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# Journal of Hearing Science®





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# Journal of Hearing Science®

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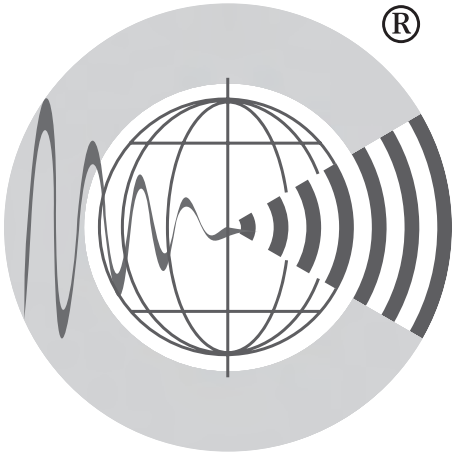



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## Abstracts

Dear Colleagues,

It is with great pleasure that we present the September 2024 issue of the *Journal of Hearing Science*, which includes some original scientific papers and the abstracts from two conferences.

The issue opens with a hypothesis paper on the use of artificial intelligence (AI) chatbots when searching for information about mobile apps in audiology. The role that both AI and mobile apps have in our lives is growing rapidly, as it is in healthcare, but at the same time there is a great need to verify that the underlying technology is trustworthy. Some recent investigations on chatbots in audiology have highlighted problems with accuracy of information [1,2] as well as the high variability of the responses provided [3]. Such problems loom even larger because chatbots usually fail to provide sources of information, and even if specifically asked to provide them will often give incorrect or fabricated sources [4]. Clearly, this whole field needs much more scrutiny before it can be adopted more widely.

Next in the issue is a review paper on regeneration of cochlear hair cell based on stem cell therapy. It reviews a promising areas of auditory research: the potential for stem cell-based therapies to address sensorineural hearing loss. This is followed by a paper on wideband absorbance patterns in cases of tympanic membrane perforation and another on the effectiveness of bilateral fitting of the Adhear bone conduction device.

This issue also includes a conference report about the Hearing Across the Lifespan (HeAL) meeting held in Cernobbio, Lake Como, earlier this year.

Finally, there are abstracts from two highly anticipated conferences in Warsaw – first the 7th International Conference on Hyperacusis and Misophonia, and second the 59th Inner Ear Biology Workshop. Many of us here are looking forward to learning much from these meetings.

With kind regards and greetings,

*Prof. Henryk Skarzynski, M.D., Ph.D., Dr. h.c. multi*

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# Hypothesis papers

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# DO CHATBOTS PROVIDE RELIABLE INFORMATION ABOUT MOBILE APPS IN AUDIOLOGY?

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Contributions:
A Study design/planning
B Data collection/entry
C Data analysis/statistics
D Data interpretation
E Preparation of manuscript
F Literature analysis/search
G Funds collection

## Abstract

**Introduction:** In light of the growing interest in utilizing AI for information retrieval, assessing the accuracy and reliability of tools such as chatbots is essential. This study aims to evaluate the efficacy of chatbots in providing accurate information about mobile applications (apps) in the field of audiology.

**Material and methods:** The responses of the Gemini and ChatGPT chatbots to eight open-ended questions posed in Polish and English were compared. Each answer was assessed for correctness.

**Results:** Gemini_ENG achieved the highest correctness with a score of 5 points (62.5%), while ChatGPT_PL scored 2 points (25%), and both Gemini_PL and ChatGPT_ENG scored 1 point (12.5%). Chatbots were most effective in recommending apps for older adults, with three of the four chatbots providing accurate recommendations. However, they struggled when asked to recommend apps for non-English speakers, to describe apps, or to provide direct links, with none of them scoring points in these areas.

**Conclusions:** Chatbots are currently unreliable sources of information about audiology apps. Depending on the language, there is significant variability in response accuracy. A good example is that Gemini_ENG performed far better than Gemini_PL. A major issue for all of them was the frequent fabrication of data, including the creation of nonexistent app names and incorrect links.

**Keywords:** ChatGPT • Gemini • mobile apps • hearing test • Polish • English

# CZY CHATBOTY MOGĄ DOSTARCZAĆ WIARYGODNYCH INFORMACJI O APLIKACJACH MOBILNYCH W AUDIOLOGII?

## Streszczenie

**Wprowadzenie:** W świetle rosnącego zainteresowania wykorzystaniem sztucznej inteligencji do wyszukiwania informacji ocena dokładności i niezawodności narzędzi takich jak chatboty jest niezbędna. Niniejsze badanie ma na celu ocenę skuteczności chatbotów w dostarczaniu dokładnych informacji o aplikacjach mobilnych w dziedzinie audiologii.

**Materiał i metody:** Porównano odpowiedzi chatbotów Gemini i ChatGPT na osiem pytań otwartych zadanych w języku polskim i angielskim. Każda odpowiedź była oceniana pod kątem poprawności.

**Wyniki:** Gemini_ENG osiągnął najwyższą poprawność z wynikiem 5 punktów (62,5%), podczas gdy ChatGPT_PL uzyskał 2 punkty (25%), a zarówno Gemini_PL, jak i ChatGPT_ENG uzyskały 1 punkt (12,5%). Chatboty były najbardziej skuteczne w polecaniu aplikacji dla osób starszych, przy czym trzy z czterech chatbotów zapewniały dokładne rekomendacje. Miały one jednak trudności, gdy poproszono je o polecanie aplikacji dla osób nieanglojęzycznych, opisywanie aplikacji lub dostarczanie bezpośrednich linków, przy czym żaden z nich nie zdobył punktów w tych obszarach.

**Wnioski:** Chatboty są obecnie niewiarygodnym źródłem informacji o aplikacjach audiologicznych. W zależności od języka istnieje znaczna zmienność w dokładności odpowiedzi. Dobrym przykładem jest to, że Gemini_ENG działał znacznie lepiej niż Gemini_PL. Głównym problemem dla wszystkich z nich była częsta fabrykacja danych, w tym tworzenie nieistniejących nazw aplikacji i nieprawidłowych linków.

**Słowa kluczowe:** ChatGPT • Gemini • aplikacje mobilne • badanie słuchu • polski • angielski

## Introduction

Chatbots, advanced conversational tools based on artificial intelligence (AI), are able to conduct natural language dialogues [1]. Following an initial training phase on large data sets, they generate responses to queries based on a wide range of information available on the internet. Due to their potential applications in science and medicine [2], chatbots are gaining widespread interest, with researchers testing them in various health sectors [3–5].

Another example of rapidly developing technology in healthcare is mobile apps [6,7]. In the field of audiology, rapid technology development has led to the marketing of a number of apps for testing hearing [8]. The apps have good sensitivity and specificity, and a number of studies have demonstrated they have potential for screening purposes [9–11]. However, the plethora of apps can make it difficult for a user to choose the most appropriate one. Patients may therefore seek sources of information in this area. One source of information might include a chatbot, which might appeal to a patient who, for various reasons, may find it difficult to access a specialist [12]. However, the extent to which chatbots can provide relevant information about mobile apps in the field of hearing is unknown.

To date, there have been few studies in the field of audiology on the use of chatbots. Existing works have mainly concerned the extent to which AI can handle specialized questions in audiology [13–16]. The answers vary depending on the version of the chatbot used [14] and may change over time [13,15]. In addition, the accuracy of the answers can be affected by the form of the question asked, e.g. whether it is an open-ended question [13] or multiple-choice [15]. Moreover, the responses generated by chatbots can differ depending on the language used in the query, as different databases will be searched [17].

In summary, the efficacy of chatbots in providing correct audiological information is unknown. To date, no research has yet been done to verify the accuracy of information provided by chatbots about mobile apps for testing hearing. The objective of this study is therefore to test whether common chatbots are able to supply reliable information about mobile apps for testing hearing. Two types of chatbots were selected, and each was asked questions in two languages, English and Polish. The accuracy, correctness, and usefulness of the answers were assessed.

## Material and methods

Two types of free chatbots – ChatGPT version 4o, (OpenAI, San Francisco, CA, USA) and Gemini (Google LLC, Mountain View, CA, USA) – were selected for study. Questions were asked in July 2024 in English and Polish, resulting in four versions for analysis: ChatGPT_ENG, ChatGPT_PL, Gemini_ENG, and Gemini_PL.

Eight open-ended questions related to hearing test apps were formulated (**Table 1**). These questions were based on information that could be verified with reference to specific studies [8,18] or by searching the mobile app market (e.g., Google Play Store, App Store). After asking questions of the chatbots, their answers were saved and analyzed for accuracy. It was checked whether the identified apps existed (questions 1–5), whether their descriptions were correct (question 6), and which apps the chatbot recommended (questions 7 and 8).

The responses to questions 1–4 were analyzed in terms of both the number of total answers provided and the number of correct answers. An answer was considered correct if the listed app was available on at least one platform (Google Play Store or App Store) and allowed a hearing test to be done.

For question 5, it was examined whether the chatbots provided a link to a specific app, and if so, whether the link was correct.

For question 6, the evaluation was in terms of:
- app description (accuracy, completeness, incorrect information);
- user rating (quantitative/qualitative; if quantitative, whether it matched the ratings given on the platform where the app is available),
- app availability on platforms (correct indication of availability on one or both platforms).

**Table 1.** List of questions asked of chatbots

| No. | Questions |
|---|---|
| 1 | What mobile apps for testing hearing are currently available? |
| 2 | Are these apps available in Poland? |
| 3 | Are there mobile hearing test apps for children? |
| 4 | Are there any mobile hearing test apps specifically designed for children to perform so-called “play audiometry”? |
| 5 | Can you provide a link to these apps? |
| 6 | Can you point to specific examples of hearing testing apps with their description, including features, user ratings, and the platforms on which they are available? |
| 7 | Which mobile hearing test app would you recommend for a person who has manual dexterity difficulties, is unfamiliar with smartphones, or has increased reaction time? Explain your choice. |
| 8 | Can you recommend an app available in Polish for non-English speakers? |

**Table 2.** Summary of chatbot responses to questions about mobile apps for testing hearing

| Question No. | Chatbot | Number of answers given | Number of correct answers | Correctness of responses |
|---|---|---|---|---|
| 1 | Gemini_ENG | 3 | 3 | + |
| | Gemini_PL | 3 | 2 | – |
| | ChatGPT_ENG | 8 | 7 | – |
| | ChatGPT_PL | 4 | 4 | + |
| 2 | Gemini_ENG | 2 | 2 | + |
| | Gemini_PL | 2 | 2 | + |
| | ChatGPT_ENG | 5 | 3 | – |
| | ChatGPT_PL | 4 | 3 | – |
| 3 | Gemini_ENG | 1 | 1 | + |
| | Gemini_PL | 0 | 0 | – |
| | ChatGPT_ENG | 4 | 1 | – |
| | ChatGPT_PL | 4 | 1 | – |
| 4 | Gemini_ENG | 1 | 1 | + |
| | Gemini_PL | 0 | 0 | – |
| | ChatGPT_ENG | 3 | 1 | – |
| | ChatGPT_PL | 4 | 0 | – |
| 5 | Gemini_ENG | 0 | 0 | – |
| | Gemini_PL | 6 | 1 | – |
| | ChatGPT_ENG | 2 | 0 | – |
| | ChatGPT_PL | 0 | 0 | – |

For the recommendations (questions 7 and 8), it was checked whether the chatbots recommended existing apps, considered the specified user constraints, and whether their recommendations were based on certain criteria (e.g., features, method of conducting test, availability, customization options like language). The information provided by the chatbots was verified for accuracy.

The full chatbot responses to the questions in **Table 1** can be found in the online supplementary material.

## Results

### Questions 1–5: searching for mobile apps

**Table 2** is a comprehensive summary of the responses provided by all versions of the chatbots to questions 1–5. In the last column, the correctness of the responses is marked with a "+" for correct answers and a "–" for incorrect ones. A minus sign was assigned if the number of correct answers was less than the number of provided answers, or if the chatbot did not provide the name of any app or a direct link, despite the availability of this information.

Detailed responses, including the specific names of the apps identified, can be found in the supplementary material.

ChatGPT_ENG generated the greatest number of responses to questions 1–4, achieving a score of 20. ChatGPT_PL furnished a total of 16 responses. In contrast, Gemini_ENG provided just 7 responses, while Gemini_PL delivered the fewest, with only 5 in total. Notably, all of the Gemini_ENG answers were correct. Gemini_PL made an error by proposing an app that monitors music listening but cannot test hearing. Despite the fact that ChatGPT_ENG generated the greatest number of responses, its error rate was higher than the other chatbots. ChatGPT_PL demonstrated a lower error rate than ChatGPT_ENG, the only error being with the final question. Curiously, ChatGPT_PL pointed to the same app in response to two questions, giving its name once in Polish and once in English; however, because the app exists in both languages these answers were considered correct.

All chatbots were asked to provide links directing a user to the respective apps. Both Gemini_ENG and ChatGPT_PL reported that they were unable to provide direct links. Gemini_ENG argued that this was for security reasons, while ChatGPT_PL said it was because of not having access to the internet and not being able to view up-to-date sources. Nevertheless, Gemini_ENG included a direct link to a specific app in its response to the first question (see Appendix 1 of the supplementary material). On the positive side, both chatbots, Gemini_ENG and ChatGPT_PL, furnished step-by-step instructions on how to search for a specific app. Gemini_ENG provided examples of existing apps, while ChatGPT_PL gave the name of an app that didn't exist. Strangely, ChatGPT_ENG and Gemini_PL

**Table 3.** Summary of chatbot responses to questions about app descriptions

| Chatbot | Number of apps provided | Number of correct apps | Platforms* (chatbot answer compared to availability on platform)** | Correct description of app** | Quantitative user rating: concordance of responses** | Concordance of all responses |
|---|---|---|---|---|---|---|
| Gemini_ENG | 3 | 3 | 4/3 | 3 | 0 | – |
| Gemini_PL | 3 | 2 | 4/2 | 2 | 1 | – |
| ChatGPT_ENG | 5 | 5 | 10/9 | 4 | 1 | – |
| ChatGPT_PL | 5 | 2 | 3/2 | 2 | 0 | – |

* App Store and/or Google Play Store, ** Concerning existing hearing test apps

provided direct links to apps on both platforms, but the app named by ChatGPT_ENG was not available on either platform, and incorrect links were given. At least Gemini_PL was able to provide one correct link.

**Question 6 – description of apps**

**Table 3** provides a summary of the responses given by all versions of the chatbots to the question about specific examples of hearing test apps. The analysis considered the descriptions, user ratings, and the platforms on which the apps were available. Column 4 shows data on the number of platforms (App Store and Google Play) on which apps were available (reported by chatbots and compared to their actual availability). The "Correct description of app" column shows the number of apps for which the chatbots provided descriptions containing all the correct information. The "Quantitative user rating: concordance of responses" column, on the other hand, shows the number of apps for which the chatbots provided consistent quantitative ratings, corresponding to those posted on platforms such as the App Store and Google Play. If any of these criteria were not met, the response was considered incorrect. In the last column, a plus sign is assigned if correct answers were all given in the other columns; otherwise, a minus sign is assigned.

Detailed responses, including descriptions of specific apps, can be found in the supplementary material.

Both versions of Gemini described 3 mobile apps, and both versions of ChatGPT described 5 apps each. However, Gemini_PL and ChatGPT_PL described one app that was designed for noise assessment rather than hearing testing. In addition, ChatGPT_PL described 2 apps that do not exist.

None of the chatbots correctly answered the question about the availability of the app on specific platforms (considering only existing hearing test apps). In one instance, both versions of ChatGPT and Gemini_ENG incorrectly indicated that the app was available on both platforms, despite the fact that it was, in fact, only accessible on one. Gemini_ENG made this error when describing two apps.

The descriptions given by Gemini_ENG were short, one-sentence descriptions. They were based on a description of the apps available on the platform. Although they were correct, there was a lack of detailed information about the functions of the app and the types of tests it could perform. Gemini_PL, on the other hand, focused mainly on listing the functions each app offered. The descriptions of ChatGPT_PL were also short, but the information was more accurate: the purpose of the app, the available tests, and the target group were clearly stated. The information was correct, except of course for the non-existent apps. ChatGPT_ENG gave the most comprehensive answers. They consisted of one or two sentences and gave an indication of the features of the app. All descriptions were correct, except for one in which the form of the test was incorrectly stated (pure tone audiometry instead of triplets-in-noise test).

In terms of users' evaluations of the apps, both versions of ChatGPT and Gemini_PL provided quantitative user ratings. However, only in two cases was the rating awarded consistent with the rating visible on the platform: (1) ChatGPT_ENG, giving the rating of the "Petralex Hearing Aid" app in the App Store; and (2) Gemini_PL, giving ratings for the "Mimi Hearing Test" app in the App Store. It should also be added that ChatGPT_ENG gave only a general number of ratings (e.g. thousands or hundreds), whereas ChatGPT_PL and Gemini_PL gave the exact number of times the app had been rated. In contrast, Gemini_ENG provided only a qualitative assessment, i.e. whether it was rated positively or not. However, the assessment was largely inconclusive, with the chatbot emphasizing that it was only a subjective rating made by users.

**Questions 7 and 8 – recommendations**

**Table 4** provides a summary of the recommendations provided by all versions of the chatbots. A response was deemed accurate (column 4) if the recommended app was for a hearing test. If there was even one false statement in the recommendation rationale (column 5), it was considered an error. In the last column, the correctness of the responses is marked with a plus or minus symbol. A minus sign was also assigned in cases where the chatbot did not make a recommendation, even though there was a corresponding app.

In response to question 7 (limitations of an older person), both versions of Gemini proposed two apps, whereas the two versions of ChatGPT proposed one each. However, one of the apps recommended by Gemini_PL is used for monitoring hearing during music listening and does not

**Table 4.** Summary of the recommendations given by all versions of chatbots

| Recommendations for | Chatbot | Number of answers given | Number of correct answers | Number of correct justifications provided for the assessment (no false information)* | Correctness of responses |
|---|---|---|---|---|---|
| Older person | Gemini_ENG | 2 | 2 | 2 | + |
| | Gemini_PL | 2 | 1 | 1 | – |
| | ChatGPT_ENG | 1 | 1 | 1 | + |
| | ChatGPT_PL | 1 | 1 | 1 | + |
| Non-English speaking person | Gemini_ENG | 0 | 0 | 0 | – |
| | Gemini_PL | 3 | 1 | 0 | – |
| | ChatGPT_ENG | 1 | 0 | 0 | – |
| | ChatGPT_PL | 1 | 0 | 0 | – |

* Concerning only existing hearing test apps

**Table 5.** Summary of the correctness of the chatbots' answers to all questions

| Question No. | Gemini_ENG | Gemini_PL | ChatGPT_ENG | ChatGPT_PL |
|---|---|---|---|---|
| 1 | + | – | – | + |
| 2 | + | + | – | – |
| 3 | + | – | – | – |
| 4 | + | – | – | – |
| 5 | – | – | – | – |
| 6 | – | – | – | – |
| 7 | + | – | + | + |
| 8 | – | – | – | – |
| Total points | 5 | 1 | 1 | 2 |
| **% correct responses** | **62.5** | **12.5** | **12.5** | **25** |

include additional hearing test functionality. All chatbots took into account the indicated limitations of the user and the apps they proposed are currently available on the market. Both versions of Gemini gave only brief explanations of its app choices. In contrast, both ChatGPT versions based their recommendations on detailed information about the app's features, the type of test to be conducted, and availability on different platforms.

In question 8, chatbots were asked to recommend apps for Polish users who do not speak English. Gemini_ENG indicated at the outset that it might be difficult to find such an app. It then recommended the apps previously identified, adding information that they may not be available in Polish. Chatbot also recommended using a translator or choosing an app with a simple interface. In contrast, Gemini_PL recommended 3 apps, but only one of them enables users to undertake a hearing test. It is also noteworthy that the description of this app provided by the chatbot contained incorrect information. Both versions of ChatGPT indicated, and described in detail, a selected app, but this app does not actually exist.

A detailed description of the user limitations and the exact responses of the chatbots is provided the supplementary material.

**Table 5** presents a summary of the correctness of the responses provided by the chatbots to all the questions posed. The last two lines shows the number of points obtained and the percentage of correct responses.

The results demonstrate that Gemini_ENG achieved a score of 5 points (62.5%), ChatGPT_PL attained a score of 2 points (25%), and Gemini_PL and ChatGPT_ENG scored 1 point each (12.5%). On this basis, Gemini_ENG clearly outperformed the other chatbots.

## Discussion

The objective of this study was to assess the effectiveness of chatbots in providing accurate information about mobile apps in the field of audiology. Two chatbots, Gemini and ChatGPT, were employed for the analysis of responses in both Polish and English.

The results indicate that AI cannot currently be considered a reliable source of information about mobile apps in audiology. Significant variability was observed in the correctness of responses depending on the language of the query, the type of chatbot, and the context of the question.

Among the tested chatbots, Gemini_ENG proved to be the most reliable, providing correct answers to more than half the questions posed. It outperformed other chatbots in terms of the correctness of responses, even compared to its Polish counterpart, Gemini_PL, which performed poorly. This disparity probably stems from the broader and more comprehensive training database available in English. The richer English dataset allows for better verification and validation of information, resulting in higher correctness. Similar observations were made by Jędrzejczak et al. [14], who recommend asking questions in English. Likewise, another study [19] found that ChatGPT 3.5 was unable to provide references for queries in Italian and Spanish because these versions don't include references in these languages. By way of contrast, ChatGPT (version 4o) did not exhibit significant language-related discrepancies in this study, suggesting a more consistent performance across languages.

There were noteworthy differences in the ways in which Gemini and ChatGPT provided answers. While Gemini focused on providing concise, relevant information, ChatGPT's responses were more detailed. Descriptions of apps were longer and explanations were more comprehensive. Similar observations have been reported in studies conducted by others [14]. In contrast, in questions about hypertension, Gemini gave more elaborate answers than ChatGPT [20]. This indicates that the length of the responses may depend on the specific domain of the questions.

Most chatbots demonstrated higher proficiency in recommending apps tailored to the needs of older people, with three of the four chatbots providing accurate responses. The exception was Gemini_PL, which failed in this area, probably due to restricted access to Polish-language sources. However, it was the sole chatbot to accurately identify an app suitable for non-English speakers, despite including erroneous information in the description.

In general, chatbots performed better on questions where general information was sought. The number of incorrect or unanswered answers increased for questions which included specific details, such as names, numbers, or direct links. These findings align with those of other studies, which indicate that ChatGPT has inferior performance on more specialized questions, such as those pertaining to sarcoma treatment, compared to general questions [5]. Furthermore, ChatGPT_ENG was less inclined to concede ignorance, frequently providing fictitious data [13,19].

## Conclusions

In the light of these findings, it can be concluded that, at the time of writing, chatbots cannot be considered reliable sources of information for mobile apps in the field of audiology. Among the chatbots tested, Gemini_ENG exhibited the highest level of correctness. However, the responses of the chatbots were variable and depended on the linguistic context in which the queries were posed. Notably, the Polish version of Gemini demonstrated a serious deficiency in accuracy and correctness compared to its English counterpart. A particularly concerning issue affecting all chatbots was the widespread and egregious error of supplying fabricated data, including the names of nonexistent apps and providing erroneous links to them.

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## Supplementary material

Supplementary material is available at
https://www.journalofhearingscience.com/

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# Review papers

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# COCHLEAR HAIR CELL REGENERATION BASED ON STEM CELLS: A SYSTEMATIC REVIEW

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Contributions:
A Study design/planning
B Data collection/entry
C Data analysis/statistics
D Data interpretation
E Preparation of manuscript
F Literature analysis/search
G Funds collection

## Abstract

**Introduction:** According to the World Health Organization (WHO), by 2050 at least 700 million people will need access to hearing care and hearing rehabilitation services. The search for cell or gene therapies has been intensifying, and stem cell therapy looks a promising candidate to support hearing regeneration and reduce these numbers. The aim of this study is to provide an overview of current advances in stem cell-based therapies for cochlear hair cell regeneration and the processes being developed for future applicability.

**Material and methods:** Identification and review of all articles in the databases PubMed, Web of Science, and PLoS One using the terms *stem cell*, *auditory hair cell regeneration*, and *mammalian* during February 2023 and following the PRISMA guidelines.

**Results:** 50 articles were obtained, published between 2003 and 2022 and were systematically analyzed. The current research quantity is limited and further studies are needed, particularly in human tissue.

**Conclusion:** The simultaneous use of cell therapy and gene therapy may lead to more promising results. Moreover, advances in cochlear hair cell regeneration with stem cells suggest there is a realistic potential to make the technique a useful future therapy.

**Keywords:** transplantation • stem cell • regeneration • inner ear • hair cell

# REGENERACJA KOMÓREK SŁUCHOWYCH OPARTA NA KOMÓRKACH MACIERZYSTYCH: PRZEGLĄD SYSTEMATYCZNY

## Streszczenie

**Wprowadzenie:** Według Światowej Organizacji Zdrowia (WHO) do 2050 roku co najmniej 700 milionów ludzi będzie potrzebowało dostępu usług w zakresie protetyki słuchu i rehabilitacji słuchu. Z tego względu intensyfikowane są poszukiwania odpowiednich terapii komórkowych lub genowych, a terapia komórkami macierzystymi celem wspomagania regeneracji słuchu i zmniejszenia liczby potrzebujących wydaje się obiecująca. Celem niniejszego badania jest przedstawienie przeglądu aktualnych wyników terapii opartych na komórkach macierzystych w regeneracji komórek słuchowych oraz procesów opracowywanych pod kątem przyszłego zastosowania.

**Materiał i metoda:** Dokonanie przeglądu wszystkich artykułów wyszukanych w bazach PubMed, Web of Science i PLoS One w lutym 2023 roku przy użyciu terminów *komórka macierzysta*, *regeneracja komórek słuchowych ślimaka* i *ssaki* oraz zgodnie z wytycznymi PRISMA.

**Wyniki:** Uzyskano 50 artykułów opublikowanych w latach 2003–2022 i poddano je analizie systematycznej. Obecna liczba badań jest ograniczona i potrzebne są dalsze badania, szczególnie na tkankach ludzkich.

**Wnioski:** Jednoczesne stosowanie terapii komórkowej i terapii genowej może prowadzić do uzyskania bardziej obiecujących wyników. Co więcej, postępy w regeneracji komórek słuchowych za pomocą komórek macierzystych sugerują, że istnieje realny potencjał, aby uczynić tę terapię użyteczną w przyszłości.

**Słowa kluczowe:** transplantacja • komórka macierzysta • regeneracja • ucho wewnętrzne • komórka słuchowa

**Key for abbreviations**

| | |
|---|---|
| cHCs | cochlear hair cells |
| CIs | cochlear implants |
| HCs | hair cells |
| IHCs | inner hair cells |
| OHCs | outer hair cells |
| SC | stem cell |
| vHC | vestibular hair cell |
| WHO | World Health Organization |

## Introduction

The cochlea is responsible for the processing of sound in the initial phase of the auditory pathway; it is a sound transducing organ capable of transforming the hydro/biomechanical energy coming from the middle ear through the oval window into electrophysiological energy.

In the organ of Corti there are supporting epithelial cells and specialized sensory cells called cochlear hair cells (cHCs). The cHCs of the inner ear are mechanoreceptors that transform acoustic signals into electrochemical signals through the displacement of stereocilia [1]. There are two groups of cHCs: the inner hair cells (IHCs) and the outer hair cells (OHCs). The OHCs (12,500 of them) are much more plentiful than IHCs. OHCs have long, thin stereocilia; they form later in embryonic development, and are more easily damaged than IHCs. IHCs are less numerous (3500 of them); they develop earlier and are more resilient [2].

Most cases of hearing impairment are due to the degeneration of cHCs. Damage to these cells is mainly induced by age, anoxia at birth, infection, exposure to ototoxic drugs (e.g. antibiotics, some anti-cancer drugs), genetic mutations, and exposure to high-intensity sounds. Hearing deficits may also result from damage to the neurons of the spiral ganglion that innervate the cHCs [3]. Regeneration of cHCs after damage occurs spontaneously in non-mammalian vertebrates like birds and fish but not in the mammalian cochlea, meaning that in mammals hearing loss is permanent [4].

Therapeutically, a range of hearing support technologies exist, such as hearing aids and implantable medical devices. However, in aiming to restore hearing, remarkable advances have led to other innovative therapies [5]. For cochlear implants (CIs) to be successful and effective, afferent neurons must be functional [6]; if they are not, CIs may be contraindicated, even if conventional hearing aids fail to provide any benefit. The search for new solutions has led to stem-cell therapy (SC) [7]. Continued research into the regeneration of cHCs suggests that future treatment of sensorineural hearing loss may involve a combination of gene therapy, cell therapy, molecular therapy, and CIs [8].

It is now possible to convert differentiated somatic cells into multipotent SCs that have the capacity to generate all adult cell types; this technique is called induced pluripotent stem cells. Thus, there is a wide variety of applications for this technology, including regenerative medicine, *in vitro* disease modeling, and drug screening/discovery [9].

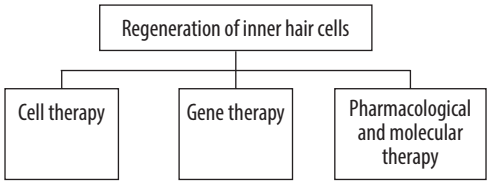


**Figure 1.** There are three main approaches to stem cell therapy: cell therapy, gene therapy, and pharmacological and molecular therapy

According to Diensthuber and Stöver, and as shown in **Figure 1**, cochlear or inner ear hair cell regeneration encompasses cell therapy, gene therapy, and pharmacological and molecular therapy [2,10].

## Stem cell-based hair cell regeneration

The inability of mammals to regenerate their hearing organ after damage is due to the postnatal decrease in SCs in the inner ear [11,12]. Generally speaking, there are two models for studying hair cell regeneration in mammals, namely cochlear organoids and cochlear organs. Hair cells within the organoids derived from pluripotent stem cells, or from a cochlear progenitor, share similar structural and functional properties to native hair cells. The inner ear and cochlear organoids can be derived from induced pluripotent SCs [13]. Induced pluripotent SCs are generated via genetic reprogramming of adult somatic cells that have limited differentiation potential but, upon reprogramming, express genes that enable them to regain plasticity and give rise to all cell types [14–16].

Previous studies have described SC therapy in which cells are transplanted into the inner ear to replace injured or dead cHCs [17–19]. To develop successful regenerative approaches for hearing loss, there must be a detailed understanding of the human inner ear, specifically its function, differentiation, and cellular mechanisms. There are currently several ongoing early-stage studies into the regeneration of cHCs.

Techniques that use SCs as a basis for cHC regeneration could play a key role in hearing restoration [5,10]. There are a large number of possible sources for obtaining SCs for hearing therapy, including: embryonic SCs, induced pluripotent SCs, mesenchymal SCs, neural SCs, and inner ear SCs. It is concluded that SC-based therapy looks especially promising for re-establishing hearing function [20–22]. There are two possible SC-based approaches to treating deafness [3,21–23]:

– endogenous regeneration or the restoration of existing HCs in the inner ear by inducing changes at the cell cycle level (administration of cell survival factors and other biologically active molecules), stimulating resident SCs within the organ of Corti to replace their own damaged cHCs;

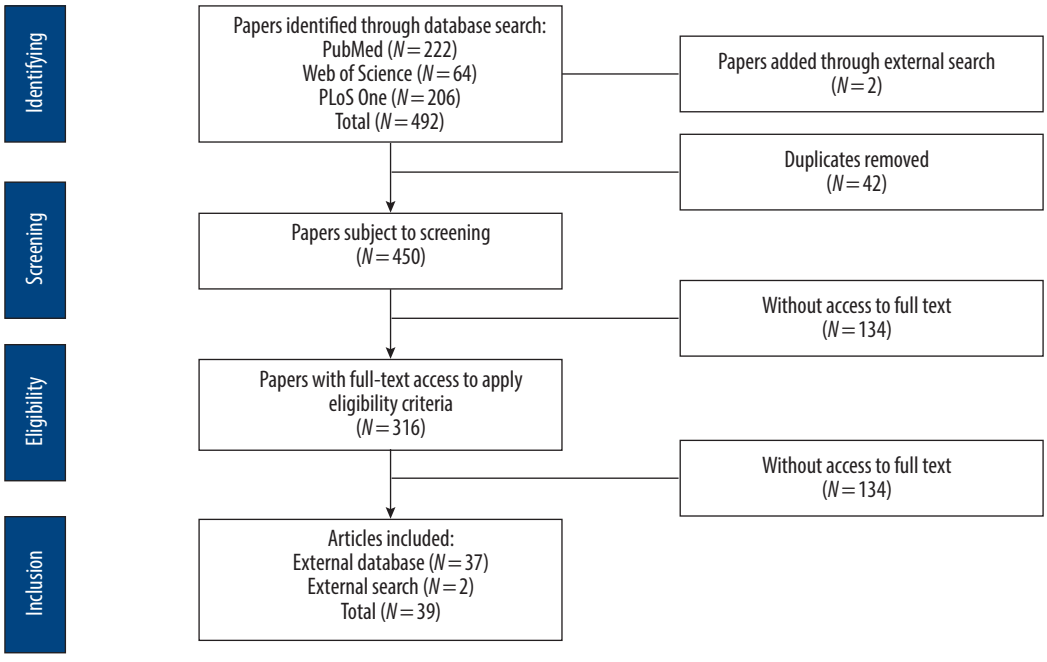


**Figure 2.** PRISMA flow chart

– exogenous delivery of SC, i.e., introduction/transplantation of SC into the inner ear.

The procedures/treatments required for endogenous activation of the inner ear in humans still remain unknown (at present); however, it is felt that SC transplantation has a higher chance of success compared to endogenous regeneration [22,23]. In order to repair the auditory sensory epithelium, identification of specific and appropriate cellular markers of inner ear SCs is needed so that endogenous inner ear SCs can be differentiated from exogenous SCs [23–25].

Cochlear support cells, once cultured and isolated *in vitro*, have the ability to express markers of the cHC, inferring that they can differentiate into this cell type. These support cells, unlike SCs, do not self-renew, so researchers have questioned if they are the best target, or if there are other undifferentiated cochlear cells with better chances of creating cHCs [26,27]. For successful regeneration of cHCs, the replacement of neural fibers is also important [28].

### Practical challenges to SC transplantation

The transplantation of SCs into the ear is complex. The literature records that there are several anatomical structures on the way to the final target site, highlighting the tympanic scala, scala media, and Rosenthal's canal [3,23], as well as the vestibular scala [11,29,30], the lateral cochlear wall [28,29,31], the perilymphatic/modiolus perforation [32,33], the round window [34,35], the lateral semicircular canal [36], and the auditory nerve [3].

Where do we stand regarding advances in SC-based therapies for ear regeneration? What processes are being developed for future applicability and what will be the impact in audiology clinical practice? These are questions that this review addresses.

## Material and methods

A systematic literature review on the topic of *Stem cell-based regeneration of cochlear hair cells* was carried out to see what processes are being tested and how the research is progressing. Articles were searched in PubMed, Web of Science, and PLoS One, using the Boolean operator AND with the terms "stem cells, auditory hair cells, regeneration, and mammalian." Other articles were also searched through external academic libraries, and 2 articles considered relevant from this external search were also included in the review.

The inclusion criteria included articles published in English, Portuguese, or Spanish, with full-text access, applicability to humans, and mentioning the term stem cell(s) in the title or abstract.

A search in February 2023 found 492 articles. Duplicates were removed and complete articles without full text online access were requested by mail direct from the first authors, leaving a selection of full text articles. Data extracted from each full text article for eligibility assessment included: Authors; Year of publication; Country; Type of therapy; Types of regenerated hair cells; Population (species); Anatomical route of cochlear transplantation; Stem-cell classification; and Findings and suggestions. The complete process is shown in **Figure 2**.

## Results

Through the database search, 492 initial papers were obtained. From them, 42 papers were eliminated as duplicates. After screening for applicability of the studies to humans and mention of the term "stem cells" in the title or abstract, 37 articles were selected for systematic analysis. To this number, 2 articles from external research were

**Table 1.** Analysis of articles

| Author (year) | Country | Regenerative therapy | Types of regenerated hair cells (HCs) | Species (translational) | Anatomical route of cochlear transplantation |
|---|---|---|---|---|---|
| **Li et al. [7] 2004** | USA | cellular therapy | cochlear | birds<br>mice | round window |
| **López-Schier [50] 2004** | USA | cellular therapy | cochlear | birds<br>mice<br>(humans) | |
| **Parker & Cotanche [56] 2004** | Switzerland | cellular therapy | cochlear | birds<br>mice | |
| **Hu et al. [49] 2005** | Switzerland | cellular therapy<br>gene therapy | cochlear | mice | tympanic scala<br>(cochleostomy) |
| **Hu & Ulfendahl [6] 2006** | USA | cellular therapy | cochlear | birds<br>mice | tympanic scala<br>vestibular scala |
| **Martinez-Monedero & Edge [27] 2007** | USA | cellular therapy<br>gene therapy | cochlear<br>vestibular | mice | tympanic scala<br>modiolus |
| **Oshima et al. [11] 2007** | USA | cellular therapy<br>gene therapy | cochlear<br>vestibular | rabbits<br>mice<br>(humans) | |
| **Edge & Chen [23] 2008** | USA | cellular therapy<br>gene therapy | cochlear<br>vestibular | birds<br>mice<br>fish | |

**Table 1 continued.** Analysis of articles

| Stem cells (SCs) classification | Study limitations | Findings and suggestions |
|---|---|---|
| embryonic<br>neural<br>bone marrow<br>inner ear | Need for more studies in the area (cochlear anatomy).<br>Correct incision procedure at the specific cochlear site.<br>Immunological barriers; possibility of immunorejection (histological incompatibility).<br>Potential use of immunosuppressants to overcome histocompatibility.<br>Tumor formation. | SCs – may have applicability in neurodegenerative diseases such as Parkinson's and others such as diabetes.<br>Injection of neural SCs.<br>Possibility of the combined use of anti-rejection drugs with cell therapy so that this situation does not occur.<br>Combination of cell therapy, gene therapy and pharmacological therapy. |
| neural<br>inner ear | Tumor formation (uncontrolled cell cycle/ excessive proliferation).<br>Need to control the orientation of new cHCs so as not to incur bad results. | Neural SCs have already generated new cHCs in the affected ear in mice. |
| neural<br>inner ear<br>embryonic<br>hematopoietic (bone marrow) | Ethical considerations (use and destruction of human embryos).<br>Tumor formation (after transplantation). | Isolating SCs from one's own ear may be a benefit for the treatment of degeneration.<br>Neural SCs – therapeutic potential for hearing loss.<br>May be preferred for treatment of neuropathy and disorders of the VIII cranial nerve. |
| embryonic<br>neural | Need for more studies and results in the area; understand cell survival and implantation. | Neural SCs have possibly better outcomes than embryonic SCs, due to the ease of differentiation into cells of functional interest to the auditory system.<br>Neural SCs can migrate to important functional structures such as the mature inner ear (along the cochlea).<br>Combination of cell therapy and gene therapy would have greater applicability for auditory cell regeneration. |
| embryonic<br>neural | Embryonic SCs: tumor formation.<br>Administration of antibiotics (reduce/control risk of rejection and inflammation).<br>Possibility of benign (teratomas) and malignant (teratocarcinoma) tumor formation; uncontrolled proliferation.<br>More research needed in the area; more tissues, applicability, sources, cell differentiation. | CIs – need for functional afferent neurons for greater success.<br>Embryonic SCs – can generate all types of cells.<br>Neural SCs – ability to restructure afferent neurons.<br>Potential ability to differentiate into neurons and glia cells during normal development and after transplantation into the nervous system. Seems to be a good bet in regenerative therapy. |
| embryonic | Type of cell choosen. Timing of infusion after damage. Differentiation state. | Neural replacement is very important in the success of cHC regeneration. |
| inner ear | Postnatal decrease of SCs at the ear level is apparently the main reason for the inability of mammals to regenerate their hearing organ after damage. | For the future, this may involve the mechanisms of action being differentiated at the cochlear and vestibular level of their SCs – discriminating between simple loss of SCs (or their ability to proliferate) and their potential as an active mechanism of repression, as well as how cochlear and vestibular cHCs act. |
| embryonic<br>exogenous<br>mesenchymal (bone marrow) | Choice and differentiation status of SCs when transplanted.<br>It has been tricky to regenerate cHC with cells transplanted from sources other than the ear. | Embryonic SCs – successful to differentiate into neurons and into cHCs.<br>Mesenchymal SCs (bone marrow) – have been used as growth factors and cellular markers for the regeneration of cHCs.<br>SC transplantation has higher chances compared to endogenous regeneration.<br>Challenge: good ordering and innervation of the cHC. |

**Table 1 continued.** Analysis of articles

| Author (year) | Country | Regenerative therapy | Types of regenerated hair cells (HCs) | Species (translational) | Anatomical route of cochlear transplantation |
|---|---|---|---|---|---|
| **Pauley et al. [46] 2008** | USA | cellular therapy<br>gene therapy | cochlear<br>vestibular | birds<br>mice<br>(humans) | |
| **Vlastarakos et al. [48] 2008** | Greece | cellular therapy<br>gene therapy | cochlear | birds<br>mice<br>(humans) | rosenthal canal<br>scala media<br>tympanic scala<br>round window<br>modiolus<br>perilymphatic perforation |
| **Brigande & Heller [47] 2009** | USA | cellular therapy<br>gene therapy<br>pharmacological therapy | cochlear<br>vestibular | birds<br>mice | scala media |
| **Jongkamonwiwat et al. [35] 2010** | United Kingdom | cellular therapy<br>gene therapy | cochlear<br>vestibular | birds<br>mice | scc lateral<br>scala media<br>tympanic scala<br>modiolus |
| **Felipe et al. [54] 2011** | Spain | cellular therapy<br>gene therapy | cochlear | birds<br>mice<br>(humans) | rosenthal canal<br>tympanic scala<br>modiolus<br>auditory nerve |

