner hair cells and synapses were spared. In contrast, the term "auditory synaptic disorder" was described the cases with damaged inner hair cell synapses and normal auditory nerve function.
At the Internal Conference on Diagnosis and Intervention of Auditory Neuropathy in Italy in 2008, a guide line for differential diagnosis and intervention of AN in children was proposed, which named this class of diseases as "auditory neuropathy spectrum disorder" (ANSD).

**Conclusions**
ANSD is a term to describe hearing loss characterized by normal or near normal cochlear hair cell function and absent or abnormal auditory nerve function. Approximately 40% of ANSD cases are related to syndromic, non-syndromic, or mitochondrial genetic factors. Inheritance patterns include autosomal dominant, autosomal recessive, X-linked, and mitochondrial.

**Keywords:** auditory neuropathy; auditory dys-synchrony; auditory neuropathy spectrum disorders.

# P117

## Hearing loss and congenital cytomegalovirus infection: a retrospective study in a tertiary paediatric hospital

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Hearing loss is one of the most common congenital anomalies, occurring in 1- 3 per 1000 newborns in the well-infant population and 2-4 per 100 in an intensive care population.Congenital cytomegalovirus (cCMV) infection is currently estimated to be the main cause of non-inherited sensorineural hearing loss. The purpose of this study was to evaluate the prevalence of hearing loss in cases of cCMV infection from Modena county. Data were retrospectively collected from the laboratory database of the University Medical Hospital of Modena.
All children undergoing urine testing for suspected CMV infection or viral DNA testing on Guthrie Card in the period between January 2004 and December 2014 were enrolled in the study. The results showed an annual prevalence of cCMV infection among suspected cases that was stable over time despite the progressive increase in subjects tested.
The prevalence of hearing loss was in line with the literature, whereas in long-term follow-up cases of moderate, medium-to-severe hearing loss with late onset were not detected. Moreover, despite all the limitations of the study, we can conclude that European epidemiological studies are needed to better define the relationship between cCMV infection and internal ear disease as the impact of environmental and genetic factors is still not entirely clarified.

# P118

**The interest of using cartilage in the surgery of chronic otitis media**

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**Objective**

Demonstrate the interest of using cartilaginous autograft by quantitatively and qualitatively assessing of morpho-functional improvement indices.

**Materiel and methods**

Target population: patients with chronic otitis media (COM) eligible for a surgical cure, from western Algeria. Size of the population 86. Inclusion criteria: simple perforation, tympanic retraction and cholesteatoma. Exclusion Criteria: antecedents of radical mastoidectomy.
Type of study: prospective study. Duration of the study: 2015-2018 (03 years).
Assessment Criteria: One-year postoperative: Morphological (néotympanum membrane integrity) and functional (AAO HNS criteria).
Surgical Technique: Tympanoplasty with or without mastoid drilling with a retro-auricular or EAC approach depending on the type of COM. The removal of the cartilage was done either from the region of the tragus or the conchae according to the chosen approach. The cartilage was used in reconstruction of the tympanum, lyses of the ossicular chain and lysis of the tympanic frame.

**Results**

1. Sociodemographic characteristics:
   Average age 36.3 years. Sex ratio 1,05.
2. Clinical characteristics:
   The most common reasons for consultation are otorrhea and hearing loss (64, 57%). Types of COM: simple perforations (38.4%), retractions (32.5%) and cholesteatomas (29.1%). The preoperative ABG was 32 +/- 1.15 dB. Ossicular chain lesions were noted in 45.3% of the cases, the most common ossicle eroded was the incus (48.9%). Among the associated bone lesions, 17.4% of the cases had a lesion on the attic wall. The tympanic membrane was reconstructed in all patients, cartilage was removed from the conchae in 82.6% of cases. Chondrostapedopexy was performed in 33.7% of cases. A reconstruction of the attic wall was carried out in 13.95% of the cases.
3. Morpho-functional evaluation:
   Overall population (all types of COM): Good morphological results: 89.5%. Postoperative ABG average: 17.47dB. Simple perforations population: Good morphological results: 89.3%. Postoperative ABG average: 14.69 dB. Retractions population: Good morphological results 96.2%. Postoperative ABG average: 19.81dB. Cholesteatoma population: Good morphological results: 81,8%. Postoperative ABG average 18.24dB. Population with chondrostpadédoxy: Postoperative ABG average: 19,86dB. Good overall morpho-functional results for all types of COM: 69,2%.

**Conclusion**

Tympanoplasty is a technique widely practiced in otology. It uses a variety of reconstruction materials. The cartilaginous autograft has ideal mechanical and acoustic properties offering satisfactory morpho-functional results, with a wide range of use according to the lesions caused by COM that are not found in the different other materials that they are autologous or synthetic.

# P119

**Young ears at risk - factors associated with failed newborn hearing screening**

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Hearing is essential and provides spatial orientation and social communication. To detect deficient hearing at the earliest, newborns are screened. Thus, adequate measures as hearing aids or cochlear implants can be taken in time to provide the best outcome in hearing.
We report on 1409 consecutive newborns from the maternity ward of the university hospital Heidelberg. The screening was performed between day 3 and 10 with otoacoustic emissions (OAE) and auditory brainstem

response audiometry (ABR). The risk factors were noted.

| Factor | Odds-Ratio (OR) | p-value |
|---|---|---|
| Weight at births below 1500g | 5,77(2,97-11,22) | **<0,001** |
| Assisted breathing more than 5 days | 11,35(3,24-39,73) | **<0,001** |
| Pre-term-retinopathy | 5,89(2,11-16,45) | **0,001** |
| Male gender | 2,05(1,23-3,43) | **0,01** |
| Birth before 32nd gestational week | 3,02(1,38-6,62) | **0,01** |
| Furosemide therapy | 6,52(1,72-24,61) | **0,01** |
| Blood transfusion | 4,88(1,34-17,68) | **0,02** |
| Intensive care | 1,73(1,06-2,83) | **0,03** |
| Intrauterine infection | 3,41(1,15-10,15) | **0,03** |
| headcircumference > 90th percentile | 2,43(1,004-5,88) | **0,05** |

As expected, the share of deliveries associated with risk factors is higher in a university hospital. The factors „birth weight below 1500g“, „pre term retinopathy“ and „assisted breathing for more than 5 days“ were in this group linked to higher occurance of hearing deficits. The factors „birth before the 32nd week“ and „furosemide therapy“ had a tendency towards higher risk. Notably, the risk for male newborns was twice as high.