**Table 1 continued.** Analysis of articles

| Stem cells (SCs) classification | Study limitations | Findings and suggestions |
|---|---|---|
| embryonic<br>adult:<br>– inner ear,<br>– hematopoietic,<br>– neural,<br>– olfactory | Possibility of immunorejection (histological incompatibility).<br>Ethical considerations (use of embryos).<br>Tumor formation.<br>Better understanding of the auditory epithelium.<br>Complex architecture of the cochlea and orientation of the new cHCs. | Embryonic SCs – studied for neurodegenerative diseases.<br>Induced pluripotent SCs – have many characteristics of embryonic and adult SCs. Induced pluripotent SCs appear to be one of the most promising cell sources for auditory regenerative therapy. |
| embryonic<br>bone marrow<br>neural | Surgical procedure – possibility of intrascalar bleeding (small amount) after drilling the cochlear base. Middle ear infection.<br>Prophylactic administration of antibiotics is a standard part of the surgical procedure (reducing risk of inflammation).<br>Possibility of immunorejection (histological incompatibility).<br>More studies are needed in the area, in terms of mechanisms, human genome, strategies. | Immunosuppression prior to the surgical procedure, to reduce the risk of rejection.<br>Neural SC transplantation – can adopt the phenotypes, morphology, inner hair cells, and outer hair cells.<br>Embryonic neuron-derived SCs – potential for synapse formation with cHCs and reinnervation of auditory epithelium.<br>Scala media – survival of implanted cHCs.<br>Modiolus – strategy used for regeneration of the spiral ganglion. |
| embryonic<br>neural<br>inner ear | The path taken by SCs to reach the cochlea.<br>Anatomical limitations of the cochlea and organ of Corti (access to structures) and cochlear chemical composition (>K+ content).<br>Activation of SCs and that they are correct in number at the correct site of damage.<br>Possibility of immunorejection (histological incompatibility). Tumor formation.<br>Need for further studies in the area (anatomical and histological). | The goal is to try to counteract tumor formation and the risk of rejection. |
| embryonic<br>mesenchymal (bone marrow)<br>neural<br>inner ear<br>induced pluripotent | Condition of the host tissue.<br>Choice of transplantation route.<br>Embryonic SCs – immunological barriers – possibility of immunorejection (histological incompatibility). Ethical considerations.<br>Need for further studies in the area of human SCs. | Source of SCs from inner ear is the utricle (has some regenerative capacity) and cochlea (more complex procedure) – up to 3 weeks after birth.<br>CIs would be complementary to therapies.<br>Best results with electrical stimulation through CIs (spiral ganglion).<br>Embryonic SCs – control of potassium homeostasis and cochlear generative potential.<br>Induced pluripotent SCs – is more indicated for immunosuppression.<br>Perilymphatic transplantation is less traumatic and can be done by cochleostomy or through the round window. Combination of SCs and CIs still needs more studies.<br>Transplantation via modiolus to gain direct access to the Rosenthal canal – best method to access the spiral ganglion. |
| induced pluripotent<br>exogenous | Anatomical limitations (access to structures) and cochlear chemical composition (>K+ content).<br>Transplanting SCs into such a complex structure with the organ of Corti.<br>SCs transplanted through the tympanic scala may have low survival ratio.<br>If it is necessary to inject SCs multiple times, infection may result. Tumor formation. Need for more studies in the area. | ABR to prove electrophysiological thresholds.<br>Induced pluripotent SCs with promising results for auditory regeneration. |

**Table 1 continued.** Analysis of articles

| Author (year) | Country | Regenerative therapy | Types of regenerated hair cells (HCs) | Species (translational) | Anatomical route of cochlear transplantation |
|---|---|---|---|---|---|
| **Parker [25] 2011** | USA | cellular therapy<br>gene therapy | cochlear<br>vestibular | birds<br>mice<br>fish<br>(humans) | |
| **Okano & Kelley [22] 2012** | USA | cellular therapy<br>gene therapy<br>pharmacological therapy | cochlear | birds<br>mice<br>fish<br>(humans) | rosenthal canal<br>scala media<br>tympanic scala |
| **Hu & Ulfendahl [28] 2013** | USA | cellular therapy<br>gene therapy<br>pharmacological therapy | cochlear<br>vestibular | mice<br>(humans) | tympanic scala<br>vestibular scala |
| **Almeida-Branco et al. [8] 2014** | Spain | cellular therapy<br>gene therapy<br>pharmacological therapy | cochlear | birds<br>mice | rosenthal canal<br>tympanic scala<br>modiolus<br>(introduction into perilymph and endolymph) |
| **Bas et al. [42] 2014** | USA | cellular therapy<br>gene therapy<br>pharmacological therapy | cochlear<br>vestibular | mice<br>(humans) | |
| **Park et al. [17] 2014** | USA | cellular therapy | cochlear | mice | scala media<br>tympanic scala |
| **Lyon [43] 2017** | USA | cellular therapy<br>pharmacological therapy | cochlear | mice<br>(humans) | middle ear |

**Table 1 continued.** Analysis of articles

| Stem cells (SCs) classification | Study limitations | Findings and suggestions |
|---|---|---|
| embryonic<br>adult | Limited potential due to widespread differentiation into cells of the organ of Corti.<br>embryonic SCs and their resources are not yet evidence with regard to auditory regeneration; however the results seem encouraging.<br>Tumor formation. | Mention retinoic acid.<br>Neural SCs – regenerate nervous tissue such as neurons and motor function. Cochlear markers faster than embryonic and mesenchymal SCs. They are faster because they are closer to cochlear tissue.<br>Neural and mesenchymal SCs – maintain the ability to migrate throughout the injured cochlea.<br>Neural and embryonic SCs – retain the ability to differentiate into neurons. So this alternative would be suitable for neurodegenerative diseases such as Alzheimer's and Parkinson's. |
| embryonic<br>adult:<br>– tissue specific<br>– hematopoietic<br>– mesenchymal (bone marrow)<br>induced pluripotent | Anatomical limitations of the cochlea (access to structures) and cochlear chemical composition (>K+ content).<br>Basilar membrane may compromise the approach by scala.<br>SC to ear transplantation by injection seems insufficient to regenerate a substantial number of cHCs and thus will not have the capacity to form a functional auditory epithelium. | Induced pluripotent SCs – overcome the possibility of immunorejection and ethical considerations.<br>Transplants of endogenous SCs will lead to further regeneration of the OHC.<br>Alternative strategies to use SCs with a spiral ganglion regeneration or with conventional therapies such as CI must be equated for the best benefit of the patient. |
| embryonic<br>neuron-derived<br>mesenchymal<br>neural<br>inner ear<br>induced pluripotent | Ethical considerations.<br>Access to the vestibule and cochlea.<br>Anatomical limitations and cochlear chemical composition.<br>Existence of neurodegeneration.<br>Molecular mechanisms still undetermined. | It is related to the CI surgery.<br>Transplantation technique via tympanic scala has less trauma to the cochlea.<br>Mesenchymal SCs – in vitro regeneration and proliferation.<br>Neural SCs – can restore hearing via exogenous transplantation. |
| embryonic<br>adult:<br>– hematopoietic<br>– mesenchymal (bone marrow)<br>neural<br>inner ear<br>induced pluripotent | Anatomical limitations of the cochlea and organ of Corti (access to structures) and cochlear chemical composition (> K+ content).<br>Possible loss of endolymph from the cochlear canal due to surgery.<br>Further studies in the area are needed. | Combination of cell therapy, gene therapy, and CIs seem interesting for the treatment of sensorineural hypoacusis.<br>CIs electrical stimulation would be complementary to therapies (cell + gene + pharm = better mid-term results).<br>Spiral ganglion regeneration and replacement would be one of the main points to restore hearing function.<br>Induced pluripotent SCs – limit cell differentiation.<br>Safest for cHC and cochlear neurons. |
| embryonic<br>mesenchymal (bone marrow and olfactory)<br>induced pluripotent | Embryonic and induced pluripotent SCs – ethical and safety considerations.<br>Choosing the most appropriate olfactory SCs, due to the existence of a wide variety of them in the epithelium.<br>Need for further studies. | Mesenchymal SCs (olfactory) – studies describe the efficiency of the cells' potential for successful brain regeneration. But there is a need for further studies for better conclusions. |
| exogenous SCs | Anatomical limitations (access to structures) and cochlear chemical composition (>K+ content in scala media). | The injection of cHCs into the tympanic scala means that they can survive, but they are unable to pass the basilar membrane into the auditory epithelium. |
| does not specify (speaks generally) | Reduced number of differentiation in cHC.<br>Need for regeneration of both inner and outer cHCs.<br>Regenerating cochlear potentials in the appropriate locations. | Cellular markers of the cochlea are similar to those of intestinal SCs. |

**Table 1 continued.** Analysis of articles

| Author (year) | Country | Regenerative therapy | Types of regenerated hair cells (HCs) | Species (translational) | Anatomical route of cochlear transplantation |
|---|---|---|---|---|---|
| **Mittal et al. [51] 2017** | USA | cellular therapy<br>gene therapy<br>pharmacological therapy | cochlear | fish<br>mice<br>(humans) | scala media<br>tympanic scala<br>modiolus |
| **Mahmoudian-Sani et al. [32] 2017** | Iran | cellular therapy<br>gene therapy | cochlear | mice | perilymphatic perforation |
| **Simoni et al. [39] 2017** | Italy | cellular therapy | cochlear | mice<br>(humans) | tympanic scala |
| **Diensthuber & Stöver [2] 2018** | Germany | cellular therapy<br>gene therapy<br>pharmacological therapy | cochlear | birds<br>mice<br>(humans) | |
| **Chen et al. [33] 2018** | China | cellular therapy<br>gene therapy | cochlear | mice | round window |
| **Lee & Park [41] 2018** | South Korea | cellular therapy<br>gene therapy | cochlear | mice | middle scala<br>tympanic scala |
| **Takeda et al. [34] 2018** | USA | cellular therapy<br>gene therapy | cochlear | mice<br>(humans) | round window<br>modiolus |
| **Tang et al. [40] 2018** | China | cellular therapy | cochlear | mice | |
| **Hyakumura et al. [31] 2019** | Australia | cellular therapy | cochlear | mice<br>(humans) | modiolus |

**Table 1 continued.** Analysis of articles

| Stem cells (SCs) classification | Study limitations | Findings and suggestions |
|---|---|---|
| embryonic<br>umbilical cord mesenchymal (bone marrow)<br>neural<br>olfactory | It is not really clear that SCs directly produce cHC. Tumor formation and apoptosis.<br>More studies are needed in the area – new strategies will emerge. | Cellular regeneration of the ear for Usher syndrome.<br>The SC option may be the future of ex vivo expansion of patient's own SCs (autologous) and their reintroduction into the injured tissue.<br>Developing cHCs from cochlea support cells – most promising method to regenerate cHCs. |
| mesenchymal<br>– bone marrow<br>– adipose tissue<br>– olfactory tissue<br>– umbilical cord | | Bone marrow SCs have the best results among mesenchymal SCs. |
| embryonic<br>hematopoietic<br>mesenchymal (umbilical cord)<br>neural<br>inner ear<br>induced pluripotent | Affecting the complex cytoarchitecture of the cochlea and residual hearing function.<br>Recover tonotopic cochlear capacity. | Inner ear SCs grow up with good prospects of restoring hearing. |
| embryonic<br>adult:<br>– mesenchymal (bone marrow)<br>– inner ear<br>induced pluripotent | Ethical considerations (use of human embryos).<br>Further human studies needed. | Induced pluripotent SCs – hold great promise for hearing regeneration.<br>CI stimulation would be complementary to therapies (such as growth factors). |
| embryonic<br>induced pluripotent | | Induced pluripotent SCs – obtained from human urine.<br>Embryonic SCs induced from urine to pluripotent induced SCs. |
| embryonic<br>induced pluripotent | Anatomical limitations and cochlear chemical composition.<br>Tumor formation – differentiation and uncontrolled development.<br>Expensive procedures. | CIs would be complementary to therapies. |
| embryonic<br>mesenchymal (bone marrow)<br>induced pluripotent | More studies in humans needed.<br>Anatomical limitations (access to structures) and cochlear chemical composition (>K+ content in the middle range).<br>Time factor – long-term effects of treatment are not known, both at the level of CCC (survival and behavior).<br>Tumor formation.<br>Differentiation status of SCs when transplanted.<br>Access route to the cochlea and its physical barriers (Reissner's and basilar membrane). | Pluripotent SCs (embryonic and induced pluripotent SCs) seem to be the most suitable to proceed to ear regeneration therapy.<br>Better therapeutic results in the tympanic scala approach compared to lateral or posterior semicircular canal.<br>Good completion of transplantation for the inner ear. |
| mesenchymal (bone marrow)<br>neural | Create a suitable microenvironment to carry out the research and results. | Electrical stimulation (by electric field) to regulate cell behavior.<br>Electrical stimulation through CIs that promotes neural SCs to differentiate into neurons. |
| embryonic<br>neural<br>pluripotent (human) | | Studies with pluripotent SCs.<br>Co-cultures concept – meaning manipulating an environment to resemble *in vivo* characteristics (microenvironment). |

**Table 1 continued.** Analysis of articles

| Author (year) | Country | Regenerative therapy | Types of regenerated hair cells (HCs) | Species (translational) | Anatomical route of cochlear transplantation |
|---|---|---|---|---|---|
| **Roccio & Edge [38] 2019** | Switzerland | cellular therapy<br>gene therapy<br>pharmacological therapy | cochlear<br>vestibular | mice<br>(humans) | |
| **Xia et al. [30] 2019** | China | cellular therapy<br>gene therapy<br>pharmacological therapy | cochlear<br>vestibular | mice<br>(humans) | cochlear lateral wall |
| **Waqas et al. [21] 2020** | Pakistan | cellular therapy<br>pharmacological therapy | cochlear | mice | scala media<br>tympanic scala (via round window) |
| **Zhang et al. [37] 2020** | China | cellular therapy<br>gene therapy | cochlear | birds<br>fish<br>mice | |
| **Zine et al. [55] 2021** | France | cellular therapy<br>gene therapy<br>pharmacological therapy | cochlear | fish<br>mice<br>(humans) | cochlear nerve<br>scala media<br>tympanic scala<br>modiolus<br>tympanic scala<br>(intraperilymphatic and intraendolymphatic) |
| **Maharajan et al. [57] 2021** | South Korea | cellular therapy<br>gene therapy<br>pharmacological therapy | cochlear | mice<br>(humans) | |
| **Guo et al. [58] 2021** | China | cellular therapy<br>gene therapy<br>pharmacological therapy | cochlear | mice | |
| **Kempfle [53] 2021** | USA | cellular therapy<br>gene therapy<br>pharmacological therapy | cochlear | mice<br>(humans) | round window (transtympanic) cochleostomy |

**Table 1 continued.** Analysis of articles

| Stem cells (SCs) classification | Study limitations | Findings and suggestions |
|---|---|---|
| induced pluripotent | Complex architecture of the sensory epithelium. Surgical access. | |
| embryonic<br>neural<br>induced pluripotent | Long-term effects of treatment are not known, both at the level of cHC (survival and behavior).<br>Anatomical limitations (access to structures).<br>More studies needed in the area. | CIs would be complementary to electrical stimulation therapies.<br>Embryonic, neural, and induced pluripotent SCs – relate to cHCs and spiral ganglion. |
| exogenous<br>endogenous<br>embryonic<br>induced pluripotent | Barriers with tight junctions.<br>Ethical considerations.<br>Tumor formation.<br>Correct incidence on specific cochlear tissue and insufficient number of SCs residing in the inner ear.<br>Expensive treatment. | Induced pluripotent SCs – obtained from human urine. |
| inner ear | More studies are needed in the area on growth factors and signals. | cHCs can be regenerated by SCs, genes, and signaling regulation. Need to inhibit apoptosis, analyze other genes and maturation – should be done in the future. |
| embryonic<br>induced pluripotent<br>pluripotent | Surgical delivery routes play a huge technical factor in the cochlea.<br>Deliver the cells into the anatomic target.<br>Establish precise cell injection through the cochlea, while minimizing surgical trauma and hearing loss.<br>Current limitations to the use of human induced pluripotent SCs – extended in vitro period of culture, reproducibility, variable efficiency of tissue derivation, incapacity to generate cochlear tissues.<br>Need for more studies in the area and humans. | Advanced in research and recent studies in human induced pluripotent SCs.<br>Approach by intraperilymphatic injection, intraendolymphatic injection, modiolar, and cochlear nerve injection.<br>In the close future, a possible regeneration of inner ear can include network vascularization and integration into microfluidic chips. |
| embryonic<br>induced pluripotent<br>mesenchymal | Successful delivery of mesenchymal SCs to the target sites, and necessary of a suitable microenvironment for survival and migration.<br>Transplanted mesenchymal SCs – may cause immunorejection, inflammation and tumor formation. | Different mesenchymal SCs isolation methods with almost similar functional characteristics. |
| autophagy<br>(does not include SC) | Complexity of autophagy mechanisms.<br>Current autophagy research – limited to cell lines, explants and animals, and few clinical trials have been examined.<br>Although excessive autophagy can lead to cell death under some conditions.<br>Need for more studies in the area. | Some proteins and mRNAs participate in the autophagy and can make them potential targets for treatment of sensorioneural hearing loss. |
| embryonic<br>induced pluripotent<br>mesenchymal | Need for more studies in the area and humans. | Endoscopic ear surgery provides a minimally invasive approach to the inner ear for regenerative therapies.<br>Possible routes: transtympanic delivery (indirect drug application into the round window membrane – in patients with residual hearing), and through the round window membrane or via cochleostomy (performed with the endoscope to target in patients without residual hearing). |

**Table 1 continued.** Analysis of articles

| Author (year) | Country | Regenerative therapy | Types of regenerated hair cells (HCs) | Species (translational) | Anatomical route of cochlear transplantation |
|---|---|---|---|---|---|
| **Kwan & White [59] 2021** | USA | cellular therapy<br>gene therapy | cochlear | mice<br>(humans) | |
| **Lee & Waldhaus [4] 2022** | USA | cellular therapy<br>gene therapy<br>pharmacological therapy | cochlear<br>vestibular | birds<br>fish<br>mice<br>(humans) | |

added, resulting in a final number of 39 articles. The flow chart following the PRISMA guidelines [37] is shown in **Figure 2**.

The distribution of papers in the databases shows a gradual increase in number over the years, with the greatest number being obtained in the period 2005–2010 and in the last half of the decade (2015–2022). The geographical distribution (by number of papers) revealed a large contribution from the United States of America (19), followed by China (5), Switzerland (3), Spain (2), South Korea (2), Australia (1), Iran (1), Pakistan (1), France (1), Greece (1), Italy (1), Germany (1), and UK (1).

## Discussion

The main focus in cochlear hair cell regeneration research is cHC regeneration [21,37], sometimes also considering vestibular (vHC) regeneration [30,38,39]. The challenge for the research covered in this review was to decide upon the most effective method for such regeneration within the human ear, since it has complex microstructure and physiology.

In regenerative therapies, there are some studies of cell therapy alone [40,41]. Others combine cell therapy with gene therapy [3,42] or with pharmacological therapy [43,44]. Finally, some studies consider all three – cell therapy, gene therapy, and pharmacological therapy [8,39].

The goal of SC-based replacement therapy in sensorineural hearing loss is to replace the lost cHCs or neurons of the spiral ganglion, with the biggest challenge being precise targeting without disrupting the cochlear architecture and damaging residual hearing function [45,46].

The complex architecture of the sensory epithelium and its difficult surgical access are anatomical limitations [39]. Transplantation involves overcoming physical barriers within the cochlea: Reissner's membrane, the basilar membrane, the organ of Corti, and the vestibule [29–31,35]. The importance of a correct incision at the specific cochlear site is vital to safe SC therapy [30]. Moreover, the growth, differentiation, and orientation of the new SCs, plus the recovery of the incisional regeneration site, are further considerations [44,46].

This review revealed that the use of embryonic SCs can trigger uncontrolled cell formation and development, with increased risk of tumor formation [30,42]. There is also the possibility of histological incompatibility (i.e. immunorejection) [3,47]. To overcome these immunological barriers, the use of anti-rejection drugs (immunosuppressants) combined with cell therapy is the favoured solution [7,48,49], and the use of autologous grafts may also help to circumvent this, as well as prophylactic antibiotic administration to reduce infection risk [49].

All procedures in regenerative therapies, specifically cell therapy, are costly, and it is also difficult to create a suitable microenvironment [3,41,42]. Therapies should be applied according to the cochlear and neural reserve of the patient [8], keeping in mind that neurodegeneration is a barrier to the entire regenerative process [6].

Lastly, the narrative review revealed that CIs can complement SC therapies, promoting electrical stimulation, and this approach may show better results in the future [30,42]. Electrical stimulation is one of the most important factors in regulating cell behavior; it influences cell proliferation, differentiation, and migration. Thus, in the future CIs have a potential role to assist SCs [30,41].

The long-term effects of treatment on both behavior and survival of cHCs are not yet known [31,35]. More studies are needed, namely of various progenitor cell populations, implications, specific factors, as well as combination and complementary therapies [3,23].

### Sources of stem cells

This review looked systematically at the sources of SCs (**Table 1**). The following are the most relevant to auditory regeneration.

**Table 1 continued.** Analysis of articles

| Stem cells (SCs) classification | Study limitations | Findings and suggestions |
|---|---|---|
| induced pluripotent<br>pluripotent | Spontaneous lineage conversion – not observed after damage (in mature mammalian cochlea). Need for more studies in the area and humans. | Advanced in research and recent studies.<br>Neonatal and even mature SCs can be genetically manipulated. |
| induced pluripotent<br>mesenchymal<br>neural<br>pluripotent<br>tissue specific | Limited regenerative capacity and the potential to isolate SC during mice postnatal development.<br>Limitations of the pluripotent SCs-based approaches – cellular output of the present-day protocols. Anatomical limitations of the cochlea and organ of Corti (access to structures) and cochlear chemical composition (>K+ content).<br>Need for more studies in the area and humans (various in vivo and *in vitro* approaches in study). | Functional hair cell regeneration: non-mammalian vertebrate (e.g. birds and fish).<br>Current research has focused on tissue specific SCs and pluripotent SCs.<br>Less explored research: neural SCs and mesenchymal SCs.<br>The continued study and use of human induced pluripotent SCs can open the way to understanding more complex diseases, like Waardenburg syndrome. |

### *Neural SCs and embryonic SCs*

Retain the ability to differentiate into neurons, and this approach may be suitable for neurodegenerative diseases such as Alzheimer's and Parkinson's. Neural SCs regenerate nervous tissue such as neurons and motor function, expressing cochlear markers faster than embryonal and mesenchymal SCs. Neural SCs are the fastest to regenerate because of their proximity to cochlear tissue [26]. Moreover, neural SCs were among the first to generate new cHCs in the ear [51]. Their use, together with electrical stimulation via CIs, leads to their differentiation into neurons, showing evidence that they can restore hearing via exogenous transplantation [29,35]. These SCs have the ability to migrate to important functional structures such as the mature inner ear [50].

### *Inner ear SCs*

May in future be capable of restoring hearing and beneficial for treating degeneration found in hearing loss, neuropathy, or disorders of the VIII cranial region [25].

### *Embryonic and induced pluripotent SCs*

Embryonic and induced pluripotent SCs related to cHCs and the spiral ganglion are thought to be the most likely to lead to therapeutic success [31]. Most studies state that pluripotent SCs (embryonic and induced pluripotent SCs) seem to be the most suitable for ear regeneration therapy [38,52].

## Possible routes for SC transplantation

Regarding the anatomical route of cochlear transplantation, perilymphatic transplantation is less traumatic and can be done by cochleostomy or through the round window [37]. Endolymph loss through the cochlear canal is possible, and bleeding is also possible due to surgery and cochlear perforation [8,49].

Developing cHCs from cochlear support cells seems one of the most promising methods for regeneration [53,54]. Alternative strategies propose using SCs for spiral ganglion regeneration together with conventional therapies such as a CI to maximise patient benefit [23]. Transplantation via the scala tympani is less traumatic to the cochlea and can be combined with CI surgery [29]. The modiolus approach can also be used at the same time as a CI operation, thus limiting the risk of residual hearing loss. Direct access to Rosenthal's canal might be the best method to access the spiral ganglion [37,55].

Overall, research into transplantation methods is still insufficient, with a need for further anatomical and histological studies [31,48,56], particularly in human tissues [10,35,54] and umbilical cord serum [57]. The question about full or partial recovery of tonotopic cochlear capacity remains to be answered [56,58].

A variety of signaling pathways, including Wnt, Notch, Hippo-YAP, Hedgehog, LIN28/Let7, key transcriptional factors (*Atoh1* or *Math1)*, and fibroblast growth factors, have been found to be involved in regulating hair cell development and regeneration by controlling the expression of various transcription factors [for review, 13].

Future research trends may involve differentiated action mechanisms at the cochlear and vestibular level, discriminating between simple loss of SCs (or their ability to proliferate) and their potential as an active regeneration mechanism [10]. The need for apoptosis inhibition, the analysis of other genes, and cell maturation must also be considered [38,55,59].

Despite ongoing challenges, embryonic and induced pluripotent SCs derived from inner ear cultures have already demonstrated potential for disease modelling and therapeutic trials. However, future continued research is required to achieve protocol optimisation and to improve applications and outcomes. The use of patient-derived cultures can facilitate the evaluation of gene therapy efficacy,

a possibility that has been trialled in other model systems, such as the eye [60].

The combined use of cell therapy and gene therapy (gene programming/editing technology) may have more promising results and strategic applicability, and are likely to become a future research trend. Regardless of the technology used, the majority of studies found support the use of pluripotent SCs. However, the continued threat of having pluripotent SCs becoming uncontrollable and inducing genetic damage and malignant cell growth is ever-present, and the potential and fate of these cells *in vivo* are under intense investigation.

## Conclusions

Inner ear pathology and therapeutic developments have traditionally relied on animal models, which usually cannot completely recapitulate the human otic system. These challenges are now being partly addressed using induced pluripotent SCs in lab cultures, which generate the sensory epithelial-like inner ear tissues.

Developing pluripotent SCs (embryonic and induced pluripotent SCs) seems to be the most promising method for ear regeneration therapy for cHCs, inserted by cochleostomy or through the round window.

The combined use of cell therapy and gene therapy appears to be the most promising method. Moreover, advances in cHC regeneration with stem cells suggest there is a realistic potential to make the technique a useful future therapy.

For implementation, timing and safe procedures are crucial to achieving an effective protocol. Further investigation is required to determine whether transplanted cells can survive long term and contribute functionally. There is also the question of whether stimulation by a CI or hearing aid promotes survival and is needed in the (re)habilitation protocol.

The current technology market may act to hinder advances in regeneration of cHCs, with low investment partly because hearing loss can usually be safely and economically rehabilitated with devices like cochlear implants and hearing aids.

If new SC treatments are introduced, the role for an audiologist will remain, as people still require diagnosis and monitoring of their hearing, both pre- and post-treatment. The use of SCs might take some decades, but we should not fear innovation. Everything that can improve quality of life, specifically hearing health, must be part of our future solutions.

## Author contributions

VC and DT were involved in the conceptualization and design of the study. VC conducted the article search and writing. FC and TP-B critically reviewed the study protocol and writing. All authors were involved in the revision of the manuscript and approved the final version.

## Conflict of interest

The authors declare no competing interests.

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# Original articles

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# WIDEBAND ABSORBANCE PATTERNS IN ADULTS WITH CENTRAL AND MARGINAL TYMPANIC MEMBRANE PERFORATION

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Contributions:
A Study design/planning
B Data collection/entry
C Data analysis/statistics
D Data interpretation
E Preparation of manuscript
F Literature analysis/search
G Funds collection

## Abstract

**Introduction:** This study aimed to investigate the impact of central and marginal tympanic membrane perforations (TMP) on wideband absorbance (WBA) and compare it with normal ears.

**Material and methods:** Three groups of individuals, aged 18 to 50 years: Group I with central TMP ($n = 65$), Group II with marginal TMP ($n = 13$), and Group III with normal middle ears ($n = 20$) were considered. WBA measurements were performed at peak and ambient pressure conditions across frequencies.

**Results:** Significant differences in WBA were observed between the groups with central and marginal TMP and the normal ear group across all frequencies. Central TMP exhibited decreased absorbance at low frequencies and increased absorbance at high frequencies, peaking at 5000 Hz. Marginal TMP showed peaks at 600, 4000, and 6000 Hz with decreased absorbance at 2000 Hz. Central TMP exhibited lower absorbance than marginal TMP at lower frequencies, while marginal TMP showed decreased absorbance at mid and high frequencies.

**Conclusions:** These findings highlight the role of WBA in differentiating normal ears from those with TMP. Understanding TM vibration patterns and frequency-dependent variations in absorbance enhances diagnostic accuracy and clinical management.

**Keywords:** wideband absorbance tympanometry • tympanic membrane • central perforation • marginal perforation • peak/ambient pressure

# WZORCE SZEROKOPASMOWEJ ABSORBANCJI U DOROSŁYCH Z CENTRALNĄ I BRZEŻNĄ PERFORACJĄ BŁONY BĘBENKOWEJ

## Streszczenie

**Wprowadzenie:** Niniejsze badanie miało na celu ocenę wpływu centralnych i brzeżnych perforacji błony bębenkowej (ang. *tympanic membrane perforations*, TMP) na absorbancję szerokopasmową (WBA) i porównanie jej z wynikami uszu z nieuszkodzoną błoną bębenkową.

**Materiał i metody:** W badaniu uczestniczyły trzy grupy osób w wieku od 18 do 50 lat: grupa I z centralną TMP ($n = 65$), grupa II z brzeżną TMP ($n = 13$) i grupa III z nieuszkodzoną błoną bębenkową ($n = 20$). Pomiary WBA przeprowadzono w warunkach ciśnienia szczytowego i ciśnienia otoczenia w różnych częstotliwościach.

**Wyniki:** Zaobserwowano znaczące różnice w WBA między grupami z centralną i brzeżną TMP a grupą z nieuszkodzoną błoną bębenkową we wszystkich częstotliwościach. Centralna TMP powodowała zmniejszoną absorbancję przy niskich częstotliwościach i zwiększoną absorbancję przy wysokich częstotliwościach, z wynikiem szczytowym na 5000 Hz. Brzeżna TMP wykazywała wartości szczytowe na 600, 4000 i 6000 Hz, a zmniejszoną absorbancję na 2000 Hz. Centralna TMP powodowała niższą absorbancję w porównaniu z brzeżną TMP na niższych częstotliwościach, podczas gdy brzeżna TMP powodowała obniżoną absorbancję na średnich i wysokich częstotliwościach.

**Wnioski:** Odkrycia te podkreślają rolę WBA w różnicowaniu uszu z nieuszkodzoną błoną bębenkową od tych z TMP. Zrozumienie wzorców wibracji TM i zależnych od częstotliwości zmian absorbancji zwiększa dokładność diagnostyczną i wpływa na postępowanie kliniczne.

**Słowa kluczowe:** tympanometria szerokopasmowa • błona bębenkowa • perforacja centralna • perforacja brzeżna • ciśnienie szczytowe/otoczenia

**Key for abbreviations**

| | |
|---|---|
| WBT | wideband tympanometry |
| WBA | wideband absorbance |
| TM | tympanic membrane |
| TMP | tympanic membrane perforation |

## Introduction

The primary function of tympanic membrane (TM) is to transmit sound waves effectively from the external auditory canal through the ossicular chain to the oval window and inner ear. This crucial process relies on the vibratory nature of the TM, i.e., it converts the ear canal's sound pressure to the ossicles' vibrations [1]. The TM can be divided into four quadrants: anterosuperior, anteroinferior, posteroinferior, and posterosuperior [2]. Studies have shown that the TM exhibits frequency-specific transmission, indicating its importance in sound transduction [3,4]. However, depending on their location and extent, tympanic membrane perforation (TMP) can have varying effects on sound transmission.

TMP can be classified according to the perforation site, whether on the pars tensa or pars flaccida. Central TMP may involve different quadrants of the TM, with anterior quadrant TMP extending from the anterior to the malleus handle, and posterior quadrant TMP extending from the posterior to the handle of the malleus. Marginal TMP involves a lack of TM borders in specific segments [5] and the location of the perforation can alter frequency-specific sound transmission to the ossicles.

Although single-frequency tympanometry with a 226-Hz probe tone is a commonly used diagnostic tool for evaluating the middle ear system, some studies have reported low accuracy in identifying middle ear pathologies [6,7]. This is because middle ear disorders produce a significant change in middle ear structures, including the ossicular mass, stiffness of the TM, and supporting structures, which leads to frequency-specific attenuation or filtering [8]. To overcome these limitations, multi-frequency tympanometry is used, which is superior to standard 226 Hz tympanometry. However, it has limitations, such as standing waves above 1500 Hz, which result in significant differences in sound pressure levels within the ear canal and, thus, can interfere with accurate readings at high frequencies [9,10]. To address these challenges, wideband tympanometry (WBT) emerged as an alternative tool to assess the middle ear system.

WBT measures the amount of sound energy absorbed or reflected by the ear. A controlled range of sounds is introduced into the ear canal, and then an analysis is performed on how much of that energy is absorbed or reflected by the eardrum at each frequency. This information provides insights into the functioning and characteristics of the middle ear [11,12].

The impact of TMP on wideband absorbance (WBA) measures on middle ear transmission has been discussed in several studies [13–18]. Karuppannan and Barman (2021) [15] and Karuppannan et al. (2024) [19] found lower absorbance values in low and mid frequencies, with higher absorbance observed beyond 4000 Hz under peak pressure conditions for individuals with TMP. Similarly, Kim et al. (2019) [17] conducted WBA on individuals with TMP and noted lower absorbance at low frequencies and higher absorbance at high frequencies. However, inconsistencies in the literature have been reported, with some studies [20,21] indicating higher absorbance at low frequencies in individuals with TMP. Overall, no consensus exists regarding frequencies with highly variable energy absorbance patterns reported in individuals with TMP.

This variability in absorbance patterns may be attributed to the inclusion of different types and locations of TMP. Even though some studies have shown that the size of the TMP has more of an impact on transmission than its location [22], the fact is that the location of a TMP significantly alters frequency-specific sound transmission [23,24]. It is therefore essential to study the effect of the location of TMP on WBA. However, no studies have compared WBA across different quadrants of the TMP, which is a research gap. Thus, the current study investigated the effect of the two types of TMP on WBA measures. The objectives were to compare, against normal ears with intact TM and under different pressure conditions, WBA across frequencies for the two TMP types.

## Material and methods

### Participants

Three groups of participants, aged between 18 and 60 years (mean age 35.2 years; *SD* = 9.7 years), were recruited for the study. Group I comprised individuals with central TMP (mean age, 36.3 years; *SD* = 6.7 years) (*n* = 65 ears); Group II included participants with marginal TMP (mean age, 32.6 years; *SD* = 4.5 years) (*n* = 13 ears), and Group III was the normal ear group, consisting of individuals with intact TM and normal hearing sensitivity (mean age 31.2 years; *SD* = 2.5 years) (*n* = 20 ears).

The study obtained ethical approval from the Ethical Committee for Bio-Behavioural Research Involving Human Subjects at the All India Institute of Speech and Hearing (approval no. WOF-0404) and adhered to the ethical principles outlined in the Helsinki Declaration (2013) for medical research involving human subjects. The study's objectives and procedures were explained in detail to each participant, and written informed consent was obtained for their voluntary participation before enrolment in the study.

### Criteria for inclusion

The normal ear group (Group III) had air conduction hearing thresholds of ≤ 15 dB HL in the octave frequencies between 250 and 8000 Hz, an air-bone gap of ≤ 10 dB HL, speech identification scores of > 90%, and uncomfortable loudness level (UCL) of > 90 dB HL. All participants exhibited normal middle ear, as confirmed by single frequency immittance findings showing an "A/As" type tympanogram with both ipsi- and contralateral reflexes present between 90 and 100 dB HL, along with the presence

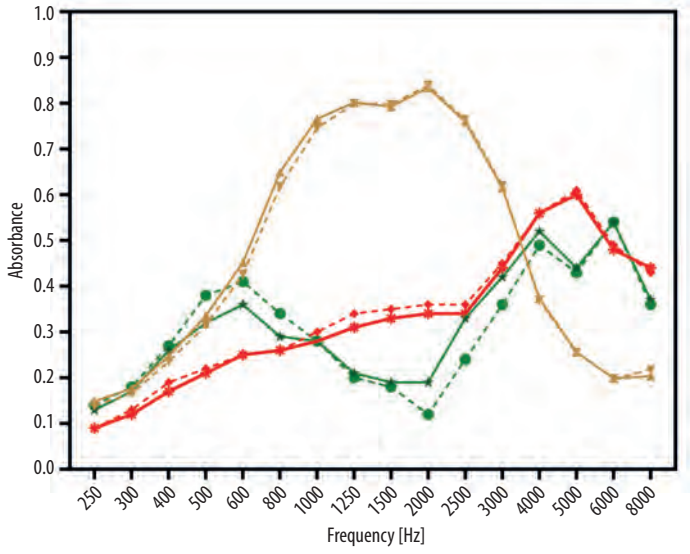


**Figure 1.** Mean WBA values across frequency of the normal ear group (brown), central TMP group (red), and marginal TMP group (green) as obtained at peak pressure (continuous lines) and ambient pressure (dotted lines)

of transient evoked otoacoustic emissions (TEOAEs) characterized by a signal-to-noise ratio (SNR) > 6 dB and reproducibility of > 85%.

Ears with central and marginal TMP (Group I and II) exhibited isolated dry TMP, i.e., without any active discharge and with no other pathologies associated with the TMP. In central TMP, the perforation was localized around the umbo or malleolar handle (*pars tensa* region), with the TM remaining intact around the bony canal. Marginal TMP was confined to the TM margins. These findings were confirmed by an experienced otologist. Ears with TMP (central and marginal) had conductive hearing loss (air-bone gap > 10 dB) and a pure tone average not exceeding 60 dB HL. Tympanometry revealed a "B" type tympanogram [11] with an absent measurable peak using a 226 Hz probe tone and absent acoustic reflexes between 500 and 4000 Hz at octave frequencies.

## Procedure

Participants were seated comfortably during the experiment without any movement. Each participant was informed of the testing methods, which involved inserting a probe tip into the ear canal to create an airtight seal, generating pressure, and presenting click stimuli. Titan Suite IMP440 v. 3.0 was used for WBA measurements. A wideband click stimulus of 100 dB peSPL was delivered while the pressure was automatically swept from +200 to –400 daPa at a medium-level pump speed of 200 daPa/s. WBA values were automatically calculated across frequencies by averaging the click stimulus response over 32 sweeps. Absorbance values at 16 individual frequencies bins, i.e. 1/3rd octave bands, were considered for analysis.

WBA values range from 0.0 to 1.0, with 1 indicating complete absorption of sound energy by the middle ear and 0 indicating total reflection. The Titan IMP/WBT440 module displays WBA across frequencies at two pressure conditions: ambient pressure (0 daPa) and peak pressure. Absorbance values were noted at both peak pressure and ambient pressure conditions extracted from WBA. Preliminary testing (PTA and immittance audiometry) and WBA testing were done on the same day.

## Statistical analysis

A non-parametric test was used for analysis because the Shapiro–Wilk test ($p > 0.05$) showed a non-normal distribution of data across frequencies. A Kruskal–Wallis test was used to determine whether or not there was a statistically significant difference between the medians of the three independent groups. Pairwise comparisons of groups were performed using the Mann–Whitney *U*-test. The Wilcoxon signed rank test was used for within-group comparisons to study the difference in absorbance between each group's peak and ambient pressure conditions. A $p$-value < 0.05 was considered significant for all statistical analyses. Cohen's $d$ was used to measure effect size and identify true significant values [25].

## Results

**Figure 1** shows the mean WBA pattern measured at peak and ambient pressure.

The normal ear group (Group III) exhibited a bell-shaped absorbance pattern. The mean WBA was lowest at 250 Hz, then increased steeply with increasing frequency to two maxima at 1250 and 2000 Hz and reduced to a minimum at 6000 Hz and above. The central TMP group (Group I) exhibited lower mean absorbance at low and mid frequencies up to 2500 Hz compared with the normal ear group. The absorbances were identical near 4000 Hz and then increased at higher frequencies (5000, 6000, and 8000 Hz) compared to the normal ear group.

Compared with the normal ear group, ears with marginal TMP showed almost similar absorbance levels up to 500 Hz, lower absorbance at mid-frequencies up to 3000 Hz, and higher absorbance at frequencies above 4000 Hz. The WBA pattern of the marginal TMP group displayed three peaks at 600, 4000, and 6000 Hz. The mean, median, and standard deviation of WBA values across frequencies and at both peak and ambient pressures are shown in **Table 1**. The test done under both peak and ambient pressure conditions displayed an almost identical pattern.

The comparison of WBA obtained across groups was performed using the Kruskal–Wallis *H*-test and revealed a significant difference ($p < 0.05$; $df = 1$) between the groups. Subsequently, pairwise comparisons between the groups were made using the Mann–Whitney *U*-test to explore whether significant differences existed between the groups at each frequency. **Table 2** summarises the results of the Mann–Whitney *U*-test.

Significant statistical differences were observed across all frequencies between the normal ear and central TMP groups. There were moderate to large effect sizes at almost all frequencies, except at 3000 Hz in the peak pressure condition and 300 and 400 Hz in the ambient pressure condition. Similarly, between the normal ear and the marginal TMP group, significant statistical differences were observed across all frequencies, except at low frequencies (250 to 500 Hz) in both pressure conditions and 600 Hz in the ambient pressure condition, with moderate to large effect sizes.

Between the central and marginal TMP groups, statistically significant differences were observed at 500, 600, and 5000 Hz in both pressure conditions and at 2000 Hz in the ambient pressure condition. Of these significant differences, moderate to large effect sizes were seen at frequencies at 500 and 600 Hz under both pressure conditions and 2000 Hz under ambient pressure conditions.

The Wilcoxon signed-rank test revealed a significant difference between the pressure conditions in the normal ear group at low frequencies up to 1000 Hz and at mid-frequencies of 2000 and 2500 Hz, with moderate to strong effect sizes. In the central TMP group, a significant difference between peak and ambient pressure was observed only for frequencies from 250 to 500 Hz, 1250 Hz, and 2500 Hz. However, the effect size was too small to be considered as significant. For the marginal TMP, a significant difference with a strong effect size was observed at 800 Hz and from 2000 to 5000 Hz.

Although the current study revealed a significant difference between the pressure conditions, the WBA pattern remained nearly identical in both pressure conditions across all groups. A summary of the Wilcoxon signed rank test is tabulated in **Table 3**.