The necessity of newborn screening and the follow-up with tracking is shown in these data.

## Tinnitus

# P120

**Comparison of two behavioral methods to detect tinnitus on a rat salicylate-induced tinnitus model**

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Tinnitus is a common consequence of damage to the auditory pathway affecting around 600 million people, due in part to modern lifestyles (overmedication, exposure to noise…) and an aging population (Mohrle et al., 2018).
Currently, no preventive or curative treatment exists to treat tinnitus (Park, 2016).
One major difficulty is the reliable detection of tinnitus in animal models; therefore, the development of new methods has become a crucial step towards the discovery of innovative drugs. Among several existing paradigms (for review see Hayes et al., 2014), one reference is a conditioned food reward (CFR) behavioral test (Stolzberg et al., 2013). However, this takes several months to establish, as animals need to be trained. Another behavioral method with no preconditioning has been developed: the gap pre-pulse inhibition of the acoustic startle reflex (GPIAS) test. Although the GPIAS test has been published by several groups, it is still contested (Turner J.G. et al. 2008). The objective of this study is to compare the results obtained with the GPIAS test and the CFR test in a salicylate-induced tinnitus rat model.

Adult male Long Evans rats were injected intraperitoneally with salicylate at 300 mg/kg/day to detect the presence of transient tinnitus using these two behavorial tests.
The CFR test consisted of 3 months of training to teach the rats to perform a central nose poke to initiate a trial. Immediately afterwards, a sound is sent laterally (left or right); the rat needs to perform a nose poke on the correct side to receive food.
The GPIAS test, developed by Turner (2006), measures the intensity of the startle reflex to a brief sound with high intensity. If a background acoustic signal was qualitatively similar to the rat’s tinnitus, poorer detection of a silent gap would be expected. After 3 months of training for the CFR test, the animals were submitted to

two GPIAS acclimations, then the baseline measures were performed (GPIAS & CFR). The next day, 2 hours after a salicylate injection, rats were submitted to GPIAS & CFR tests to evaluate the presence of tinnitus.

Behavioral results with saline or salicylate showed that: for the CFR test, the proportion of correct responses to silence was decreased with salicylate (48±11 % vs 82± 6 % for control animals, p=0.038).
For the GPIAS test, salicylate induced a decrease of % gap inhibition (-17±7 % vs 50±23 in control conditions, p=0.0043). The individual analyses confirmed the difference between control and treated rats. On the contrary, one non-responder rat for the CFR test equally did not respond to the GPIAS.

In conclusion, significant differences between the control group and the salicylate group were observed for the CFR and GPIAS test, suggesting the possible detection of tinnitus after salicylate administration by both paradigms.

**Key words**: Tinnitus, Salicylate-induced tinnitus model, GPIAS, Behavior test,

# P121

**The association between tinnitus and stress evaluated using hair cortisol and self-report measures**

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A relationship between tinnitus and stress is frequently reported in scientific and patient literature, including that online. However, the direction of any relationship is not clear, and this topic is still under research. Studies of this relationship have so far not considered that stress can be conceptualised in different ways, and instead focuses one aspect of stress only. Most studies have used measurements of perceived stress, i.e. the interaction between a stressor, an individual's appraisal, and the resources available to overcome the effect of the stressor. These studies reported a correlations between self-reported tinnitus and perceived stress measurements [(1)]. Other studies measured stress biomarkers. Cortisol was the most commonly assessed biomarker and showed a blunted and delayed response comparing to non-tinnitus participants [(2)]. Although many patients associated the onset of tinnitus with stress, or reported stress worsened their tinnitus, measurements of stress as a stimulus (number and strength of events) has never been used in tinnitus research.
The relationship between tinnitus and stress was evaluated by assessing more than one concept of stress; as stimulus (number and severity of stressful events), perceived (self-reported measure), and response (cortisol level). We also compared stress levels between people with and without tinnitus, and assessed factors that are hypothesis to mediate any relationships.
Participants completed questionnaires including Depression, Anxiety and Stress Scale (DASS), Daily Hassles Scale (DHS), and Recent Life Changes Scale (RCL). Hair samples were collected to measure cortisol secretion over the previous 1-5 months (1 cm of hair representing 1 month's growth). Tinnitus was assessed using the Tinnitus Functional Index (TFI) and tinnitus tester (Pitch matching and loudness matching). As of June 2019, 32 of a required 92 participants have completed baseline assessment; a preliminary analysis will therefore be presented.

**Keywords:** Tinnitus, Stress, Cortisol

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# P122

**Axonal sprouting in the dorsal cochlear nucleus plays a role in the development of tinnitus**

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One of the primary theories of the pathogenesis of tinnitus involves maladaptive auditory-somatosensory plasticity in the dorsal cochlear nucleus (DCN), which is presumed to arise in a form of axonal sprouting. Some evidence suggests that a disrupted balance between auditory and somatosensory inputs occurs after hearing damage and may induce tinnitus. However, most studies investigating this phenomenon have employed a model of hearing damage in which the causal relationship between these changes and tinnitus is not accounted for. Thus, the present study investigated changes in auditory-somatosensory innervation and the role that axonal sprouting plays in this process by comparing findings between animals with and without tinnitus. Rats were exposed to a noise-inducing temporary threshold shift (TTS) and then divided into tinnitus and non-tinnitus groups based on the results of gap pre-pulse inhibition of acoustic startle (GPIAS) recordings. Subsequently, the DCNs were collected from rats categorized into three groups, based on the number of weeks (1, 2, or 3) after noise exposure and the protein levels of vesicular glutamate transporter 1 (VGLUT1), which is associated with auditory inputs to the DCN, and VGLUT2, which is primarily associated with somatosensory inputs, were assessed. Additionally, factors related to axonal sprouting, including growth-associated protein 43 (GAP43), postsynaptic density protein 95 (PSD95), synaptophysin, alpha-thalassemia/mental retardation syndrome X-linked homolog (ATRX), growth differentiation factor 10 (GDF10), and leucine-rich repeat and Ig domain containing 1 (Lingo1), were measured by Western blot analyses. Compared to the non-tinnitus group and the noise-unexposed control group, the tinnitus group exhibited a significant decrease in VGLUT1 at 1 week and a significant increase in VGLUT2 at 3 weeks. Additionally, the tinnitus group exhibited significant increases in GAP43, ATRX, and GDF10 levels in the DCN at 3 weeks after noise exposure. The present results provide further evidence that changes in the neural input distribution to the DCN can cause tinnitus and that axonal sprouting underlies these alterations.