## Discussion

The WBA pattern observed in the normal ear group was smooth, broad, and double-peaked. Individuals with normal TMs exhibited low absorbance at 250 and 8000 Hz, with maxima at 1250 and 2000 Hz. The present study's finding coincides with those of Karuppannan and Barman (2021) [15]. These frequencies reflect where the middle ear's stiffness and mass dominate [26]. Reduced absorbance below 1000 Hz reflects the stiffness-controlled middle ear system, whereas at higher frequencies the middle ear system is mass-dominated; both lead to impedance mismatch and reflection of sound energy. Between 1000 and 3000 Hz, the stiffness and mass components cancel, allowing energy to pass into the middle ear [20].

For both pressure conditions, the central TMP group exhibited lower absorbance values at low and mid-frequencies, with medium values around 2500 Hz and higher absorbance beyond 3000 Hz compared with the normal TM group. These findings are consistent with previous studies [15,17,19], which reported reduced absorbance values in the low and mid-frequency range for ears with TMP. This decreased absorbance may be attributed to the lower ratio of the affected eardrum area to the oval window area and to the buckling effect. Tonndorf and Khanna's (1972) classic study on frequency-specific vibration of the TM [3] revealed that the membrane vibrated cohesively at lower frequencies but that TMP disrupts this normal vibratory pattern, necessitating a larger area for low frequencies. This requirement could lead to decreased absorbance, potentially reduced absorbance at low frequencies.

Contradictory results are also apparent and documented in the literature, with studies reporting increased absorbance at low and mid frequencies in ears with TMP in comparison to individuals with intact TM [20,21,27]. Previous authors with contradictory findings to those displayed here have attributed this phenomenon to an increase in mass within the middle ear caused by TMP, which allows low-frequency energy to easily enter the middle ear [26]. At higher frequencies, there are some inconsistencies between our work and that of previous authors, with some studies reporting normal or near-normal absorbance [27,28] although others, consistent with the current work, see increased absorbance at higher frequencies [15,20,29]. One animal study has found reduced absorbance at higher frequencies [24]. The increased absorbance measured in the current study at higher frequencies can be ascribed to shorter wavelengths easily passing through large TMPs.

To our knowledge, no studies have measured WBA in individuals with marginal TMPs. The present study revealed they had similar absorbance to the normal ear group up to about 500 Hz, low absorbance at mid-frequencies up to 3000 Hz, and higher absorbance at frequencies above 4000 Hz. The WBA pattern of the marginal TMP group displayed three peaks at 600, 4000, and 6000 Hz, together with a dip at 2000 Hz. The rationale behind this can be understood by examining the physiology of sound transmission through the TM. Most participants had marginal TMP in the posterior quadrant. Since the resonant frequency of the posterior quadrant of the TM is approximately 2000 Hz [3], damage to this quadrant can lead to a reduction in sound transmission at 2000 Hz and give a significant decrease in absorbance. Most of the TM responsible for low-frequency transmission remains intact, perhaps accounting for an absorbance pattern similar to that of

**Table 1.** Descriptive statistics (mean, *SD*, and median) of WBA obtained at peak and ambient pressure conditions in the normal ear group, central TMP group, and marginal TMP group

| Pressure condition | Frequency [Hz] | Normal ear group (*n* = 20 ears) | | | Central TMP (*n* = 65 ears) | | | Marginal TMP (*n* = 13 ears) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean | *SD* | Median | Mean | *SD* | Median | Mean | *SD* | Median |
| Peak pressure | 250 | 0.14 | 0.14 | 0.05 | 0.09 | 0.09 | 0.06 | 0.13 | 0.12 | 0.08 |
| | 300 | 0.17 | 0.17 | 0.06 | 0.12 | 0.13 | 0.09 | 0.17 | 0.17 | 0.12 |
| | 400 | 0.24 | 0.24 | 0.07 | 0.17 | 0.16 | 0.14 | 0.26 | 0.26 | 0.20 |
| | 500 | 0.33 | 0.32 | 0.1 | 0.21 | 0.18 | 0.17 | 0.32 | 0.24 | 0.24 |
| | 600 | 0.45 | 0.44 | 0.12 | 0.25 | 0.21 | 0.21 | 0.36 | 0.21 | 0.30 |
| | 800 | 0.64 | 0.66 | 0.12 | 0.26 | 0.24 | 0.20 | 0.29 | 0.25 | 0.21 |
| | 1000 | 0.76 | 0.78 | 0.08 | 0.28 | 0.31 | 0.16 | 0.28 | 0.28 | 0.22 |
| | 1250 | 0.8 | 0.8 | 0.07 | 0.31 | 0.35 | 0.10 | 0.21 | 0.23 | 0.16 |
| | 1500 | 0.79 | 0.8 | 0.09 | 0.33 | 0.36 | 0.13 | 0.19 | 0.22 | 0.17 |
| | 2000 | 0.83 | 0.86 | 0.11 | 0.34 | 0.32 | 0.14 | 0.19 | 0.24 | 0.03 |
| | 2500 | 0.76 | 0.8 | 0.18 | 0.44 | 0.32 | 0.24 | 0.33 | 0.34 | 0.23 |
| | 3000 | 0.61 | 0.61 | 0.18 | 0.44 | 0.28 | 0.49 | 0.42 | 0.25 | 0.41 |
| | 4000 | 0.37 | 0.38 | 0.17 | 0.56 | 0.22 | 0.54 | 0.52 | 0.18 | 0.53 |
| | 5000 | 0.25 | 0.22 | 0.11 | 0.60 | 0.16 | 0.57 | 0.44 | 0.21 | 0.54 |
| | 6000 | 0.19 | 0.18 | 0.09 | 0.48 | 0.19 | 0.44 | 0.54 | 0.22 | 0.50 |
| | 8000 | 0.2 | 0.18 | 0.1 | 0.44 | 0.23 | 0.43 | 0.37 | 0.17 | 0.34 |
| Ambient pressure | 250 | 0.13 | 0.13 | 0.04 | 0.09 | 0.10 | 0.07 | 0.14 | 0.12 | 0.14 |
| | 300 | 0.16 | 0.16 | 0.05 | 0.13 | 0.12 | 0.10 | 0.18 | 0.16 | 0.17 |
| | 400 | 0.23 | 0.23 | 0.07 | 0.19 | 0.16 | 0.16 | 0.27 | 0.26 | 0.20 |
| | 500 | 0.31 | 0.3 | 0.09 | 0.22 | 0.18 | 0.20 | 0.38 | 0.23 | 0.29 |
| | 600 | 0.42 | 0.42 | 0.12 | 0.25 | 0.21 | 0.20 | 0.41 | 0.24 | 0.32 |
| | 800 | 0.61 | 0.63 | 0.11 | 0.26 | 0.25 | 0.16 | 0.34 | 0.29 | 0.20 |
| | 1000 | 0.74 | 0.73 | 0.09 | 0.30 | 0.32 | 0.16 | 0.28 | 0.28 | 0.23 |
| | 1250 | 0.79 | 0.8 | 0.07 | 0.34 | 0.32 | 0.19 | 0.20 | 0.23 | 0.16 |
| | 1500 | 0.79 | 0.81 | 0.09 | 0.35 | 0.36 | 0.28 | 0.18 | 0.23 | 0.17 |
| | 2000 | 0.83 | 0.88 | 0.11 | 0.36 | 0.36 | 0.21 | 0.12 | 0.20 | 0.19 |
| | 2500 | 0.76 | 0.82 | 0.18 | 0.45 | 0.32 | 0.31 | 0.24 | 0.32 | 0.27 |
| | 3000 | 0.61 | 0.64 | 0.17 | 0.45 | 0.27 | 0.47 | 0.36 | 0.22 | 0.34 |
| | 4000 | 0.36 | 0.38 | 0.16 | 0.56 | 0.21 | 0.54 | 0.49 | 0.17 | 0.51 |
| | 5000 | 0.25 | 0.22 | 0.12 | 0.61 | 0.18 | 0.59 | 0.43 | 0.21 | 0.54 |
| | 6000 | 0.19 | 0.17 | 0.1 | 0.49 | 0.17 | 0.47 | 0.54 | 0.21 | 0.48 |
| | 8000 | 0.21 | 0.21 | 0.1 | 0.43 | 0.17 | 0.40 | 0.36 | 0.16 | 0.34 |

**Table 2.** Pairwise comparison between the groups (Mann–Whitney *U*-test) of WBA obtained between the normal ear, central TMP, and marginal TMP groups, together with effect size at peak and ambient pressure conditions. The bolded font indicates a significant difference with medium to large effect size. Significance levels: **p* < 0.05; ***p* < 0.01

| Pressure condition | Frequency [Hz] | Normal vs central TMP | | | Normal vs marginal TMP | | | Central vs marginal TMP | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *z* | *p* | *r* | *z* | *p* | *r* | *z* | *p* | *r* |
| Peak pressure | 250 | **3.81** | **< 0.01**** | **0.41** | 1.43 | 0.15 | 0.25 | 1.06 | 0.28 | 0.12 |
| | 300 | **3.45** | **< 0.01**** | **0.37** | 0.88 | 0.37 | 0.15 | 1.33 | 0.18 | 0.15 |
| | 400 | **3.33** | **< 0.01**** | **0.36** | 1.03 | 0.30 | 0.18 | 1.13 | 0.25 | 0.12 |
| | 500 | **4.03** | **< 0.01**** | **0.44** | 1.62 | 0.10 | 0.28 | **2.29** | **0.02*** | **0.35** |
| | 600 | **4.64** | **< 0.01**** | **0.51** | **1.95** | **0.05*** | **0.34** | **2.15** | **0.03*** | **0.34** |
| | 800 | **5.14** | **< 0.01**** | **0.56** | **3.64** | **< 0.01**** | **0.63** | 0.23 | 0.81 | 0.02 |
| | 1000 | **4.80** | **< 0.01**** | **0.52** | **4.01** | **< 0.01**** | **0.70** | 0.04 | 0.96 | 0.00 |
| | 1250 | **4.99** | **< 0.01**** | **0.54** | **4.76** | **< 0.01**** | **0.82** | 0.68 | 0.49 | 0.07 |
| | 1500 | **4.47** | **< 0.01**** | **0.49** | **4.76** | **< 0.01**** | **0.83** | 1.12 | 0.26 | 0.12 |
| | 2000 | **5.02** | **< 0.01**** | **0.55** | **4.79** | **< 0.01**** | **0.83** | 1.10 | 0.27 | 0.12 |
| | 2500 | **4.53** | **< 0.01**** | **0.49** | **3.13** | **< 0.01**** | **0.54** | 0.00 | 0.99 | 0.00 |
| | 3000 | 2.43 | 0.01* | 0.26 | **2.17** | **< 0.01**** | **0.38** | 0.22 | 0.82 | 0.02 |
| | 4000 | **3.08** | **< 0.01**** | **0.33** | **2.26** | **< 0.01**** | **0.39** | 0.30 | 0.75 | 0.03 |
| | 5000 | **6.29** | **< 0.01**** | **0.69** | **2.46** | **0.01*** | **0.43** | 2.10 | 0.03* | 0.23 |
| | 6000 | **5.79** | **< 0.01**** | **0.63** | **4.38** | **< 0.01**** | **0.77** | 0.63 | 0.52 | 0.07 |
| | 8000 | **4.36** | **< 0.01**** | **0.47** | **2.87** | **< 0.01**** | **0.50** | 0.76 | 0.44 | 0.08 |
| Ambient pressure | 250 | **2.99** | **< 0.01**** | **0.32** | 0.51 | 0.60 | 0.09 | 1.35 | 0.17 | 0.15 |
| | 300 | 2.39 | < 0.01** | 0.26 | 0.11 | 0.91 | 0.02 | 1.34 | 0.17 | 0.15 |
| | 400 | 2.06 | 0.03* | 0.22 | 0.33 | 0.74 | 0.03 | 0.99 | 0.32 | 0.11 |
| | 500 | **3.24** | **< 0.01**** | **0.35** | 0.24 | 0.81 | 0.03 | **2.76** | **< 0.01**** | **0.31** |
| | 600 | **4.43** | **< 0.01**** | **0.48** | 1.01 | 0.31 | 0.18 | **2.75** | **< 0.01**** | **0.31** |
| | 800 | **4.91** | **< 0.01**** | **0.53** | **2.80** | **< 0.01**** | **0.52** | 0.93 | 0.34 | 0.10 |
| | 1000 | **4.44** | **< 0.01**** | **0.48** | **4.09** | **< 0.01**** | **0.76** | 0.04 | 0.96 | 0.00 |
| | 1250 | **4.64** | **< 0.01**** | **0.53** | **4.80** | **< 0.01**** | **0.83** | 1.22 | 0.21 | 0.13 |
| | 1500 | **4.37** | **< 0.01**** | **0.47** | **4.77** | **< 0.01**** | **0.83** | 1.74 | 0.08 | 0.19 |
| | 2000 | **4.94** | **< 0.01**** | **0.54** | **4.82** | **< 0.01**** | **0.83** | **2.25** | **0.02*** | **0.35** |
| | 2500 | **4.38** | **< 0.01**** | **0.48** | **3.57** | **< 0.01**** | **0.62** | 1.29 | 0.19 | 0.14 |
| | 3000 | **2.38** | **0.01*** | **0.26** | **2.87** | **< 0.01**** | **0.50** | 1.01 | 0.31 | 0.11 |
| | 4000 | **3.35** | **< 0.01**** | **0.36** | **2.04** | **< 0.01**** | **0.35** | 0.92 | 0.35 | 0.10 |
| | 5000 | **6.09** | **< 0.01**** | **0.66** | **2.35** | **0.01*** | **0.41** | 2.36 | 0.01* | 0.26 |
| | 6000 | **5.85** | **< 0.01**** | **0.64** | **4.31** | **< 0.01**** | **0.75** | 0.63 | 0.52 | 0.07 |
| | 8000 | **3.88** | **< 0.01**** | **0.42** | **2.78** | **< 0.01**** | **0.52** | 0.65 | 0.51 | 0.07 |

**Table 3.** Wilcoxon signed rank results, its significant level of WBA, and effect size obtained across frequencies between pressure conditions (peak and ambient) across the groups. The bolded font indicates a significant difference with medium to large effect size. Significance levels: *$p < 0.05$; **$p < 0.01$

| Frequency [Hz] | Normal ear group | | | Central TMP group | | | Marginal TMP group | | |
|---|---|---|---|---|---|---|---|---|---|
| | *z* | *p* | *r* | *z* | *p* | *r* | *z* | *p* | *r* |
| 250 | **2.57** | **0.01*** | **0.57** | **1.89** | **0.05*** | **0.23** | 0.71 | 0.47 | 0.19 |
| 300 | **2.57** | **0.01*** | **0.57** | **2.13** | **0.03*** | **0.26** | 0.81 | 0.41 | 0.22 |
| 400 | **1.95** | **0.05*** | **0.43** | **1.94** | **0.05*** | **0.24** | 0.51 | 0.95 | 0.14 |
| 500 | **2.75** | **< 0.01**** | **0.61** | **2.40** | **0.01*** | **0.29** | 1.57 | 0.11 | 0.43 |
| 600 | **2.84** | **< 0.01**** | **0.63** | 0.80 | 0.42 | 0.09 | 1.24 | 0.21 | 0.34 |
| 800 | **2.93** | **< 0.01**** | **0.65** | 1.00 | 0.31 | 0.12 | **2.01** | **0.04*** | **0.55** |
| 1000 | **2.84** | **< 0.01**** | **0.63** | 0.46 | 0.64 | 0.05 | 0.30 | 0.75 | 0.08 |
| 1250 | 0.05 | 0.95 | 0.01 | **1.90** | **0.05*** | **0.23** | 0.59 | 0.55 | 0.16 |
| 1500 | 0.96 | 0.33 | 0.21 | 1.07 | 0.28 | 0.13 | 0.85 | 0.93 | 0.23 |
| 2000 | **2.53** | **0.01*** | **0.56** | 0.31 | 0.75 | 0.03 | **1.68** | **0.03*** | **0.46** |
| 2500 | **1.98** | **0.04*** | **0.44** | **2.11** | **0.03*** | **0.26** | **2.49** | **0.01*** | **0.68** |
| 3000 | 1.6 | 0.1 | 0.36 | 1.59 | 0.11 | 0.19 | **2.43** | **0.01*** | **0.67** |
| 4000 | 1.3 | 0.18 | 0.29 | 1.09 | 0.27 | 0.13 | **2.43** | **0.01*** | **0.67** |
| 5000 | 0.49 | 0.62 | 0.11 | 1.36 | 0.17 | 0.16 | **1.86** | **0.05*** | **0.51** |
| 6000 | 0.89 | 0.37 | 0.2 | 1.70 | 0.08 | 0.21 | 0.05 | 0.95 | 0.01 |
| 8000 | 0.89 | 0.37 | 0.2 | 0.35 | 0.72 | 0.04 | 1.07 | 0.28 | 0.29 |

normal ears. Additionally, the absorbance peaks at 4000 and 6000 Hz may be attributed to the complex vibratory pattern of the TM as discussed by Tonndorf and Khanna [3]. Moreover, higher absorbance at high frequencies might be linked to shorter wavelengths, enabling sound energy to pass through the TMP more effectively.

When the central and marginal TMPs are compared, it appears that marginal TMP exhibited greater absorbance at lower frequencies (up to about 1000 Hz). Conversely, central TMP showed greater absorbance at mid and high frequencies (except at 6000 Hz) under both peak and ambient pressure conditions. This difference may be attributed to the intact central portions in individuals with marginal TMP, which are responsible for transmitting lower frequencies. In contrast, greater transmission of higher frequencies in individuals with central TMP might be linked to the shorter wavelengths involved.

The current study also analyzed the difference in WBA between peak and ambient pressure conditions. Generally, the presence of a measurable peak is not commonly observed with a TMP. However, the present study obtained WBA measurements at peak pressure for the TMP group, possibly due to use of a wide-frequency stimulus. Our results align with the findings of other studies [15,17,19].

In the normal ear group, WBA measured at peak pressure was significantly higher at 250 to 1000 Hz compared to ambient pressure, while no difference was found at high frequencies. These findings are in line with previously reported studies [30,31]. The difference is attributed to the greater mobility of the TM at peak pressure, a condition where maximum energy enters the middle ear [32]. Under ambient pressure conditions, differences in the ear canal and in middle ear pressure can induce either positive or negative pressure, affecting the TM's stiffness and generating larger impedance, leading to reduced absorbance at low frequencies [33,34].

The study's findings on central TMP revealed a significant difference in WBA between the pressure conditions at 250 to 500 Hz and at about 2500 Hz, although there was a small effect size. The difference might be due to the presence of a large TMP. In contrast, individuals with marginal TMP exhibited a significant difference between ambient and peak pressure conditions at 800 Hz and 2000 to 5000 Hz, with medium to large effect sizes. This difference in pressure conditions may be due to factors such as TMP size and the point of contact between the TM and the sulcus in marginal TMP.

## Conclusion

This study demonstrates the effectiveness of WBA in distinguishing between central and marginal TMP. The observed distinct WBA patterns among different types of TMP highlight its potential as a valuable diagnostic tool in clinical settings. Furthermore, the study's outcomes point to possible reasons behind the contradictory findings in

previous studies, suggesting the need for more stringent subject selection criteria. Furthermore, the study underscores the importance of TMP types and highlights the need for further investigation into the size and precise location of perforations within specific quadrants, although it did not address size variations within TMP groups.

## Disclosure statement

There are no potential conflicts of interest.

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# Case studies

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# EFFECTIVENESS OF THE ADHEAR BONE CONDUCTION DEVICE FITTED BILATERALLY IN A CHILD WITH CONDUCTIVE HEARING LOSS: CASE STUDY

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Contributions:
A Study design/planning
B Data collection/entry
C Data analysis/statistics
D Data interpretation
E Preparation of manuscript
F Literature analysis/search
G Funds collection

## Abstract

**Introduction:** Frequently, bone conduction hearing aids on a softband are not tolerated by children due to pressure on the head or visual esthetics. By way of contrast, a non-surgical hearing system – Adhear (Med-El, Innsbruck, Austria) – allows the sound processor to be attached using a noninvasive adhesive adapter. The objective of this study was to evaluate the effectiveness of the Adhear system and assess its subjective benefits in a child who had bilateral conductive hearing loss.

**Case report:** The Adhear system was tested in a 13-year-old child with bilateral conductive hearing loss. Pure tone audiometry and speech audiometry in quiet were performed without and then with a pair of devices placed bilaterally. Word recognition scores (WRS) at 50 and 65 dB SPL in quiet were measured using the Pruszewicz monosyllabic Polish word test. After 4 weeks, subjective hearing benefit and experience with the Adhear system was done using the APHAB (*Abbreviated Profile of Hearing Aid Benefit*) questionnaire.

**Results:** With Adhear, WRS at 50dB SPL increased significantly from an unaided score of 10% to 80%. The result of free-field audiometry with Adhear on both sides indicated a hearing level within the normal range.

**Conclusions:** Adhear is an effective rehabilitation option for children with bilateral conductive hearing loss.

**Keywords:** Adhear • bone conduction hearing aid • bilateral conductive hearing loss

# SKUTECZNOŚĆ URZĄDZENIA NA PRZEWODNICTWO KOSTNE ADHEAR ZAŁOŻONEGO OBUSTRONNIE U DZIECKA Z NIEDOSŁUCHEM PRZEWODZENIOWYM: OPIS PRZYPADKU

## Streszczenie

**Wprowadzenie:** Aparaty słuchowe na przewodnictwo kostne na elastycznej opasce często nie są tolerowane przez dzieci ze względu na ucisk na głowę lub estetykę wizualną. Natomiast niechirurgiczny system słuchowy – Adhear (Med-El, Innsbruck, Austria) – umożliwia przymocowanie procesora dźwięku za pomocą nieinwazyjnego, samoprzylepnego elementu mocującego. Celem niniejszego badania była ocena skuteczności systemu Adhear i subiektywnych korzyści z jego stosowania u dziecka z obustronnym niedosłuchem przewodzeniowym.

**Opis przypadku:** System Adhear został przetestowany u 13-letniego dziecka z obustronnym niedosłuchem przewodzeniowym. Audiometrię tonalną i audiometrię mowy w ciszy wykonano najpierw bez urządzeń, a następnie z parą urządzeń, umieszczonych obustronnie. Wyniki rozpoznawania słów (WRS) przy 50 i 65 dB SPL w ciszy mierzono za pomocą testu identyfikacji polskich słów jednosylabowych Pruszewicza. Po 4 tygodniach subiektywne korzyści słuchowe i doświadczenia z systemem Adhear zostały ocenione za pomocą kwestionariusza APHAB (*Abbreviated Profile of Hearing Aid Benefit*).

**Wyniki:** Dzięki systemowi Adhear wynik WRS przy 50 dB SPL wzrósł znacząco – z 10% do 80%. Wynik audiometrii w polu swobodnym z Adhear po obu stronach wykazał poziom słyszenia w zakresie normy.

**Wnioski:** Adhear jest skuteczną opcją rehabilitacji dla dzieci z obustronnym niedosłuchem przewodzeniowym.

**Słowa kluczowe:** Adhear • aparat słuchowy na przewodnictwo kostne • niedosłuch przewodzeniowy obustronny

## Introduction

Early identification of hearing loss and ear diseases is key. According to hearing aid guidelines, a child with hearing impairment should be diagnosed before 3 months of age and fitted with a hearing aid before 6 months [1]. The selection and fitting of hearing aids for young patients is a complex diagnostic process that requires the cooperation of specialists in various areas [2,3]. The most important element in the selection of hearing aids is proper fitting and assessing the device's effectiveness.

Not all patients can be compensated for their hearing loss with classic air-conduction hearing aids. They include those with defects in the outer or middle ear, chronic otitis, or other inflammatory conditions. Typically, these subjects will have conductive or mixed hearing loss and require the use of bone conduction to allow the external sound to reach the inner ear directly, in this way bypassing damaged structures at the level of the outer and/or middle ear.

Bone conduction involves the transmission of sound through the bones of the skull to the inner ear. A bone conduction hearing aid changes the captured sound signal into vibrations of the bones of the skull, stimulating the fluids in the inner ear directly [4]. Unlike the many different models of classic hearing aids, there are only a few bone conduction devices available. They can be mounted on the patient's head using soft bands or eyeglass frames. These solutions are often not well accepted by children with conductive hearing loss because of head pressure, skin irritation, sweating, discomfort during long use, poor sound quality, or cosmetic stigma [5,6].

Another solution for patients with conductive hearing loss is a non-surgical hearing system, Adhear. The Adhear system (Med-El, Innsbruck, Austria) is a nonsurgical bone conduction hearing aid, available since 2017, which uses an adhesive patch to connect the sound processor to the skull. The device has a symmetrical design so it can be used on either ear. It is intended for patients with conductive hearing loss or unilateral deafness, either temporary or permanent. There are no age restrictions to using the device, and it is suitable for children as young as a few months. The Adhear system consists of a bone conduction audio processor that is held in place with an adhesive adapter placed over the mastoid behind the auricle (**Figure 1**). An integrated transducer in the sound processor converts sound into mechanical vibrations, which are carried by the adhesive adapter and transmitted through the skin to the mastoid and then directly to the inner ear.

The audio processor has dual microphones and is powered by a single P13 battery. A button allows the user to switch between programs, the number depending on the age and expectations of the user. There is a volume control, which can be turned off for the youngest users. The signal processor uses an automatic classifier to control the adaptive directional microphone system and suppress feedback. The proprietary adhesive adapter uses a non-toxic, non-allergenic medical adhesive tape to attach the adapter to the skin and provide good sound quality without pressure on the head or skin. It is water resistant and breathable, and can be used continuously for 3–7 days [7,8]. In comparison, bone conduction hearing aids on soft bands are often not well accepted by children because of head pressure, sweating, or visual stigma. The lack of a headband makes the system less conspicuous and more comfortable.

**Figure 1.** Right ear showing Adhear adapter (left) and processor in place (right)

Westerkull and colleagues presented the principles of a self-adhesive adapter and its capabilities and advantages in 2018 [9]. During development, the device was called Adjoin, but later marketed under its current name, Adhear. Two papers discussing the physics of adhesive transmission in bone conduction and summarising results from several other authors have confirmed its effectiveness [7,10]. These two works set out the results of pre-clinical testing and provide comparisons to the established soft-band arrangement.

Implantable devices using bone conduction of sound can also be used to improve hearing in patients with conductive hearing loss. Bone conduction implants are indicated for candidates who could not benefit from conventional hearing aids. The available implantable hearing devices nowadays are subdivided into two major categories: passive (e.g., BAHA – either Connect/Attract – or the Ponto device by Oticon) and active (e.g., Med-El Bonebridge and the Osia system by Cochlear). These devices are indicated in patients with stable bone conduction hearing thresholds within the recommended manufacturer's range. Bone conduction implants are a solution for patients over 5 years old [11]. Bilateral bone conduction fitting was successfully done and audiological benefits and patient satisfaction were shown [11].

For patients for whom previous surgical procedures have not given adequate benefits and for whom classic hearing aids cannot be used (or for various reasons decide against implantable solutions), bone conduction hearing aids are the only option to improve hearing.

For both non-invasive and implantable devices, research has emphasized the importance of early auditory rehabilitation for normal age-appropriate quality of life. Each case needs to be analyzed individually, looking at audiological aspects as well as the patient's subjective assessment. The emotional and behavioral difficulties involving children and adolescents with mild to profound hearing loss are primarily linked to concerns about relationships with peers. In this context, language and communication are important for the psychosocial development of children, as they are the main means of establishing and maintaining social interactions [12].

This paper presents a case report. Due to the specificity of the disorder, it is difficult to collect large material, and at present only a few papers have been published showing the bilateral use of the Adhear system in children. There is a need for more detailed research in this area. The purpose of this study was to evaluate the safety and efficacy of bilateral fitting of the non-invasive Adhear bone conduction device in a child with conductive hearing loss.

## Case report

This case concerns a 13-year-old female with bilateral conductive hearing loss due to congenital defect of the middle ears (**Figure 1**). The patient had used bilateral hearing aids. She had a history of chronic middle ear disease and interventions including ventilation tubes, but with no improvement in hearing. Due to chronic inflammation, the child could not use conventional hearing aids during treatment. Attempts were made to use bone conduction hearing aids on a soft band, but these were rejected due to the child's discomfort and reluctance. Due to deformity of the auricles, the parents are considering reconstruction and they do not want an implantable solution at this stage. After reviewing various bone conduction hearing aids, and based on audiometry and medical history, the Adhear system was selected bilaterally.

## Methods

Hearing tests for air and bone conduction were done. After the bone conduction devices were selected and set up, sound field thresholds and a word recognition test with and without the device were done. Sound field thresholds with the devices on both sides were measured using warble tones at 0.25, 0.5, 1, 2, 3, and 4 kHz with loudspeakers placed 1 m in front of the patient. Word recognition scores at 50 and 65 dB SPL in quiet were measured with speech coming from the front using the Pruszewicz monosyllabic Polish word test. Subjective evaluation of benefits from the Adhear were assessed using the APHAB questionnaire (*Abbreviated Profile of Hearing Aid Benefit*). APHAB comprises 24 questions about auditory functioning grouped into four categories: EC (*Ease of Communication*); BN (*Background Noise*); RV (*Reverberation*); and AV (*Aversiveness*). The patient completed the questionnaire before the devices were fitted and again one month after the Adhear system was fitted.

## Results

Results of pure tone audiometry, free-field audiometry, and speech audiometry in quiet are shown in **Figures 2**, **3**, and **4**. The result of free-field audiometry with Adhear on both sides indicated a hearing level within the normal range, with results better than 25 dB HL (**Figure 3**). With Adhear in place, WRS at 50 dB SPL increased significantly from an unaided score of 10% to 80% (**Figure 4**).

The results of the APHAB questionnaire confirm the benefits of the Adhear system (**Figure 5**). The most significant benefit was in the category of speech understanding in difficult acoustic conditions (RV), a factor that is particularly important for children. The AV scores were higher with the device, probably because the child needed more than a month to adapt to the new sounds. Adaptation time is individual and can take several months.

## Discussion

The audiological performance of the new device benefits from the low weight of the adhesive adapter, improved mechanical transmission, and, compared to a softband, a better position for stimulation close to the ear canal [13]. In 2019, Neumann and colleagues presented the first study evaluating the audiological and clinical outcomes of Adhear [14], where, in short- and mid-term follow-ups in children under 10 years of age, it was compared with conventional bone conduction devices integrated in softbands. The comparisons established that sound field thresholds (in quiet and noise) and WRSs were statistically indistinguishable between the devices. However, compared to the softband users, the Adhear children achieved

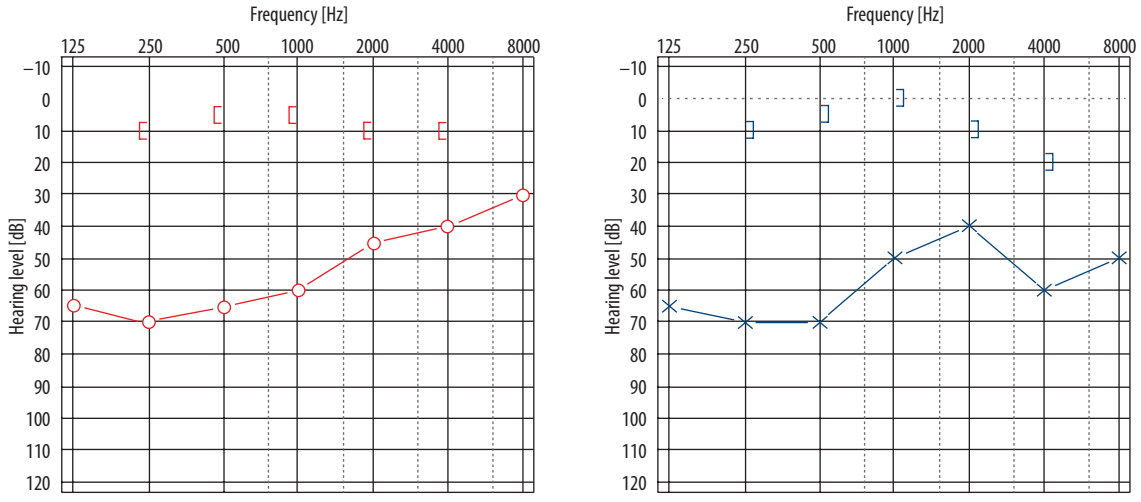


**Figure 2.** Pure-tone audiometry

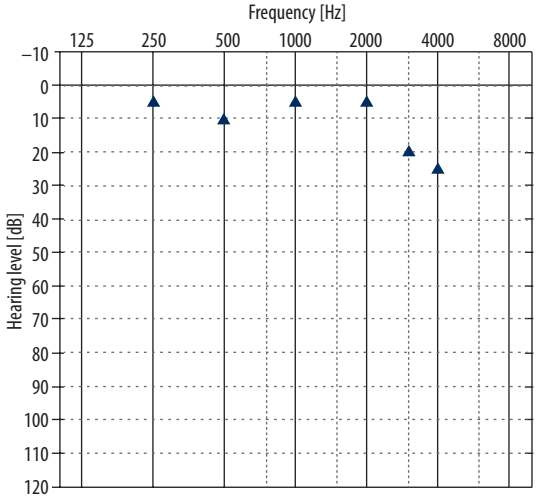


**Figure 3.** Free-field audiometry with Adhear system on both sides

significant improvements in thresholds at 1 and 8 kHz immediately after the first attachment, and needed no acclimatization time. The Adhear users achieved a mean WRS within normal limits (91%) in quiet and experienced only slight difficulties (78%) in noise.

Dahm et al. also reported good hearing benefits in 12 cases of Adhear use [15]. The aided threshold averaged 30.8 dB HL (*SD* ± 7.1) compared to an unaided threshold of 45.1 dB HL (*SD* ± 7.0). Speech reception threshold in quiet was 56.8 dB (± 6.1) and improved to 44.5 dB (± 6.4) in the aided condition, while WRS improved by about 30% at 65 dB SPL. Two questionnaires, SSQ12 and AQoL-8D, demonstrated a statistically significant improvement following 2 weeks of device use.

A clinical study by Skarzynski and colleagues [7] compared Adhear with a softband solution as well as with a magnetically attached bone conduction implant. Users of the implant received comparable hearing benefits to those who used Adhear. Mean aided sound field thresholds and speech understanding in quiet and noise were similar.

A study by Urik et al. [16] compared the results of patients with Bonebridge implants and with those using the Adhear system. There were 15 children with conductive hearing loss and using the Adhear device who were included in the study. In 5 cases, the Adhear device was used bilaterally. In this group, mean free-field outcomes improved from the unaided condition of 28.1 ± 0.9 dB HL to 17.3 ± 2.9 dB HL. In our presented case, free-field outcomes with bilateral Adhear were also within the range of normal hearing (**Figure 3**). Urik and colleagues also measured speech outcomes in quiet in 13 of their Adhear patients and this revealed a mean benefit of 23.1 ± 2.6. Speech-in-noise outcomes resulted in a mean benefit of 16.4 ± 12.0 for the Adhear group. Patient quality of life was also assessed and, as in the present study, confirmed the benefits of the device: the AQoL-6D utility score for the Adhear group was 0.75 ± 0.17 and improved to 0.85 ± 0.15 with use of the device.

A study by Liu et al. [17] has demonstrated the high effectiveness of the Adhear device in children with bilateral conductive hearing loss. The aim of their study was to characterize the auditory benefit and sound localization accuracy of bilateral bone conduction adhesives devices compared to unilateral devices. The mean unaided sound field hearing threshold was 57.9 ± 5.1 dB HL, while the mean aided hearing threshold for the right ear was 32.4 ± 5.3 dB HL, for the left ear it was 32.0 ± 5.6 dB HL, and for both ears it was 27.8 ± 5.3 dB HL. The mean unaided WRS was 4.6 ± 13.1%, while the mean aided WRS was 83.0 ± 10.1% for the right ear, 81.6 ± 14.5% for the left ear, and 90.3 ± 10.2% for both ears. In terms of sound localization accuracy, the MAE (mean absolute error) was 43.5 ± 19.0° in the unaided condition, 70.0 ± 8.5° for the right ear, 69.3 ± 9.4° for the left ear, and 51.2 ± 14.8° for both ears. It is worth noting that the average MAEs increased (worsened) significantly under unilateral fitting conditions. The results of Liu et al., like the present paper,

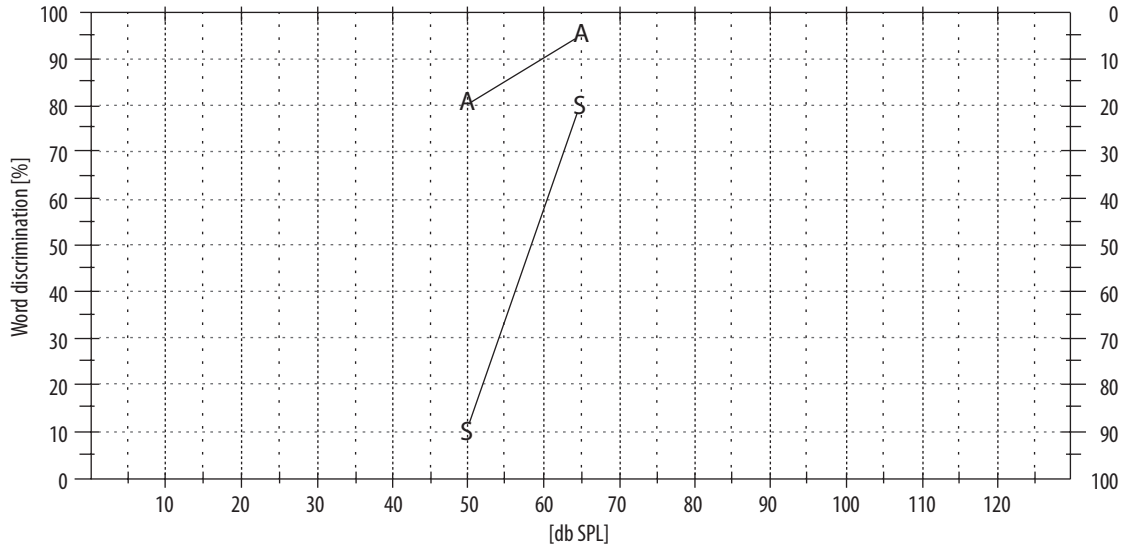


**Figure 4.** Speech audiometry. **A**, with Adhear on both sides; **S**, without the devices

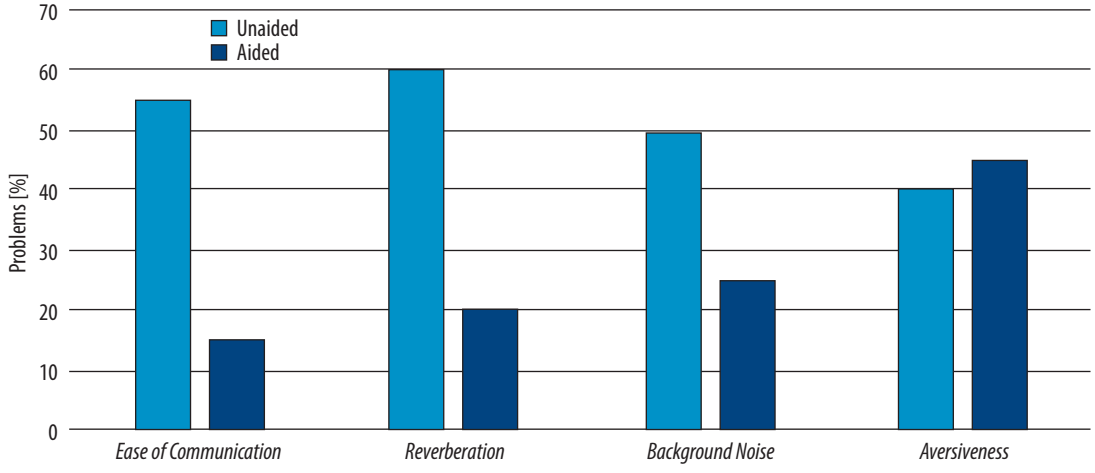


**Figure 5.** Results of APHAB questionnaire after 1 month of Adhear use

suggest that bilateral use of the Adhear system has a significant beneficial effect on speech perception; in addition, the study showed a better ability to localize sound in the bilateral mode compared to unilateral.

There are now many papers showing the results of using the Adhear system in children and adults [7,8,14,18–22]. Our results complement existing studies in the area of bilateral use of Adhear in children, and confirm the system's effectiveness in improving hearing, speech understanding, and quality of life.

## Conclusions

With the Adhear device, the 13-year-old child in this report showed significant improvements in hearing thresholds, speech recognition in quiet, and quality of life, confirming the effectiveness of the system. The Adhear device appears to be a good alternative to other bone conduction devices for children with conductive hearing loss.

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# Conference reports

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# REPORT FROM HEARING ACROSS THE LIFESPAN (HEAL 2024), 6–8 JUNE 2024, CERNOBBIO, LAKE COMO, ITALY

**Ewelina Bukato[1E], Aleksandra Kołodziejak[1E], Rita Zdanowicz[1E], Natalia Czajka[1E], Piotr H. Skarzynski[1,2E]**

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Following the last conference two years ago, the latest HeAL conference took place on 6–8 June 2024. This biennial conference brings together delegates from around the world, offering an opportunity to share experiences and expand their knowledge. This year's conference focused on contemporary audiology and related hearing sciences. Speakers presented results of centre-based studies, multicentre clinical trials, and research protocols.

The Institute of Physiology and Pathology of Hearing (IPPH) was represented by Prof. Piotr H. Skarzynski, Natalia Czajka, PhD, Aleksandra Kołodziejak, MSc, Ewelina Bukato, MSc, and Rita Zdanowicz, MSc.

At the opening ceremony, Dr Ferdi Grandori introduced the objectives of the conference, and in the early auditory training session, Dr Natalia Czajka presented two papers on Skarzynski's Stimulator of the Polymodal Sensory Perception. First she outlined ways in which therapy in our centre is conducted and set out what the Stimulator could do. In the second part, she presented the results of in-patient and home therapy. She demonstrated

Natalia Czajka presenting the results of in-patient and home therapy

the statistically significant effectiveness of both in-patient and remote therapy.


IPPH representatives at the HeAL conference: (from left) Rita Zdanowicz, Aleksandra Kołodziejak, and Ewelina Bukato

On the second day of the conference, Aleksandra Kolodziejak presented a paper on *Symptoms of auditory processing disorders (APD) in children with tinnitus*, Ewelina Bukato presented one on *Summary of the implementation of the 12 years of hearing screening programs among first and sixth grades attending the primary school*, and Rita Zdanowicz gave a pesentation on *Normative values of tests assessing auditory processing disorder (APD) for children aged 6 to 12*. All three received a lot of interest from the audience due to the impressive numbers of patients examined. There were also questions from the audience, who were interested to learn about the working and research methods at our centre.

Dr Sofya Vikhnina presented interesting results in her paper, *Central auditory processing disorders in children with congenital cytomegalovirus infection*, which focused on children with cochlear implants. The results showed that children with congenital CMV and a cochlear implant

Ewelina Bukato and Rita Zdanowicz at the poster session

perform worse than other CI users. The poorer outcomes may be due to CMV involvement in the nervous system as well as central auditory processing disorders.

In the Teleaudiology session, Matthew Bush compared results of clinical tonal audiometry with those of three hearing testing apps: EarTrumpet, Hearing Test & Ear Age Test, and Shoebox. There was a good correlation, both in patients with hearing loss and in those with normal hearing. The next presentation was *Telehealth and simulated patient learning environments: preparing students for the changing face of healthcare*. The main themes of the paper were an overview of Aston University's telehealth activities including the development of telehealth, examples of innovative telehealth practice, and challenges and the future in telehealth teaching. Next, Jamiee Rich presented *Healthy Ears: A telehealth-facilitated randomised-controlled trial utilising the "Blow, Breathe, Cough" health promotion programme to resolve otitis media with effusion in children*. The aim was to assess whether implementation of Blow, Breathe, Cough into an innovative telemedicine service model would increase the effectiveness of treatment of OME.

In the final part of the session, Greta Barnabei presented *Ear Portal: Using asynchronous teleaudiology to improve access to ear, nose and throat services for children with otitis media in an urban area*. The aim was to provide an introduction to the Ear Portal telehealth system which is a hospital-based system designed to reduce waiting times for children wanting to see an ENT specialist. The system has been proven to be work well: it is cost-effective and, as expected, has reduced waiting times to see a specialist.

In the poster session, the Institute of Physiology and Pathology of Hearing team presented six papers:

*Organisational aspects and results of the hearing screening programme in first-grade children in the Mazowieckie voivodeship,*
*Hearing screening in school-aged children from Kyrgyzstan: results of screening and observations,*
*Pilot hearing screening in school-aged children from Armenia, Russia, Kyrgyzstan and Azerbaijan,*
*Use of portable music players and other noise-related risks among children aged 11–12 years,*
*Aptitude of parental suspicion of hearing loss in children,*
*Prevalence of tinnitus in a sample of 43,064 children in Warsaw.*

All were of great interest due to the impressive size of the study groups and the unexpected results.

# 7th International Conference on Hyperacusis and Misophonia, 15–17 September 2024, Warsaw, Poland

Dear Colleagues,

The "*Journal of Hearing Science*" is pleased to publish the abstracts submitted for the 7th International Conference on Hyperacusis and Misophonia (ICHM7).

The ICHM7 takes place on 15–17 September 2024 in Warsaw, Poland. Three days of the conference will be filled with talks from a multi-disciplinary group of world-renowned keynote speakers bringing together studies of audiology, ENT, mental health, neuroscience, epidemiology, psychoacoustic, psychometry, neurology, and other areas. The program will feature key medical, surgical, rehabilitative, and mental health opinion leaders from the clinical and laboratory research sphere. We will also hear from persons who have lived with the experience of hyperacusis and misophonia and their families.

We are happy to welcome to Warsaw oto-rhino-laryngologists, audiologists, psychiatrists, psychologists, speech and language therapists, hearing aid dispensers, social workers, neuroscientists, researchers, and other professionals who are involved in research or care for patients with tinnitus, hyperacusis, and misophonia.

We wish you a productive and exciting meeting!

*Prof. Henryk Skarzynski, MD, PhD, dr. h.c. multi*
*Honorary President*

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# 7TH INTERNATIONAL CONFERENCE ON HYPERACUSIS AND MISOPHONIA, 15–17 SEPTEMBER 2024, WARSAW, POLAND

## Keynote Lectures

### Hyperacusis in Fragile X model of autism and chronic stress-induced hyperacusis

Salvi R.

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

**Objective 1**

**Introduction:** The biological mechanisms underlying hyperacusis are poorly understood, but medical conditions and genetic factors associated with this disorder could provide insights on underlying causes and treatments. Human investigations suggest that chronic stress may contribute to hyperacusis, but experimental evidence for this view is limited.

**Material and methods:** We pharmacologically induced chronic stress in rats by chronically administering corticosterone (CORT) stress hormone in drinking water. Loudness measures were obtained by measuring rat reaction time-intensity (RT-I) functions before and after chronic CORT treatment.

**Results and conclusions:** CORT treatment significantly reduced reaction times at suprathreshold intensities, behavioral evidence of loudness hyperacusis. Electrophysiological studies showed no change in the neural output of the cochlea in CORT-treated rats; however, sound evoked neural responses from higher auditory centers were significantly enhanced, evidence of sound-evoked neural hyperactivity in the central auditory pathway. Rats were tested for sound avoidance hyperacusis using an Active Sound Avoidance Paradigm (ASAP) in which sounds of increasing intensity were used to "drive" a rat from a preferred dark enclosure to a bright, open "avoidance" arena. Following Chronic CORT treatment, a 90 dB broadband noise was much more effective in "driving" the rat from the preferred dark enclosure into the bright, open "avoidance" arena, evidence that chronic stress had induced sound avoidance hyperacusis.

**Objective 2**

**Introduction:** Many individuals with autism and autism spectrum disorder (ASD) exhibit hyperacusis. Fragile X (FX) syndrome, a leading genetic cause of ASD, is associated with CGG expansions near the *Fmr1* gene resulting in FMRP protein deficiency.

**Material and methods:** We tested for loudness hyperacusis in FX syndrome by comparing RT-I loudness growth functions from male rats lacking the *Fmr1* gene with WT rats.

**Results and conclusions:** Fmr1 knockout and WT rats had similar thresholds in quiet, however, *Fmr1* knockout rats had significantly shorter reaction times at suprathreshold intensities than WT rats for pure tones and broadband noise, behavioral evidence of loudness hyperacusis in *Fmr1* knockout rats. Temporal integration of loudness was evaluated by measuring reaction time as a function of stimulus duration. Reaction times decreased as duration increased from 50 to 300 ms in WT rats, evidence of temporal integration of loudness. Reaction time in Fmr1 knockout rats were shorter than WT rats and RTs showed little change with duration, evidence of aberrant temporal integration in *Fmr1* rats. The MTEP-mGlu5 receptor has been implicated in autism. To investigate its role in hyperacusis, reaction-time intensity functions were measured in Fmr1 knockout rat and WT rats before and after treatment with an MTEP mGlu5 receptor antagonist. The MTEP mGlu5 receptor antagonist had no effect on the reaction times of WT rats, whereas it dose-dependently increased reaction times in *Fmr1* KO rats to values similar to those WT rats. These results suggest that MTEP-mGlu5 antagonists might be clinically effective at suppressing loudness hyperacusis in individuals with ASD.

***Prof. Richard J. Salvi*** *has conducted numerous studies on plasticity of the central auditory system, sensory hair cell loss and regeneration, noise-induced hearing loss, tinnitus, hyperacusis, auditory perception, brain imaging, cell death and neuroprotection.*

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### Exploring the neurochemical and psychophysiological basis of misophonia and hyperacusis

Ward J.[1], Rinaldi L.[1], Ronen I.[2], Agbude R.[1], Forster S.[1], Makowski D.[1], Simner J.[1]

[1] *School of Psychology, University of Sussex, Brighton, UK*
[2] *Brighton and Sussex Medical School, Brighton, UK*

Misophonia and hyperacusis are typically defined in terms of a narrow versus broad range of trigger sounds and are often linked to different aspects of a sound (sound meaning for misophonia, loudness for hyperacusis). However, co-morbidity between these conditions suggests possible mechanistic overlap. In two studies we examine the neurochemical and psychophysiological basis of misophonia (using a validated questionnaire) and hyperacusis (based on uncomfortable loudness thresholds). The technique of magnetic resonance spectroscopy (MRS) uses MRI to estimate the concentration of excitatory and inhibitory neurotransmitters in the brain, enabling us to explore whether either type of sound intolerance is linked to general levels of excitability in key regions of the brain (auditory cortex, insula). No differences were found. In a separate experimental study, participants engaged in an attention-demanding visual task whilst concurrently measuring responsiveness to task-irrelevant sounds (in terms of level

of distraction, psychophysiological responsiveness). Here we do find that different aspects of the task are differentially sensitive to misophonia and hyperacusis. Misophonia is linked to heightened responsiveness to sounds whereas hyperacusis is linked hyper-vigilance.

**Jamie Ward** *is a Professor of Cognitive Neuroscience in the School of Psychology at the University of Sussex, UK. He specialises in individual differences in perception and its neural and cognitive basis.*

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**Loudness hyperacusis: mechanisms of loudness perception and their breakdown**

Moore B.C.J.

*Cambridge Hearing Group, Department of Psychology, University of Cambridge, UK*

There may be several different forms of hyperacusis (Tyler et al., 2014). This presentation is concerned with "loudness hyperacusis", for which sounds with medium and high levels appear to be louder than normal. The normal perception of loudness can be understood using a model that takes into account the processing of sounds in the peripheral auditory system (Moore et al., 1997). This model has been modified to take into account the perception of loudness by people with cochlear hearing loss (Moore and Glasberg, 2004). The model predicts the loudness recruitment that typically is associated with cochlear hearing loss, and it also predicts that hearing loss can sometimes be associated with "over-recruitment", so that some sounds appear louder than normal. However, the model does not account for abnormalities of loudness perception, like hyperacusis, that can occur for people with normal or near-normal audiograms. This suggests that factors associated with higher levels in the auditory system need to be taken into account. The effects will be discussed of several factors that are not currently taken into account in the loudness model, but that can influence the perception of loudness. These include: the functioning of the efferent system that regulates the active mechanism in the cochlea; central plasticity and adaptation effects; the influence of visual stimuli; and the influence of the perceived properties of the sound source.

***Brian Moore*** *is Emeritus Professor of Auditory Perception in the University of Cambridge. His research focuses on the perception of sound by people with normal and impaired hearing, and on the design and fitting of hearing aids. He is a Fellow of several prestigious societies, such as the Royal Society of London and the Academy of Medical Sciences. He has received the Silver and Gold medals from the Acoustical Society of America, and awards from the American Academy of Audiology, the Association for Research in Otolaryngology, and the American Auditory Society. He has an Honorary Doctorate from Adam Mickiewicz University, Poland. He has published 22 books and over 700 refereed journal articles.*

## Workshops

***4C Hyperacusis/Misophonia Management Questionnaires*: a method to enhance patient's readiness for therapy**

Aazh H.

*Hashir International Specialist Clinics & Research Institute for Misophonia, Tinnitus and Hyperacusis, London, UK*

Patients may not choose therapies involving talking, such as cognitive behavioural therapy (CBT), as the first choice for managing issues related to their health, including hyperacusis and misophonia, despite the research evidence supporting the effectiveness of such therapies. The first choice is usually to seek a medical cure. In the absence of a medical cure, patients with hyperacusis and misophonia believe that only way to cope is by using avoidance and ritualist behaviours (e.g., over use of ear protection, isolating themselves, avoiding certain environments or people, telling people not to make noise, and so on). Therefore, the decision to take part in a therapy programme instead of engaging in avoidance and ritualist behaviours constitutes a change. One of the reasons in favour of the status quo and against the change is the patient's lack of confidence in their ability to make the change (i.e., they are not confident that they can abandon avoidance behaviours and rituals and learn a different way of managing their problem). For example, a patient with misophonia may think that "I do not think that I can control my anger even with CBT. It is easier to avoid people at mealtimes" or a person with hyperacusis may think "I can avoid pain in my ears by avoiding loud sounds, but I doubt that I can use CBT to avoid pain." This workshop aims to: (1) discuss factors related to improving patient's readiness and motivation for therapy, (2) practise utilising *4C Hyperacusis and Misophonia Management Questionnaires*. Using 4C questionnaires helps the clinician to encourage the patient to explore their own strengths, motivations and resources that can help them to learn and apply the CBT skills in order to manage their hyperacusis or misophonia without relying on avoidance and ritualist behaviours, (3) report psychometric properties of the 4C questionnaires.

***Dr. Hashir Aazh*** *is an academic clinician and over the last 20 years he has developed and managed several Tinnitus Clinics in the UK. His clinical and research interest is on rehabilitative therapies for tinnitus, hyperacusis and misophonia for children and adults. He was the head of the specialist tinnitus clinic at the Royal Surrey County Hospital NHS Foundation Trust UK for over a decade (2010–2021). He has written over 50 scientific papers in the field of Audiology and has trained over 1000 audiologists, psychologists and other healthcare professionals in his Tinnitus Masterclass. Hashir is Honorary Hearing Research Consultant at the Royal Surrey NHS Foundation Trust (UK), Affiliate Associate Professor at Florida Atlantic University (USA) and Visiting Research Fellow at the University of Surrey (UK). He has served as Managing Editor of the journal Noise and Health, Associate Editor of the International Journal of Audiology, Editor-in-Chief of the journal Iranian Audiology, a member of the Editorial board of the journal Auditory and Vestibular Research, and the Secretary of the British Society of Audiology.*

**Differential diagnosis between misophonia and mental health disorders**

Jaffe J.J.

*Private Practice, Sherman Oaks, CA, US*

Assist audiologists in distinguishing misophonia from other mental health issues that would most appropriately be referred to mental health services. Audiologists are increasingly learning to use CBT (cognitive behavioral therapy) to assist their patients with misophonia management, following a structured plan developed for their implementation. While this approach might be helpful for patients, it is incumbent upon providers to recognize limitations when deeper issues may be present. Misophonia typically presents with several features that could also, or instead, indicate a more complex mental health condition. For example, misophonia patients commonly exhibit high degrees of perfectionism, rigidity, and excessive preoccupation with rules, including harsh judgment of others who – in their view – are disrespectful of their rules. But these can also be symptoms of OCD (obsessive compulsive disorder) or OCPD (obsessive-compulsive personality disorder), which need to be addressed by a qualified mental health professional. There are other frequent concurrent symptoms indicative of underlying co-occurring psychological problems involving depression, anxiety, self-injury, or possible history of trauma. Several case studies will illustrate these distinctions and highlight the significant role of psychotherapists in collaboration with audiologists or ENTs in treatment of misophonia and tinnitus.

***Jaelline Jaffe**, PhD, has been a licensed psychotherapist in Southern California since 1976, working with individuals, couples, and medical issues, which led to her establishing LemonAidCounseling.com. For the past dozen years, her practice has focused almost exclusively on sound sensitivity disorders, mainly Tinnitus and Misophonia. She has worked with many hundreds of Misophonia patients from age 8 to 70, mostly with teens and young adults, who often find her via her website, SensitiveToSound.com. Dr. Jaffe often works in conjunction with audiologists across the US to assist their patients with the intense emotional and family issues associated with Tinnitus, Misophonia, and Hyperacusis. She has presented on these topics at numerous professional conferences for audiologists as well as for psychotherapists. Dr. Jaffe is a cofounder and Board member of the Misophonia Association, and also program coordinator for their annual convention for hundreds of patients and families. The 11th annual convention will take place in November 2024 in Atlanta, GA. Dr. Jaffe is author of the upcoming book "These Sounds are Driving Me Crazy!" Training for Mental Health Professionals in Treating Sound Sensitivity Disorders.*

**Using the *Duke Misophonia Questionnaire* and *Duke Misophonia Interview* in evidence-based treatment planning for adults**

Rosenthal M.Z.

*Duke University, Durham, NC, USA*

This 90-minute workshop will provide an overview of clinical procedures used at the Duke University Center for Misophonia and Emotion Regulation to assess and develop treatment plans for adults with misophonia. Dr. Rosenthal will review how to use the *Duke Misophonia Questionnaire* (Rosenthal et al., 2021) and *Duke Misophonia Interview* (DMI; Guetta et al., 2022) as psychometrically validated assessment measures for misophonia in the context of other assessment approaches. Using a combination of quantitative and qualitative measurement, treatment planning will be discussed using an evidence-based transdiagnostic model for behavioral health. Specific candidate interventions will be outlined using approaches that are brand specific (e.g., *Unified Protocol adapted for Misophonia*; McMahon et al., 2023) as well as those that are brand agnostic and individually tailored to each patient (e.g., *Process based therapy for Misophonia*; Rosenthal et al., 2023). All behavioral health treatment planning will be discussed within the broader framework of a multi-disciplinary model of care (e.g., audiology, occupational therapy, psychiatry, etc.). The primary goal of this workshop is to provide attendees with a clear framework to assess and treat misophonia that combines the use of psychometrically validated measures with qualitative functional analyses to develop transdiagnostic and evidence-based individualized treatment plans for adults with misophonia, all within a multi-disciplinary framework.

***Dr. M. Zachary Rosenthal** is a clinical psychologist, Associate Professor, and Director of the Duke Center for Misophonia and Emotion Regulation. He is a clinician, scientist, educator, mentor, and advocate and has a lived experience as a loved one of someone with Misophonia.*

## Oral Presentations

### Audiologic assessment in misophonia

Campbell J., Feeley A.

*Department of Speech, Language, and Hearing Sciences, Central Sensory Processes Laboratory, University of Texas, Austin, TX, USA*

The recent misophonia consensus definition states "Misophonia is a disorder of decreased tolerance to specific sounds or stimuli associated with such sounds" (Swedo et al., 2022). Although designated as a decreased sound tolerance disorder, little information is provided on the typical audiologic profile of these patients (Campbell et al., 2023; Ralston and Campbell, 2024). There is some evidence that extended high frequency pure tone testing may reveal significantly better thresholds in adults with normal thresholds and minimal tinnitus (Campbell, 2019), suggesting that in normal hearing, atypical auditory perception may be related to a 'heighted awareness' of sound (Campbell et al., 2023). Thus, the goal of this study is to complete an audiologic battery on normal-hearing listeners with misophonia to determine whether heightened awareness of sound may be likely (via extended high frequency thresholds and speech-in-noise outcomes), without hyperacusis being present (via ULL measures and the Khalfa HQ). Twelve individuals with misophonia were compared to twelve normal-hearing controls for right and left PTA, high frequency PTA, extended high frequency PTA, and QuickSIN scores. In the misophonic group, audiometric measures were correlated with the Duke Misophonia Questionnaire subscales. No significant group differences were found, nor correlations between audiometric data and misophonic symptoms. However, it was of interest that all participants with misophonia scored positive on the Khalfa HQ, indicative of hyperacusis, while ULL results were in the normal range. Clinical implications of these preliminary findings and future research are discussed.

***Julia Campbell*** *obtained a clinical doctorate in Audiology (AuD) and triple research doctorate in Speech, Language, and Hearing Sciences, Cognitive Neuroscience, and Behavioral Neuroscience (PhD) from the University of Colorado at Boulder. Currently she is a Clinical Associate Professor in the Department of Speech, Language, and Hearing Sciences at the University of Texas at Austin, where she serves as the Audiology Program Director and PI of the Central Sensory Processes Laboratory. Julia Campbell also serves as the chair of the scientific advisory board for the Misophonia Research Foundation. Julia's research interests are focused on the identification of an objective measure of tinnitus using EEG, and she has published on this and related topics in the "American Journal of Audiology", "Journal of Speech, Language, and Hearing Research", and the "Journal of the American Academy of Audiology", among others.*

### Clinical assessment of hyperacusis

Branco-Barreiro F.C.

*Department of Phonoaudiology, Federal University of São Paulo, Brazil*

Hyperacusis is a hearing disorder characterized by an increased sensitivity to everyday environmental sounds. There is no objective test to confirm its existence. Therefore, it needs to be assessed by subjective measures. There is no standard protocol for evaluating hyperacusis. A minimum protocol should include audiological tests, loudness discomfort levels and self-report questionnaires.

***Prof. Fátima Cristina Alves Branco-Barreiro*** *has a degree in Audiology and Speech Therapy, Master's Degree in Audiology and PhD in Neuroscience and Behavior. She is a Professor of Audiology and Otoneurology at the Federal University of Sao Paulo, Brazil.*

### Combining psychoeducation, sound exposure and counseling in hyperacusis therapy

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[2] *Department of Child Health, The Netherlands Organization for Applied Scientific Research TNO, Leiden, The Netherlands*

**Introduction:** Hyperacusis is characterized by an increased sensitivity to everyday sounds. Patients with hyperacusis find sounds that are normal to others intolerably loud and uncomfortable. This can cause emotional distress and avoidance of sounds and noisy environments. So far, there is a clinical need in finding a hyperacusis therapy. Extensive efforts have been made to develop treatments for hyperacusis, including cognitive behavioral therapy, tinnitus retraining therapy, counseling, hearing devices, pharmacological therapy, and more. Nevertheless, there is no established clinical treatment for hyperacusis.

**Objective:** Our main objective was to investigate if combining psychoeducation, sound exposure and counseling in a clinical setting could possibly result in an useful clinical tool to help people with hyperacusis.

**Material and methods:** All patients were referred by General Practitioners and Ear, Nose and Throat Specialist to the Speech and Hearing Centers situated in Hengelo and Zwolle, The Netherlands. The patients primary complaint was an intolerance to sounds. A total of 30 patients without hearing loss, 15 males and 15 females, aged between 24 and 76 years were included in this study. First of all, the patients received psychoeducation about the functioning of the auditory system and counseling and information associated with hyperacusis. After the auditory assessment, they made a selection of their five most disturbing sounds from our database with daily sounds. During the therapy sessions, the social worker

carefully fine-tuned the volume to reach a safe maximum level, consistently monitoring the patient's physical and emotional reactions during the procedure. The sound intensity on the audiometer was incrementally raised until a range of 70–80 dB sound pressure level was reached across the sessions. Subsequent therapy sessions occurred biweekly. Short--term effects between the start and the end of therapy were based on tolerable level of sound exposure, subjective level hinderance of hyperacusis and sensitivity to sound using the *Hyperacusis Questionnaire*. The long-term effect was based on *Hyperacusis Questionnaire* six months after the end of therapy. Linear mixed effects and regression models were applied to study outcomes over time.

**Results:** Results showed a significant increase of exposure level, a significant decrease in sensitivity to daily sounds, and a significant decrease in *Hyperacusis Questionnaire* between the start and the end of therapy. The mean number of sessions during therapy was six and ranged between four and eight. There was no significant change in *Hyperacusis Questionnaire* after the end of therapy and 6 months later. This study has been evaluated for people without hearing loss. Furthermore, it is recommended to clarify the influence of both tinnitus and hearing loss on the therapy result.

**Conclusions:** The therapy decreased short- and long-term sensitivity to sound in patients with hyperacusis. The therapy had a positive impact on the daily life of patients with hyperacusis by reducing auditory sensitivity, not only for the sounds used in the therapy sessions, but also a transfer to daily sounds, therefore making this exposure therapy an effective therapy.

***Sandrien Thieren*** *started her study Speech-Language Pathology and Audiology at the University of Leuven, Belgium. After she received her Master's degree in Speech-Language Pathology, she obtained her Master's degree in Audiology. After completing her studies, she started working in the Netherlands as a clinical audiologist. Since 2019, she works as an audiologist at Pento Hearing and Speech Centers, where she sees children and adults from diagnosis to rehabilitation. Her area of expertise and research interests is on tinnitus and hyperacusis.*

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**Diagnosis and treatment of misophonia and hyperacusis based on the neurophysiological model**

Jastreboff M.M.[1,2], Jastreboff P.J.[1,2]

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[2] *Department of Otolaryngology, Emory University School of Medicine, Atlanta, GA, USA*

Hyperacusis and misophonia are subcategories of decreased sound tolerance. They may occur individually, but frequently they take place concurrently. In both disorders people exhibit reduced tolerance to sounds normally not bothersome for the average listener and reactions to bothersome sounds are very similar, even identical. However, there are distinctive differences between these conditions which are essential in their differential diagnosis and their treatments. Detailed patients' interview is necessary, with questions focusing on description of bothersome sounds, situations associated with sound, reactions to sound, and how they affect everyday activities, identifying pleasant sounds and other factors influencing the responses to bothersome sounds. Reactions to sound of hyperacusis patients depend predominantly on physical characteristics of the sound, particularly to sound energy (linked to its intensity). The meaning of the sound is irrelevant as well who/what produces it and in which environment. The loudness discomfort levels (LDLs) are lower than normal average value of 100 dB HL and they are typically below 80 dB HL. Unfortunately, low values of LDLs cannot be used as a proof of hyperacusis presence as low values can be due to misophonia as well. It is postulated that the problems experienced by patients arise from abnormal amplification of sound-evoked activity within the auditory system, with other systems in the brain activated only as secondary consequence. In misophonia sound evoked activity within the auditory system is normal, but improper connections between the auditory system and other systems in the brain develop at subconscious level as a result of temporal association of the presence of misophonic triggers with negative emotional or physical situation. The values of LDLs can be from as low as 20 dB HL to 120 dB HL and cannot be used to diagnose the presence of misophonia. Detailed patients' interview is essential. Desensitization with the variety of sounds has been proposed and successfully used to reverse abnormal gain within the auditory pathways and by this treat hyperacusis for many years already. The sounds and the protocols of their use are modified to follow guidelines of the neurophysiological model. The advice about the use of sound is combined with counseling focusing on explanation of mechanisms of hyperacusis. For misophonia treatment based on passive and active extinction of subconscious conditioned reflexes is implemented, with stress on the principle of complex conditioned stimuli and on involvement of generalization principle. Detailed counseling, tailored to the case of a specific patient, is crucial to achieve a successful outcome of the treatment. The treatments for hyperacusis and misophonia are distinctively different and treatment for hyperacusis is not successful for misophonia and treatment for misophonia has limited effectiveness for hyperacusis. Analysis of over 200 patients with decreased sound tolerance showed an over 80% success rate of significant improvement.

***Margaret M. Jastreboff****, PhD has been involved in tinnitus and decreased sound tolerance research since 1984, and on a full-time basis since 1991 while working at the University of Maryland School of Medicine. Her experimental work encompassed the study of the mechanisms of tinnitus using molecular biology, pharmacology, and behavioral techniques, including testing drugs for their effectiveness for tinnitus attenuation. She has been involved in clinical work for over 30 years while working first as an Associate Professor at Emory University, later as a Visiting Research Professor at Towson University and currently at JHDF, Inc. Collaborating closely with her husband Pawel J. Jastreboff, PhD, in 2001 she proposed a concept, name and treatment for specific a version of decreased sound tolerance – misophonia – when patients exhibit negative reactions to specific for a given patient patterns of sound. After over 35 years in academia, where she was involved in basic science and clinical research, teaching Au D students and treating patients, she become a President of JHDF, Inc., a non-profit foundation dedicated to research and education in the field of tinnitus and decreased sound tolerance as well as treating patients.*

**Evaluation of hearing in noise performance in patients with misophonia: preliminary results**

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[2] *Vestibular and Speech Disorders Diagnosis and Rehabilitation Center, Ankara University İbn-i Sina Hospital Audiology, Ankara, Turkey*
[3] *Department of Audiology, Faculty of Health Sciences, Ankara University, Ankara, Turkey*

**Introduction:** Misophonia is a condition of showing excessive and negative emotional reactions to certain sounds. People with this condition experience intense emotions such as extreme anger, disgust or anxiety to sounds that are common in everyday life, such as chewing, clicking, breathing, swallowing or clicking a pen. Although it has been reported that misophonia may affect hearing, sleep, concentration and emotional well-being, it has not been determined exactly how hearing skills are affected.

**Aim:** The aim of this study was to evaluate the hearing in noise performance in patients with misophonia.

**Material and methods:** The participants consisted of 8 adult individuals who presented to the Audiology, Balance and Speech Disorders Diagnosis and Rehabilitation Unit of Ibn-i Sina Hospital, Ankara University Faculty of Medicine, Ankara, Turkey, with the complaint of misophonia and 8 adult controls. Each participant underwent audiologic evaluation (pure tone audiometry, speech audiometry, immitansmetric examination, speech tests) and decreased sound tolerance assessment (scales and questionnaires, loudness discomfort level measurement). *Khalfa Hyperacusis Scale* (HQ), *Misophonia Symptom List* (MSL) and loudness discomfort level measurement (LDL) were used together to differentiate decreased sound tolerance. Individuals who were reported to have normal hearing on audiologic evaluation and who were moderately or severely disturbed by at least three sounds on the MSL were included in the misophonia group. The Turkish hearing in noise test (HINT) adaptive, test in noise front condition was administered to all participants in two conditions, with and without the presence of the misophonic stimulus. The HINT test was initiated at 65 dB, whereas the misophonic stimulus was presented at the same level of intensity as in daily life. The signal-to-noise ratio of the two HINT conditions were compared within themselves and with the control group.

**Results:** Hearing in noise performance was worse in the individuals with misophonia compared to the control group ($p < 0.05$). Hearing in noise performance was better in the presence of a misophonic stimulus than in the absence of a misophonic stimulus. However, this difference was not statistically significant ($p > 0.05$).

**Conclusions:** Individuals with misophonia may experience hearing in noise problems, especially in the presence of triggering stimuli. Future studies should be planned to evaluate this situation and should be taken into consideration when evaluating the effects of misophonia.

***Nazife Öztürk Özdeş*** *is a research assistant in audiology at Ankara University, Turkey and a PhD student in her thesis period. Her main academic interests are tinnitus, decreased sound tolerance and auditory processing. She is currently working in the audiology clinic of Ankara University, Turkey, where she is interested in the diagnosis and rehabilitation of tinnitus and decreased sound tolerance. In addition, she has been working on the development of a rehabilitation program for individuals with hyperacusis and misophonia. She is also a volunteer research assistant at Hashir International Specialist Clinics & Research Institute for Misophonia, Tinnitus and Hyperacusis. She is a student member of the International Society of Audiology.*

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**Examining cognitive, emotional, auditory, and family functioning in children with misophonia**

Siepsiak M., Turek A., Michałowska M., Gambin M.

*Faculty of Psychology, University of Warsaw, Poland*

Misophonia typically emerges during childhood, yet research in this demographic is limited. This study aimed to investigate a wide range of characteristics in young individuals with misophonia. We examined 90 Polish children between the ages of 7 and 18, including a control group, along with their parents, utilizing interviews, audiological assessments, questionnaires, and performance-based tests. The first set of results has already been published. Among the main findings, it was observed that children with misophonia did not significantly differ from their peers in terms of developmental disorders, emotional and social competencies, head injuries, epilepsy, tinnitus, or perinatal factors. However, they exhibited higher levels of anxiety and depression, an increased incidence of OCD, migraines, and psychosomatic complaints. Additionally, their mothers more frequently self-reported postpartum depression compared to mothers in the control group. The results not yet published can be categorized into three aspects of functioning: 1) Regarding hearing loss and the seven assessed tests of so-called central auditory processing, no significant group differences were found ($p > .05$). However, the misophonia group demonstrated faster reactions to visual stimuli tests. 2) Concerning cognitive functioning, children with misophonia outperformed the control group in divided attention ($p = .038$, Cohen's $d = .62$) and inhibitory control ($p = .004$, Cohen's $d = .76$). In logistic regression, better inhibition emerged as a significant predictor for the likelihood of having misophonia, even after adjusting for gender and IQ. No significant group differences were observed in cognitive speed processing, auditory short-term memory, abstract reasoning, verbal reasoning, mean grades for school performance, and behavior. 3) The data on parental stress and the relationship between the child and parent are currently under analysis and will be presented during the conference. The results of this study are in line with most of the previous preliminary reports and the most recent outcomes. However, they also shed light on new aspects of the early development of misophonia and indicate the need for further exploration, particularly in examining better attentional and inhibitory aspects as potential mechanisms underlying misophonia.

***Anna Turek*** *is a PhD candidate in the Individual Differences Department at the Faculty of Psychology, University of Warsaw, Poland. She is actively involved in research on misophonia and provides therapeutic services to individuals affected by the disorder.*

**Exploring hyperacusis through art: a journey of connection and understanding**

Lawton I.

*Department of Art and Music, Solent University, Southampton, UK*

As an artist and someone who suffers from Hyperacusis, I have delved into my own creative journey to explore and express my personal experience of living with this condition. My goal is to connect with others through my art, aiming to communicate the intricacies of what it is like to live with Hyperacusis. Arts Based Research, as Barone and Eisner (2012) argue, seeks to create expressive work that allows viewers to empathetically engage with the lives of others, fostering a deeper understanding of the studied situation. Incorporating art forms into research processes, as highlighted by Boydell et al. (2012), serves multiple purposes such as evoking emotional responses, promoting shared storytelling, and constructing alternative forms of representation, thus offering different ways of knowing. In my presentation, I will be sharing the ongoing visual outcomes of my artistic exploration. Barone and Eisner (2012) emphasise that art-based research does not aim to provide a single correct answer but rather to illuminate complex interactions and raise questions about important social and cultural issues. Similarly, Heidegger (1935) posits that art is not merely about creating objects but is a process through which artists engage with the world, deepening their understanding of existence. Through my artwork, I aim not only to understand my own experiences but also to connect with fellow sufferers, as Rubin (2023) suggests that art has the power to transcend language barriers and foster healing connections between the artist and the audience. Additionally, my involvement in the "Sound Off Competition" and running workshops with my students have provided opportunities to explore the transformative potential of participatory arts research. Hogan et al. (2015) and Charon (2021) highlight the benefits of participatory research, including the ability to explore difficult subjects, create something new, and provide relief and recognition for sufferers. By sharing experiences and creating a sense of community, participatory research, including arts-based approaches, plays a vital role in restoring well-being and fostering understanding among individuals with similar experiences (Van Der Kolk, 2014). In summary, my artistic journey not only deepens my understanding of Hyperacusis but also aims to connect with others, foster empathy, and contribute to the broader discourse on health and well-being through the visual arts.

***India Lawton*** *following her graduation with a BA (Hons) in Photography from The Arts University Bournemouth, India pursued further studies, obtaining an MA in Photographic Studies from Westminster University, a Postgraduate Certificate in Education (PGCE) from Oxford Brookes University, and subsequently an MA in Education, also from Oxford Brookes University. India is a full-time lecturer within the Department of Art and Music at Solent University, UK, and a Senior Fellow of the Higher Education Academy. Additionally, she is a part-time research student at Solent University, where she is embarking on a practice-based photography PhD. India's photographic work encompasses themes of trauma, personal experiences, family history, and more recently, the visualisation of others' experiences. Her work has been showcased in exhibitions at local, national, and international levels.*

**Hyperacusis and hearing rehabilitation in adults with cochlear implants (CI) – case series**

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**Introduction:** There are cases of hyperacusis (Hy) among CI patients (CI candidates and CI users) and the level of suffering is high. However, prevalence data are not available. Furthermore, unlike tinnitus, little is known about how this condition should be treated in CI aftercare. We have therefore initiated case series with the primary goal of providing information on the frequency with which the diagnosis of Hy is encountered in CI patients in the clinics in Salzburg and Wels, and association between Hy, and CI aftercare, without specific treatment of Hy.

**Material and methods:** At least 10 individual cases are planned. "Cases" of these prospective case series are all adult CI patients with bilateral sensorineural hearing loss and with the additional diagnosis of hyperacusis. Clinical measures are: (1) four frequency hearing thresholds (PTA), (2) Data Logging, (3) *Freiburg Monosyllables*, (4) HSM sentences in noise, (5) uncomfortable loudness levels (ULL), (6) *Abbreviated Profit of Hearing Aid Benefit* APHAB (subjective hearing), (7) HIQ, (8) SSSQ, (9) *Visual Analog Scale* (VAS), (10) *Tinnitus questionnaire*, (11) *Hospital Anxiety and Depression Scale*, (12) *Hearing Stress Questionnaire*.

**Results:** Preliminary results in Salzburg, over a period of 9 months, Hy was observed in 10.3 percent of all CI patients who met the study criteria. All Hy cases were older than 56 years, 83 percent of them were male. Examples are the following two cases (case description not yet complete).

***Case 1:*** Man, 57 years old: The causes of progressive hearing loss include chronic otitis media and granulomatosis with polyangiitis (GPA). He was fitted with hearing aids 6 years ago. Before the CI, he had a severe hearing loss on the left and a slight hearing loss on the right, furthermore a strong tinnitus and Hy. Six months after the CI (left), subjective hearing and tinnitus have improved. However, social withdrawal and Hy scores have deteriorated.

***Case 2:*** Woman, 61 years old, had her first sudden hearing loss 15 years ago, 13 years ago she was fitted with hearing aids. After a stapesplasty 10 years ago, she lost her residual hearing in her right ear. On the left she suffered from a slight hearing loss. She also suffered from tinnitus (not clear which side). Two months after the CI fitting 8 years ago, Hy

developed and at the same time the left ear deteriorated (moderate hearing loss) and the tinnitus (both sides) increased dramatically. Hearing training and a change in the fine-tuning of the speech processor reduced Hy. After a viral infection, Hy worsened again. At present, five years after the first CI, the second ear is within the CI indications and the impairment due to the recurring Hy has increased to the maximum. The tinnitus had also improved considerably in the meantime. However, it has now increased significantly again, especially without hearing aid and CI (50% speech recognition at quiet, CI ear (*Freiburg Monosyllables*, 65 dB)). Last year, she had to quit her stressful job because of her hearing problems and the negative impact on her subjective well-being.

***Dr. Maria Huber*** *is a health psychologist, clinical psychologist and psychotherapist (behavioral therapist, psychoanalyst). For many years, she is associated with the CI-Center, Department of Otorhinolaryngology, Head and Neck Surgery, University Clinic Salzburg. As a clinical psychologist, she has many years of clinical experience with normal hearing and hearing impaired children, adolescents and adults. She has also worked for many years as a researcher and leader of multicenter scientific projects. Her current scientific interests include cognitive status, depressive status and hyperacusis in adult cochlear implant (CI) patients.*

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**Hyperacusis and misophonia in the ASD population**

Danesh A.A.

*Florida Atlantic University, Boca Raton, FL, USA*

Is it hyperacusis, sound oversensitivity, or something else? Whatever we want to call it, we have seen children and adults in the ASD population who show over-reaction to auditory signals. This over-reaction can be due to the loudness or the content of the auditory stimuli. The underlying pathophysiology of hyperacusis is not entirely clear. For some, it is due to neuronal and anatomical variations and for others it can be related to a specific reason such as those with Bell's palsy, superior canal dehiscence, or aneurysm of the middle cerebral artery. The pathophysiology of misophonia seems to be totally different. The neurodiversity in the ASD population can cause challenges for clinicians and make it difficult to understand, diagnose and manage hyperacusis and misophonia in this population. This presentation will address decreased sound tolerance (DST) disorders in the ASD and will discuss diagnosis and management strategies.

***Ali A. Danesh****, MS, PhD, CCC-A, FAAA, is currently a Professor at the Department of Communication Sciences and Disorders, and also has a Secondary appointment as Professor of Integrated Medical Sciences in the Charles E. Schmidt College of Medicine, Florida Atlantic University (FAU), Boca Raton, Florida. Dr. Danesh has affiliate positions in the College of Science, Department of Psychology and the Department of Electrical Engineering and Computer Science at the College of Engineering and Computer Science. He has faculty appointments at the Audiology Department of Salus University and Department of Otolaryngology, Miller School of Medicine, University of Miami. His research interests are in the areas of tinnitus, hyperacusis, misophonia, auditory evoked potentials, auditory processing disorders, and vestibular assessment. Dr. Danesh obtained his BSc in audiology from Iran University of Medical Sciences, Tehran, Iran, his MS in audiology from Idaho State University, Pocatello, Idaho, and his PhD in audiology, with an emphasis on auditory electrophysiology, from the University of Memphis, Memphis, Tennessee. Dr. Danesh is an American Board of Audiology board certified practicing audiologist. His current clinical work concentrates on patients with tinnitus, vertigo and sound sensitivity disorders (e.g., hyperacusis and misophonia).*

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**Hyperacusis in tonic tensor tympani syndrome (TTTS)**

Bezerra C.

*São Paulo University, São Paulo, Brazil*

One of the symptoms observed in tonic tensor tympani syndrome is hyperacusis. Manual therapy on the palatine aponeurosis can help treat this symptom. The physiotherapist's performance is fundamental for treatment.

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***Hyperacusis Assessment Questionnaire*** **– a new tool for assessment hyperacusis in tinnitus patients**

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[4] *Oto-Ryno-Laryngology Surgery Clinic, World Hearing Center, Institute of Physiology and Pathology of Hearing, Warsaw/Kajetany, Poland*

**Introduction:** Hyperacusis is a kind of decreased tolerance to sound and is difficult to measure objectively. It often co-occurs with tinnitus. There is a need for valid and reliable patient-reported outcome measures to capture this subjective phenomenon.

**Aim:** The aim of the study to create a questionnaire capturing hyperacusis in terms of loudness, fear, and pain and then to evaluate its psychometric properties.

**Material and methods:** The study group consisted of 106 patients, made up of 51 men and 55 women. They were aged between 19 and 72 years, mean 45.2 years (*SD* = 12.4). An initial pool of 33 questions capturing hyperacusis was subjected to expert evaluation and pilot testing. Then, a shortened 19-item version of the tool was checked out. Medical interview, audiological examination and a set of questionnaires: *Tinnitus Handicap Inventory*, *Hyperacusis Questionnaire*, *State-Trait Anxiety Inventory*, and *Visual Analogue Scale* was completed by all subjects.

**Results:** The final 14-item *Hyperacusis Assessment Questionnaire* showed an appropriate three-factor structure that explained 70.5% of the variance. Convergent validity and divergent validity were confirmed by correlations with other measures of hyperacusis, anxiety, tinnitus severity,

misophonia, and hearing thresholds. Internal consistency as assessed with Cronbach's alpha was excellent (α = 0.91) as was reproducibility (intra-class correlation, ICC = 0.96).