# P123

**A genetic study for sensorinueral tinnitus based on genome-wide association & endophenotype study: Development of genetic diagnostic kit for precision medicine**

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**Background**
Tinnitus is the perception of sound that does not occur from external sources which is interferes with daily life by causing insomnia, loss of concentration, and depression. It is common condition affecting between 10 and 15% of the general population. Despite its high prevalence, the genetic research of the chronic primary tinnitus is still in its early stages.

**Objectives**
This study was performed to find genetic variation in patients with chronic sensorineural tinnitus using the genome-wide association study (GWAS) analysis method.

**Methods**
Among those who complained of subjective or objective tinnitus, patients diagnosed with chronic sensorineural tinnitus by an expert were registered in the experimental group. We genotyped blood of 111 cases of tinnitus patients and 133 controls with Korean Biobank Array, which was optimized for the Korean population.

**Results**
Out of more than 800,000 single nucleotide polymorphisms (SNP), 4 loci (rs1924089, rs1039239, rs11064191, and rs9682978) were identified as significantly associated with tinnitus ($P<10^{-5}$). Three of them were located in intergenic regions; one was located in intronic region of a coding gene, SORBS2.

**Conclusion:** We could not find any previous reports of the four SNPs we discovered with respect to tinnitus. Subsequent functional validation would be needed.

**Keywords**: GWAS (genome-wide association study); Tinnitus; genetics; precision medicine

# P124

**In vitro and in vivo attenuation of salicylate-induced tinnitus by memantine**

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Memantine, a noncompetitive antagonist of the *N*-methyl-D-aspartate (NMDA) receptor, suppresses the release of excessive levels of glutamate that may induce neuronal excitation. So far, there are only few reported studies, mainly behavioral studies, on the effects of memantine therapy on tinnitus. For a better understanding of the therapeutic effect of memantine on salicylate-induced tinnitus, observations at the cellular and auditory cortex levels would be ideal. The purpose of this study was to investigate the effects of memantine on the expression of the activity-regulated cytoskeleton-associated protein (*ARC*), early growth response 1 (*EGR-1*), c-Fos, and tumor necrosis factor-alpha (*TNFα*) genes; as well as the *NMDA receptor subunit 2B (NR2B)* gene and protein, in the SH-SY5Y cell line and in vivo. We also used gap-prepulse inhibition of the acoustic startle reflex (GPIAS) and noise burst prepulse inhibition of acoustic startle, and the auditory brainstem level (electrophysiological recordings of auditory brainstem responses, ABR) and NR2B expression level in the auditory cortex to evaluate whether memantine could reduce salicylate-mediated behavioral disturbances. *NR2B* was significantly upregulated in salicylate-treated cells, but downregulated after memantine treatment. Similarly, expression of the inflammatory cytokine genes *TNFα* and *NR2B* and immediate-early gene *ARC* was significantly increased in the salicylate-treated cells, and decreased when the cells were treated with memantine. These results were confirmed by NR2B immunocytochemistry. GPIAS was attenuated to a significantly lesser extent in rats treated with a combination of salicylate and memantine than in those treated with salicylate only. The mean ABR threshold in both groups was not significant different before and 1 day after the end of treatment. Additionally, NR2B protein expression in the auditory cortex was markedly increased in the salicylate-treated group, whereas it was reduced in the memantine-treated group. These results indicate that memantine is useful for the treatment of salicylate-induced tinnitus.

# P125

**Role of preoperative air-bone gap in tinnitus outcome after tympanoplasty for chronic otitis media with tinnitus**

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**Background & Objective**

Previous reports indicated that middle ear surgery might partially improve tinnitus after surgery. However, until now, no influencing factor has been determined for tinnitus outcome after middle ear surgery.The purpose of this study was to investigate the association between preoperative air-bone gap and tinnitus outcome after tympanoplasty type I.

**Materials and Methods**

Seventy-five patients with tinnitus who had more than 6 months of symptoms of chronic otitis media on the ipsilateral side that were refractory to medical treatment were included in this study. All patients were evaluated through otoendoscopy, pure tone/speech audiometer, questionnaire survey using the visual analog scale and the tinnitushandicap inventory for tinnitus symptoms before and 6 months after tympanoplasty. The influence of preoperative bone conduction, preoperative air-bone-gap, and postoperative air-bone-gap on tinnitus outcome after the operation was investigated.

**Results & Conclusions**

The patients were divided into two groups based on preoperative bone conduction of less than 25dB (n=50) or more than 25dB (n=25). The postoperative improvement of tinnitus in both groups showed statistical significance. Patients whose preoperative air-bone-gap was less than 15dB showed no improvement in

postoperative tinnitus using the visual analog scale (p=0.889) and the tinnitus handicap inventory (p=0.802). However, patients whose preoperative air-bone-gap was more than 15dB showed statistically significant improvement in postoperative tinnitus using the visual analog scale (p<0.01) and the tinnitus handicap inventory (p=0.016). Postoperative change in tinnitus showed significance compared with preoperative tinnitus using visual analog scale (p=0.006). However, the correlation between reduction in the visual analog scale score and air-bone-gap (p=0.202) or between reduction in tinnitus handicap inventory score and air-bone-gap (p=0.290) was not significant. We suggest that the preoperative air-bone-gap can be a predictor of tinnitus outcome after tympanoplasty in chronic otitis media with tinnitus.

# P126

## Relationship Between Tinnitus Loudness Measure by Visual Analogue Scale and Psychoacoustic Matching of Tinnitus Loudness

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### Introduction

Because tinnitus is mostly subjective, there are no clinical diagnostic tests to objectively measure the condition. Psychoacoustic measures are able to define the auditory attributes of tinnitus using the psychoacoustic method of matching its pitch and loudness. Although the clinical value of the psychoacoustic method of matching of tinnitus pitch and loudness is rather equivocal, it is still used as a counselling tool to assess tinnitus “numerically”. A patient’s own description rating of tinnitus loudness and annoyance — as measured on a Visual Analogue Scale (VAS) — is another possible approach to estimate either the patient’s perception (loudness) or their negative reaction to the problem (annoyance).

### Objective

As no such studies have previously addressed this topic, the objective of our study was to assess the relationship between psychoacoustically measured tinnitus loudness and tinnitus loudness measures with VAS in people with normal hearing and hearing loss.

### Material and methods

Study participants comprises of 140 adult patients (46.4% women, 53.6% men) aged from 19 to 81 years old who had had tinnitus for at least 6 months. The most frequent reported localization of their tinnitus sensation was bilateral (48.6%); 40% experienced unilateral tinnitus; and 11.4% heard tinnitus in the head. All participants were first asked to complete a VAS to indicate their tinnitus loudness. Hearing thresholds were then determined for each patient at frequencies from 0.125 to 8kHz; loudness and frequency of the tinnitus were also matched psychoacoustically.

### Results

Tinnitus loudness measured in dB SL was significantly lower in patients with bilateral hearing loss than in patients with unilateral hearing loss or in patients with normal hearing. Tinnitus loudness measured with VAS was significantly higher in patients with bilateral hearing loss than in patients with normal hearing. In patients with normal hearing there was a relationship between psychoacoustic matches of tinnitus loudness and tinnitus loudness measured with VAS, but this relationship did not hold for the hearing loss patients.

### Conclusions

The VAS scale for tinnitus loudness does not generally correspond to psychoacoustic measures of tinnitus loudness. Only in patients with normal hearing it could be somewhat indicative of tinnitus loudness measured psychoacoustically.

**Keywords:** tinnitus; visual analogue scales; psychoacoustics

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# P127

**Tinnitus severity in patients qualified for stapes surgery**

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**Introduction**

Otosclerosis is one of the most complex causes of progressive hearing loss in middle-aged adults. Apart from progressive hearing loss, tinnitus is one of the basic symptoms of otosclerosis. Current audiometric tests are poorly related to reported tinnitus severity.

**Objective**

The aim of the current study was to assess the prevalence and severity of tinnitus among a group of Polish otosclerosis patients who qualified for stapes surgery. In addition, the relationship between the air and bone conduction thresholds and tinnitus severity was evaluated.

**Material and methods**

This study included patients qualified for surgical treatment of otosclerosis in a single tertiary referral center. The main eligibility criteria were: age ≥ 18 years; preoperative audiological diagnosis indicative of otosclerosis (the air-bone gap > 10 dB in pure-tone audiometry test and no stapedial reflex in the impedance audiometry test); no previous stapes surgery in the ears; no contraindication to take part in a questionnaire study; signing an informed consent for participation in the study; complete documentation, including medical interview, preoperative pure-tone audiometry, and tinnitus questionnaire. The patients for whom otosclerosis was not confirmed intraoperatively were excluded. Patients who were diagnosed with tinnitus were asked to fill in the Tinnitus Functional Index (TFI). For statistical analysis, IBM SPSS Statistics v.24 software was used.

**Results**

Based on the medical interview, tinnitus was the first symptom of otosclerosis in 35% of the participants and 65% of all patients with otosclerosis experienced clinically significant, chronic tinnitus before stapes surgery. For 59% of patients, tinnitus was a significant or severe problem. The degree of hearing loss seemed to be marginally related to the severity of tinnitus reported by the patient.

**Conclusions:** Tinnitus is a common complaint among patients with otosclerosis, being a significant or severe problem for more than half of them. For this reason, it is worth considering in the future the implementation of standardized questionnaires for the assessment of tinnitus severity as a routine procedure in the diagnostic process of patients with otosclerosis, as well as in the postoperative period, which will be the next stage of our study.

**Keywords:** tinnitus; otosclerosis; questionnaires; stapes surgery

# P128

**Different recovery pattern between ocular vestibular evoked myogenic potentials and caloric tests in superior vestibular neuritis**

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**Introduction**
Superior vestibular nerve is more commonly involved in acute vestibular neuritis (VN). Caloric tests and ocular vestibular evoked myogenic potentials (O-VEMPs) are used both in diagnostic process, to identify the side and to give a topographic classification of involvement, and during the follow-up to verify the recovery of the vestibular function.

**Objectives**
To assess the recovery of vestibular function in acute superior vestibular neuritis (VN) at caloric tests and O-VEMPs. Additionally, we wish to evaluate if a correlation between the functional improvement and the patient's perception of the illness itself exists and to establish the role of the age as a discriminating factor for the perception of the recovery process.

**Methods**
41 cases of superior VN have been recruited between 2011 and 2016, at the Regional Vertigo Center of ENT clinic of the University Hospital of Padova. Anamnesis and DHI questionnaire were collected; pure-tone audiometry, a computerized videonystagmography (VNG) inclusive of bi-thermal stimulation of the ears, and air-conducted ocular vestibular evoked myogenic potentials (AO-VEMPs) were performed in all patients. Subsequently, patients have been re-evaluated using the same set of tests at a distance of at least 6 months.

**Results**
83% out of the patients exhibited a partial/complete recovery at caloric test, conversely only 33% exhibited a partial/complete recovery at AO-VEMPs. The mean DHI of the patient with a partial/complete recovery was significantly inferior than who didn't recovery at AO-VEMPs (1±1 versus 33±27, p=0,019), and it was significantly inferior in patients with partial/complete recovery at both caloric test and AO-VEMPs compared to partial/complete recovery at only caloric test (1±1 versus 26±27, p=0,038). The age of the patients doesn't seem to play a significant role in determining the recovery of the condition.

**Conclusions**
We found a discordance between the recovery pattern of ocular vestibular evoked myogenic potentials and caloric tests in the follow up of superior vestibular neuritis. We discussed the possible explanation of these findings underlying the different type of stimulation of the two tests (low and high frequency) on different vestibular neurons (tonic or phasic) and the possible role of the utricular function in patient's perception of recovery.