**Conclusions:** The new *Hyperacusis Assessment Questionnaire* is a psychometrically sound and brief tool that can assess the severity of hyperacusis in terms of loudness, fear, and pain. It can be used in clinical practice and scientific research for patients with hyperacusis and tinnitus.

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### *Hyperacusis Questionnaire* (Mini-HQ9) – a valid short tool for use in a clinical tinnitus population

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**Introduction:** The demands on psychometric procedures for therapy research in hyperacusis (HC) and their differentiation from phonophobia, misophonia and recruitment have increased. The widely used noise hypersensitivity questionnaires (GÜF, Nelting and Finlayson, 2004, Germany; *Hyperacusis Questionnaire*, HQ, Khalfa et al., 2002, France) are partly invalid and repeatedly criticized (Bläsing et al., 2010; Fackrell et al., 2015), partly without instructions and without quartiles, implausible cut-point of the HQ.

**Material and method:** We retrospectively evaluated 216 tinnitus-inpatients (47% women, age 49 ± 10) with the evaluated *Structured Tinnitus Interview* (STI, Goebel and Hiller, 2002): 60% HC; 15% phonophobia/misophonia; 24.4% without HC. At T1 and T2, we evaluated the sensitivity to change of the individual items of GÜF and HQ, numerical analog scales (NAS), *Tinnitus Questionnaire* (TF, Goebel and Hiller, 1998), discomfort thresholds (UBS), factor analysis, internal consistency, retest reliability (rtt), d, convergent and discriminative validity, BDI, BDI-II, BSI.

**Results:** Reevaluation GÜF: reliability: α = .93; best test quality item 5, 6, 10, 12, 13; quartiles 0–45, cut-off (AUC) > 16; correlation NAS-HK *r* = .68, HQ *r* = .88; TF *r* = 0.44; Pearson: rtt.83; ICC.93; UBS ≤ .2; d: *r* = .57. Reevaluation HQ: reliability: α = .92; best test quality item 5, 8, 9, 12t; quartiles 0–42, cut-off (AUC) > 18; correlation NAS-HK *r* = .63, GÜF *r* = .95; TF *r* = 0.35; Pearson: rtt.84; ICC.87; UBS ≤ .2; d: *r* = .47. The multidimensional structure of the GÜF and HQ could not be confirmed. Evaluation Mini-HQ9: after a comprehensive individual item analysis, we merged the 9 most change-sensitive (Pearson) and most stable (rit) items from GÜF and HQ into a new "Mini-HQ9": reliability: α = .93; quartiles 0–27; cut-off (AUC) > 11; corr. CFT *r* = .95; HQ *r* = .88; common variance 77%; corr. NAS-HK *r* = .63; TF *r* = .35; Pearson: rtt.83; ICC.79; UBS ≤ .2; test-retest d: *r* = .48. One-dimensional structure.

**Conclusions:** The Mini-HQ9 summarizes the advantages of the GÜF and HQ and has proven itself as a standard instrument for graduation and effect studies of the HK in pilot studies with only 9 items. Women are more sensitive to noise (*r* = .21). HC instruments are not suitable for misophonia. The UBS only for recording relevant side differences.

***Gerhard Goebel** (1946) is Prof. of Otology at the Medical Faculty of the Technical University of Munich. Since 1985 he has been a senior physician at the Roseneck Clinic, Hospital for Behavioral Medicine in Prien am Chiemsee. He has been a specialist in internal medicine since 1983 and in psychosomatic medicine since 1994. From 1999 to 2012, he was head physician at Prien Hospital and developed cognitive behavioral therapy (CBT) for tinnitus and hyperacusis patients in the inpatient sector. Numerous publications on these topics and habilitation in 1999 (ENT Clinic of the TUM, Prof. Dr. Wolfgang Arnold). Since 2001 lecturer in advanced training for CBT and behavioral medicine at the University of Zurich, supervisor for the Bavarian State Medical Association (BLÄK), German Medical Association for Cognitive Therapy (DÄVT) and the Bavarian Academy for Psychotherapy (BAP). Together with Wolfgang Hiller he published: Tinnitus Questionnaire (TF) (1994, 1996), Structured Tinnitus Interview (STI) (1999, 2000) as well as Mini-Tinnitus Questionnaire (Mini-TF12, 2004) and Mini-Hyperacusis Questionnaire (Mini-HQ9, 2014). Since 2013 he has been conducting diagnostic and therapeutic research on hyperacusis and misophonia therapy in his own practice.*

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### Imaging hyperacusis and misophonia: present and future

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Brain imaging of humans with loudness hyperacusis or misophonia remains one of the primary methods to discover the brain networks underlying these sound tolerance disorders, to differentiate them from each other and to dissociate them from related comorbid conditions of tinnitus or hearing loss. Despite the challenges associated with the expense and noise of some imaging tools, results of such studies have informed the existing experimental and theoretical framework of hyperacusis and misophonia. In a pioneering functional MRI study, Melcher and colleagues (2010) parsed out the contribution of co-occurring hyperacusis to brain imaging findings

of tinnitus. They noted that while both subcortical and cortical auditory areas were responsive in those with hyperacusis (relative to controls), such an elevated response was only noted in the auditory cortex for those with tinnitus. Recent studies (e.g., Hofmeier et al., 2021; Koops and van Dijk, 2021) further explored this dissociation using both fMRI and auditory brain-stem responses (ABRs). Brain imaging studies of misophonia (e.g., Kumar et al., 2021; Schroder et al., 2019) are more recent but have begun to change how we think of the condition, pointing to regions beyond the auditory cortex (such as motor cortex, insula) as playing a key role in this condition. In a recently completed study in my lab, we have collected both auditory brainstem response and fMRI data on young adults with hyperacusis or misophonia and their controls, results of which will be reported at the meeting. In summary, current and future non-invasive brain imaging studies continue to expand our understanding of the pathophysiology of hyperacusis and misophonia and will allow us eventually to develop and test new therapies that help patients.

***Dr. Fatima T. Husain*** *is a Professor in the Department of Speech and Hearing Science, and Associate Dean in the College of Applied Health Sciences, University of Illinois Urbana-Champaign, United States of America. She is also faculty in the Neuroscience Program and the Beckman Institute for Advance Science and Technology at the same university. Dr. Husain's research program is centered around three major themes: (1) normal audition and speech perception, (2) disorders of the auditory system, particularly hearing loss, tinnitus, hyperacusis and Misophonia, and (3) effects of aging on audition and cognition. She uses a multidisciplinary approach combining behavioral, brain imaging, and computational neuro-modeling tools. She is the immediate past Chair of the Scientific Advisory Committee, American Tinnitus Association and serves on the scientific boards of Hyperacusis Research and SoQuiet Foundation. She is presently an Editor of the "American Journal of Audiology".*

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**Investigating cognitive reappraisal as a treatment for misophonia**

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Misophonia presents significant challenges for those affected. While potential interventions have emerged and are gaining momentum, there is still no evidence-based treatment for this disorder. Cognitive reappraisal (CR), a psychotherapeutic technique that alters the perception of triggering sounds, sources, and context to regulate emotional responses, holds promise in reducing misophonic reactions. However, its effectiveness in misophonia, as well as the expectations and experiences of misophonia sufferers with this method, remain unexplored. This study aims to address these gaps. The proposed intervention employs a process-based treatment approach, examining one technique at a time in the context of individual needs, possibilities, and characteristics. Preliminary data from the ongoing main study, which includes a control group, will be presented at the conference, along with the pilot quantitative and qualitative data and protocol characteristics. Below are selected results from the pilot study. Twenty-three participants completed the pilot study. Misophonia, anxiety, and depression symptoms, among others, were assessed at three time points: twice before the four weekly sessions and after the treatment. The first session was a 90-minute online group session focused on psychoeducation about CR and increasing motivation. This was followed by three weekly individual 30-minute sessions tailored to each participant's needs, such as finding the optimal time for CR implementation, the most accessible situations, and the most acceptable types of reappraisals, as well as reinforcing motivation to use CR. All sessions were audio-recorded and analyzed for protocol adherence. After completing the study, volunteering participants engaged in additional audio-recorded focus groups to discuss the most and least valuable and challenging aspects of the study, the most suitable time points for using CR (before, during, or after the trigger), and potential adverse effects of the treatment. The main study, currently ongoing, includes a separate group of 108 participants, including a control group practicing Schultz's Autogenic Training, allowing for more rigorous pre- and post-treatment assessments. In both the pilot and the main study, apart from assessing misophonia symptoms, we conducted semi-structured interviews for psychiatric and personality disorders, cognitive and audiological tests, and various psychological questionnaires. In the pilot study, there was a significant reduction in misophonia symptoms post-treatment. We observed a significant decrease ($p < .001$; partial eta-squared = .41) in the impact of misophonia, as measured by the S-Five scale. There were similar effects on the remaining scales. Eighteen participants (78%) experienced at least a 10% reduction in symptoms, while three showed no change, and two experienced a worsening of symptoms. Qualitative analysis indicated that meeting others with the same disorder was perceived as beneficial, as was the awareness that "somebody is researching Misophonia", "misophonia is real" or "being a part of the misophonia community." Indeed, while misophonia symptoms decreased only after the treatment, we observed a significant decrease in anxiety and depression not only after the treatment but also between the first and second assessments (yet before the treatment began). In addition to the pilot study findings, preliminary results from the ongoing randomized controlled trial (RCT) will be presented at the conference.

***Marta Siepsiak*** *is a psychologist, CBT psychotherapist, music therapist, and researcher. Over the last 8 years, she has led multiple research projects related to sound over-responsivities, currently examining the effectiveness of psychotherapeutic interventions in Misophonia – a study financed by the REAM Foundation. Outside of academia, she works as a clinician with children, adolescents, and adults, including individuals suffering from misophonia. Her primary research and clinical interests include misophonia and other sensory over-responsivities, so-called central auditory processing disorder, psychology of music, anxiety disorders, abnormal child psychology, and psychotherapeutic interventions.*

**Making sense of treatment research for misophonia: an overview and call to the field**

Rosenthal M.Z.

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There are few studies evaluating treatment approaches for Misophonia, no specific evidence-based treatments known through systematic and replicated research to be efficacious, and no field-wide consensus about how to help patients. At the same time, patients are suffering and need help. What are we to do? As clinicians, we need to follow evidence-based principles of change and, at the same time, be flexible and individually tailor treatments appropriately. In this presentation, (a) published research studies investigating Misophonia will be reviewed and synthesized, (b) recommendations will be made for a research-based agenda to identify appropriate treatments for Misophonia, and (c) pragmatic and reasonable guidelines will be outlined about how to help patients until there are known efficacious treatment protocols. A practical and humane approach will be offered that is multi-disciplinary, client-centered, collaborative, measurement-based, accounts for co-occurring psychiatric diagnoses, and is grounded in known biopsychosocial processes of change and interventions shown to effectively target these processes transdiagnostically.

***Dr. M. Zachary Rosenthal*** *is a clinical psychologist, Associate Professor, and Director of the Duke Center for Misophonia and Emotion Regulation. He is a clinician, scientist, educator, mentor, and advocate and has a lived experience as a loved one of someone with Misophonia.*

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**Measurements of sound sensitivity: lessons from the citizen science project**

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**Introduction:** Environmental noise is recognized by the World Health Organization as a significant public health concern, negatively impacting human health and well-being. To address and better understand these effects, the "De Oorzaak" citizen science project was initiated in Flanders, Belgium.

**Objective:** This study aims to assess the effects of environmental noise on health, stress, and sleep among the Flemish population. It also seeks to explore the relationship between sound sensitivity and sociodemographic factors.

**Material and methods:** A comprehensive population-level questionnaire was administered to thousands of Flemish residents over a one-month period. This survey included questions on quality of life, health status, stress levels, sleep quality, and detailed sociodemographic information. Additionally, a subset of 100 participants from Antwerp underwent an extensive audiological test battery. This included self-report questionnaires, audiometry, and a newly developed diagnostic test for hyperacusis utilizing natural sounds. Participants were categorized into two groups based on their scores from the *Hyperacusis Questionnaire* (HQ): with or without hyperacusis.

**Results:** Data collection is ongoing, and preliminary analysis focuses on the correlation between various measures of sound sensitivity and other demographic variables.

**Conclusions:** The "De Oorzaak" project leverages citizen science to generate a rich dataset that will enhance our understanding of the public health implications of environmental noise.

***Prof. Dr. Laure Jacquemin*** *is an audiologist with clinical and research experience at the Tinnitus Treatment and Research Centre Antwerp (TINTRA). Her clinical work focuses on psycho-education and cognitive behavioural therapy for patients with tinnitus, hyperacusis and misophonia. Her current research focuses on diagnosis and treatment of hyperacusis.*

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**Metacognitive Interpersonal Therapy for Misophonia: a single-case study**

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Misophonia is a chronic condition in which the exposure to specific sounds increase the arousal and the recurrence of specific intense negative emotions. Patients generally think that the trigger sounds are intentionally produced by others and because of this interpretation they usually feel anger and disgust. We hypothesized that misophonia may be strongly related to maladaptive interpersonal schemas which create difficulties in interpersonal relationships. Maladaptive interpersonal schemas are typically present in personality disorder. Persons with maladaptive interpersonal schemas think that other people try to subjugate, criticize, dominate, exploit, deceive, disregard and humiliate them. Furthermore, these patients typically endorse a representation of self as mistreated, constricted, harmed, damaged, humiliated, impotent, inadequate or fragile. We describe the course of a treatment of Metacognitive Interpersonal Therapy (MIT) with a young man presenting misophonia and co-occurrent obsessive-compulsive personality disorder (OCPD) and avoidant personality disorder (AVP), with narcissistic traits. He presents self-criticism, perfectionism and emotional overcontrol. Therapy aimed increasing awareness of maladaptive interpersonal schemas and promoting a healthy self. Behavioural experiments were used to increased quality of social relationships and tolerance to the trigger sounds. Qualitative and quantitative (*Amsterdam Misophonia Scale*, structured clinical interview for DSM-IV personality disorders, *Beck's Depression Inventory-II*, *State-Trait Anxiety Inventory*) outcomes at the intervention are summarized.

***Eleonora Natalini*** *is a Psychologist and a Cognitive Behavioural Therapist. She is also a Metacognitive Interpersonal Therapist and EMDR Therapist. Her main areas of expertise*

*are: Personality Disorders, Anxiety and Mood Disorders, Misophonia and Tinnitus. She works with adolescents and adults.*

**No time for triggers: objective misophonic avoidance measurement using behavioral timing data**

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This study presents and evaluates the *Misophonic Behavioral Avoidance Test* (M-BAT), a standardized tool designed to objectively measure misophonic avoidance behavior towards trigger sounds. Employing recent psychometric models, the M-BAT leverages response times and behavioral decisions in assessing reactive and anticipating avoidance strategies. Reactive avoidance, characterized by immediate escape from triggers, is measured through auditory exposure to triggers, affording respondents the choice to voluntarily stop or endure the exposure. Anticipating avoidance, involving the prevention of expected trigger exposure, is assessed through responses to sound descriptions, where respondents decide to approach or omit a trigger exposure. The central behavioral measures are denoted as sound endurance and anticipating sound avoidance tendency. The study aims to evaluate and optimize the M-BAT, assessing psychometric properties and evaluating the validity of the behavioral measures. An online survey and behavioral testing procedure were employed to collect data from a large sample of individuals clinically or sub-clinically experiencing misophonia. The extensive validation approach gives insight into various dimensions of validity including nomological, differential, and criterion validity. Beyond clinical characterization and advances in understanding the nature and causes of misophonia, the findings hold potential implications for informing treatment approaches and tailored strategies to alleviate the impact of misophonia. This research establishes a foundation for advancing and refining the measurement and understanding of misophonic avoidance behavior. The M-BAT introduces a valuable tool for researchers and clinicians, fostering avenues for future investigations into misophonia and its treatment.

***Nico Remmert** is a researcher and fourth-year PhD candidate in psychology at Freie Universität Berlin, specializing in the department of Methods and Evaluation/Quality Assurance. Nico earned his MSc in Psychology from Freie Universität Berlin in 2020. His research revolves around advancing psychometric methods to assess misophonia, with a specific emphasis on understanding misophonic avoidance behavior through the analysis of process data, including response times. Currently, Nico is engaged in collaborative work with the Misophonic Clinical Academic Research Group at King's College London and Svetlana Shinkareva's research group at the University of South Carolina. This multidisciplinary collaboration underscores Nico's dedication to fostering innovative approaches to misophonia research. Nico is known for his pivotal role in developing the Berlin Misophonia Questionnaire Revised, a symptom-oriented tool for the comprehensive assessment of misophonia.*

**Noise sensitivity: strategies for a systematic review**

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In the psychological, psychoacoustical and public-health literature, noise sensitivity is seen an important predictor of human attitudes and behaviour towards noise. But what exactly is noise sensitivity, and how is it measured? To address this question, we are currently performing a systematic review of the different conceptualizations and ways of measuring noise sensitivity. That is done across different domains of investigation (e.g., engineering, environmental science, medicine, physics, psychology, and public health). In a first step, we are aiming to establish a comprehensive overview of the psychometric quality of the different noise-sensitivity measures currently available. To that effect, documentation written in the English, French, German or Korean language is scrutinized, including grey literature and unpublished materials. The search is implemented with a wide focus, not limited to scientific journal databases: it includes querying experts, governmental and funding agencies, as well as scanning pertinent conference proceedings. The synthesis will yield a map of instruments available to measure noise sensitivity in different languages and cultures, as well as identify gaps of conceptual specification. The outcome of this study may be used to hone the definition of noise-sensitivity and to differentiate it from related concepts such as noise annoyance, hyperacusis, phonophobia and misophonia.

Research funded by Fondation pour l'Audition (FPA RD-2022-6).

***Karin Zimmer** is Professor of Empirical Educational Research at the University of Vechta. She has worked at various universities in Germany and Denmark, and has co-ordinated the German national educational report. Karin Zimmer has been senior analyst of PISA at OECD in Paris, France. Her academic background is in experimental psychology, pedagogy, and statistics.*

**Objective functional biomarkers to find druggable targets for tinnitus and hyperacusis**

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Currently, conflicting views on the neural correlate of tinnitus hinder the development of effective diagnosis and therapy for tinnitus (Knipper et al., Ruttiger, 2020). Although hyperacusis often co-occurs with tinnitus, it is until now considered neither in clinical diagnosis nor for targeted, individualized therapies. On the basis of findings with objective functional biomarkers (PTT, ABR, fMRI, EEG) used in patients, we challenge the hypothesis that co-occurance of hyperacusis worsens tinnitus percept towards a disease that most requires treatment and therapy. In this context, the hypothesis of the successful use of cognitive therapies for hyperacusis and tinnitus is particularly interesting for future therapy development.

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***Marlies Knipper*** *has been a Professor at the Hearing Research Center of the Ear, Nose and Throat Clinic, Eberhard Karls University of Tübingen, since 2008 and has headed the "Molecular Hearing Physiology" working group since 1993. Marlies Knipper has been working in the field of hearing research for over 30 years. Her research focuses on auditory processing disorders, age-related hearing loss and neuropathies, as well as tinnitus and hyperacusis in animals and human clinical studies. Her research aims to contribute to the relationship between cognition and hearing. She is also particularly interested in creating an infrastructural platform for more efficient use of cross-system research into the various senses. She is a member of the Leopoldina – National Academy of Sciences (Halle/Saale), Leibniz-Sozietät der Wissenschaften zu Berlin and AcademiaNet.*

**Preliminary results of tinnitus therapy using bimodal stimulation with the Lenire device in patients with tinnitus and reduced sound tolerance**

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**Introduction:** Tinnitus is defined as the perception of sound without an external acoustic stimulus. Currently, there is no single effective treatment method for tinnitus that works for all patients. One of the newer approaches is bimodal stimulation, which combines acoustic stimulation with somatosensory pathway stimulation. The goal of therapy is to reduce the severity of tinnitus and improve the quality of life for patients suffering from tinnitus.

**Aim:** The aim of this study was to evaluate the effectiveness of bimodal stimulation using the Lenire device in reducing tinnitus severity in 15 patients.

**Material and methods:** The authors present the results of their own research on the use of bimodal stimulation therapy in 15 patients. The results of the *Tinnitus Handicap Inventory* (THI) questionnaire were analyzed.

**Results:** The results of the authors own research on the use of bimodal stimulation with the Lenire device showed an effectiveness of 78%, with an average decrease in THI score of 34 points.

**Conclusions:** Bimodal stimulation using the Lenire device may be an effective treatment method for tinnitus in patients with reduced sound tolerance.

**Quality of sexual life in individuals with misophonia and their partners**

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**Introduction:** Misophonia, a selective 'hatred of sound' has been often misunderstood new mental disorder which is characterized by intense negative emotional reactions, feelings, thoughts with or without impulsive behavior to a source of specific irritating trigger sound. Triggers usually produced by the human mouth, throat or nose and include chewing, crunching, slurping, sniffing etc. Cutlery, environmental, or machine sounds can also be the triggers. The term 'misophonics' has been used for the name of misophonia sufferers. One of the main self-reported effects in misophonics is destructive impact on constant close romantic relationships with aggression, 'fight or flight' response and worsening/ruining love affair.

**Objective:** We aimed to study the quality of sexual life (QoSL) in misophonics both sexes and their close partners.

**Material and methods:** Into our study we enrolled 88 misophonics with male-to-female ratio 1:2.9 (23/65) and 88 their partners with male-to-female ratio 2.9:1 (65/23) aged 25.1 ± 5.6 years old. For evaluation of the quality of sexual life in males we used *International Index of Erectile Function* (IIEF) and *Sexual Quality of Life-Male* (SQOL-M) self-report questionnaires. In females we used the *Sexual Quality of Life-Female* (SQOL-F) self-report questionnaire and *Female Sexual Function Index* (FSFI). Obtained total scores according to questionnaires in misophonics we compared with the survey results of controls, their peers (88 males and 88 females who were couples in love) without misophonia or any distinct mental/urological/gynecological pathology aged 25.3 ± 4.9 years old. Mann-Whitney *U*-test to compare two groups was used. Statistical significance was judged at $p < 0.05$.

**Results:** Misophonia only, without concomitand mental disorders was diagnosed in 30 (34.1%) individuals: 20 (66.7%) females and 10 (33.3%) males. Comorbid mental disorders in misophonics were registered in 58 (67.4%) patients: 45 (77.6%) females and 13 (22.4%) males, and included generalized anxiety disorder (GAD), $n = 21$ (23.6%): 17 females, 4 males; depressive disorder (DD), $n = 12$ (13.5%): 8 females, 4 males, panic disorder (PD), $n = 11$ (12.4%): 8 females, 2 males; obsessive compulsive disorder (OCD), $n = 8$ (9.0%): 6 females, 2 males; and relationships obsessive-compulsive disorder (ROCD), $n = 6$ (6.7%): 6 females, 0 males. We noted that Individual total scores distinguishing QoSL in female and male misophonics as well as in their sexual partners were statistically lower comparing with Controls, their peers without misophonia or any distinct mental disorder. Total score of *International Index of Erectile Function* scale in males with misophonia only was 40.9 ± 5.4 vs 68.1 ± 3.7 in Controls, $p < 0.05$. These findings show decreased erectile function in misophonic males. Moreover, we have found that healthy males without misophonia who are the partners of females misophonics also have decreased IIEF total score comparing with Controls 49.3 ± 4.9 vs 68.1 ± 3.7 respectively, $p < 0.05$. The small sample of enrolled males with misophonia might be considered the main limitation of our study.

**Conclusions:** Misophonia, a selective sound sensitivity syndrome, is decreasing the quality of sexual life in sufferers both sexes and their partners. Males misophonics are suffering from erectile dysfunction as well as healthy males who are the partners of females misophonics. We consider that these aspects should be taken into account by psychiatrists, psychotherapists, psychologists during the management of couples with misophonia in one of the partners.

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**Relationship between hyperacusis and anxiety in men and women with tinnitus**

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**Introduction:** Some research has indicated a possible link between hyperacusis and anxiety, suggesting that psychological factors may play a role in the severity of auditory sensitivity. However, the nature of this relationship in subjects with tinnitus and how it may differ between men and women remains unclear.

**Aim:** The aim of the study is to investigate the relationship between hyperacusis and anxiety in men and women with tinnitus.

**Material and methods:** The study group consisted of 51 men and 55 women with tinnitus and hyperacusis in similar age (men: $M = 45.4$; $SD = 12.4$; women: $M = 44.9$; $SD = 12.8$). The study was conducted in the Tinnitus Clinic of the tertiary Ear, Nose, and Throat Center in Kajetany (near Warsaw, Poland). The audiological examination included pure-tone audiometry, impedance audiometry, and measurement of uncomfortable

loudness level. The *Hyperacusis Assessment Questionnaire* (HAQ), *State-Trait Anxiety Inventory* (STAI), and *Tinnitus Handicap Inventory* (THI) were used.

**Results:** Men and women exhibited similar levels of hyperacusis, anxiety, and tinnitus severity. However, gender differences emerged in the relationship between hyperacusis and anxiety. The correlation was stronger in men (*rho* = 0.57; $p < 0{,}001$) than in women (*rho* = 0.27; $p = 0.073$). Anxiety was a significant predictor of hyperacusis ($\beta = 0.35$; $p = 0.05$) only in men, while in women the significant predictor of hyperacusis was tinnitus severity ($\beta = 0.32$; $p = 0.046$).

**Conclusions:** The relationship between hyperacusis, anxiety, and tinnitus severity in individuals with tinnitus varies by gender. In men, anxiety plays a more significant role in hyperacusis, whereas in women, tinnitus severity is a more crucial factor.

***Assoc. Prof. Elżbieta Gos**, PhD is a psychologist specializing in psychometrics. She works in the Teleaudiology and Screening Department of the Institute of Physiology and Pathology of Hearing. She develops and validates measurement tools and research methodology and performs statistical analyses. Her scientific interests involve the subjective aspects of hearing disorders, especially in tinnitus, hyperacusis, and misophonia.*

## The neurophysiological model for hyperacusis and misophonia

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Based on the observations of several hundred patients with decreased sound tolerance the existence of unrecognized earlier auditory disorder with distinctive and different from hyperacusis characteristics has been identified. Patients with this disorder exhibited negative emotional and autonomic reactions evoked by a specific for a given patient patterns of sound. Consequently, the name "misophonia" for this previously not described disorder and its definition based on data gathered from our patients was proposed in 2001. Furthermore, clinical observations pointed out that misophonia requires a different approach for diagnosis and treatment than hyperacusis. Although the proposed definition was independent of the etiology of misophonia, nevertheless, on the basis of analysis of its features, including reported reactions to bothersome sounds ("misophonic triggers") it was possible to propose potential mechanisms of misophonia and the neurophysiological model for both misophonia and hyperacusis. The model postulates that a key characteristic of misophonia is the formation of inappropriate, pattern-specific subconscious connections between the auditory system and other systems of the brain, particularly with the limbic and autonomic nervous systems. These connections are governed by the principles of conditioned reflexes. Notably, the brain systems and connections involved in misophonia are the same as in the case of tinnitus. However, in the case of tinnitus the abnormal neuronal activity is generated within the auditory pathways and spreads to other systems in the brain, while in case of misophonia normal sound-evoked activity which is the same as in case of subjects without misophonia, is spreading and incorrectly activates various systems in the brain, yielding emotional and autonomic reactions to misophonic triggers. The mechanism of hyperacusis is based on abnormally increased gain within the subconscious part of the auditory pathways. This yields a high level of neuronal activity, equivalent to activity evoked by much stronger sound in normal subjects. The activation of the other systems in the brain is a consequence of spreading this abnormally enhanced sound-evoked activity by normally functioning neuronal connections from the auditory to other systems in the brain. In misophonia sound-evoked signals within the auditory pathways are normal, but incorrect pattern-specific connections yield abnormally strong activations of various systems in the brain.

***Pawel J. Jastreboff**, PhD, ScD, M.B.A, is currently Professor Emeritus of Otolaryngology – Head and Neck Surgery at Emory University School of Medicine, Atlanta, Georgia, and Visiting Professor sine die at University College London, London, UK. Dr. Jastreboff is recognized for his development of the first accepted animal model of tinnitus, the neurophysiological model of tinnitus and based on its clinical method of tinnitus and DST treatment, known as TRT. Furthermore, collaborating with Dr. Margaret M. Jastreboff, PhD, he proposed a concept, name and treatment for specific version of DST – misophonia – when patients exhibit negative reactions to particular for a given patient patterns of sound. Dr. Jastreboff received a PhD in Neurophysiology and Doctor of Sciences degree (habilitation) in Neuroscience from the Polish Academy of Sciences. He is a co-author of over 140 papers, 180 abstracts and three books. In 1993 he received the prestigious Robert W. Hocks award for his contribution to the field of tinnitus and in 2014, at 11th International Tinnitus Seminar the Award for Clinical Excellence, for 25 years of work with TRT. Currently he continues his work in JHDF, Inc.*

## The role of family dynamics in misophonia: proposed topics for formal study

Jaffe J.J.

*Private Practice, Sherman Oaks, CA, US*

As a solo practice clinician, over the past dozen years I have seen many hundreds of individuals and families affected by misophonia. I have taken their stories to heart and have conducted several informal surveys to consider whether their experiences are shared by others dealing with this complex condition. As I am not in a university or medical setting, I do not have the equipment or staff to conduct formal studies to test my observations, questions, and conclusions. This session will present the results of some of my informal surveys in the hope that researchers might take this raw data and conduct studies to verify or dismiss my informal results. Included will be my observations and surveys in the US addressing the following questions:

1. Is there a larger percentage of people with misophonia in the US who were raised in families of origin with more highly structured religions (mainly Catholicism, but also other more rigidly structured religions such as Orthodox Jewish, Seventh Day Adventist, Southern Baptist, etc.) than

in other less structured religions? [Reasoning: Possibly more rigidity in family rules around behavior, especially at the table, emphasis on right/wrong, guilt, shame].
2. Is there a larger proportion of Neanderthal DNA in people with misophonia than those without misophonia? [Reasoning: Misophonia appears to be survival brain interpretation of certain sounds as danger, as ancient peoples would have reacted].
3. Are people with misophonia more likely to have Myers-Briggs types IJ (Introversion/Judgment) than the average reported percentages of those types in the general population? Would they have been in those categories prior to or only resulting from misophonia?
4. Are people with misophonia more likely to have a family lineage including this or other neurological disorders, as compared to families without misophonia? [Reasoning: there may be a genetic component to misophonia].
5. Do people with misophonia have higher ACEs (Adverse Childhood Experiences) scores than comparison groups? Is misophonia related to occurrence of a traumatic event or ongoing? [Reasoning: may be a genetic link, but epigenetics – stressful family circumstances – may have set off initial reactivity].
6. Is the area of the brain in which misophonia has been mapped to occur in some way connected to the areas that have been mapped in earlier OCD studies? [Reasoning: there are many similar symptoms that may be related].
7. Are girls more often affected by misophonia in part because they are acculturated to be less assertive and to have weaker boundaries around other people? [Reasoning: girls tend to suppress negative emotions until they overflow].

My hope is that researchers may take an interest in exploring some of these speculations that have grown from observations and informal surveys to determine if any validity to these theories.

***Jaelline Jaffe**, PhD, has been a licensed psychotherapist in Southern California since 1976, working with individuals, couples, and medical issues, which led to her establishing LemonAidCounseling.com. For the past dozen years, her practice has focused almost exclusively on sound sensitivity disorders, mainly Tinnitus and Misophonia. She has worked with many hundreds of Misophonia patients from age 8 to 70, mostly with teens and young adults, who often find her via her website, SensitiveToSound.com. Dr. Jaffe often works in conjunction with audiologists across the US to assist their patients with the intense emotional and family issues associated with Tinnitus, Misophonia, and Hyperacusis. She has presented on these topics at numerous professional conferences for audiologists as well as for psychotherapists. Dr. Jaffe is a cofounder and Board member of the Misophonia Association, and also program coordinator for their annual convention for hundreds of patients and families. The 11th annual convention will take place in November 2024 in Atlanta, GA. Dr. Jaffe is author of the upcoming book "These Sounds are Driving Me Crazy!" Training for Mental Health Professionals in Treating Sound Sensitivity Disorders.*

## Posters

### Creating and using mobile applications for tinnitus and hyperacusis assessment and therapy – a scientific literature review

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**Introduction:** Audiological aliments like tinnitus and hyperacusis require multidisciplinary care. Therapy for these ailments should be available on a daily basis because of their troublesomeness in everyday functioning. To meet this challenge, specialists are looking for solutions in increasingly common access to the Internet and increasingly widespread use of mobile devices. Furthermore, smartphones have an application ecosystem that can be extended by new apps programmed for a particular health problem.

**Aim:** Assessment of the scale and direction of creating and using mobile applications to diagnose and treat tinnitus and hyperacusis.

**Material and methods:** Google Scholar and PubMed were searched for the 13 years 2010–2023. The search strategy used the following keywords: "tinnitus mobile applications", "hyperacusis mobile applications", "smartphone-based treatment tinnitus/hyperacusis", "smartphone-based diagnostic tinnitus/hyperacusis", "CBT apps", "sound and relaxation therapy apps", "tinnitus/hyperacusis therapy smart". The results of the review were catalogued and organized into themes.

**Results:** Results were organized into the following themes: (1) ranking evaluation and analysis of applications supporting tinnitus/hyperacusis therapy existing in the Internet space, (2) applications supporting the diagnosis of tinnitus, with particular emphasis on the EMAs (*Ecological Momentary Assesments*), (3) applications supporting the therapy of tinnitus/hyperacusis (4) a look into the future – the use of sensors built-in or connected with mobile devices, the use of artificial intelligence (AI), bid data techonology.

**Conclusions:** Smartphone-based applications with EMAs, sensors, possibility of using different wearable diagnostic devices can be helpful in better understanding the tinnitus variability and its causes. Combining the mobile applications with a mobile crowdsensing, central database and the support of AI techniques is a valuable source for developing scientific research. Clinically verified methods provided by mobile applications can become a part of the therapeutic process proposed by specialists and enable easy, cost-free and wide range of therapeutic support in dealing with tinnitus and hyperacusis. In tinnitus/hyperacusis therapy multifunctional smart devices managed by mobile applications such as: smart hearing

aids, cochlear implants, hearables may be equally important. The development of mobile technologies and AI techniques will contribute to the creation of smart therapy platforms for tinnitus/hyperacusis in the future.

## Cross-cultural adaptation and validation of the Danish version of *Inventory of Hyperacusis Symptoms* (HIS)

Steen Nemholt S.[1,2], Helge C.[3], Scutt P.[4,5], Baguley D.M.[†], Schmidt J.H.[6,7]

[†] David M. Baguley passed away in 2022. This work is dedicated to his memory.

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**Objective:** As there are currently no validated hyperacusis questionnaires available in Denmark, the aims of the present study were to (i) cross-culturally adapt a Danish version of the *Inventory of Hyperacusis* (IHS) and (ii) investigate its usability, validity, and reliability for Danish adults with hyperacusis.

**Material and methods:** The *Inventory of Hyperacusis Symptoms* was translated into Danish using recently established good practice guidelines. We carried out a two-phase study to translate and culturally adapt the IHS into Danish and to evaluate its clinometric properties (test-retest reliability and convergent construct validity). Procedure and study sample: the IHS consists of 25 items grouped into five dimensional factors. We followed a six-step guideline for the process of translating and culturally adapting questionnaires. Steps 1-3 included translation into Danish, step 4 involved a committee review to achieve cross-cultural equivalence in the forward translation, step 5 involved cognitive debriefing interviews to investigate the participants' comprehension of the questions, and step 6 finalized the process. In the second phase, the adjusted questionnaire was tested for consistency over time in a small sample of patients ($n = 32$).

**Results:** The overall scale exhibited high internal consistency, indicated by a Cronbach's alpha coefficient of 0.95, suggesting good internal reliability. The internal reliabilities of the subscales *Psychosocial*, *Functional impact*, *Communication*, and *Emotional* arousal were deemed adequate, with coefficients of 0.92, 0.85, 0.80, and 0.75 respectively. However, the subscale *General loudness* displayed lower internal reliability with a coefficient of 0.66. The test-retest reliability analysis revealed a strong positive correlation between the test and retest of the IHS ($r = 0.93$, $p < 0.001$). A paired $t$-test indicated no significant difference between the total scores of the test and retest ($p = 0.10$). Most items demonstrated acceptable test-retest reliability, with Cohen's Kappa coefficients ranging from 0.40 to 0.82. However, eight items did not meet the recommended cut-off, with one item (item 2) having a kappa value of 0.19, and seven items (items 3, 4, 5, 9, 10, 11, 22) having kappa values ranging from 0.25 to 0.40. Four factors showed acceptable reproducibility based on the ICC analysis.

**Conclusions:** We have demonstrated that the Danish translation of the IHS seems to be a reliable and valid general measure of hyperacusis-related issues with potential for clinical use. With respect to the subscales, further studies using the IHS-DK and similar measures should clarify whether specific hyperacusis distress measures can be identified.

**Susanne Steen Nemholt** *is a Senior Researcher, PhD, with a background in audiologopedics and psychotherapy. With extensive experience in the field of communication disorders, particularly in tinnitus and hyperacusis among children and adolescents, she has been involved in various research projects, presentations, and advisory boards. Susanne has focused on clinical guidelines, prevalence studies, and interventions for these conditions in her publications. Her academic journey includes a PhD from the University of Southern Denmark and a Master's degree from the University of Copenhagen.*

## Effectiveness of combined psychological and behavioural therapy in a person with audiological and neurological ailments

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**Introduction:** The aim of this study was to evaluate the effectiveness of psychological and behavioural therapy in improving the quality of life of a person after tetraparesis with comorbidities: tinnitus, hypersensitivity.

**Case report:** This case study concerns a 37-year-old patient with tinnitus, hypersensitivity to noise and chronic arm pain. The complaints occurred after a cervical spine reoperation. The reoperation was performed because of a C1 to TH 2 epidural haematoma and tetraplegia after the first surgery. The patient's quality of life and psychosocial functioning were perceived to be significantly impaired. A diagnostic audiological, psychological and neurophysiological assessment (QEEG study) was performed. The patient was qualified for HRV (heart rate variability) therapy and ACT (acceptance and commitment therapy). After the interactions, changes in brain bioelectrical activity in terms of Alpha-Beta1 and Beta2 waves and an improvement in psychosocial state were observed.

**Conclusions:** The individualized, holistic approach applied to the patient's problems related to chronic complaints allowed an improvement in his quality of life.

***Małgorzata Fludra*** *is Psychologist employed at the Department of Tinnitus of the Institute of Physiology and Pathology of Hearing, Warsaw/Kajetany, Poland.*

**Evidence of validity for the English-translated MisoQuest**

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**Introduction:** Although misophonia is characterized by distress and impairment, it is not yet classified as a psychological disorder in diagnostic manuals, largely because it is unclear how it should be defined and assessed. The MisoQuest, developed by Siepsiak and colleagues (2020), is a psychometrically promising self-report measure for misophonia. However, the MisoQuest was written in Polish and validated in Polish-speaking samples before being translated into English.

**Objective:** The current study aimed to evaluate the psychometric properties of the English MisoQuest, establish preliminary evidence of convergent and discriminant validity of the MisoQuest, and investigate whether MisoQuest scores predict meaningful outcomes.

**Material and methods:** We recruited participants with ($n = 44$) and without ($n = 95$) misophonia to complete a series of psychological measures, including the MisoQuest, the GAD7 for anxiety, and the SHS for sensory hypersensitivity. Participants also completed an online cognitive task where they read three short stories, one in each sound condition (silence, aversive, and trigger). Following each story, participants answered ten multiple-choice questions about the content of the story. Their reading comprehension accuracy was quantified as the percentage of questions answered correctly.

**Results:** We demonstrated that the English MisoQuest has excellent internal consistency, strong test-retest reliability, and that scores specifically tap misophonia symptom severity rather than generalized anxiety or broader sensory sensitivities. Additionally, we established evidence of criterion validity, demonstrating that higher MisoQuest scores predict lower reading comprehension accuracy in the presence of trigger sounds, but not aversive sounds or silence. Overall, this study indicates that the MisoQuest is a reliable and useful measure for identifying misophonia in English-speaking individuals and that scores on this measure are related to clinically relevant outcomes.

***Kate Raymond,*** *I learned about misophonia through my own lived experience. For as long as I can remember, I have been enraged by the sound of chewing gum. In high school, I found the label for that experience, and during my undergraduate degree at McMaster University, I began to study it. I quickly realized that there was little empirical research on misophonia and that the condition affected many other people. My motivation to understand my own experience turned into scientific curiosity and a drive to make an impact through research. I earned a Master of Science degree in clinical psychology at Western University, where I evaluated the reliability and validity of misophonia assessments. I am currently a PhD student at Western University, aiming to investigate the neural mechanisms underlying misophonia using naturalistic paradigms that emulate real-world listening. Through this work, I hope to contribute to a better understanding and treatment of misophonia.*

**Exploring the cognitive dimensions of misophonia: affective inflexibility and rumination**

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Misophonia is an under-researched condition characterized by a sensitivity to certain sounds and related stimuli. Misophonic reactions depend heavily on the context surrounding the triggers and their sources, pointing to the importance of a cognitive aspect of the disorder, which is not yet fully understood. Thus, the primary aim of this study is to explore the relationship between misophonia and affective inflexibility, a hypothesized cognitive feature of the disorder that involves difficulties in shifting the way one responds in the face of emotional stimuli. A secondary focus is a potential association between misophonia and different forms of rumination, which is a mental process associated with affective inflexibility that involves repetitive negative thinking. In the current study, 140 participants (mean age = 29.98 years; *SD* = 6.72; 49 female/ 91 male) with and without symptoms of misophonia were recruited online via Prolific and misophonia-related newsletters. Participants completed the recently developed *Memory and Affective Flexibility Task* (MAFT), designed to assess affective flexibility, as well as a battery of self-report measures to evaluate misophonia severity, rumination, and cognitive inflexibility, as well as symptoms of anxiety and depression. We found positive correlations between affective inflexibility indices – specifically, decreased accuracy and increased reaction time on the MAFT – and overall misophonia severity. Honing in on specific symptom domains of misophonia as measured by the S-Five, we found a significant correlation between the affective inflexibility index of decreased switch accuracy and the impact, externalizing, and (although outlier-dependent) internalizing domains of misophonia severity. Further, self-reported cognitive inflexibility was also positively associated with misophonia severity. Based on multivariate regression analyses, three forms of rumination – perseverative cognition, anger rumination, and brooding – were all positively associated with misophonia severity, even when controlling for symptoms of anxiety and depression. These findings contribute to the understanding of misophonia from a cognitive perspective, potentially elucidating avenues for targets of future treatments.