**Keywords**: superior vestibular neuritis, vestibular evoked myogenic potentials, caloric tests, utricular function, vestibular recovery

# P129

**Relationship of MR spectroscopy and cerebral gray matter volume in the compensation of vestibular neuritis patients**

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**Objective**
In compensation process after unilateral vestibular loss, the peripheral vestibular deficit alone does not seem to determine functional recovery, central compensation have been suggested to account for the variability of

clinical recovery in Vestibula neuritis(VN) from Gray matter volume(GMV) change and alteration of connectivity of the functional imaging study during compensation. But the mechanism of these structural change of brain during compensation period is not clear until today, So we performed this study to define the relationship between the alteration of Brain metabolite and structural change of Brain

**Method**
This study examined 3D T1 image of MRI for GMV change and MRspectroscopy(MRS) for taking brain metabolite in 15 normal controls and 27 unilateral Right handed VN patients (Right: 14, Left: 13), from 2016 to 2018. We analyzed GMV and MRS of the primary vestibular cortex.

**Results**
Right VN patients showed increased significantly ipsilateral glutamate(Glu) and glutamine(Gln) concentration when compared with those of controls. (p=0.012) They also showed increased contralateral Glu and Gln concentration rather than controls. (p=0.049). Their GMV of the ipsilesional vestibular cortex was increased than controls. On the other hand, Left VN patients didn't show statistical significance in ipsilateral Glu and Gln concentration when compared to control. But, there were significant increase in Glu concentration on the contralateral side. (p=0.017). We could not found any significant GMV change in Lt VN

**Conclusion**
We found the dominance of the right side vestibular cortex in the compensation of vestibular neuritis patients through GMV and MRS. Also, we could see the similarity between GMV changes and MRS metabolite concentration changes.

# P130

**A possible role for the video-HIT to detect semicircular canal involvement in BPPV with positional downbeating nystagmus**

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**Objective**
Positional downbeating nystagmus (PDN) is a relatively frequent finding in clinical practice. Though universally considered of central origin, thanks to the use of refined imaging techniques (brain MRI and high-resolution CT scans of temporal bones) and magnification of torsional patterns of nystagmus with video-oculography (VOG), nowadays PDN is mostly attributed to peripheral pathologies. Benign paroxysmal positional vertigo (BPPV) involving the non-ampullated arm of the posterior canal (PC) or the anterior canal (AC) have demonstrated to represent the most frequent condition. Torsional components of DPN and intensity reduction with visual fixation, though peculiar features of peripheral DPN, are not always clearly detectable. Therefore, differential diagnosis with central DPN and, in case of otolithic etiology, detection of the semicircular canal involved by BPPV (non-ampullated arm of PC versus contralateral AC) is frequently not easy. The aim of this case series, in accordance with the physiopathogenetic mechanism responsible for peripheral DPN, is to provide potential contributions to distinguish peripheral from central pathologies and, in case of otolithic involvement, to suggest useful clues to detect the canal affected.

**Materials and methods**
4 females (mean age 51.8 years, range age 43 – 58 years) presenting with abrupt onset of positional vertigo were enrolled. Three patients had an apogeotropic variant of PC-BPPV and one patient an AC-BPPV, three of them showing PDN whereas in 1 case a spontaneous downbeating nystagmus enhanced by positioning tests could be detected. VOG findings, VEMPs data and video-head impulse test (vHIT) measurements before and after canalith repositioning procedures (CRP) for vertical canals-BPPV were considered.

**Results**
All patients showed symmetrical cervical and ocular vestibular-evoked myogenic potentials (VEMPs) and selective deficit of the vestibulo-ocular reflex (VOR) gain for a single vertical canal at the vHIT. Each patient was treated with CRP for BPPV involving the hypofunctioning canal and post-treatment evaluation showed resolution of vestibular symptoms, receding of PDN and restitution of vertical canal function in all cases.

**Discussion**
In peripheral PDN, debris within a canal may alter endolymphatic fluids dynamics and cupular response

mechanisms resulting in a VOR gain reduction for the involved canal. This condition is likely to occur in apogeotropic forms of PC-BPPV in which otoliths floating within the non-ampullated arm of the PC could remain partially entrapped. This situation may account for an incomplete canalith jam, similarly to what has been already described for horizontal canals. The peculiar spatial orientation of the non-ampullated arm of PC and the distance between the otolith clot and the cupula could not lead, in most cases, to a continuous cupular displacement explaining the lack of spontaneous downbeating nystagmus in most patients.

**Conclusions**
PDN can be due to both peripheral and central vestibular pathologies. In case of otolithic genesis, involvement of the non-ampullated arm of PC is not easy to distinguish from contralateral AC involvement. In these cases, vHIT could play a key role in the differential diagnosis. Considering these preliminary results, we propose the use of the vHIT to detect semicircular canal involvement in case of BPPV with PDN.

# P131

**Effects of hypergravity on histamine receptor expression in vestibular nuclei of rats**

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**Background**
Humans without vestibular function never suffer from motion sickness, indicating that the vestibular system is essential for the development of motion sickness. Motion sickness occurs as a result of a mismatch or conflict between the information arising from the vestibular system, the visual and proprioceptive inputs. Many different environments can cause motion sickness including travelling on land, sea or space. H1 blockers are clinically effective in preventing motion sickness. Histaminergic neuron system is important in the developing of motion sickness.

**Objectives**
In this study, we studied the expression of histamine receptors and vestibule-histaminergic interaction during the hypergravity stimulation.

**Methods**
we use our gravity system named Inha G-simulator which is a centrifuge device for animal. With 4G hypergravity stimulation was exposed for 24hrs, 1week, 2weeks and 4weeks with SD male rats( aged 7~8weeks, weighing 250-300g). We checked the the vestibular function with animal rotator (VOR responses). And we did western blotting and immunohistochemistry analysis to quantify the protein expression of histamine receptors in medial and lateral vestibular nuclei.

**Results**
We analyzed the VOR responses after the hypergravity stimulation. Under 4G for 24hrs stimulation for 4weeks, the gain at 0.04, 0.08, 0.16, 0.32 HZ were 0.572±0.113, 0.638±0.095, 0.660±0.093, 0.756±0.198 respectively. They showed significant reduction on VOR gain compared to the control group. Decreased VOR gains were recovered to normal range on 3~4days after stopping the hypergravity stimulation. The expressions of histamine receptor (H1 & H2) were increased significantly compared to control group in vestibular nuclei after 4G 4weeks stimulation of hypergravity. Also 4weeks exposure group showed higher expression than 24hrs and 2weeks exposure group significantly in H1 receptor expression.

**Conclusion**
We can induce the results that hypergravity stimulation affect the vestibular function and modulation of histamine receptors expression in vestibular nuclei.

**Key Words** : space motion sickness, hypergravity, vestibular system

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# P132

**New proposal regarding mechanism of positional alcohol nystagmus**

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**Background and Objectives**

Positional alcohol nystagmus (PAN) is specific findings observed in acute alcohol intoxication, which is shows characteristic nystagmus findings with two phases. Initial phase exhibits a persistent geotropic positional nystagmus (PGPN) in a head-roll test occurring about 30 minutes after alcohol intake (PAN 1), followed by a quiescent period without nystagmus. Then, persistent ageotropic positional nystagmus (PAPN) occurs at approximately five to six hours after alcohol intake (PAN 2). Aschan et al. suggested that ethanol diffuses to cupula from capillaries faster than to endolymph resulting in “lighter cupula” than endolymph (PAN 1). However, the mechanism underlying PAN is still unclear. The aim of present study is to investigate the possibility of serum osmolality change by alcohol intake as a cause of PAN.

**Materials and Method**

Nine healthy adults without previous history of vertigo or normal caloric test were recruited voluntarily. Each volunteer drank 37.5% alcohol of 2.5ml/kg four times every 10 minutes. Positional nystagmus was checked before alcohol intake, and every 1 hr after the last alcohol intake until 7 hours. Serum osmolality was measured before alcohol intake, and 1 and 7 hours after the last alcohol intake.

**Results**

Prior to alcohol intake, the serum osmolality of all participants was 285.9 ± 4.4 mosm/kg. At 1 hour after drinking, all participants exhibited PGPN in a head-roll test, and serum osmolality increased to 302.9 ± 8.9 mosm/kg. At 6~7 hours after drinking, PAPN was observed in all participants, and serum osmolality decreased to 289.1 ± 9.4 mosm/kg.

**Conclusions**

Recently, it has been proposed that density difference between perilymph and endolymph provokes PGPN or PAPN, and alteration in serum osmolality may cause this density difference. The present study demonstrated that alcohol intake increased serum osmolality, which may serially increase osmolality of perilymph and then endolymph with a time lag, causing PGPN at the early stage (PAN 1). Over the time, blood alcohol is cleared, reducing serum osmolality, which may serially decrease osmolality of perilymph and then endolymph with a time lag, causing PAPN at the late stage (PAN 1). Although it has long been accepted that PAN is caused by relative difference in diffusion rate of alcohol between cupula and endolymph, the present study suggests that change in serum osmolality due to alcohol intake may be responsible for the PAN and alcohol-associated dizziness.

# P133

**Juvenile Ménière's disease and delayed endolymphatic hydrops**

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**Objectives**

Symptoms in Ménière's disease (MD) usually start in middle age, particularly between 30 and 50 years of age. Classical MD is rarely found in children and the associated literature is scarce. In general, the frequency of MD in children is only 0.4-7.0% of that in adults.

**Methods**

Here, we selected 7 cases of juvenile MD and 1 case of delayed endolymphatic hydrops (DEH) among 57 juvenile patients with vertigo who were referred to the neuro-otological clinic of Aichi Medical University Hospital during the period from January 2015 to December 2017. Ages varied between 13 and 18 years (mean, 14.6 years). The 8 patients comprised 1 male and 7 females. Diagnosis of MD was based on the criteria of the Japan Society for Equilibrium Research for Meniere's Disease.

**Results** Clinical features of juvenile MD patients were characterized as follows. Most MD patients complained of intensive frequent headache, ear fullness, nasal symptoms and sleep disorder except vertigo, especially were needed for treatment of headache.

Among the 7 MD patients with fluctuating hearing loss, 5 patients showed a positive response to the glycerol test. Based on this finding, benign paroxysmal vertigo (BPV) was distinguished from migraine-related dizziness and other pathologies.
In general, juvenile MD patients often complain of persistent headache, whereas dizziness and hearing loss in juvenile MD show good progress. Actually, all of our cases showed hearing improvement, but vertiginous attacks persisted for a long period in 2 cases and transtympanic ventilation was subsequently performed. The frequency of vertigo reduced in one case, but the remained unchanged in the other case.

**Conclusions**
In juvenile Ménière's disease, insomnia and refusal to attend school can occur due to dizziness, frequent headaches and other symptoms, so interventions such as child psychiatry and counseling may be necessary, depending on the case.

# P134

**Effect of intratympanic steroid injection in light cupula**

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**Objective**
To evaluate the effects of intratympanic steroid injection (ITS) in light cupula.

**Methods**
A total of 47 patients showing persistent geotropic direction-changing positional nystagmus with null point (light cupula) were randomly classified into three groups: ITS (N=15), vestibular suppressant (VS, N=16) and canalith repositioning procedure (CRP, N=16). Positional nystagmus and dizziness severity by dizziness handicap inventory (DHI) and visual analogue scale (VAS) were conducted before and 3 days and 1 week after first treatment to compare the effect of each treatment.

**Results**
DHI and VAS scores had decreased after each treatment; however, there were no differences among the three groups. A week after the first treatment, 7, 6 and 7 patients showed resolution of direction-changing positional nystagmus (DCPN) in the ITS, CRP and VS groups, respectively. There were no significant differences between the three groups. In the ITS group only, however, reversal of the stronger side on head roll test was observed in 6 patients, and 2 of them showed resolution of DCPN at the third day.

**Conclusions**
ITS was not effective for patients with light cupula at 1-week follow-up. However, some patients in the ITS group showed resolution of DCPN at earlier follow-up.