***Vivien Black*** *is a recent graduate of the University of California, Berkeley, where she earned an undergraduate degree with Honors in Psychology. She is presenting her thesis project where she investigates affective flexibility and rumination as they relate to misophonia symptoms. Outside of her research, Vivien is involved with misophonia advocacy and helps lead a peer support program with the organization SoQuiet.*

**Investigating sensory gating in misophonia: evidence from a paired-click EEG paradigm**

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**Introduction:** Modern environments are filled with a complex array of sounds. Since humans cannot attend to all these auditory inputs simultaneously, the brain must suppress irrelevant sounds (Kaya and Elhilali, 2017). Typically, people suppress repetitive sounds like chewing and pen clicking, but these same sounds can elicit strong emotional reactions in people with misophonia (Enzler et al., 2021). This suggests that misophonia may be characterized by deficits in auditory processing related to the suppression of repetitive sounds. Auditory sensory gating is a neural process wherein behaviourally irrelevant sounds are filtered out to retain cognitive resources for meaningful sounds (Mayer et al., 2009). Notably, reduced sensory gating capacity is observed in clinical samples with sensory and emotional deficits, such autism spectrum disorder (Crasta et al., 2021).

**Objective:** The current study used EEG to investigate reduced sensory gating capacity as a mechanism underlying the experience of misophonia.

**Material and methods:** We recruited individuals with varying degrees of self-reported misophonia severity (assessed using the MisoQuest) to complete questionnaires about their sensory and emotional experiences. Additionally, they participated in a standard paired-click ERP paradigm, which is commonly used to assess sensory gating capacity (Boutros and Belger, 1999). EEG data were acquired using a 32-channel Biosemi system while participants sat upright and listened to paired clicks delivered binaurally through headphones. Sensory gating was quantified as a reduction in the P50 potential evoked by the second of the two consecutive click sounds. A reduction in the amplitude of the second P50 waveform (S2) relative to the first (S1) is taken as a measure of sensory gating capacity.

**Results:** We demonstrated that higher MisoQuest scores are associated with larger S2:S1 ratios in the P50 amplitude, suggesting that greater misophonia severity is associated with poorer sensory gating. MisoQuest scores were also positively correlated with scores on the sensory gating inventory, indicating that misophonia severity is associated with subjective sensory gating deficits.

**Conclusions:** This research demonstrates that misophonia may be characterized by reduced sensory gating capacity, suggesting that people with misophonia may have bottom-up deficits in auditory processing that influence the way they perceive sound.

***Kate Raymond**, I learned about misophonia through my own lived experience. For as long as I can remember, I have been enraged by the sound of chewing gum. In high school, I found the label for that experience, and during my undergraduate degree at McMaster University, I began to study it. I quickly realized that there was little empirical research on misophonia and that the condition affected many other people. My motivation to understand my own experience turned into scientific curiosity and a drive to make an impact through research. I earned a Master of Science degree in clinical psychology at Western University, where I evaluated the reliability and validity of misophonia assessments. I am currently a PhD student at Western University, aiming to investigate the neural mechanisms underlying misophonia using naturalistic paradigms that emulate real-world listening. Through this work, I hope to contribute to a better understanding and treatment of misophonia.*

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**Misophonia and school functioning of children and adolescents: teachers' and parents' perspectives**

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Misophonia, characterized by hypersensitivity to specific sounds, significantly impacts family dynamics and children's school experiences. This study aims to understand this condition from teachers' and parents' viewpoints, providing insights into coping strategies within educational and family contexts. Research Questions: 1. Are teachers able to recognize symptoms of misophonia in students at school? 2. How do teachers manage the challenges posed by misophonia? Do they view it as a challenge affecting their professional experience? 3. How do parents perceive and respond to their children's symptoms of misophonia at home? 4. What coping strategies do parents employ to manage misophonia in both family and school settings? 5. What support and intervention strategies do teachers and parents prefer for dealing with misophonia? Research Methodology: Participant Selection: The study involves teachers and parents. Teachers working with students of various age groups and parents of school-aged children are included. Sample: Approximately 300 individuals will be surveyed, including parents (both mothers and fathers). Additionally, 20 participants will be selected for in-depth interviews. Surveys: Participants will complete anonymous surveys. In-Depth Interviews: Selected participants will engage in detailed interviews to discuss their experiences and coping strategies. Expected Outcomes: This study aims to provide information on how teachers and parents perceive misophonia and identify effective coping strategies for managing this disorder in school and family settings. This exploratory research addresses a significant gap, as little is known about the experiences of those dealing with misophonia in children and adolescents. Study Objectives: The primary objective is to investigate the perceptions and experiences of misophonia in children and adolescents from the perspectives of teachers and parents. The study will explore its impact on family functioning and children's school performance. Significance of the Study: 1. Children with Misophonia: Highlighting the need for support due to the disorder's relative obscurity and the subsequent impact on students. 2. Parents' Experience: Examining how parents perceive their children's symptoms and their connections with school functioning, including interactions with teachers regarding misophonia. 3. Teachers' Perspective: Assessing teachers' awareness of misophonia, their experiences with affected students, and their observations on how these children function in the school environment. Project Outcomes: 1. Final Report. 2. Conference Presentations. 3. Scientific Publication. 4. Public Dissemination. Dissemination: The study's findings will be shared through participation in student conferences, promotion on social media by student organizations, and

dissemination among students at the Faculty of Psychology, University of Warsaw. By the time of the conference, preliminary results on parents' and teachers' perspectives and their coping strategies for dealing with misophonia symptoms in children will be available, with the qualitative interview phase still to be conducted.

### Reliability and validity of the *Sound Sensitivity Symptoms Questionnaire* (SSSQ) and *Hyperacusis Impact Questionnaire* (HIQ) in adults with hyperacusis

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**Introduction:** Hyperacusis is defined as a reduced tolerance to sound(s) that are perceived as normal to the majority of the population or were perceived as normal to the person before their onset of hyperacusis. It can be complicated and challenging to live with as every aspect of life, work, education, can be affected. A clear measure of sensitivity to sound is important. Several questionnaires for hyperacusis have been developed, including the *Hyperacusis Impact Questionnaire* (HIQ) and *Sound Sensitivity Symptoms Questionnaire* (SSSQ). However, these questionnaires need to be assessed for use online.

**Objective:** To independently evaluate the validity and reliability of these questionnaires in a general adult population reporting hyperacusis.

**Material and methods:** We evaluated the factor structure using exploratory factor analyses, internal consistency using Cronbach's Alpha, convergent and discriminant validity through their correlations with established measures (e.g., *Inventory of Hyperacusis Symptoms* (IHS), *Hyperacusis Questionnaire* (HQ), *Tinnitus Function Index* (TFI), *Patient Health Questionnaire-9* (PHQ-9), and *General Anxiety Disorder Scale-7* (GAD-7)) and floor and ceiling effects.

**Results:** Exploratory factor analyses revealed the HIQ has unidimensional structure with high internal consistency (Cronbach's $\alpha = 0.93$). The SSSQ had a two-factor structure (item 4 on 1 factor measuring misophonia) with moderately high internal consistency (Cronbach's $\alpha = 0.64$). Discriminant validity was established for both HIQ and SSSQ with low to moderate correlations with the TFI ($r = 0.1$), PHQ-9 ($r = 0.4$), and GAD-7 ($r = 0.4$). Convergent validity was demonstrated for both HIQ and SSSQ through moderately high correlations with the HQ ($r = 0.6$) and IHS ($r = 0.6$). Both questionnaires also showed potential sensitivity issues with floor and ceiling effects.

**Conclusions:** Overall, both instruments demonstrated acceptable internal consistency and construct validity, confirming their utility for assessing sound sensitivity and hyperacusis impact. However, SSSQ item 4 measured a different construct than the other items in the SSSQ due to it being misophonia-based, although its inclusion could be deemed as informative for clinical use but should not be included in the overall score. Future studies should explore additional validations and conduct longitudinal assessments to enhance these tools further.

**Magdalena Sereda** *is an Associate Professor at the NIHR Nottingham Biomedical Research Centre, University of Nottingham. Her research focuses on assessing the effectiveness of NHS contracted sound therapy options for tinnitus, clinical management of hyperacusis, and non-invasive brain stimulation for tinnitus. Magdalena graduated from Warsaw University in Biology and obtained a PhD in Neuropsychology from the Institute of Experimental Biology, Warsaw. As a Guest Researcher at Humboldt University, Berlin, she researched animal models of tinnitus. Later, she worked as a Career Development Fellow at the MRC Institute of Hearing Research in Nottingham to look at objective characterisation of tinnitus using magnetoencephalography. Over the years Magdalena's research has concentrated on several aspects of the functioning of the auditory system, including cochlear implant technology and tinnitus. She has over 20 years' experience of working with people with different hearing disorders including tinnitus sufferers, cochlear implant users and deaf adolescents. She has gained experience in various audiological, neuropsychological and psychophysiological techniques as well as in vitro animal electrophysiology and brain imaging methods (MEG).*

### Investigation of the relationship between hyperacusis and misophonia severity and depression and anxiety

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**Objective:** Hyperacusis and misophonia are frequently associated with psychiatric comorbidities. The relationship between symptom severity and depression and anxiety has not been directly demonstrated.

**Aim:** The aim of this study was to investigate whether there is a correlation between the severity of hyperacusis and misophonia and depression and anxiety.

**Material and methods:** Participants consisted of 12 adults who presented to the audiology clinic with complaints of decreased sound tolerance. Each participant underwent audiologic evaluation (pure tone audiometry, speech audiometry, immittancemetric examination, speech tests) and decreased sound tolerance assessment (scales and questionnaires, loudness discomfort level measurement). The *Khalfa Hyperacusis Questionnaire* (HQ), *Misophonia Symptom List* (MSL) and loudness discomfort level measurement (LDL) were used together to differentiate decreased sound tolerance. Individuals with both hyperacusis and misophonia who were reported to have normal hearing on audiologic evaluation were included in the study. *Hyperacusis Handicap Questionnaire*, *Misophonia Scale*, *Beck Depression Scale* and *Beck Anxiety Scale* were administered to all participants. The correlation

between the severity of hyperacusis and misophonia and anxiety and depression scores were evaluated.

**Results:** A moderate positive significant correlation was found between hyperacusis severity and depression score ($r = 0.633$; $p = 0.03$). No significant relationship was observed between hyperacusis severity and anxiety score ($p > 0.05$). No significant correlation was observed between the number of misophonic triggers and the score of misophonia scale and depression and anxiety ($p > 0.05$).

**Conclusions:** Increased severity of hyperacusis may increase depression. Future studies should be continued by increasing the number of data for a reliable interpretation of the findings. Keywords: anxiety, depression, hyperacusis, misophonia.

***Nazife Öztürk Özdeş*** *is a research assistant in audiology at Ankara University, Turkey and a PhD student in her thesis period. Her main academic interests are tinnitus, decreased sound tolerance and auditory processing. She is currently working in the audiology clinic of Ankara University, Turkey, where she is interested in the diagnosis and rehabilitation of tinnitus and decreased sound tolerance. In addition, she has been working on the development of a rehabilitation program for individuals with hyperacusis and misophonia. She is also a volunteer research assistant at Hashir International Specialist Clinics & Research Institute for Misophonia, Tinnitus and Hyperacusis. She is a student member of the International Society of Audiology.*

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**Multidisciplinary management of misophonia: our algorithm**

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**Introduction:** Misophonia as a selective sound hypersensitivity has been relatively new recognized mental disorder that characterized by different negative emotions and behavior inspired by specific triggering sounds. Among medical specialists misophonia sufferers have been called "misophonics". Misophonics have been experiencing different grades of anxiety, disgust, rage and/or fear as well other negative feelings when they hear individual specific sound triggers. The negative emotions of sufferers complicate of even destroy their ability to perform usual daily activities and thus may impact their quality of life. Although there is a certain quantity of misophonics in general population, many general practitioners, ear, nose and throat (ENT) doctors, and other medical specialists face the difficulties of diagnosing and management misophonia. It is newly recognized pathology without strict diagnostic/treatment protocol and follow-up. Basing on our own experience we aimed to assess the quality of life in misophonics and create a multidisciplinary algorithm for misophonia management.

**Material and methods:** We analyzed our experience of management 293 individuals with sound hypersensitivity. The results of routine audiological tests and self-report questionnaires were gathered retrospectively from the records of the patients. Measures included pure tone audiometry, uncomfortable loudness levels (ULLs), and responses to the tinnitus impact questionnaire (TIQ), the hyperacusis impact questionnaire (HIQ), and the screening for anxiety and depression in tinnitus (SAD-T) questionnaire.

**Results:** Audiological testing with subsequent ENT counseling let us separate misophonics from people with other hearing disorders characterized sound hypersensitivity. We revealed hyperacusis 212 (72.4%) pts with sound hypersensitivity. Their average A-MISO-S total score was 0. Other 81 (27.6%) pts were characterized by normal audiometric parameters while their average A-MISO-S total score was 9.6 ± 4.8.

**Conclusions:** Misophonia can occur both in isolation and in combination with certain mental illnesses. It is worsening overall quality of life as well as quality of sexual life in sufferers. Proposed own multidisciplinary algorithm of management can be a helpful tool for doctors in different specialties to provide proper care for misophonics. Considering the negative influence of misophonia on the quality of overall and sexual life in sufferers, urologists/gynecologists and sexologists should be included into the multidisciplinary team for correct medical support of sufferers.

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**Tinnitus reduction after stapedotomy**

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**Introduction:** Otosclerosis is a bone remodeling disorder within the otic capsule of the temporal bone. According to the literature, 65–90% of patients with otosclerosis experience

tinnitus. The most commonly used and most effective treatment for otosclerosis is surgery, including stapedotomy or formerly stapedectomy, with both these techniques providing satisfactory results in hearing improvement. Many clinicians and investigators report a substantial reduction of tinnitus after stapes surgery in patients with otosclerosis.

**Aim:** To assess tinnitus reduction after stapedotomy.

**Material and methods:** The study population was 95 patients with otosclerosis suffering from tinnitus. They completed the *Tinnitus Functional Index* (TFI) before stapedotomy and 3 months after the surgery. The minimal important change was estimated with the *Clinical Global Impression* (CGI) *scale* as the external criterion (anchor). The mean change method and the receiver operating characteristic (ROC) method were used to determine a minimal important change in tinnitus sensation.

**Results:** The improvement in tinnitus after stapedotomy was reported by 69.4% of the patients with otosclerosis. Minimal important change in tinnitus was estimated as a reduction of 8.8 points in the TFI.

**Conclusions:** The value of 8.8 points in the TFI can be used as a benchmark of stapedotomy effectiveness in otosclerosis patients suffering from tinnitus.

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**The use of heart rate variability (HRV) biofeedback therapy in the treatment of misophonia**

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**Introduction:** Misophonia is characterized by an extreme sensitivity to specific sounds that provoke intense emotional and physiological responses. Despite significant interest, many questions about the origins of misophonia and effective treatment methods remain unresolved. Current treatment approaches often include cognitive behavioral therapy, relaxation techniques, and sound therapy, which collectively aim to diminish hypersensitivity and enhance patients' quality of life.

**Objectives:** This study aimed to evaluate the potential efficacy of heart rate variability (HRV) biofeedback therapy in the treatment of misophonia, based on a case study of a 30-year-old woman.

**Material and methods:** The case study focuses on a patient with auditory hypersensitivity and misophonia, who is irritated by specific sounds such as slurping, gurgling, dripping water, clattering shoes, and barking dogs. She has been experiencing these symptoms for about 15 years and has noticed an increase in sensitivity to sounds during her pregnancy, leading to tension, anger, and helplessness. Due to the patient's pregnancy, pharmacological treatment was not an option. Instead, a quantitative analysis of brain bioelectrical activity (quantitative electroencephalogram, QEEG) was conducted, revealing an overexpression of fast Beta 1 and Beta 2 waves in most recordings. The patient underwent heart rate variability (HRV) therapy, with optimal respiratory parameters (7.5 breaths/minute) determined and applied during therapy. Fifteen HRV training sessions were conducted in three series of five sessions each, held from Monday to Friday with a weekly break between series (1.5 months in total). Concurrently, the patient participated in psychological therapy. QEEG testing was repeated after the therapy.

**Results:** After therapy, there was a reduction in the relative power of QEEG in the Theta (4–8 Hz) and Alpha (8–12 Hz) waves and fast Beta2 (20–34 Hz) waves in the frontal and central brain regions, which was particularly evident in the signal recorded with the eyes closed. These changes were consistent with the patient's subjective feelings – she sleeps better, is relaxed and calm. She is also able to ignore sounds that previously irritated her.

**Conclusions:** The utilization of HRV therapy led to a prompt enhancement in the patient's daily functionality as well as changes in QEEG, with a notable improvement observed within a period of 1.5 months. The potential application of HRV biofeedback therapy in the treatment of misophonia is a promising avenue for further investigation.

***Małgorzata Pastucha*** *is an Audiologist and Special Educator. She graduated from the Faculty of Physics and Educational Studies at the A. Mickiewicz University in Poznań. Her current field placement is with the Experimental Audiology team in the Institute of Physiology and Pathology of Hearing. She is interested in objective tests of the auditory organ and electroencephalography.*

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**What would be the factors linked to hyperacusia in schools?**

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**Introduction:** The integrity of the auditory system is crucial for students to have optimal settings for the learning process. Nevertheless, certain auditory alterations can impede this progress, such as hyperacusis, which is characterized by a diminished ability to tolerate common and/or ambient sounds.

**Objectives:** The aim of this study was to examine the occurrence of sound intolerance and identify the related factors in school-aged children.

**Methods:** The present investigation was conducted as a cross-sectional, prospective, and descriptive observational study, involving the evaluation of 60 youngsters of both genders. The evaluation was conducted via a questionnaire that was completed by parents and/or guardians via the virtual platform.

The inquiries centered on sociodemographic information on past and present health, as well as auditory well-being. Additionally, it is noteworthy that the children were also inquired about their self-perception of tinnitus, hearing, and hypersensitivity to sounds.

**Results:** The findings of the current study revealed that 48% of the students assessed experience annoyance from certain sounds, and in 15% of them, the discomfort is triggered by more than five auditory stimuli. The sounds that were most frequently reported as causing discomfort were screams, accounting for 17.4% of the reports, followed by motorbikes at 7.8%. In addition, there was an observed correlation between various discomforts such as odors/lights (15%) and motion sickness (23.3%). An analysis comparing auditory hypersensitivity and parents' opinion of their children's perception revealed that 8.3% of children exhibited hypersensitivity.

**Conclusions:** The findings indicated that 8.3% of schoolchildren have a sound intolerance. When it comes to elements linked to hyperacusis in children, it has been noted that students who experience discomfort from light or scents also exhibit higher levels of aural hypersensitivity. Furthermore, motion sickness had no bearing on hyperacusis instances.

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# 59th Inner Ear Biology Workshop, 15–17 September 2024, Warsaw, Poland

Dear Colleagues,

We present abstracts submitted for the 59th Inner Ear Biology Workshop (IEB 2024), which will take place in Warsaw from Sunday, 15 September to Tuesday, 17 September 2024.

The Inner Ear Biology Workshop is organized annually by a group of scientists interested in and actively pursuing the research of inner ear biology. The annual meetings are held in different European academic centers with the objective of a free exchange of scientific accomplishments.

In 2024, the meeting is hosted jointly by the World Hearing Center and the Institute of Sensory Organs.

We wish you a productive and exciting meeting!

*Prof. Piotr H. Skarzynski, MD, PhD, MSc*
*General Secretary of the 59th IEB Workshop*

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# 59TH INNER EAR BIOLOGY WORKSHOP, 15–17 SEPTEMBER 2024, WARSAW, POLAND

## Invited Lectures

### Cellular senescence in inner ear physiology and disease

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Cellular senescence, a state of permanent cell cycle arrest, is vital in embryonic development, including inner ear formation. During this process, senescent cells aid morphogenesis by regulating tissue remodeling and ensuring proper cell differentiation. Senescent cells are characterized by distinctive morphological and physiological changes, including enlarged cell size, increased β-galactosidase activity, altered gene expression, and the secretion of various inflammatory cytokines, chemokines, and proteases, collectively known as the senescence-associated secretory phenotype (SASP). Senescence cells secrete factors that shape the development of auditory structures like the cochlea and vestibular apparatus. Deficits in embryonic senescence can lead to malformations and functional impairments in the inner ear. This highlights the importance of regulated senescence for proper inner ear development. Vestibular schwannomas, age-associated benign tumors from Schwann cells of the vestibular nerve, further illustrate senescence's role in ear health. These tumors can cause hearing loss, tinnitus, and balance issues. In tumors, senescence can both suppress and promote growth. While senescence halts uncontrolled cell proliferation, the senescence-associated secretory phenotype (SASP) can create a pro-tumorigenic environment, enhancing tumor survival and progression. Understanding senescence's dual roles in embryonic development and tumorigenesis is crucial for targeted therapies. In embryonic contexts, promoting proper senescence could prevent anomalies. In vestibular schwannomas, modulating senescence and SASP factors may improve outcomes, indicating the potential for senescence-targeted interventions in developmental and neoplastic inner ear conditions.

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### Chronic electrical stimulation may slow down deafness-induced neural degeneration but does not change responsiveness of the auditory nerve

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**Introduction:** The auditory nerve degenerates after severe damage to the organ of Corti including loss of hair cells and/or synapses. It is often assumed, based on the use it or lose it principle, that activation of the auditory nerve by chronic electrical stimulation (CES), as delivered by a cochlear implant (CI), halts further neural degeneration. However, data in both animal and human literature (e.g., Seyyedi et al., 2013) do not convincingly support that assumption. In the present study we examined the effect of CES on the auditory nerve in deafened guinea pigs, applying both structural and functional measurements.

**Material and methods:** Normal-hearing guinea pigs received an intracochlear electrode array and were ototoxically deafened four weeks later by co-administration of kanamycin and furosemide. CES treatment started either one or five weeks after deafening, and it was applied for 6 days/week during 2 weeks. The CES stimuli consisted of asymmetric charge-balanced current pulses of 300 μA presented at quasi-random variable pulse rate (0.7–1.9 kHz; 1 kHz on average). Using a MED-EL PULSAR stimulator, awake eCAP recordings were weekly performed as described in Ramekers et al. (2022). Following the final eCAP recording session the animals were sacrificed, and their cochleas were processed for histological quantification of the spiral ganglion cells (SGCs).

**Results:** SGC survival was similar for the implanted right and the non-implanted left ears in control animals. The SGCs in the implanted ear were significantly larger than those in the non-implanted ear. Animals receiving CES showed a moderate but statistically significant increase in SGC survival in their implanted/stimulated ear compared to the contralateral ear; cell size across ears was similar in these animals. We did not observe a difference in any of the several eCAP outcome measures between CES treated animals and untreated control animals.

**Conclusions:** CES slows down, but does not stop SGC degeneration, which is consistent with previous studies in animal models and CI users. CES is nor beneficial neither detrimental for the nerve's responses to electrical stimuli as delivered by a CI.

**Cochlear stress granules: potential regulators of stress and survival in the inner ear**

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Sensory hair cell death is a primary cause of adult-onset sensorineural hearing loss. It is well established that, in mammals, cochlear hair cells are not regenerated and thus hearing loss resulting from hair cell death is permanent. Understanding the mechanisms that determine hair cell death is therefore essential to provide effective therapies for protecting those cells. Stress granules are membrane-free aggregates of mRNA and RNA-binding proteins that form during cellular stress. By controlling the fate of mRNAs, SGs play a key role in the post transcriptional regulation of gene expression during stress. Stress granules assemble rapidly when cells are exposed to stress and normally disperse when the stress is resolved. I will discuss our research on stress granules and their potential role in the homeostatic response of cochlear cells to ototoxic drugs that cause hair cell loss and deafness.

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**Deciphering the genetic background of autosomal dominant hearing loss**

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**Introduction:** Autosomal dominant hearing loss (ADHL) is the second most common form of inherited HL with an onset usually after the first decade of life. It affects mainly high frequencies and progresses over time. Autosomal-dominant genes are responsible for about 20% of cases of hereditary non-syndromic deafness, with 63 different genes identified to date.

**Material and methods:** In this study, 105 families with a vertical inheritance pattern of hearing impairment were recruited. Genomic DNA was isolated from peripheral blood samples or buccal swabs of available family members. In all probands targeted next-generation sequencing (NGS) using a targeted multi-gene panel (237 genes) was performed. In 6 largest unsolved families linkage analysis and whole genome sequencing (WGS) were performed. Presence of the selected probably pathogenic variants and their segregation with HL within the family were confirmed by standard Sanger sequencing.

**Results:** Genetic cause of ADHL was identified in 43.8% (46/105) of the examined families. Among the 46 identified HL variants only 26% (12/46) have been previously reported and the remaining 74% are novel (34/46). We identified missense variants (27/46; 58.7%), splice site variant (9/46; 19.5%), stop-gain variants (5/46; 10.9%) as well as frameshift variants (5/46; 10.9%). Among the most common causative genes were *MYO6* ($n = 8$), *TBC1D24* ($n = 5$), *KCNQ4* ($n = 4$), *GSDME* ($n = 4$), *POU4F3* ($n = 4$) and *WFS1* ($n = 4$). Pathogenic variants causative of HL in the *NLRP3*, *LMX1A*, *FGFR3*, *CD164*, *GRHL2*, *TMC1*, *COCH*, *ATP2B2* and *CEACAM16* genes were detected in single families. Implementation of linkage analysis and WGS resulted in the identification of the non-coding variants in the *EYA4* and *ATP11A* genes and two novel candidate genes.

**Conclusions:** Our custom multigene panel has demonstrated good diagnostic performance. Considering frequent identification of novel genetic variants it is necessary to perform thorough clinical examination and variant segregation analysis with ADHL in all available family members. The use of linkage analysis and WGS increases the detection rate of causative variants, especially located in the non-coding regions, and provides the opportunity to identify novel genes.

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**History of the Inner Ear Biology Meeting**

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The IEB's inaugural assembly convened under the name of "Arbeitstagung für Innenohrbiochemie" (Ear Biochemistry) in 1964 within the environs of Düsseldorf, Germany, orchestrated by Sigurd Rauch (1916–2003), gathering a select cohort of burgeoning otolaryngologists with a proclivity for scholarly inquiry. Spanning the period from 1964 to 1967, four symposia on Inner Ear Biochemistry were convened in Germany, marking the nascent stages of a burgeoning scholarly endeavor. However, the intellectual purview of these gatherings swiftly transcended national boundaries, metamorphosing into an international discourse. The seminal year of 1968 witnessed a pivotal milestone as the venue shifted beyond the confines of Germany, alighting upon Zürich, concurrently accompanied by an appellation modification from "Workshop on Inner Ear Biochemistry" to the more encompassing "Workshop on Inner Ear Biology". Since its seminal expansion, the convocation has traversed a series of locales and nations annually, responding to invitations from a diverse array of hosts. Attendance burgeoned precipitously, only to plateau subsequently, stabilizing at a robust cadre numbering between 100 to 200 attendees. The workshops have perennially exuded a distinctive ambiance, meticulously nurtured and preserved throughout successive iterations. Their paramount objective, both then and now, remains the fostering of an environment conducive to candid exchange, characterized by affability interwoven with rigorous intellectual debate. This ethos facilitates the cultivation of enduring professional connections, even amongst dissenting viewpoints. Notably, the workshop serves as a veritable nexus for biologists and otolaryngologists alike, fostering interdisciplinary discourse and collaborative problem-solving. Otolaryngologists, notwithstanding their clinical commitments, assume a pivotal role in fostering cohesion amidst this interdisciplinary amalgamation, thereby augmenting the workshop's enduring legacy.

**Identification of feedforward/feedback contributions to age-dependent hearing loss and tinnitus using OPM-MEG**

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Acquired auditory processing disorders including age dependent hearing loss, speech discrimination deficits, tinnitus or hyperacusis, require a personalized diagnosis to assign the individual cause within the auditory hierarchy to either the periphery, subcortical or distinct cortical or cortico-fugal neuronal dysfunctions. The well-functioning feedforward and feedback PV-IN network is an essential precondition for temporal intracortical network function in audition that above all senses relies on high speed of information flow (Zajac I.T. and Nettelbeck T., 2018). We hypothesize disease-specific deficits in temporal intracortical network function in auditory circuits. Therefore, the diagnostic of those should have a special significance. We used time-sensitive MEG-OPM measurements and aimed to study different auditory stimulus paradigms to detect fast auditory processing in different groups of tinnitus with and without hyperacusis or presbycusis. We expect this method to become an efficient diagnostic strategy to fathom peripheral or central contribution of the distinct auditory impairments in the future to improve individualized targeted interventional therapies. Here we will present preliminary results demonstrating the usability and function of the OPM-MEG for hearing research.

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**Irx3/5 null deletion in mice blocks cochlea-saccule segregation**

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**Introduction:** A gene cadre orchestrates the normal development of sensory and non-sensory cells in the inner ear, segregating the cochlea, the organ of Corti, and five vestibular endorgans. Evolution transforms the basilar papilla in sarcopterygians into the cochlea in mammals. However, the role of genes driving the ear development is largely unknown.

**Material and methods:** We used double null mice for Iroquois homeobox 3 (Irx3) and 5 (Irx5) transcription factors (Irx3/5 DKO). Mice can survive to about E16.5, after that they occasionally can reach at E17.5.

**Results:** We show that double deletion of (Irx3/5 DKO) mice leads to the fusion of the saccule with the cochlear base. The medial rows of cochlear HCs in the expanded sensory epithelium assumed vestibular-like hair cells near the modiolus while others seem like cochlear hair cells. The otoconia and tectorial membranes are needed for normal function but are absent in the Irx3/5 DKO inner ear. The mutant cochlea showed a reduced spiral ganglion neuron population, which projects fibers to both saccular- and cochlear-like HCs. The central projections from the cochlear apex-base contour are not fully segregated into a dorsal and ventral innervation in the Irx3/5 DKO cochlear nucleus. An expansion of the cochlear dorsal nuclei in the brainstem reaches vestibular fiber connections only in the Irx3/5 DKO. Additionally, the auditory and vestibular systems in Irx3/5 DKO mice are interconnected, characterized by the formation of bilateral connections between the descending vestibular and ascending apex that also demonstrates a unique interconnectedness between the cochlear apex and the vestibular neurons, a "vestibular-cochlear" nerve (VCN) in the mouse inner ear.

**Conclusions:** We suggest that it indicates the mammalian cochlear apex, which is a derived from the lagena. Further, a newly bilateral connection between the vestibular and apex are reminiscent of sarcopterygians based on fibers and neurons.

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**The cognitive ear: the emerging role in the aging**

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Hearing impairment is known as a major clinical risk factor for cognitive decline, with relevant clinical implications for dementia prevention, diagnosis, and treatment. However, the complex pathophysiological relation between hearing impairment and dementia remains to be fully defined. Consistently with the concept of an "hearing health trajectory," beginning at conception/birth and continuing throughout life in which environmental factors, such as noise, medicaments, and lifestyles (e.g., alcohol, smoking, diabetes, and weight gain), contribute to affect hearing. Several studies identified the exposure noise-induced hearing loss (NIHL) as a risk factor for sensory aging and cognitive decline processes. Although the association among age related hearing loss (ARHL), NIHL, and cognitive impairment has been clinically widely documented the molecular mechanisms underlying this association are not fully understood, and it is not known how these risk factors (sensory aging and noise) can interact, affecting brain functions. We recently found that early noise exposure in an established animal model of ARHL (C57BL/6 mice) accelerates the onset of age-related cochlear dysfunctions. While an animal model of Alzheimer's disease (AD), that is the 3 × Tg-AD mice we found that NIHL before that phenotype is manifested, caused persistent synaptic and morphological

alterations in the auditory cortex and earlier hippocampal dysfunction, increased tau phosphorylation, neuroinflammation, and redox imbalance, along with anticipated memory deficits compared to the expected time-course of the neurodegenerative phenotype. Furthermore, in the WT mice also HL, can accelerate ARHL onset inducing persistent synaptic alterations in both auditory cortex and hippocampus affecting memory performance and oxidative-inflammatory injury. Collectively, our experimental data confirm the existence of "cognitive ear" that can be early affected, thus midlife HL can be responsible for a hippocampal-dependent memory dysfunction. Considering that memory dysfunction is usually the first cognitive symptom of dementia (like AD) onset, from a translational point of view, our results support the hypothesis that associating auditory and memory screenings could represent a powerful non-invasive tool to potentially identify subjects with a high risk to develop dementia, allowing early diagnosis and treatment.

**The impact of OAEs on hearing science and the inner ear research**

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Otoacoustic Emissions (OAEs) are vibrational patterns which are recorded in the external meatus, upon stimulation of the ear by a transient or sinusoidal signal. They were discovered by David Kemp in 1978 but their true potential in gauging the functional status of the cochlear amplifier never truly shined and thus this tool never made the impact we were hoping to make, back in the 80s. This lecture will present an excursus of some interesting developments the last 45 years, based on otoacoustic emissions in three key areas of hearing science, such as: (i) screening for hearing deficits (neonatal screening, adult screening, drug efficiency monitoring etc.); (ii) pharmacology testing models; (iii) inner ear modelling.

**The resident mast cells are a component of the cochlear immune system**

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The immune system is often conceptualized as the body's internal police force. Its innate components (macrophages, dendritic cells, neutrophils, eosinophils, basophils, mast cells, and the complement system), in conjunction with the acquired immunity (T cells, B cells), are responsible for the surveillance of the body, discrimination between "self" and "non-self," recognition of pathogens and toxins, and appropriate defense if needed. In addition to the classic lymphoid tissues, such as the thymus, spleen, and lymph nodes, immune system cells are found throughout the body, including the cochlea. Several publications have reported the existence of resident immune cells in the human and rodent cochlea, but their role in cochlear biology remains unclear. Our research group has concentrated on characterizing two cochlear cell types representing the innate immune system: the microglia and the mast cells. Microglia develop from yolk-derived mesodermal precursors and reside exclusively in the central nervous system. Recently, research has identified microglia in the cochlea based on the expression of the Iba1 surface molecule. To gain further insight into the nature of these cells, we employed immunofluorescence, confocal microscopy, and Western blot techniques to characterize them using another marker for microglia (TMEM119). Our findings indicate that in the murine cochlea, Iba1+ TMEM119+ double-positive cells are absent before and after the onset of hearing and after acoustic trauma in adult mice. In contrast, the second cell type, mast cells, were present in the developing and adult rodent cochleae, as evidenced by the expression of several markers, including heparin, mast cell tryptase, mast cell chymase, and IgE receptor. Their numbers were sparse, but inhibiting their degranulation reduced cisplatin-induced damage to auditory hair cells. Furthermore, adding the supernatant from degranulated mast cells resulted in the loss of inner and outer hair cells. Moreover, we identified all four receptors for the major mast cell mediator, histamine, in the murine cochlea. Furthermore, our systematic review demonstrated the association between allergies, sudden sensorineural hearing loss, Meniere disease, and acute low-tone hearing loss. We conclude that mast cells, but not microglia, are resident cells in the rodent cochlea and that their activation can contribute to cochlear pathologies.

## Oral Presentations

### 350 families' whole genome sequencing in early onset hearing loss: the French Reference Centre's experience

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**Introduction:** Up to recent years, diagnosis of the genetic aetiology of early onset hearing loss (EOHL) relied on targeted gene panel analyses, with a diagnosis rate of about 50%. French clinical geneticists have now access to trio whole genome sequencing (WGS) through the Plan France Medecine Genomique 2025 sequencing platforms for most patients presenting with EOHL.

**Methods:** Testing criteria for WGS differ according to the subgroup of EOHL indications. The strategy is genome-first for syndromic hearing loss (HL), WGS after normal array-CGH for HL associated with malformation(s) and WGS after normal GJB2/GBJ6 and HL gene panel for patients presenting with isolated HL.

**Results:** From 2020 to mid-2024, 350 families assessed in the French Reference Center for genetic HL underwent whole genome sequencing on the SeqOIA platform. The diagnosis yield was 40%. We will present the main genes identified, the diagnostic rate according to subgroups of the EOHL indication, clinical vignettes, along with the advantages and limitations of our strategy.

**Conclusions:** WGS allows for identification of additional causes (class 4 and 5 variants) and candidate genes, but variants interpretation is more complex than exome or gene panel analysis. We show with our four-and-a-half years' experience that WGS is an enticing new tool for patient diagnosis, with its advantages and setbacks.

### A mouse model of unilateral stereotactic radiosurgery-induced hearing loss

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**Introduction:** Stereotactic radiosurgery (SRS) is a precise, single-session irradiation technique commonly used to treat vestibular schwannomas. However, SRS can lead to irreversible hearing loss, most probably due to irradiation-induced damage to the nearby inner ear. Currently, no preventive or therapeutic options exist, highlighting the need for the development and experimental testing of novel treatments. To enable this research, we developed a protocol for inducing unilateral hearing loss in mice through targeted unilateral cochlear irradiation.

**Material and methods:** We used 6-week-old C57BL/6J mice and administered precise unilateral irradiation in the vicinity of the cochlea using a Leksell Gamma Knife® Icon device. The precision and reproducibility of the targeted area were ensured through radiological imaging for each mouse using the integrated cone beam CT scan and co-registering these images with MRI and CT mouse atlas images. To ensure meaningful translational data, we placed a single 4 mm isocenter lateral to the cochlea with the 80% isodose line passing through the modiolus to deliver 8 ($n = 3$), 16 ($n = 5$), 24 ($n = 8$), and 32 ($n = 8$) Gy. Auditory brainstem responses (ABR) were measured one day prior to irradiation (baseline) and at one and four weeks post-irradiation. Statistical analysis was performed using two-way repeated measures ANOVA with Bonferroni correction.

**Results:** In all experimental groups, the irradiation dose received by the non-irradiated cochlea was less than 15% of that received by the irradiated cochlea. In the 32 Gy group, irradiation of cochlea yielded significant threshold shifts, compared to the non-irradiated ear, at 22.6 and 32 kHz on day 7 and to a greater degree on day 28. Similar but less pronounced effects were observed in the 24 Gy group. Furthermore, we observed a unilateral decrease in the *p-p* and wave I amplitudes, following 72–78 dB SPL click stimulation, in both groups. No hearing loss was detected in the 8 and 16 Gy groups. Histopathological studies are ongoing.

**Conclusions:** Targeted near-cochlear irradiation in mice induces unilateral dose-dependent high-frequency hearing loss,

evidenced by increased threshold shifts and decreased ABR wave I amplitudes. This model provides a valuable tool for exploring the radiobiological mechanisms underlying SRS-induced hearing loss and for testing potential radioprotective agents.

**A one-year time course of electrocochleography in cochlear implant users**

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**Introduction:** Electrocochleography (ECochG) has become increasingly valuable in cochlear implant (CI) surgery as it allows intraoperative monitoring of effects of electrode array insertion on residual hair cell function. Additionally, ECochG has the potential to portray postoperative cochlear function. Here we examine the postoperative time course of residual hair cell function by recording ECochG in CI recipients intraoperatively and at several time points up to one year postoperatively.

**Material and methods:** Twenty-three patients with severe sensorineural hearing loss receiving a CI participated in a trial comparing electrode arrays (SlimJ and Mid-Scala of Advanced Bionics) and surgical approaches (Jwair et al., 2021). Here, the data are analysed by investigators blinded to the randomisation. ECochG recordings were performed at 5 time points using the active insertion monitoring (AIM) system of Advanced Bionics: intraoperatively, at 4–6 weeks, 3–4 months, 6–7 months and 12–14 months postoperatively. Responses to pure-tone stimuli with frequencies varying from 125 to 4000 Hz, at sound levels 100–115 dB HL, were recorded at each of the 16 available electrodes, in two opposite phases. The difference and sum of the recordings to the opposite phases were computed as estimates of cochlear microphonics (CM, reflecting hair cell potentials) and auditory nerve responses, respectively.

**Results:** Significant CM responses (>1.5 μV) were found in 12 out of 22 patients intraoperatively, and postoperatively in 10/22 at 4–6 weeks, 12/16 at 3–4 months, 11/16 at 6–7 months, and 7/11 at 12–14 months. Notably, 7 intraoperative non-responders showed significant responses in the first postoperative session. Averaged across frequencies the largest responses were observed recorded at the apical electrodes during the intraoperative session. The responses were smallest at 4–6 weeks postoperatively, then recovered after 3 to 7 months, and decreased again up to one year after implantation. Intraoperatively, the dominant frequency was 500 Hz, postoperatively it was 125 Hz.

**Conclusions:** Residual hair cell function gradually declines over the course of one year. Three months after cochlear implantation a short period of recovery is seen, which may be attributed to natural reduction of the acute local inflammatory response to the electrode array.

**AAV-mediated precision treatment of SchABE8e in the pou4f3Q113*/+ mouse model**

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Genetic mutations can cause hereditary deafness, among which DFNA15 caused by mutations in the transcription factor *POU4F3* is a clinically common autosomal dominant non-syndromic deafness, and studies have been conducted to partially restore hearing in model mice by small molecule inhibitors. However, this strategy fails to address the root cause of hearing impairment. Base editors are capable of editing mutation sites precisely and efficiently, and have the potential to completely restore hearing damage. In order to verify the effectiveness of gene editing for the treatment of this deafness disease, taking the Chinese DFNA15 (POU4F3Q113*) deaf family line as an example, we successfully constructed the Pou4f3Q113*/+ mouse model with the progressive hearing damage of this type of patients, which provides a good animal model for the study of this type of genetic deafness disease. On the basis of the three highly efficient Cas9 types discovered in the laboratory in the previous stage, a series of novel ABE toolboxes were developed through the fusion of multiple types of TadA deaminases. The test screened SchABE8e base editor in vitro can realize precise and efficient editing at the pathogenic mutation (up to 50% efficiency). Compared with ABE8e, SchABE8e has higher editing efficiency and lower off-target mutations and indels, which is safer and more precise. Subsequently, by analyzing the secondary structure of SchABE8e protein, the optimal splitting site 4 was screened to achieve similar protein expression as WT SchABE8e in the HEK293T cell. Delivery of split SchABE8e/NNGR-Target3 into the cochlea of neonatal mice by double AAV-Anc80L65 achieved long-term effective hearing recovery (hearing level close to that of WT mice) in Pou4f3Q113*/+ mice. This project provides a new strategy for the treatment of DFNA15 disease in the clinic, and the development of SchABE8e also provides a novel tool option for gene therapy of other hereditary deafness diseases.