# P135

**Somatosensory, visual and vestibular sensory systems evaluation in a population of visually impaired patients**

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**Introduction**
It is well known that multiple sensory informations, namely visual, vestibular and somatosensory (mostly proprioceptive) are involved in a complex processes necessary to both spatial orientation and balance control. In the last 2 decades many moderate to strong evidences were provided about sensory interaction in postural control organization, demonstrating that failures in any of these systems could interfere with other systems, potentially producing a global postural control disturbance. However, although this multi-sensorial integration has long been hypothesized to play a crucial role in balance system organization, its nature still awaits a more accurate characterization. The aim of this study was to investigate the relationship between

vision and vestibular function, assessing a postural control ability/strategy in a population of normal and visual impaired patients.

**Methods**
A total of 30 patients were enrolled in our study. They were assigned to three different experimental groups. Control group (A): 10 patients ranging from 40 to 75 years of age with normal vision and no vestibular impairment. Group B: 10 patients (38-75 years of age), affected by maculopathies, characterized by a significant reduction in central visual acuity and a third group (C), consisting in 10 patients ranging from 38 to 82 years of age, affected by mixed, predominant peripheral low vision due to pathologies affecting the extrafoveal retina or the optic nerve in which the patient's capacity for spatial perception is altered. All patients underwent a complete ophthalmological and otoneurological assessment, consisting in a clinical vestibular evaluation standard otofunctional analyses and a Sensory Organization/Motor control test analysis through Neurocom Equitest hardware/software platform.

**Main Results**
In our observation, no patients reported the presence of vertigo. Equitest Postural Composite score analysis showed no statistically significant differences among groups (p=0,0618). SOT analyses indicated more complex results. No statistically significance was achieved by analysis of SOT1 (values of 88±4,01; 89,00± 3,40; 87,83±4,76, for groups A,B anc C respectively), SOT2 (83,2±2,83; 82,51±10,03; 82,34±3,96; groups A,B,C), SOT 3 and 6 (77,91±7,34; 76,70±9,47; 78,42±6,10; groups A,B,C). Condition 4 were significantly reduced in groups B and C as compared to CTRL (p=0,01). SOT5 was unaffected in group C, while a significant reduction was detected in group B (50,97±12; 67,98±8,02; group B and C respectively).

**Conclusion**
The literature provides numerous evidences about patients with vestibular disorders subdued to optokinetic stimulation, indicating that visual stimulation through repetitive movement of retinal images, could induce a vestibular responses adaptation leading to progressive postural control recovery, providing more strong evidences about the role of complex multi-sensory integration/interaction in postural control. Main findings of our study indicated that, as expected, visual sensory contribution was significantly reduced in all visual impaired patients as compared to ctrl, while only in central vision impaired patients, a significant reduction in vestibular sensory contribution was detected, suggesting that the visual defect distribution could play a key role in vestibular sensory contribution modulation.

# P136

**Regenerative Research for Vestibular Disorders**

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In our aging society, it is very important to keep healthy balance condition. However, the risk of falling gradually increases with age, because of various hypofunctions such as vestibular disorders. In birds, hair cells are known to regenerate after the damage of ototoxic drugs, while it is difficult to regenerate in mammals. Up to date, no curative therapy for vestibular disorders has been established yet. So, the development of new treatment is one of the most important themes in our aging society. Vestibular hair cells and vestibular ganglion cells are the main regions of peripheral vestibular damage. Both are related with age - related equilibrium disorders, as the number of those cells decrease with aging. Recently, cell transplantation therapy might be a promising tool for severe damage of inner ear. We reported that induced pluripotent stem cells (iPSCs) and human neural stem cells (hNSCs) can differentiate into hair cell and vestibular ganglion cell like cells respectively in vitro. So those cells have possibilities as donor cells for transplantation therapy of vestibular disorders. Next, we established animal models of vestibular disorders using ototoxic drugs. For the evaluation of vestibular function, we also examined the vestibulo ocular reflex (VOR) and observed the decrease of VOR gain after application of ototoxic drugs. At present, we are investigating the effectiveness of cell transplantation using animal model in vivo. Morphologically, we could confirm the survival of transplanted cells in normal mouse, but no differentiation into desired cells. We are confirming the functional effectiveness of cell transplantation for the damaged animal model. We have to examine what most suitable conditions are for both of donor and recipient conditions. Although lots of further investigations are required, cell transplantation might have a possibility as a new regenerative therapy for vestibular disorders.

# P137

**Computational simulation of the cupula behavior in vestibular pathologies of the inner ear**

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Vertigo is reported as one of the most common symptoms in the world, commonly related with vestibular disorders. It is considered the third most frequent complaint in medicine, transmitting a sense of inadequacy and insecurity, mainly in elders. The aim of this work is to contribute to a better understanding on how the vestibular system works, mainly during vestibular rehabilitation process. This knowledge will help in the development of new techniques that will facilitate a more efficient rehabilitation. Vestibular rehabilitation consists in a set of exercises, known as maneuvers, that can reduce and even eliminate the symptoms of dizziness and imbalance associated with a vestibular disorder.

The finite element method (FEM) is a computational tool that allow the development and simulation of biological structures such as the inner ear. The FEM aim is to find solutions for a complex problem, usually dividing the problem domain into small parts called elements, with the same mechanical properties; which allows the analysis of displacements, stresses, torsions or other measurements in the structure after the simulation with the defined conditions.
The complexity of the vestibular labyrinth structures is a challenge for the model development, additionally, the fluid-structure interaction due the endolymph inside the canals is one of the main concerns in the simulation process.

A three-dimensional computational model of the vestibular system, containing the bio-fluids that promote the body balance, as the endolymph, will be built using the smoothed-particle hydrodynamics (SPH) method to simulate the fluid behavior. In SPH the domain is discretized by particles possessing constant mass. The other vestibular components, as the semicircular canals structure, the cupulas in each canal and the otoconia debris, in the case of a pathological case, which simulate the benign paroxysmal positional vertigo disease, will be discretized using the finite element method. The components will be meshed accordingly and boundary conditions will be applied mesmerizing the inner ear environment. All the considered material properties for the vestibular system components including the endolymph were obtained from the literature.
After all the conditions defined it will be possible to obtain all the biomechanical results related with the model simulation. Using ABAQUS software the numerical model of the vestibular system will be analyzed. The final computational model will be built using images from Magnetic Resonance Imaging (MRI), that will help to obtain a more realistic model, which allow to get more accurate results.

The vestibular numerical model will be used to optimize the standard maneuvers, which will permit to reduce the number of maneuvers and modify the intensity of the movements involved, and consequently attenuate the unbalance symptoms. Additionally, it will be studied the influence of the otoconia migration in the cupula behavior.

**Keywords**: Biomechanics, Vestibular System, Computational, Finite Element Method

# P138

**W276S/W276S mutation in KCNQ4 causes vestibular dysfunction after acceleration stimulation via hair cell degeneration**

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**Objective**

KCNQ4 mutation causes autosomal dominant progressive hearing loss. A vestibular phenotype of this kind of mutation have not been clearly identified. This study was performed to investigate if a mutation of W276S/W276S in KCNQ4 causes vestibular dysfunction in animal model and human.

**Methods**
W276S/W276S KCNQ4 mutation mouse was created with CRSPR gene editing technique. For evaluating the contribution of KCNQ4 in maintaining vestibular function after excessive acceleration stimulation, acceleration challenges (4G and 6G for 24 hours) were applied to wild type, hetero, and homo mice. First, in the wild type mice, 60µl (5mg/ml conc) of retigabine, KCNQ4 activator, was injected i.p. before stimulation. After the challenges, vestibulo-ocular reflex was measured by anima rotator. The difference in the value of VOR gain, time constant, and mean slow phase velocity during off-vertical axis rotation between the mice with retigabine injection and without injection was compared. Second, the difference of VOR gain, time constant, and and mean slow phase velocity during off-vertical axis rotation among wild, hetero, and homo mice was compared. The Changes in the sensory epithelium were observed by confocal microscopy after immunostaining. The hair cell loss was calculated by FIJI. The human phenotype of vestibular dysfunction was investigated with video head impulse test and cVEMP.

**Results**
After the challenge, the wild type mice without retigabine injection showed lower gain and shorter time constant than those in wild type mice with retigabine injection (p=0.03). Homo mice showed significant decrease in the vestibular function (p = 0.001) at the parameters of gain in slow harmonic acceleration test from 0.04 to 064 Hz (mean 0.83 ± 0.1vs. 0.52 ± 0.03, for wild and homo p=0.01), time constant in step velocity test (2.2 ± 0.4 sec vs. 1.0 ± 0.5 sec for wild and homo, p=0.02) and slow phase velocity of nystagmus at off-vertical axis rotation (1.6 ± 0.7 vs. 1.0 ± 0.5, p=0.005). Immunohistochemistry showed significant hair cell loss in the ampullar of each semicircular canal. In human (n=4), gain of vHIT and cVEMP loss were observed in 50% of the patients with KCNQ4 mutation.

**Conclusion**
W276S/W276S KCNQ4 mutation can cause vestibular dysfunction, either after acute excessive acceleration stimulation or during daily activities.

# P139

**West Nile virus infection impairing hearing and balance**

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**Background**
West Nile virus (WNV) is a neurotropic mosquito-borne virus first isolated in humans in Uganda in 1937. WNV has spread in the last 20 years, becoming common also in Europe, the Middle East, North America, and East Asia, and causing disease outbreaks. In Italy, WNV is now considered endemic in several regions, and the infectious peak usually occurs in August and September. In most cases, human infection is asymptomatic. When clinically manifest, the spectrum of disease may range from a mild infection with flu-like symptoms to a debilitating illness with development of neuro-invasive disease. WNV-associated sensorineural hearing loss (SNHL), albeit rare, has been previously reported. Poor balance has been also described, but data regarding vestibular function in terms of clinical and instrumental analysis are lacking. We herein illustrate two cases of SNHL and balance impairment due to WNV infection.

**Method**
A 56-year-old and a 75-year-old male patients with very recent diagnosis of WNV infection came to our attention complaining hearing loss and balance disorders. They were investigated with pure tone audiometry, word recognition scores, auditory brainstem responses and, for the first time, they were also studied in terms of vestibular function using videonystagmography.

**Results**
In both cases a bilateral SNHL was documented. Unlike findings in the few other published cases, an improvement in audiometric thresholds and vestibular function was documented in both of our patients.

**Conclusion**
Hearing and balance disorders are reported not rarely among patients with WNV infections. It would be appropriate to study prospectively a large series of patients with WNV infection, in order to better define the

epidemiology of cohlear-vestibular involvement and understand the alterations to peripheral and central auditory and vestibular functions. In cases with cochlear-vestibular involvement, a long term follow-up of more than 6 months should be preferred.

# P140

**Inner ear pathology without vestibulocochlear nerve involvement**

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**Background**

Inner ear pathologies usually involve the vestibulocochlear nerve (CN VIII), causing hearing loss, fullness, tinnitus and vertigo. However, tumors of the inner auditory canal portion of the facial nerve (CN VII) and of the geniculate ganglion may arise as isolated peripheral facial nerve palsy, without cochlear symptoms.

**Methods**

26 years old caucasic female patient with right CN VII paralysis for about two years. Initially diagnosed at other site as Bell's palsy and treated with oral corticosteroid therapy, the deficit had progressively worsened from III to VI House Brackmann grade, without hearing loss. Electromyography and electroneurography of mimic muscles showed no spontaneous or voluntary activity of the right orbicular muscles of the mouth and the eyelid and unexcitable motor conduction of the right facial nerve. Brain MRI with gadolinium and Temporal bone CT showed focal contrast enhancement in the geniculate fossa with extension to the inner auditory canal, characterized by irregular margins, bone spicules inside it and rarefaction of the surrounding bone matrix, strongly suspected for hemangioma of the geniculate ganglion. On March 2016 patient underwent tumor removal with middle cranial fossa approach and facial nerve reconstruction with great auricolar nerve graft. Histopathological examination resulted facial nerve schwannoma.

**Results**

The middle cranial fossa approach allowed the complete preservation of the hearing. No evidences of recurrence were detected at MRI follow-up performed every 6 month for two years after surgery. Facial nerve activity was progressively improved from VI to III HB grade during the two years after surgery.

**Conclusions**

Despite the rarity, some inner ear pathologies can lead to an isolated facial nerve palsy without hearing loss. Brain MRI is mandatory if the paralysis has not improved after 3 weeks of corticosteroid therapy. The timeliness of diagnosis and treatment influences the rehabilitation chances. An important role is also played by grafting for facial nerve reconstruction and by post-operative physiotherapy.

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