**AAV-OTOF gene therapy for autosomal recessive deafness 9: a multicenter, multiage, non-randomized controlled intervention study**

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**Introduction:** Autosomal recessive deafness 9 (DFNB9) is a congenital auditory neuropathy with clinical features including congenital or prelingual, bilateral symmetry, severe to complete deafness, caused by OTOF mutations. We previously reported the safety and efficacy of adeno-associated virus (AAV) mediated OTOF (AAV-OTOF) delivery in children for the first time worldwide. It is believed that AAV-OTOF can restore hearing in infants and children with DFNB9, but there have been no safety and efficacy studies in elder participants. This study enrolled the adolescent and adult participants and aimed to investigate the association of age with safety and efficacy of AAV-OTOF gene therapy.

**Material and methods:** This study is a multicenter, open-label, single-arm and intervention trial. We recruited 9 DFNB9 participants with age diversity (1.8- to 23.9-year-old) from 4 Chinese sites. All participants carry biallelic OTOF mutations with severe to complete hearing loss, unilateral or no cochlear implantation. Participant 3 received two rounds of AAV-OTOF injection. Single injection of AAV-OTOF into the inner ear was performed in other 8 participants. The follow-up period was from July 2023 to May 2024. The primary outcomes were safety and tolerability. Secondary outcomes included auditory function assessments.

**Results:** We present a relevant evaluation of safety and efficacy in 9 participants 2–9 months after AAV-OTOF treatment. No serious adverse events (AEs) occurred in the dose of AAV-OTOF at $8.4 \times 10^{11}$ to $1.12 \times 10^{12}$ vg. No serious drug-related AEs (AEs) were observed with a total number of 8 of grade I and II AEs. Hearing recovered in 8 participants after surgery. At 1 month after surgery, the mean click ABR threshold for participants decreased from >99 dB at baseline to 56.7 dB. Notably, the thresholds of click-ABR, tone-burst ABR (TB-ABR) and pure tone audiology (PTA) thresholds for the 23.9-year-old adult participant decreased from >100 dB, >100 dB, and 93.6 dB at baseline to 70, 81, 67.9 dB at 1 month, respectively. We found that the hearing recovery effects showed a potential age correlation. Participants with hearing recovery were divided into three groups by age (1: 1–2 years; 2: 5–8 years; 3: >14 years). The thresholds of click-ABR (PTA) in the 3 groups were improved by 45 dB (7.9 dB), 51.8 dB (56.7 dB) and 20 dB (25.7 dB) at 1 month, respectively. The thresholds of TB-ABR in group 2 and 3 improved from >99 dB at baseline to 57 dB and 69 dB at 1 month, respectively. Overall, participants aged 5–8 years had a better hearing recovery.

**Conclusions:** In this trial, AAV-OTOF gene therapy was proved as a safe and effective treatment for infants to adult patients with DFNB9, which appears to be age-related therapeutics and indicated a large treatment window of AAV mediated gene therapy.

The trial has been registered on ClinicalTrials.gov, NCT 05901480, and is ongoing.

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**Ageing, noise, and ER stress: exploring stereocilia fusion pathology in the cochlear outer hair cells with super-resolution expansion microscopy**

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Healthy hearing is fundamentally dependent on the stereocilia bundles of cochlear hair cells. Recent studies on human post-mortem cochleas have shown that ageing is associated with structural impairment of these hair bundles, specifically in outer hair cells (OHCs) (Wu and Liberman, 2022). Genetic

defects and environmental noise challenge the maintenance of the OHC hair bundle structure, contributing to age-related hearing loss. Several prior studies have described stereocilia fusion as a hair bundle pathology, yet often without a clear definition of this abnormality and its molecular mechanisms. Here, we aimed to elucidate the molecular anatomy of OHC stereocilia fusion by studying mouse models of ageing, prolonged noise-exposure, and cell-intrinsic stress caused by genetic perturbations. We utilised a novel imaging method to cochlear research, expansion microscopy, to generate super-resolution data of the OHC hair bundle structure and protein expression. Ageing in the C57BL/6J mice exhibited mild OHC stereocilia fusion, in most cases restricted to the lateral edges of hair bundles, indicating gradual progression of the fusion pathology. OHCs of young adult C57BL/6J mice exhibited elevated likelihood of stereocilia fusion following eight hours of daily, moderate-level noise-exposure (90 dB SPL) over the course of a week, with no evidence of recovery over one-month post-trauma period. Most severe phenotype was found in the genetic mouse model of perturbed endoplasmic reticulum homeostasis (ER stress), exhibiting adult-onset OHC stereocilia fusion with rapid progression to prominent fusion covering the whole hair bundle (Herranen et al., 2020; Ikäkeimo et al., 2021). This severe pathology correlated with reduced FM1-43-dye uptake through the mechanotransduction channels, loss of key stereociliary proteins (neuroplastin, PMCA2, myosin 7a, BAIAP2L2), and increased expression of the calcium buffer oncomodulin in the stereocilia, indicative of a major disturbance to mechanotransduction and to the $Ca^{2+}$-balance required for stereocilia maintenance (Ikäheimo et al., 2024). These hair bundle abnormalities preceded OHC death, suggesting a window of opportunity to intervene with the maintenance of hair cell survival. We conclude that understanding the molecular anatomy of the hair bundle pathology might facilitate the development of targeted therapies for maintaining bundle integrity or to promote bundle repair.

**Alteration of the gut microbiome causes sensorineural hearing loss by increasing blood-labyrinth barrier permeability and cochlear inflammation through the gut-cochlear axis**

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Recent advances in neuroscience have revealed a bidirectional communication between the gut microbiota and the central nervous system (CNS), known as the "gut–microbiota–brain axis." Imbalance in the gut microbiota, called dysbiosis, can increase intestinal permeability, allowing pathogens to trigger inflammation in distant organs. Despite these established connections, no research has explored the link between gut microbiota alterations and inner ear function. To address this gap, this study delved into the molecular mechanisms underlying a potential association between gut microbiota alterations and sensorineural hearing loss (SNHL). To this aim, we used a mouse model of gut dysbiosis induced by dextran sulfate sodium (DSS) treatment, supplemented with fecal microbiota transplantation (FMT) from donor patients with active (aUC) or remissive (rUC) ulcerative colitis. This enabled us to exacerbate or ameliorate the microbiome imbalance, respectively. Auditory brainstem responses (ABRs) were conducted alongside morphological, immunofluorescence, and molecular analyses. ABR results revealed a significant increase in auditory thresholds in mice subjected to DSS and FMT-aUC treatments. Conversely, FMT from rUC donors exhibited a protective effect on auditory function, highlighting the beneficial impact of microbiota restoration. Morphological evaluations revealed loss of outer hair cells (OHCs), degeneration of spiral ganglion neurons (SGNs), and atrophy of the stria vascularis in mice with gut dysbiosis. Conversely, FMT from rUC donors displayed a protective effect on cochlear structures. Immunofluorescence and Western blot analyses unveiled increased oxidative stress and inflammation in cochlear tissues of mice with gut microbiota alterations, while restoration of microbiota composition exerted otoprotective effects. These findings were associated with disruptions in the integrity of the blood-labyrinth barrier (BLB), characterized by altered expression of tight junction proteins (ZO-1 and Occludin), $Na^+/K^+$-ATPase levels, along with increased of pericytes damage and vascular permeability in mice with altered microbiota composition. Overall, these results suggest a mechanistic link between gut microbiota alterations and SNHL through oxidative stress and inflammation mediated by changes in BLB permeability. This study provides experimental evidence supporting the existence of a gut-cochlear axis and highlights the potential therapeutic implications of restoring gut microbiota balance in mitigating hearing impairment associated with gut dysbiosis.

**Antisense oligonucleotides for dominantly inherited hearing impairment DFNA9: from cells models to humanized mice**

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The c.151C>T (p.P51S) mutation in *COCH* is highly prevalent in the Dutch/Belgian population and causes DFNA9 (hearing loss and vestibular dysfunction) in > 1500 individuals. The initial symptoms manifest between the 3rd and 5th decade of life, which leaves ample time for therapeutic intervention. The clear non-haploinsufficiency disease mechanism indicates that blocking or reducing the p.P51S mutant cochlin protein levels may alleviate or prevent the DFNA9 phenotypes.

Considering the broad expression of *COCH* by the fibrocytes of the inner ear, we designed "gapmer" antisense oligonucleotides (ASO) to specifically induce RNase H1-mediated degradation of *COCH* transcripts containing the c.151C>T mutation. We established several model systems to investigate the molecular efficacy of ASOs targeting the c.151C>T mutation or low-frequency mutant allele-specific SNPs.

Using overexpression models, we identified several ASOs that efficiently induce the degradation of mutant *COCH* transcripts. By introducing chemical modification to the oligonucleotide bases, we can alter the affinity and selectivity for the mutation transcript. We identified several ASOs with a strong preference for the mutant transcript in overexpression models. To investigate allele-specificity under physiological expression levels, we exposed patient-derived otic progenitor cells (iPCS-OPCs) to different ASOs for 8 days. In parallel, we developed a genetically humanized mouse model for DFNA9 in which human sequence-specific therapeutic strategies can be evaluated. Phenotypic follow-up of mice of all genotypes indicate that the genetic humanization has no adverse effects, and removal of the Cdh23ahl allele is mandatory to observe the late-onset auditory phenotype: the first signs of high-frequency hearing loss emerged at 12 months of age.

Studies in iPSC-OPCs indicated that the ASOs identified in overexpression studies also effectively reduce mutant *COCH* transcript levels in patient-derived cells with physiological expression levels. Unfortunately, variation between replicate wells of OPC differentiation is relatively high, making it difficult to draw conclusions on allele-specificity. We selected a candidate ASO, directed against a rare mutant allele-specific intronic SNP, for subsequent studies in our humanized mouse model. First intracochlear injections will be conducted in May 2024, after which we can collect the first in vivo data on gapmer ASO uptake and efficacy in fibrocytes of the mammalian inner ear.

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**Assessing the cognitive decline post hearing loss**

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Hearing loss is known to exacerbate cognitive decline in humans. However, the variability in the impact of cognitive function is large, making predictions and drawing causal links difficult. We tested cognitive function in mice using an 8 arm radial maze (8ARM) that has no auditory cues and found that deafened mice showed a dramatic deficit in working memory. We used a hearing loss model that placed the human diphtheria toxin receptor (HDTR) in inner hair cells (IHCs) so that upon exposure to diphtheria toxin (DT), IHCs were selectively ablated. Despite this very uniform and complete deafening, there was a large variance in cognitive function loss, implicating a biological cause to the variance. We are creating a battery of behavioral tests to better investigate this variance and to test the fundamental hypothesis that the impact of hearing loss is in part dependent on how the individual animal weights hearing in sensory integration circuits. This battery of tests has the added advantage of being utilized for assessing the impact of hearing restoration treatments and for defining thresholds of hearing loss that lead to cognitive deficits. We will include the Y-maze and Novel object recognition test (NORT) as tests for memory both short term and consolidation and with the added advantage of repeated measures. We are including visual and auditory training to assess both learning and acuity as a proxy for sensory weighting. And finally, we are measuring auditory evoked potentials in awake animals in an attempt to identify correlations that can be used as predictors of cognitive function sensitivity.

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**Biosafety and biodistribution study of vesicle-enriched secretome fractions in cochlear implantation trauma**

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**Introduction:** Cochlear implantation induces trauma leading to immunological reactions compromising the performance of the implant. Vesicle-enriched secretome fractions (VSF) derived from human umbilical cord mesenchymal stromal cells is a new class of biological therapeutic targeting cochlear cells to modulate immunological processes. Previously, efficacy in spiral ganglion cell culture and attenuation of threshold shifts and protection of hair cells in vivo were shown. Safety, biodistribution and neuroprotective effects of VSF to maintain gross structural integrity of the cochlea after implantation trauma has been evaluated.

**Material and methods:** Hearing guinea pigs (GP) were implanted with a cochlear implant followed by administration of VSF and hearing was assessed until sacrifice after 4 weeks. A long-term group of hearing GP received VSF with a six month follow up of monthly hearing measurement. A third group of hearing GP received labelled VSF investigating distribution of VSF in the inner ear 1–2 hours post application. All groups underwent detailed histological assessment.

**Results:** Treatment with VSF improved hearing after cochlear implantation compared to control animals. Fibrosis 4 weeks post implantation was at similar level to control animals. Electrophysiological and histological findings of the long-term group revealed no adverse effects of VSF and ABR thresholds remained on a similar level compared to control. Positive fluorescent signal of labelled VSF was confirmed in cells of the inner ear.

**Conclusions:** Uptake of labelled VSF into the guinea pig cochlea has been demonstrated and treatment with VSF seems to mediate immunological processes to a healthier state and maintain gross structural integrity. Application of

VSF associated with cochlear implantation seems to be a safe and solid combination to prevent post implantation trauma and preserve residual hearing.

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**Can you hear without FIRE: The impact of microglia loss on cochlear function**

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The immune system is known to critically regulate the central nervous system during development, homeostasis, as well as during injury and repair. More recently, much work has been undertaken to characterize the role of immune cells in the cochlea. Macrophages are the resident innate immune cells of the cochlea. Several studies on mice have reported that macrophages mediate drug uptake via the stria vascularis, promote synaptic repair after noise exposure, and participate in tissue remodeling after cochlear implantation. In other organs macrophages can be heterogenous. Whether there are subpopulations of macrophages in the cochlea and the function of such subgroups are unknown. Here we report molecular heterogeneity among cochlear macrophages which include a population resembling microglia, the primary innate immune cells of brain and two molecularly distinct subpopulations of macrophages resembling two recently identified macrophage population in peripheral nerve. To begin to ascertain the function of one of these populations we examined FIRE mice (Csf1rΔFIRE/ΔFIRE), a mouse model that is entirely microglia- deficient but retains other macrophages which lacks embryonic macrophages and microglia We examined cochlear histology at postnatal days 5, 14, 6 weeks and 6 months of age. Surprisingly, FIRE mice had lower auditory brainstem response thresholds and higher wave I amplitudes when compared to aged-matched control mice at both 6 weeks and 6 months, suggesting less aged-related hearing loss. However, the FIRE mice number,organization of inner and outer hair cells and inner hair cell synapse were similar to aged-matched control C57/Bl6 mice. Taken together, these findings suggest that microglia are dispensable for cochlear development but may play a role in age-related hearing loss, a role which requires further investigation.

**Causes of bilateral sensorineural hearing loss in 838 patients according to degree of progression of hearing loss**

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**Purpose:** This study aimed to investigate the etiology of hearing loss (HL) based on the rate of its progression.

**Material and methods:** Pure tone audiometry was conducted on 42,744 tests (9,269 cases) at Shinshu University Hospital between 2012 and 2022. Cases with unilateral HL, conductive HL, postoperative ear issues, functional hearing loss, and those with bilateral scaling-out at initial examination or lacking detailed data were excluded. A retrospective review was performed on 838 cases followed for over 5 years. Hearing progression was categorized as "Stable" (0–1 dB HL/year), "Slow progression" (1–3 dB HL/year), and "Fast progression" (>3 dB HL/year). Causes were classified as genetic, middle ear disease, Meniere's disease-related, other known causes, and unknown etiology.

**Results:** Of the 838 cases, 302 were classified as "Stable", 402 as "Slow progression", and 134 as "Fast progression". Across all groups, unknown etiology was the predominant cause of HL, followed by genetic factors in Stable and Slow progression groups, and others in the Fast progression group. Regarding genetic etiology, the *GJB2* and *STRC* genes were most prevalent in the Stable group, while genes such as *SLC26A4* and *CDH23* were identified across all groups.

**Conclusions:** Regardless of progression rate, over half of bilateral HL cases had an unknown etiology. Genes such as *GJB2* and *STRC* were identified to exhibit stable hearing, consistent with our findings. This study suggests that genes responsible for progressive HL manifest in an intermittent, rather than continuous, manner, highlighting the clinical utility of genetic testing, particularly in cases lacking long-term follow-up data.

**Cochlear health in a cohort of cochlear implant users carrying the p.Pro51Ser variant in the *COCH* gene (DFNA9): a cross-sectional study evaluating the changes in the electrically evoked compound action potential (eCAP)**

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The present study focuses on DFNA9, an autosomal dominant disorder caused by pathogenic variants in the *COCH* gene. These mutations induce the formation of aggregates that are toxic to the fibrocytes in the extracellular matrix, ultimately leading to degeneration of spiral ganglion neurons (SGNs), which are crucial for transmitting auditory signals from the cochlea to the brain. An important tool for evaluating the function of the SGNs, which are the target cells of a cochlear implant (CI), is the electrically evoked compound action potential (eCAP). Therefore, the main objective is to evaluate the eCAP to describe the function of the SGNs and study cochlear health in CI patients with DFNA9.

For this reason, we included 15 carriers of the p.Pro51Ser variant in the *COCH* gene who received a Med-El CI (DFNA9 group) and 15 matched control CI subjects without DFNA9 to compare the impedances and subsequently the threshold, amplitude and slope of the eCAP amplitude growth function (AGF). These parameters were evaluated from intraoperative autoART recordings (Med-El) during CI surgery. Matching of the two groups was based on sex, age at implantation, duration of deafness, and type of implant. The first results, regarding the difference in impedance between DFNA9 and non-DFNA9 patients, show a significant interaction between time and group in the middle and basal electrodes, indicating that electrode impedances were similar in the early phase after implantation between the two groups, but increased significantly more for the DFNA9 group up to one year after implantation. Secondly, the results show that the success rate (present or absent) to record eCAP responses is lower in the DNFA9 group: eCAPs were detectable in 75.5% of the intraoperative measurements (145/192) in comparison to 96.9% (186/192) in the group without DFNA9. ECAP absence in the DFNA9 group was observed across the whole electrode array, but more pronounced in the basal region (channels 11 and 12). Additionally, comparing the parameters of the AGF, the maximum eCAP amplitude was consistently smaller and the AGF slope consistently shallower for the DFNA9 group compared to the control group throughout the entirety of the electrode array. Finally, the eCAP thresholds in patients with DFNA9 were higher compared to those in the control patients for all cochlear locations. To our knowledge, this is the first study to investigate the eCAP measurements in patients with DFNA9. As proven in the literature, eCAP measures correlate well with the health and survival of SGC. This means that the results of our study predominantly suggest that DFNA9 leads to an even stronger reduction in excitability and neuronal health than seen in other causes of deafness.

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**Development of a ouabain-induced hearing loss guinea pig model for preclinical efficacy assessment**

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Ouabain is a cardiac glycoside acting as a $Na^+/K^+$-ATPase inhibitor. It is widely used in the treatment of congestive heart failure and arrythmia, but currently less used due to its narrow therapeutic index. Several animal studies demonstrated ouabain's ototoxicity, its contribution to spiral ganglion neuron (SGN) loss and subsequent hearing loss. The objective of this study was to compare various methods of ouabain administration in the middle and inner ear to characterize its ototoxic effects in guinea pigs. Different routes of administration and doses of ouabain were tested: 1, 3 and 10 mM administered via round window injection (RWI), a round window membrane deposit (RWM) or transtympanic administration (TT). Several parameters were assessed, from the injection volumes (5, 10, 20 µL), to the injected ear (L/R) and the repetition of administration (up to 3 times). Specific care was provided to the treated guinea pigs with consideration to hydration and food supplementation. ABR thresholds and DPOAE amplitudes were measured between baseline (BL) and T+7DAYS or T+14DAYS to evaluate hearing impairments. These functional measures were correlated with histological analyses, SGN counts, inner hair cell (IHC) and outer hair cell (OHC) counts. Both RWI and RWM, at 3 and 10 mM, induced important hearing loss demonstrated by a significant increase of ABR thresholds followed by considerable SGN loss. Moreover, a decrease of DPOAE amplitudes and some inflammatory processes were observed, even at the lowest dose of all the ouabain administrations. Interestingly, IHC and OHC numbers were similar after a 1 mM ouabain injection whereas DPOAE amplitudes dramatically decreased. However, a 1 mM dose of ouabain administered via TT generated a decrease of DPOAE amplitudes, without affecting ABR thresholds and neither the SGN nor the HC count. Apart from the TT ouabain administration, all the tested approaches generated various health issues, such as major weight loss, ear necrosis, rectal prolapse and high mortality. Ultimately, ouabain administration in the middle or inner ear of guinea pigs is ototoxic with heterogenous results, not limited to SGN loss. Concomitantly, most of the tested routes of administration generated variable health issues.

**Discovery of NOX3 inhibitors for the prevention of acquired hearing loss**

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The accumulation of reactive oxygen species (ROS) in cells and tissues contributes to the development and the progression of numerous diseases such as cancers, metabolic syndromes, or sensorineural disorders. NAPDH oxidases, a family of enzymes which sole function is to produce reactive oxygen species (ROS), appeared as relevant therapeutic targets in the treatment of oxidant-mediated pathologies. More specifically, the NOX3 isoform is only expressed in the inner ear and, although its physiological role in the cochlea is not known, there is increasing evidence that NOX3 is involved in different forms of acquired hearing loss. Thus, the inhibition of NOX3 would provide an efficient otoprotective strategy, notably by preventing ROS-induced damages to the auditory synapse. This project aims at discovering NOX3 small molecule inhibitors for the prevention of acquired sensorineural hearing loss. We developed a cell-based high-throughput screen using an inducible system allowing the expression of NOX3 upon 24 h treatment with tetracycline. NOX3 activity was assessed through the detection of generated extracellular superoxide radical anion ($O2^{\bullet-}$) using the colorimetric assay WST-1. The non-specific NOX inhibitor diphenyleneiodonium chloride (DPI) was used as a reference compound for maximal inhibition. Among the 15,511 compounds screened, 115 showed an inhibitory activity on NOX3 equal to or higher than 50% and were considered as hits. These hits were further tested in dose-response using WST-1 and validated using orthogonal assays detecting hydrogen peroxide (Amplex Red/HRP and CBA fluorometric assays) and cytotoxicity. The specificity of the validated hits for NOX3 over the 6 other isoforms was also assessed. This critical early drug discovery step paves the way to the development of new small molecule therapeutics for the prevention of sensorineural hearing loss.

**EDNRB2 is a novel marker for hair cell precursors in chick auditory epithelia**

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Although mammalian cochleae have no capacity for hair cell (HC) regeneration, the avian auditory epithelium, namely basilar papilla (BP), can regenerate HCs through direct conversion of supporting cells (SCs) or mitotic proliferation of SCs. To explore molecular mechanisms for HC regeneration in chick BP, we previously established an explant culture model of chick BP, in which HC regeneration occurs predominantly via direct conversion of SCs (Matsunaga et al., 2020) and performed single-cell RNA sequencing (Matsunaga et al., 2023) by using our model. A pseudotime trajectory analysis for the process of SC-to-HC conversion revealed that SCs were once reprogrammed to the precursor state, followed by differentiation into HCs (Matsunaga et al., 2023). In addition, we observed temporal upregulation of EDNRB2 encoded endothelin receptor b2 in reprogrammed SCs as differentially expressed genes during SC-to-HC conversion. In the current study, we examined the expression patterns of EDNRB2 in the developing chick BP to determine which stage progenitor or precursor populations express EDNRB2. The expression of EDNRB2 in BP was found only in the time points when HC differentiation was initiated, not in common progenitors for HCs and SCs or immature HCs and SCs after fate determination. The result supports our hypothesis that EDNRB2 is a marker for HC precursors and indicates that SCs are reprogrammed to the precursor state just before fate determination, not to the common progenitor state. We also assessed the roles of EDNRB signaling during SC-to-HC conversion in regenerating chick BP explants using a selective inhibitor for EDNRB signaling. The pharmacological inhibition of EDNRB signaling significantly reduced the number of regenerated HCs, not reduced the number of SCs, which indicates that EDNRB signaling may play a role in the differentiation of precursors into HCs. Further, RNA sequencing is underway to identify the critical molecules downstream of EDNRB signaling. In conclusion, EDNRB2 is a novel marker for HC precursors in chick auditory epithelia and may be involved in HC differentiation.

**Efficacy of cochlear implantation in patients with severe to profound hearing loss without prior hearing aid use**

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**Introduction:** Previous studies have suggested a negative association between the duration of hearing loss and post-cochlear implantation (CI) audiometry performance. Consequently, it has been assumed that cochlear implantation may be less effective in ears without prior hearing aid (HA) use. However, some cases of CI recipients with no history of HA use (non-aided) have demonstrated favorable hearing outcomes. This study aimed to explore the relationship between the duration of hearing loss in non-aided ears and postoperative auditory performance, as well as to compare CI outcomes between non-aided and aided ears.

**Material and methods:** This retrospective study included 153 ears (127 cases) with postlingual hearing loss that underwent CI for bilateral severe to profound hearing loss between April 2011 and March 2023. Among them, 28 patients (29 ears) received CI in non-aided ears, while 100 patients (125 ears) received CI in aided ears. One patient (2 ears) underwent CI in both non-aided and aided ears, and six patients (12 ears) received CI in non-aided ears initially, followed by

CI in aided ears. Audiometry performance was assessed using the Japanese monosyllable test (CI2004).

**Results:** No significant correlation was found between audiometry performance at 1 year postoperatively in non-aided ears and the duration of hearing loss without HA use. Additionally, there was no significant difference in audiometry performance between non-aided and aided ears at 1 year after surgery.

**Conclusions:** This study revealed that irrespective of the duration of hearing loss, postoperative CI outcomes in non-aided ears were comparable to those in aided ears. Patients with severe-to-profound hearing loss often exhibit asymmetric hearing loss, leading to requests for CI on the worse hearing ear. While surgeons may hesitate to provide CI to ears without prior HA use, our findings suggest that such hesitancy may not be warranted, assisting the decision-making process for CI in patients with postlingual hearing loss.

## Epigenetic landscape of supporting cell reprogramming toward hair cell regeneration in chick auditory epithelia

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Sensorineural hearing loss is usually intractable because of virtually no capacity for hair cell (HC) regeneration in mammals. In contrast to mammals, lower vertebrates including birds have the capability for HC regeneration. Elucidation of precise mechanisms for HC regeneration in avian auditory epithelia will contribute to exploring key molecules for inducing HC regeneration in mammals. We previously established an explant culture model of the chick auditory epithelium, namely basilar papilla (BP), showing total HC loss and consecutive HC regeneration through direct conversion of supporting cells (SCs) to HCs (Matsunaga, 2020). Single-cell RNA sequencing using this model illustrated dynamic changes in expressed genes in BP SCs during direct conversion, which indicates that reprogramming of SCs to the precursor state occurs before differentiation into HCs (Matsunaga, 2023). To explore mechanisms of SC reprogramming toward hair cell regeneration in chick BP, we performed an integrated analysis of RNA- and ATAC-seq of chick BP explant cultures during the early phase (three time points) of HC regeneration. ATAC sequencing detected 80,366 peaks. Based on the distance from the transcription start site, and correlations between read counts and peak depths and between their alterations in a time course, we determined enhancer candidate loci for differentially expressed genes. We focused on a set of temporally upregulated genes during SC reprogramming in our previous single-cell RNA sequencing data (Matsunaga, 2023). In temporally upregulated genes during SC reprogramming, enhancer candidate loci were identified in nine genes. Motif enrichment analysis of these loci indicated transcription factors that may control chromatin remodeling in SC reprogramming. In the near future, we will examine spaciotemporal expression patterns of these transcription factors in regenerating chick BP.

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## Essential role of *ISL1* in the development and survival of spiral ganglion neurons in the inner ear

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*ISL1*, a LIM-homeodomain transcription factor, is expressed during embryonic development of the inner ear in both hair cells and spiral ganglion neurons (SGNs), but its function is still unknown. For the first time, we created a mouse model with Isl1 conditional deletion in the SGNs, and in our previous study of adult Isl1CKO mice, we identified the significant role of Isl1 in the phenotype and function of the SGNs. Here, we present the results of our cellular and molecular assessment of the SGNs' embryonic development in the Isl1CKO. Using immunolabeling, we studied the 3D structure of the cochlea by confocal and light-sheet microscopy through different stages of development. Exploiting the reporter protein, we inspected the axon projection characteristics in the Isl1CKO by time-lapse imaging of the cochlear explant. At the molecular level, we performed RNA sequencing to assess the transcriptional regulation impact of *ISL1*. In addition, we investigated the involvement of *ISL1* in epigenetics regulation by performing the CUT&Tag assay for important histone markers. We observed that from early developmental stages, the SGNs of the Isl1CKO manifested a problem in migration and axonogenesis. And while the proliferation of SGNs was not affected a gradual increase in apoptosis was detected. In Isl1CKO, despite the wrong location, SGNs projected axon fibers towards the target cells, however, axonogenesis started earlier and advanced faster than the control, resulting in disorganized and overshooting axons. Similarly, our RNA sequencing of the Isl1CKO's SGNs showed massive changes in the expression of the genes essential for neuronal communication, migration, survival, and axon projection including *DCC*, *Robo*, *Unc-5*, *Nrp*, members of the *EphA* family, *Ntrk2*, *Ntrk3*. Interestingly, we identified a sizeable overlap between the downregulated genes and the genes with altered histone modification, such as *EphA5*, *Unc5b*, and *Grin3a*, indicating that *ISL1* not only directly interacts with downstream genes to regulate their expressions, but also employs the epigenetics machinery to demethylase H3K27me3, and promote the expression of genes essential in neuronal development, axonogenesis, and synaptogenesis. Here, we unfold the prominent

regulatory role of *ISL1* in developing SGNs and provide insight into its direct and indirect targets.

**Extended high frequency thresholds and their association with otoacoustic emissions and demographic factors**

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**Objectives:** Hearing assessments typically cover frequencies up to 8 kHz, although testing can extend to 16 or 20 kHz. The range beyond 8 kHz is commonly referred to as the extended high frequency (EHF) range. This study aimed to investigate the connection between EHF hearing thresholds (HTs) and distortion product otoacoustic emissions (DPOAEs) in adult subjects. Factors such as the presence of spontaneous otoacoustic emissions (SOAEs), gender, ear side, and aging were of interest.

**Material and methods:** Participants consisted of 95 adults. The age ranged from 21 to 77 years, with an average of 42 ± 14. There were 55 women, comprising 58% of the whole group. All subjects had normal middle ear function verified by 226 Hz tympanometry. None had any known history of otologic disease. DPOAEs were measured using the HearID system (Mimosa Acoustics Inc., Champaign, IL, USA) with an ER-10C probe (Etymotic Research, Elk Grove Village, IL, USA). DPOAEs were measured at 9 selected frequencies for F2 of 1, 1.5, 2, 4, 6, 8, 10, 12, and 16 kHz. Only ears which gave a signal-to-noise ratio (SNR) greater than 6 dB at 3 of the 4 frequencies from 2, 3, 4, and 6 kHz were analyzed. SOAEs were acquired using the in-built routine (SOAE50) provided by the HearID system, resulting in a measurement of so-called synchronized SOAEs (SSOAEs).

**Results:** The key findings indicate that DPOAEs, both within the standard frequency (SF) range (0.125–8 kHz) and the EHF range (10–16 kHz), decrease as thresholds deteriorate. Age significantly influences DPOAEs and HTs in both ranges, with EHFs being particularly affected. The presence of SOAEs was the only other significant factor influencing DPOAE level. Gender and ear side had minor and non-significant effects on both DPOAEs and HTs.

**Conclusions:** In conclusion, DPOAEs in the EHF range emerge as reliable predictors of EHF HTs, and given their correlation with age, they may serve as suitable markers for early signs of presbycusis.

**From cells to cures: hiPSC-derived inner organoids and RNA therapy to resolve genetic hearing loss**

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Genetic hearing loss impacts millions worldwide, yet effective treatments remain unavailable, leaving patients reliant on technological aids such as hearing aids or cochlear implants. One major obstacle in therapy development is the lack of representative in vitro models of the human inner ear capable of mimicking genetic inner ear diseases and facilitating treatment validation. In this study, we present a novel approach to address this challenge. We differentiated human induced pluripotent stem cells (hiPSCs) derived from patients with genetic hearing diseases into 3D self-organizing inner ear organoids. Specifically, we focused on two genes associated with significant auditory impairments: *USH2A*, hereditary deafblindness, and *COCH*, implicated in late-onset genetic hearing loss, the latter presenting a window for intervention. We successfully generated disease-specific inner ear organoids by growing patient hiPSCs through precise modulation with small molecules and growth factors at distinct intervals. With immunohistochemistry we showed the presence of organ-specific cell structures within both *USH2A*- and *COCH*-inner ear organoids, including otic vesicles, hair cells and periotic mesenchymal cells. We compared the disease-specific inner ear organoids with healthy inner ear organoids through molecular and structural analyses and confirmed the presence of mutant transcripts in the patient-derived inner ear organoids. Moving beyond characterization, we demonstrate the clinical relevance of the model by countering the disease phenotype with antisense oligonucleotides (ASOs) in vitro. ASOs can specifically target and modify RNA transcripts and slow down or halt genetic disease progression. We applied ASOs to late-stage disease-specific inner ear organoids via gymnotic delivery and observed its effect on mutant transcript expression through PCR analysis following ASO therapy. This study underscores the potential of human inner ear organoids as a platform for modelling genetic inner ear diseases and evaluating potential therapeutic interventions. Our findings offer promising avenues for increasing treatment options for individuals affected by genetic hearing loss, offering hope for improved outcomes and quality of life.

**HMGA2 mediates tonotopic identity in the developing mouse cochlea**

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*HMGA2* belongs to the non-histone chromosomal high--mobility group (HMG) protein family and binds to DNA at promoter and enhancer regions. The chromatin modifier plays a role in recruiting other factors such as histone acetylases, which together with *HMGA2* form a structure called the enhanceosome that has impact on local chromatin structure and therefore gene expression. In the cochlea, *HMGA2* is tonotopically expressed. However, its relevance for frequency specific hearing remains to be determined. Sonic hedgehog, a tonotopic morphogen, forms an apex-to-base decreasing gradient in the cochlea. To study the impact of hedgehog signaling on *HMGA2* expression, the hedgehog pathway was activated in vivo using a gain-of-function mouse model. On the other hand, retinoic acid forms a base-to-apex decreasing morphogen gradient, thereby opposing sonic hedgehog. To modulate retinoic acid levels in vivo, gain- and loss-of-function mouse models were used. Furthermore, conditional knockout of *Hmga2* from the otocyst stage was established to study the relevance of *HMGA2* using histological staining and hearing measurements. Constitutive activation of the HH pathway resulted in ectopic expression of *HMGA2*. Retinoic acid in turn was found to limit the extension of the *HMGA2* gradient towards the base. This finding indicates that positional information mediated by tonotopic morphogen gradients establish the *Hmga2* gradient in vivo. Conditional knockout of *Hmga2* did not affect the cellular composition of the organ of Corti. However, adult mice fail to develop normal low-frequency hearing as determined by ABR and DPOAE measurements. In this work, we used different transgenic mouse lines to establish that embryonic patterning via retinoic acid and sonic hedgehog establish the *HMGA2* gradient along the tonotopic axis. Also, loss of *Hmga2* demonstrated that the chromatin modifier is necessary for the development of low-frequency hearing in the adult animal. Together, these results indicate that *HMGA2* is an integral part in mediating tonotopic identity in the mouse cochlea.

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**Human pluripotent derived auditory neuron progenitors (LCTANP1) for the treatment of auditory neuropathy spectrum disorder**

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**Introduction:** Loss of auditory nerve cells can lead to auditory neuropathy, even when the hair cells and the cochlear nucleus remain intact. Cell-based therapy for replacing lost or dysfunctional auditory neurons may restore hearing in these cases and enhance the degree of success of a cochlear implant procedure by repopulating the cochlea with transplanted, functional auditory neurons. We developed a novel proprietary differentiation process to manufacture LCTANP1 composed of Auditory Neuron Progenitors from clinical grade line of pluripotent human stem cells.

**Material and methods:** The manufacturing process starts with a series of differentiation cues, in specific time frames, and ends by harvesting the auditory neuron progenitors cells, and cryopreserving them as LCTANP1 drug product in a ready to administer format. LCTANP1 cells were characterized by biological and functionally relevant sets of markers, using different quantitative methods that we newly developed and customized such as analysis of specific protein marker expression by flow cytometry, and immunofluorescence and expression profiles, including RNA sequencing. Functional in-vitro assays were developed to measure neuronal properties of LCTANP1, such as the ability to elicit calcium influx. Fluorescent labeled LCTANP1 cells were delivered to the base of the cochleae of eight ouabain-treated guinea pigs by a cochleostomy and via the scala tympani, or the modiolus. Seven days later, animals were euthanized and labeled LCTANP1 cells were visualized within the cochlea using a fluorescence stereoscope and by human specific immunofluorescent staining.

**Results:** LCTANP1 cells were successfully manufactured at scale, met pre-set release criteria, and demonstrated relevant activity in in-vitro functional tests. LCTANP1 cells were cryopreserved in a ready-to-administer, thaw and inject format and were successfully thawed, successfully transplanted and survived a 7-days study in an in-vivo Guinea pig model. LCTANP1 cells are currently being evaluated in a functional model of hearing simultaneous with additional manufacturing enhancements.

**Conclusions:** LCTANP1 is a novel cell-based product composed of Auditory Neuron Progenitors derived from clinical grade pluripotent stem cells. LCTANP1 completed initial CMC and Preclinical POC.

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**In situ 3D fluorescence microscopy mapping in the Prphp-mCherry mouse line differentiates inner ear afferent populations**

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The inner ear facilitates hearing and balance sensory encoding through its complex 3D structure and heterogeneity of primary afferent neurons. In the cochlea, spiral ganglion neurons (SGN) directly encode sound via the inner hair cell-Type I SGN circuit and regulate cochlear amplification feedback via the outer hair cell-Type II SGN circuit. Vestibular ganglion neurons (VGN) are divided into calyx afferents (Type I hair

cells), dimorphic (both Type I and Type II hair cells), and bouton afferents (Type II hair cells), that map to regions of the cristae and otolith organs to encode features of head position and acceleration. Both Type II SGN and vestibular bouton afferents are marked by the Type III intermediate filament protein Peripherin (Prph). We developed a transgenic mouse model using Prph promotor elements which demonstrated mCherry reporter expression (Prphp-mCherry) in SGN and VGN throughout postnatal development, characterised using CUBIC1/PEGASOS clearing and Lightsheet fluorescence microscopy. We found overlap of the Prphp-mCherry and Prph immunopositive populations in the hook and basal regions of the cochlea, but significant mismatch in mid-apical regions. In the vestibular ganglion, mCherry immunolabelling was confined to small diameter afferent somata by adulthood, colocalising with Prph positive bouton afferent fibres, although mismatch in fibre staining suggests a subpopulation has been identified. Using nanopore sequencing, the integration site of the Prphp-mCherry transgene cassette was located within the *Grm8* gene encoding metabotropic GluR8, where exon reshuffling was evident. Intriguingly, *Grm8* is a marker of Type Ic SGN, which synapse on the modiolar face of the inner hair cells and are particularly vulnerable to noise and aged-related hearing loss. Significant overlap of Type Ic SGN markers and Prphp-mCherry neurons has been quantified, indicating the integration site of the transgenic construct may have influenced transgene expression. Mapping the distribution of type and subtype markers in the cochlea SGN and under-resolved landscape of the VGN in 3D has revealed new protein marker compartmentalization, uncovering broader afferent heterogeneity in the inner ear.

**Influence of impeded biomechanics after implantation**

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The hearing preservation has become a standard goal of cochlear implantation. A new method of monitoring cochlear function has become available using the intracochlear electrocochleography or intracochlear ECochG. This measurement allows via intracochlear electrodes of the cochlear implant itself to record the response to acoustic stimuli. The feasibility of such recordings was presented at the XXXII World Congress of Audiology in 2014. Intracochlear ECochG is a sensitive measurement of cochlear function that allows for detection of basilar membrane contact in Flex arrays. Our previous study (Lorens et al., 2019) showed that the measurements of acoustically evoked intracochlear potentials via the location dependent intracochlear electrodes are systematically recordable in a wide range of postoperative hearing abilities of cochlear implantees. The most sensitive location within the cochlea to record CM potentials depends on the frequency tone used. The deeper in the cochlea the mean maximum CM peak-to-peak amplitude is, the lower the stimulating tone frequency will be. Multiple recordings along the cochlea provide a method for assessing cochlear mechanics. In this study, we use this approach to test for electrophysiological evidence of basilar membrane fixation, demonstrated by a substantial basal or apical shift of maximum ECochG response away from the characteristic frequency of the stimulus. The hearing preservation rates and speech outcomes will be presented and compared in 16 cochlear implantees.

**Input ear impedance and eardrum energy reflectance variations related to increase in intracochlear and intracranial pressure**

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Variations of the intracranial pressure (ICP) are expected in particular pathologies, in microgravity conditions, and associated with postural changes. Lumbar puncture is a direct but highly invasive ICP diagnostic method. Mechanical models of the inner and middle ear suggest an indirect non-invasive method based on acoustic measurements. Since the cerebro-spinal fluid is in contact with the intracochlear fluid, a change of the pressure in the lymphatic fluid of the peripheral hearing system gives information about variations of the ICP. In particular, the increase in the cochlear fluid pressure is measurable as increase of the middle ear reflectance and, consequently, the otoacoustic emission (OAE) phase (Buki et al., 1996; Avan et al., 2018; Voss et al., 2010). Avan et al. (2000) theoretically characterized the enhancement of the middle ear stiffness in terms of stapedius reflex activity as a reduction of the stapes compliance and observed the relative increase of the distortion product OAEs (DPOAEs) phase. As ICP changes can be induced by postural changes, we use theoretical models of middle ear transmission to discuss how and why the reactance of input ear impedance Z changes with the body posture, showing correlation with the increase and the frequency shift of the eardrum energy reflectance, and the DPOAE phase changes. Specifically, in the low-frequency range, the reactance of Z is stiffness-dominated, thus it is a negative function of (and inversely proportional to) frequency. When a tested subject changes position from orthostatism to clinostatism, the negative term of reactance decreases and the frequency of null reactance increases. This evidence is in accordance with the reduction of the stapes compliance which is most relevant at very low frequency. These results help providing a theoretical basis to (and a connection among) the empirical methods for the indirect estimate of ICP variations based on the monitor of different observable consequences of the variation of the middle ear transmission, which can be applied in microgravity conditions or also in cerebrospinal diseases.

**Intracochlear administration methods across species**

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**Introduction:** The delivery of therapeutic molecules to the inner ear represents a challenge due to the blood-labyrinth barrier, necessitating local delivery approaches, particularly for expensive medications or drugs with limited therapeutic range or susceptibility to systemic side effects. This is particularly relevant for viral gene transfer and cell-based therapies, but could be meaningful for small molecules, peptides or antibodies. Adeno-associated virus (AAV) vector emerges as a prominent choice for gene therapy due to its infection efficiency, low toxicity, sustained gene expression, and cost-effectiveness.

**Purpose:** This study aimed to compare different intracochlear (IC) injection techniques in mice and guinea pigs.

**Material and methods:** Mice were administered via posterior semicircular canal (PSCC) and round window (RW) injections, while guinea pigs received cochleostomy infusion, RW injection (RWI) or deposit (RWD) due to anatomical differences. Auditory function effects of IC administration were evaluated in both species via auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE) measurements. Following AAV delivery, GFP expression from AAV-mediated cell transfection enabled the identification of transfected cochlear regions. Both PSCC and RW injections successfully targeted hair cells, albeit with variations in transfection patterns and intensity depending on the injection method and AAV subtypes. Regarding molecules delivery, the continual renewal of inner ear fluid may necessitate chronic infusion for maintaining active concentrations, assessable through pharmacokinetics evaluation. Finally, impacts on auditory function varied among injection methods, with PSCC and IC routes showing less effect on DPOAE amplitudes and ABR thresholds compared to the RW route.

**Conclusions:** In conclusion, our study highlights the importance of refined intracochlear delivery method in animals accordingly to the delivered product and targeted inner ear disorders. While all methods effectively enabled to reach cochlear hair cells, variations in transfection patterns or in product concentration at targeted cells, and effects on auditory function highlight the need for careful consideration of injection strategies. It is worth mentioning that the posterior semicircular canal (PSCC) and cochleostomy route, though effective in animals, lacks translational relevance to humans. Moving forward, further research into optimizing delivery methods and understanding their specific effects on cochlear function will be essential for advancing therapeutic interventions in auditory disorders.

**Investigating the genetic bases of age-related hearing loss – human GWAS to mouse models**

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The most common form of sensory disability is age-related hearing loss (ARHL), a complex disorder involving genetic and environmental factors. While not life-threatening, ARHL causes communication difficulties and is associated with social isolation, depression and reduced physical and cognitive function. Moreover, there is a growing literature suggesting causal links between ARHL and dementia. Presently, there are no biological therapies for the condition, and our limited knowledge of the underlying genetic mechanisms of ARHL is a severe impediment to the design of interventions.

As with other complex disorders, genome-wide association studies (GWAS) have had limited success in identifying susceptibility genes. However, the advent of the UK Biobank, with ~500k participants, is a game-changer having the statistical power to identify genome-wide significant loci. Indeed, a recent study reported 44 significant loci associated with self-reported hearing difficulty or hearing aid use. These data represent an opportunity to increase our understanding of ARHL, but first the gene responsible for the association at each locus needs to be confirmed.

Mice are the predominant model organism for hearing research. Similarities in auditory structure and physiology with humans, close evolutionary relationship of genomes and the available genetic toolkit, make mice an ideal system for studying the functional genomics of hearing. To elaborate upon the genetics of ARHL, we are generating knockout mice for genes located in close proximity to the strongest associations and assessing their hearing; we are utilising the IMPC programme, importing knockout lines and undertaking recurrent auditory phenotyping up to 15-months of age.

In addition to *Clrn2*, using this approach we have validated *Baiap2l2* and *Klhdc7b* as important for mammalian hearing. However, no overt hearing phenotype was evident in *Arhgef28*, *Nid2* or *Fto* mutant mice.

These findings highlight the potential of the UKBB data to elaborate upon the genetics of mammalian hearing, but also the difficulty of translating results from human GWAS to animal models. Importantly, here we have only investigated gene loss-of-function, which may not be appropriate in all cases. Validated mouse models will provide essential information regarding the pathobiology of ARHL, and this knowledge will lay the foundation necessary for developing preventive strategies.

**Isolated early onset hearing impairment? Diagnosis of syndromic forms by whole genome sequencing**

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**Introduction:** Clinical geneticists involved in the diagnosis of early onset hearing loss can to encounter young patients with apparently isolated hearing loss or cochleovestibular pathology and a diagnosis of a syndromic form. For example, diagnosis in a young child of Usher syndrome, that associates congenital hearing loss (and vestibular areflexia in the type 1) and later onset retinitis pigmentosa, is not rare in our practice.

**Material and methods:** In France, patients presenting with early onset isolated or syndromic hearing loss have access to diagnostic trio whole genome sequencing through the Plan France Medecine Genomique 2025 sequencing platforms. From 2020 to 2023, almost 500 families have undergone whole genome sequencing for this indication on the SeqOIA platform only.

**Results:** Among patients assessed in the Paris Reference Center for Genetic Deafness and presenting with apparently isolated early onset hearing loss or cochleovestibular pathology, whole genome sequencing identified one patient with Usher syndrome (*USH2A*) and four patients with very rare syndromic forms. Syndroms identified are Heimler syndrome (*PEX6*), a Perrault syndrome phenocopy (*NARS2*), Dystonia Deafness Cerebral Hypomyelination syndrome (*BCAP31*) and Hypoparathyroidism Deafness Renal syndrome (*GATA3*).

**Conclusions:** Diagnostic whole genome sequencing enables the diagnosis of ultra rare conditions, which would not have been possible with usual panel sequencing testing strategies. For the patients, the diagnosis of a syndromic form in an apparently isolated presentation allows for better care and genetic counselling but can also be distressing. Indeed, the delay between the result and the manifestation of additional symptoms and the severity of these symptoms can be uncertain.

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**Lef1 and Tcf7l2 are Wnt signalling effectors with contrasting functions during inner ear sensory organ formation**

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The inner ear is composed of several sensory organs responsible for the detection of sound, head position and acceleration. During embryonic development, these organs originate from neurosensory-competent domains within the otocyst, but the molecular signals controlling sensory organ formation remain unclear. The transcription factor *Sox2* is required for neurosensory specification since its deletion abolishes the differentiation of sensory organs and their associated neurons. *Sox2* is initially present throughout the otocyst, but it becomes restricted to the ventro medial aspect. Our recent work suggests that this restriction is regulated in a dose-dependent manner by a dorso ventral gradient (from high to low) of canonical Wnt activity. Dorsally, high levels of Wnt activity inhibit sensory organ formation whereas ventrally, low levels are needed to maintain prosensory specification. To find out how Wnt signalling can exert these two contrasting functions, we analysed the expression and function of four members of the Tcf/Lef family of transcription factors (*Lef1*, *Tcf7*, *Tcf7l1* and *Tcf7l2*) in the chicken otocyst. We found that all members of the family are expressed in the otocyst but that each factor has a unique expression pattern. Our functional studies suggest that only *Lef1* and *Tcf7l2* contribute to prosensory specification. The expression pattern of *Lef1* and its gain-of-function effect reflect high levels of Wnt activity, while the distribution of *Tcf7l2* and the effect of its overexpression are consistent with low levels of Wnt activity. In summary, our results suggest that *Lef1* and *Tcf7l2* transcription factors are key effectors of the Wnt activity gradient during inner ear sensory organ formation.

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**Lgr5+ endogenous progenitor cells in the adult (deafened) cochlea**

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**Introduction:** *LGR5* positive supporting cells (SCs) in the cochlea give rise to hair cells (HCs) during embryonic development. Neonatal SCs have increased progenitor potential compared to adult and only a few studies (including ours) have shown survival of SCs with progenitor cell markers after severe HC loss in adult mice. In mammals, there is no evidence for spontaneous HC regeneration in adulthood. However, three-dimensional cultures have allowed the expansion and experimentation of human (and mouse) inner ear organoids. Here, we evaluated HC differentiation from human cochlear organoids and from adult normal-hearing and deafened mice.

**Material and methods:** Adult patients undergoing surgery for skull base tumors were included. Sensory epithelium of the cochlea and vestibular organ was collected in medium and tissue was digested to single cell suspension. Adult Lgr5-eGFP-IRES-creERT2 heterozygous mice were used. Mice were deafened with a single dose of furosemide in combination with kanamycin and deafening was confirmed by auditory brainstem responses (ABRs). Cochleas were harvested and digested to single cell suspension and after filtering, 3D drops were made with Matrigel. Cells were grown on expansion medium (EM) for 10 days and differentiation medium (DM) for 3–10 days after. Organoids were fixed, permeabilized and processed for immunofluorescence and whole-mounted for imaging in a confocal microscope.

**Results:** Vestibular-organ-derived organoids were generated in EM from all seven patients so far included. Cochlea--derived organoids were generated in five out of seven patients. After exposure to DM, vestibular organ-derived and cochlea-derived organoids produced MYO7A+ HC-like cells. Cochlear organoids from normal-hearing mice expressed *LGR5* and Ki67 in EM and *MYO7A* after differentiation. Significantly less cochlear organoids were produced from deafened mice; however the organoids reached similar size as NH-cochlear organoids, expressed *LGR5* and *Ki67* in EM and *MYO7A* after differentiation.

**Conclusions:** Cochlear and vestibular tissue from adult patients (and adult normal-hearing and deafened mice) possess progenitor potential and the capacity to generate inner ear organoids in vitro. After differentiation, HCs were visible in tissue derived from human cochlea, human vestibular organ, and adult mouse cochlea. The adult inner ear has (limited) regenerative capacity and can produce new MYO7A+ HCs.

## Loud low frequency sound-induced pathophysiology of cochlear sound transmission and sound transduction

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**Introduction:** Prolonged exposure to loud sounds can result in noise-induced hearing loss. Among sounds of different frequencies, loud high frequencies are damaging to base of the cochlea while loud low frequencies induce damage in the larger range of cochlea. Research has predominantly focused on the effects of high-frequency noise, though the consequences of loud low-frequency sounds (LFS) on cochlear function have received almost no attention.

**Objective:** The primary objective of this study was to investigate adverse effects of loud-LFS on cochlear sound transduction and sound transmission in guinea pigs.

**Material and methods:** Under anesthesia, guinea pigs were exposed to 200 Hz at 120 dB SPL for 40 mins, while controls received similar exposure at 55 dB SPL. The distortion product otoacoustic emissions (DPOAEs) and compound actional potential (CAP) thresholds were measured to evaluate outer hair cell (OHC) function and sound transmission to brain, respectively. In a different cohort of surgical guinea pigs, organ of corti (OoC) vibrations were recorded at the apex of cochlea, using optical coherence tomography. All measurements were taken before and after the sound exposure.

**Results:** We present that loud-LFS knocks out CAP for all tested frequencies, from 0.5 to 32 kHz. However, DPOAEs amplitudes remain unchanged to similar exposure. These findings suggest that loud-LFS selectively impairs sound transmission to brain without altering the hearing threshold, particularly in the cochlear region specific to middle to high frequencies. However, subsequent investigations of loud-LFS impact on apical cochlear mechanics revealed that loud-LFS alters apical frequency resolution by causing an upward shift in OoC best frequency. Our results also show a decrease in OoC vibrations at all frequencies, particularly more pronounced at frequencies below and above the best frequency. Vibration magnitude reductions were largest at low stimulus levels while alterations at intense stimulus levels were insignificant, reflecting a loud-LFS mediated impairment of OHC driven amplification at the apex of the cochlea. These response-magnitude changes were accompanied by morphological alterations within the cochlea.

**Conclusions:** These findings suggest that loud-LFS causes selective damage, impairing cochlear sound transmission across a wide range while preserving OHC transduction at cochlear base and damaging it at the apex.

## Mapping human inner ear development: insights from single-nucleus transcriptomics

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The inner ear, essential for auditory perception and balance, relies on specialized cell types whose developmental mechanisms are incompletely understood. While animal models provide valuable insights, human inner ear development remains predominantly characterized by descriptive approaches. The advent of single-cell transcriptomics offers a promising approach to decode the complexities of human inner ear development. In this study, we introduce the Human Inner Ear Developmental snRNAseq Atlas (HIEDRA), a comprehensive single-nucleus RNA transcriptomic dataset that covers the entire membranous human inner ear at nine developmental stages from fetal weeks 7 to 15. Our analysis of over 55,000 cells reveals the molecular dynamics that drive the differentiation of epithelial, neuronal, and mesenchymal cell populations in both vestibular and cochlear regions. We provide new insights into specific gene markers that differentiate cochlear from vestibular cell types. We additionally find the involvement of canonical signaling pathways such as Notch, Wnt, and Hippo in sensory cell type development – a correlation previously established in non-human models. Moreover, our data reveal the contribution of additional signaling pathways, including TNF, to hair cell formation in silico. We also identify the role of ErbB, Notch and Hippo signaling pathways in the specification of key nonsensory epithelial cell types: vestibular dark cells and cochlear marginal cells, which are crucial for inner ear function. Our findings not only clarify the complex landscape of human inner ear development but also highlight the diverse roles of otic mesenchymal cells,

previously underappreciated in this context. This characterization deepens our understanding of human inner ear development and offers potential to explore pathophysiological mechanisms that lead to hearing loss and balance disorders. Furthermore, leveraging these insights to improve culture models, such as human pluripotent stem cell-derived inner ear organoids, could significantly boost their applicability for in vitro studies of developmental processes and their efficacy as disease models.

**Mechanotransduction molecules regulate stereocilia membrane mechanics**

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Hair cells are the mechanoreceptors of the auditory and vestibular sensory systems. The sensory hair bundle is the organelle that senses movement and converts this movement to an electrical signal using mechanosensory ion channels. These channels can operate at high frequencies and with sensitivities at molecular dimensions. How is this achieved? A growing body of data supports the hypothesis that the stereocilia membrane plays a role in modulating mechanotransduction. Biochemical modulations happens with PIP2, for example, regulating permeation and conductance. Mechanical modulation is implicated by fluorescent recovery after photobleaching which demonstrated that the MET open probability co-varied with membrane diffusivity; lower diffusivity correlated with more open channels. We now demonstrate that the MET channel complex directly regulates membrane viscosity using a newly developed viscosity sensor, BODIPY 1c. This sensor shows a strong correlation between MET channel activity and membrane viscosity both during development and in mutant mice that disrupt mechanotransduction. Biophysically dissecting current, voltage and calcium demonstrates that scramblase activity associated with the MET channel is responsible for the lower membrane viscosity. Conventional MET channel blockers block the scramblase activity resulting in an elevation in viscosity. We suspect an as yet undefined membrane flippase/floppase system creating an asymmetric membrane that is then regulated by the MET channel's scramblase activity. The reduced viscosity will directly impact MET channel kinetics and sensitivity.

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**NEUROD1 orchestrates cell fate changes and neurogenesis**

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NEUROD1, a basic helix-loop-helix (bHLH) transcription factor, plays an essential role in neurogenesis within both the central and peripheral nervous systems. Recent studies demonstrate its capability to directly reprogram various cell types into neurons. However, these cell-reprogramming experiments indicate that NEUROD1 possesses the ability to instigate cell-fate changes but only under specific conditions that remain incompletely characterized. To gain further insights into the ability of NEUROD1 to change cell-fate and promote neurogenesis in vivo, we used three distinct gain-of-function of Neurod1 mouse models. Each model was designed to explore different temporal and spatial overexpression of NEUROD1. Overexpression of NEUROD1 in non-neuronal progenitors resulted in the induction of neurogenesis and neuron generation, while in neuronal progenitors it yielded no discernible changes. Our study provides new evidence that NEUROD1 is an efficient reprogramming factor of non-neuronal progenitors into neurons and confirms its potential for facilitation of reprogramming therapeutic strategy.

**Novel antisense therapy for USH2A patients**

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**Perilymph proteome in prelingual deafness**

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**Introduction:** Inner ear liquid biopsy would help in understanding the molecular environment of the diseased organ. In cases of prelingual deafness it is postulated that cochlea' insufficiency, both congenital and acquired in early postnatal period is connected with remarkable changes in the perilymph proteome and the protein pathways. In cases in which deafness results from genetic change of the cochlea structures, like *GJB2*-related deafness, it is probably expected that proteins encoded by the defected gene should not be detected in the perilymph or should be detected at much different levels. Analogically, in cases of deafness acquired due to ototoxic agents exposure one could expect that proteome characteristics would be altered, among others towards inflammatory products and proteins involved in oxidative stress reactions. Having known the proteome status of the perilymph we could approach to more precise prognosis of the disease, especially in cases of residual hearing.

**Material and methods:** We have collected perilymph samples using glass capillaries from preliminary group of prelingually deaf children during the procedure of cochlear implantation in Institute of Physiology and Pathology of Hearing, Warsaw. Each sample was no less than 2 µl and was harvested via round window approach. Method: using mass spectrometry (MS) protein quality analysis was performed in each sample.

**Results:** Number of detected proteins across samples varied from 1324 to 2103. Further samples taken from patients with different kinds of deafness are needed to continue the analyses.

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**PHOENIX – an animal free platform to accelerate the development of new therapeutics against sensorineural hearing loss**

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Cochlear hair cells and their associated auditory neurons do not regenerate after injury, leading to irreversible sensorineural hearing loss. This lack of regenerative potential of cochlear progenitors is a major obstacle to developing efficient in vitro models, delaying new therapeutic advancements for hearing loss treatment. Consequently, from early preclinical stages, testing new treatments relies on animal-based models, resulting in low throughput, significant variability, and limited predictive value. We have recently identified the signaling pathways that can reprogram stemness in senescent auditory neuroprogenitors. By synergistically targeting the WNT and TGFβ/Smad pathways using small molecules or genetic means, we achieved virtually unlimited expansion of auditory neuroprogenitors in vitro. This reprogramming does not compromise their ability to differentiate into mature and functional auditory neurons, even after 40 passages and a thousand-fold amplification. The so-called phoenix auditory neuroprogenitors can be frozen and thawed, leading to the creation of a cell bank and offering an efficient alternative to animal-based models. The phoenix platform provides numerous advantages, including suitability for high-throughput technologies, low experimental variability, single-cell resolution, and a significant reduction in the number of animals used. Furthermore, it maintains the phenotype of auditory neurons in a primary culture-like setup. Initially developed using mouse neural cells, we are currently implementing this model with human fetal otic neural stem cells. This reprogramming method represents a significant breakthrough in overcoming a major bottleneck in auditory research. The phoenix platform offers an efficient, high-throughput, cost-effective, and 3R-compatible approach for in vitro screening of potential otoprotective and otoregenerative drug candidates. In addition, the precise investigation of the mechanisms leading to phoenix proliferation opens new avenues in the field of inner ear regeneration.

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**Quantitative RNA-scope study of the expression patterns of Tcf/Lef transcription factors in the otocyst**

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Canonical Wnt signalling is a critical morphogenic pathway regulating cell fate choices and tissue patterning during embryogenesis. In the developing inner ear, Wnt signalling is implicated in both otic induction and hair cell differentiation. Our recent work revealed that it also regulates prosensory specification, which is the formation of inner ear sensory organ precursor cells. We observed a dorso-ventral gradient of Wnt signalling activity throughout the early otocyst and showed that it has two opposing functions: inducing and repressing prosensory specification in a manner that is dose-dependent. Wnt signalling operates through the activation of different sets of target genes, expression of which is regulated by four transcription factors from the Lef/Tcf family. Thus, to better understand the process and mechanisms of Wnt gradient activity during prosensory specification, we analysed the expression patterns of *Lef1*, *Tcf7*, *Tcf7l1*, and *Tcf7l2* in the early chicken otocyst using RNA-scope fluorescent in situ hybridisation and developed a quantification pipeline to better determine their activity levels. Our results show that *Lef1*, *Tcf7*, *Tcf7l1* and *Tcf7l2* exhibit distinct expression patterns across the dorsal and ventral axes of the otocyst. The different levels and patterns of expression quantified using our pipeline suggested that *Lef1* and *Tcf7* are candidates for effectors of

high Wnt signalling activity and *Tcf7l2* is an effector of low activity. Further functional validation confirmed that *Lef1* and *Tcf7l2* act downstream of Wnt signalling to regulate its two contrasting functions during otic prosensory specification and thereby substantiate our RNA-scope pipeline as a valuable tool for quantitative spatial analysis.

**Sensory transduction plays an essential role in the maturation of inner hair cells, afferent ribbon synapses and auditory nerve fibers**

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Inner hair cells (IHCs) convert sound stimuli into electric signals through activation of mechanosensory transduction channels (*TMC1* and *TMC2*) localized at the tip of the stereocilia (Pan et al., 2013, 2018; Kurima et al., 2015). Hair cells acquire transduction, progressively, from the basal end of the cochlea to its apical end, during the first postnatal week in mice (Lelli et al., 2009). Before the onset of hearing, IHCs also fire spontaneous action potentials which are believed to play a role in the development and maturation of the auditory system (Kros et al., 1998; Trish et al., 2007; Johnson et al., 2011, 2017). Alteration in sensory transduction has been shown to affect hair cell physiology (Marcotti et al., 2006, Corn et al., 2018) and maturation of IHC synapse morphology (Lee et al., 2021). Here we further investigate how sensory transduction affects IHC physiology, afferent ribbon synapses, as well as downstream type-I auditory nerve fibers (ANF) properties. To tackle this question, we took advantage of several mouse models with altered sensory transduction: mice lacking or carrying dominant mutation in TMC proteins. We performed single cell electrophysiological recordings to assess voltage-dependent calcium currents and exocytosis and examined ribbon synapse with immunostaining and transmission electron microscopy at 2 weeks and 3 weeks. We assessed the spontaneous and evoked firing properties of ANF, in vivo, using single fiber recording in anesthetized mice (2–4 months). Finally, we assessed RNA expression of the ANF fibers by RNA single cell sequencing in P24–P28 mice. Our work demonstrates preservation of synaptic properties and features in *Tmc2* KO mice and alterations in fast and sustained exocytosis along with impairment of voltage-dependent calcium currents *Tmc1* KO and double *Tmc1*/*Tmc2* KO mice. These changes are also associated with alteration in the morphology of the synapse as we demonstrated previously (Lee et al., 2021) and further validated in this study. Our work demonstrates that sensory transduction plays an important role in the development and maturation of hair cells, their afferent synaptic machinery as well as maturation of the ANF.

**Single-cell transcriptomic atlas reveals increased regeneration in diseased human inner ear balance organs**

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Mammalian inner ear hair cell loss leads to permanent hearing and balance dysfunction. In contrast to the cochlea, vestibular hair cells of the murine utricle have some regenerative capacity. Whether human utricular hair cells regenerate in vivo remains unknown. Here we procured live, mature utricles from organ donors (9 ears from 6 organ donors) and vestibular schwannoma patients (24 ears from 24 patients), and presented a single-cell transcriptomic atlas at unprecedented resolution. We validated marker genes using immunostaining and RNAscope in situ hybridization and described previously unknown markers of 13 sensory and non-sensory cell types. In addition, we compared and found partial overlap and correlation between transcriptomes of human and mouse hair cells and supporting cells. We further uncovered transcriptomes unique to hair cell precursors, which are validated in both organ donor and vestibular schwannoma utricles. Unexpectedly we found 14-fold more hair cell precursors in vestibular schwannoma utricles, demonstrating the existence of ongoing regeneration in humans. Lastly, supporting cell-to-hair cell trajectory analysis revealed 5 distinct patterns of dynamic gene expression and associated pathways. Our dataset constitutes a foundational resource, accessible via a web-based interface, serving to advance knowledge of the normal and diseased human inner ear.

**Styrene ototoxicity is associated with memory impairment and hippocampal dysfunctions**

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Styrene is an organic solvent commonly used in industries with well-documented ototoxicity. Although styrene toxicity on cochlear structures has been extensively documented, its detrimental effect on the central nervous system and on brain structures involved in the auditory (i.e. auditory cortex) and extra-auditory (i.e. hippocampus) pathway has not been established yet. Our recent study reveals that styrene exposure increases oxidative stress in both the cochlea and auditory cortex activating macrophages and glial cells (Paciello et al., 2024). Considering that alterations in the auditory cortex induced by peripheral damage can be linked with cognitive impairment and altered hippocampal functions (Paciello et al., 2021; Paciello et al., 2023), we wondered if the ototoxic effect of styrene could also be associated with cognitive dysfunctions. Therefore, the aim of our study was to investigate the relationship between styrene ototoxicity and cognitive impairment. To this aim, adult male Wistar rats were exposed to styrene for 5 days a week during 3 weeks at a dose of 400 mg/kg. Hearing loss and damage to neural transmission were assessed by recording auditory brainstem responses (ABR) and by performing wave II latency and amplitude analysis. At the end of treatment (day 21) animals underwent behavioural test (Novel object recognition test-NOR) to evaluate recognition memory. Then, we performed morphological analyses and western blot assays in hippocampal samples to evaluate the level of oxidative stress, macrophage infiltration, glial cell activation and inflammation in the hippocampus. Results revealed a decrease in auditory threshold in styrene-exposed animals compared to control animals. Hearing loss was associated with memory deficits and hippocampal dysfunction with increased oxidative stress, lipid peroxidation, inflammation, and *Iba-1* and *CD68* expression suggesting microglia-induced inflammation. Overall, the present study suggests that styrene can exert an oto/neurotoxic effect not only in the cochlea but also in brain structures involved in auditory and extra-auditory pathways, leading to altered hippocampal functions and memory impairment.

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**Supporting cell responses to sensory cell damage: novel insights from a quantitative analysis of cyclodextrin--induced ototoxicity in mice**

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**Introduction:** The cochlea is vulnerable to various pathological conditions, with sensory cells typically being the primary target of damage. However, supporting cells also experience significant impacts. Despite their critical role in maintaining the structural and functional integrity of the sensory epithelium, the responses of supporting cells to cochlear damage are not well understood. This study aims to characterize the reactions of supporting cells to sensory cell damage in mouse cochleae.

**Material and methods:** The study utilized a mouse model of cochlear damage induced by cyclodextrin to simulate the ototoxicity of the cochlea. A single dose of cyclodextrin treatment caused cochlear damage with a damaging pattern similar to that caused by aminoglycoside antibiotics. The cochleae were examined at various time points after the treatment to evaluate supporting cell survival patterns and the roles of various types of supporting cells in the repair process of the organ of Corti. We also examined the vulnerability of different supporting cell populations and cochlear responses to supporting cell pathogenesis.

**Results:** Cyclodextrin exposure caused considerable sensory cell loss, particularly outer hair cell loss. Despite significant sensory cell damage, most supporting cells survived. These surviving cells not only helped maintain the structure of the organ of Corti but also expressed immune molecules. However, the basal end of the cochlea exhibited noticeable supporting cell death, with quantitative analysis indicating that pillar cells were the most vulnerable, followed by Deiters' cells. This supporting cell death triggered the local expression of immune molecules in the surrounding supporting cells. Additionally, macrophages were observed in areas where supporting cells were absent at the chronic phase but not in regions with sensory cell loss at the acute stage of cochlear damage.

**Conclusions:** This study elucidates the complex dynamics of supporting cell responses in the cochlea following damage, demonstrating that while most of these cells retain their structural integrity and initiate immune responses, they exhibit varied vulnerability to cochlear insults. The findings emphasize the importance of supporting cells in cochlear recovery processes and the potential of targeting these cells for therapeutic strategies aimed at restoring auditory function.

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**The border and inner-phalangeal cells are required to synchronize the calcium action potentials in developing inner hair cells**

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**Introduction:** Developing cochlear inner hair cells (IHCs) elicit sensory-independent $Ca^{2+}$ action potentials (APs) that propagate along the auditory pathway and is required for the maturation of the IHCs and for the refinement of the neural circuitry. The AP activity in IHCs is believe to the synchronized by spontaneous ATP-induced $Ca^{2+}$ waves originating in the non-sensory supporting cells (SCs). This ATP signalling triggers fluid secretion from the SCs by activating $Ca^{2+}$-activated $Cl^-$ channel (TMEM16A), which has been reported having bipolar influence on the IHC excitability.

Whether TMEM16A channels are involved in the functional maturation of IHCs is still unclear.

**Material and methods:** We used conditional Tmem16afl/flPlp1-cre mice in which the expression of TMEM16a was downregulated specifically in the inner phalangeal and inner border cells (IPhC and IBC), which are the SCs adjacent to the IHCs. Cell-attached path-clamp electrophysiology was used to monitor the SAP activity from ex-vivo cochlear tissue, while whole-cell patch-clamp was used to record current and voltage responses in pre- and post-hearing IHCs.

**Results:** We showed that the absence of TMEM16A in IPhC and IBC significantly prolonged the inter-spike intervals (ISIs) of spontaneous APs in the IHCs. High-frequency burst of APs (≥10 Hz) in IHCs were almost completely eliminated in the absence of TMEM16a channels. Calcium imaging also revealed a significantly reduced correlation in APs between nearby IHCs from Tmem16afl/flPlp1-cre mice. Although the IHCs from Tmem16afl/flPlp1-cre mice appeared to experience an initial delay in the maturation of their basolateral membrane currents, they were indistinguishable from control IHCs.

**Conclusions:** We showed that IPhCs and IBCs mediate the synchronization of APs in nearby IHCs and that the activation of TMEM16A channels is critical to elicit high-frequency burst (>10 Hz) in developing IHCs. We also found that IHCs from Tmem16afl/flPlp1-cre mice show a delay in their maturation, highlighting the possible role of ATP signalling from the SCs is driving the normal development of IHCs.

## The clinical effect of steroids on hearing preservation in PDT patients in cochlear implantation

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**Introduction:** Recent advances in cochlear implantation which consist of: the design of the electrodes, atraumatic surgical techniques and monitoring of cochlear function during implantation, draw attention also to administration of steroids and other anti-inflammatory drugs for preserving residual hearing.

**Aim:** The main aim of this study was to assess the clinical effect of steroids (dexamethasone and prednisone) on hearing preservation in patients who underwent cochlear implantation with different cochlear implant systems (Oticon®, Advanced Bionics®, Med-El®).

**Material and methods:** 147 adult patients met the inclusion criteria and were enrolled to the study and divided into three groups depending on the brand of cochlear implant they received and participated in all follow-up visits regularly. They were also randomly divided into three subgroups depending on the steroid administration regime: (1) intravenous dexamethasone (0.1 mg/kg body weight twice a day for three days); (2) combined intravenous and oral steroids (dexamethasone 0.1 mg/kg body weight twice a day plus prednisone 1 mg/kg weight once a day); and (3) no steroids (control group).

**Results:** The results were measured by pure tone audiometry (PTA) at three time points: (i) before implantation, (ii) at processor activation, and (iii) 12 months after activation. A hearing preservation (HP) figure was also calculated by comparing the preoperative results and the results after 12 months. Further measures collected were electrode impedance and hearing threshold in the non-operated ear. The highest HP measures were obtained in the subgroups who were given steroids. Of the 102 patients given steroids, HP was partial or complete in 63 of them (62%). In comparison, partial or complete HP was achieved in only 15 patients out of 45 (33%) who were not given steroids. There were differences between the three cochlear implant groups, with the Med-El and Advanced Bionics groups performing better than the Oticon group (45% and 43% of the former two groups achieved partial or complete HP compared to 20% in the latter). Hearing thresholds in the non-operated ear were stable over 12 months.

**Conclusions:** Pharmacological treatment with steroids in patients undergoing cochlear implantation helps to preserve residual hearing.

## The effect of brain-derived neurotrophic factor and neurotrophin-3 on the auditory nerve response to cochlear implant stimulation

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Cochlear implants rely on the integrity and correct functioning of auditory nerves. However, after sensorineural hearing loss there is a well-characterised degeneration of auditory nerves. One proposed mechanism of this degeneration is a reduction in the levels of neurotrophins, which are naturally occurring proteins that aid the growth and maintenance of neurons. Neurotrophin application to the cochlea of animals prevent morphological degeneration of the auditory nerves after deafness, making it a promising treatment for improving cochlear implant outcomes. The current study examined the effects of two major neurotrophins present in the cochlea, brain-derived neurotrophic factor and neurotrophic factor-3, on the function of individual auditory nerve fibres to cochlear implant stimulation.

Guinea pigs were ototoxically deafened and then divided into groups that received treatment for four weeks with either

brain derived neurotrophic factor, neurotrophin factor-3 or Ringer's solution as a control. Treatments were administered one week after deafening and delivered to the left cochlea through a combined cannula-electrode array that was attached to a mini-osmotic pump. Additional control groups included guinea pigs deafened for five weeks and an acutely deafened group. At the end of the treatment period responses of individual auditory nerve fibres to acute electrical stimulation of the cochlea at rates from 200–5000 pulses per second were recorded.

Both brain derived neurotrophic factor and neurotrophin-3 treatment generally normalized the reduction in spike latency of auditory nerve fibres observed after deafness. BDNF significantly reduced thresholds compared to all control groups, while NT-3 did not. Both BDNF and NT-3 increased the first-spike dynamic range compared to untreated groups. These results were largely similar regardless of the stimulus rate used.

These results suggest that neurotrophin treatment of the cochlea after deafness appears to preserve the latency of auditory nerve fibres, but may alter the response threshold and dynamic range. Further investigations are required to determine if neurotrophins are likely to preserve or improve auditory nerve function when used with a cochlear implant.

## The expression and functional role of histamine receptor 3 in the mammalian inner ear c57BL/6 mice

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**Introduction:** Histamine receptor 3 (H3R) is known for its regulatory functions in the central nervous system, but its role in the mammalian inner ear is poorly understood. This study investigates the expression and functional implications of H3R in the inner ear of postnatal c57BL/6 mice.

**Material and methods:** Immunofluorescent staining was employed to determine the localization of H3R in the cochlea of postnatal day 3–5 (P3-5) mice. Cochlear explants were cultured for 24 hours in the presence of one of two H3R agonist/antagonists, Ciproxifan or Pitolisant, at various concentrations (10 μM, 50 μM, 100 μM). The effect of Ciproxifan and Pitolisant on hair cells (HCs) and spiral ganglion neurons (SGNs) morphology was assessed using fluorescent microscopy.

**Results:** H3R expression was detected in HCs and SGNs. Exposure to Ciproxifan induced significant damage and loss of inner and outer HCs in a concentration-dependent manner. Moreover, the typical apex-to-base directional growth of some type II SGN fibers appeared to be reversed (base-to-apex). Exposure to Pitolisant did not reduce the number of HCs. Still, it caused morphological changes in HCs cilia and a reversed directional growth of type II SGNs but to a lesser extent than the equivalent concentrations of Ciproxifan.

**Conclusions:** This study validates the expression of H3R in the inner ear of P3-5 c57BL/6 mice and suggests its potential role in the development and maintenance of hair cells. The differential effects of H3R antagonists underscore the necessity of pharmacovigilance for this class of drugs, particularly in the fields of otology and audiology. Further research is necessary to elucidate the mechanisms underlying the role of H3R in auditory development and to explore its potential as a therapeutic target for hearing and balance disorders.

## The functional integrity of the mechanoelectrical transduction complex in the hair cells of the mature cochlea requires MYO7A

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**Introduction:** The transduction of acoustic information into electrical signals depends on the mechanically induced displacement of stereociliary bundles projecting from the apical surface of the sensory hair cells. Hair bundle deflection opens mechanoelectrical transducer (MET) channels located at the tips of the shorter rows of adjacent stereocilia. The gating of the MET channels requires force supplied by the tensioning of tip links during sound-induced bundle displacement. The motor protein MYO7A, which is an unconventional myosin responsible for syndromic (Usher 1B) or non-syndromic recessive deafness in humans when mutated, has long been associated with tip-link tensioning, but conclusive evidence is still lacking. In this study, we investigated the role of MYO7A in mature hair cells using conditional knockout mice.

**Material and methods:** The role of MYO7A in mature hair cells was investigated using conditional Myo7afl/flMyo15-cre mice in which the delayed downregulation of the protein allowed normal cochlear development and hearing function up to about postnatal day 20. Patch clamp electrophysiology was used to record the MET current, which was elicited by displacing the hair bundles of the IHCs and OHCs with a piezo-driven fluid jet. The morphology of the stereociliary bundles and their molecular composition was investigated using immunofluorescence microscopy and scanning electron microscopy. Hearing function was measured using auditory brainstem responses.

**Results:** We found that mature hair cells from MYO7A-deficient mice progressively lose their MET current while still having normal hair bundle morphology (up to at least 1 month of age), albeit with a considerably reduced stiffness. Surprisingly, the resting open probability of the MET channel and its sensitivity to intracellular and extracellular $Ca^{2+}$ were not affected in the absence of MYO7A. By 2 months of age, the hair bundles of the hair cells started to become disorganised and by 7 months the organ of Corti was almost completely devoid of hair cells. We also found that the

progression of hearing loss and deterioration of the stereociliary hair bundles in Myo7a-deficient mice was accelerated by noise insults. Finally, transcriptomic analysis showed that the absence of MYO7A in 1 month-old mice caused the downregulation of a number of genes known to be essential for mechanoelectrical transduction.

**Conclusions:** We found that MYO7A is required for maintaining the functional integrity of the stereociliary hair bundles, but it is not essential for setting the resting tension on the mechanoelectrical transduction complex in mature cochlear hair cells.

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## The hidden truth of hereditary hearing loss: gaining insight into the genetic basis of non-syndromic mimics

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**Introduction:** The definition of a molecular diagnosis for patients affected by hereditary hearing loss (HHL) is significantly hampered by the extreme clinical and genetic heterogeneity that characterise the condition. In particular, peculiar and understudied cases are those of non-syndromic mimics (NSM), meaning patients with particularly mild forms of syndromic HHL or initially presenting isolated deafness and delayed onset of other clinical signs.

**Material and methods:** In the last 18 months, a cohort of 73 apparently non-syndromic Italian HHL patients has been enrolled in the study. All the individuals were negative at *GJB2* and *STRC* genetic tests and underwent whole-exome sequencing, aiming to define a molecular diagnosis and eventually identify NSMs.

**Results:** A molecular diagnosis was provided for 36/73 patients (49.3%), and 12 of them could be classified as NSMs. In detail, two groups of patients could be highlighted: (1) patients presenting subtle additional signs that were missed during the first clinical evaluation and (2) patients whose molecular diagnosis suggests the future development of additional clinical features. In Group 1, two patients were identified, and they carried pathogenic variants within the *MITF* and *GATA3* genes, which are associated with Waardenburg and Barakat syndromes, respectively. As regards Group 2, ten patients were detected, and the involved genes were *CDH23* (one patient), *USH2A* (six patients) and *ADGRV1* (three patients). Thus, these results suggest that Usher syndrome type 2 accounts for the vast majority of NSMs (75%). Moreover, these considerations further confirm our previous findings regarding the high prevalence of Usher syndrome type 2 carriers in the Italian population (1: 70).

**Conclusions:** Identifying patients within Group 1 of NSMs highlights the importance of a critical re-evaluation of the diagnostic criteria of each condition and provides crucial insight into the clinical characteristics of very mild forms of syndromic deafness. On the other hand, the clinical condition of Group 2 NSM patients will be evaluated by a multidisciplinary team in order to provide personalised follow-up and specific preventive strategies.

## The pharmacological action of Pimozide on vestibular Type-I and Type-II hair cells

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Pimozide is a conventional antipsychotic of the diphenylbutylpiperidine class widely used for treating schizophrenia, delusional disorders, and managing motor and phonic tics in Tourette's syndrome. Its primary mechanism of action in the central nervous system is as a dopaminergic D2 receptor antagonist. Additionally, Pimozide is known for blocking various types of voltage-gated calcium and potassium channels. Among its side effects, dizziness and balance disorders are the most observed.

This study delved into the effects of Pimozide on ionic currents in vestibular hair cells. Using the patch-clamp whole-cell technique, we studied the effect of Pimozide at a concentration of 3 μM on the ionic currents expressed by chicken embryo vestibular Type-I and Type-II hair cells, as well as on mammalian Type-II hair cells. Consistent with a previous report on chicken embryo, Pimozide significantly increased the delayed outward rectifying K+ current of Type-II hair cells on mouse. In chicken embryo, the drug also notably reduced the inward (anomalous) rectifying K+ current and the mixed Na+/K+ (Ih) current.

In Type-I hair cells, Pimozide showed no significant effect on IK,L, a large low-voltage activated outward rectifying K+ current absent in Type-II cells, nor on the small delayed outward rectifying K+ current. The latter result suggests that the delayed rectifying K+ current involves different channel subunits in the two hair cell types. Additionally, Pimozide did not alter the inward Na+ current expressed by Type-I hair cells.

In conclusion, these findings highlight that Pimozide selectively impacts potassium channels in Type-II, but not Type-I, hair cells. The drug acts as a delayed outward rectifying potassium channel opener in Type-II cells, potentially leading to a decrease in afferent signal transmission from these cells to primary sensory neurons. While providing a possible explanation for the vestibular side effects of Pimozide, the above results also open up possibilities for its use in reducing altered vestibular input in various vestibular disorders.

**Unraveling age-related cellular and molecular mechanisms associated with vestibular sensory epithelium and its prolonged resilience compared to cochlear aging**

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Aging of the inner ear contributes to age-related hearing loss (ARHL) and vestibular dysfunction (ARVL). Many studies have examined the underlying mechanisms involved in ARHL, while ARVL remains poorly understood. ARVL is the gradual loss of bilateral vestibular function accompanied by interruptions to visual and proprioceptive inputs, increasing the risk of imbalance, geriatric dizziness, and injurious falls. According to the National Institute of Health, age-related falls account for 50% of all accidental deaths, and it is the 6th leading cause of death in the elderly, highlighting the urgency of understanding the molecular basis to develop targeted therapeutics. Evidence from human and animal studies indicates age-related functional and morphological alterations in the vestibular sensory epithelia with a slow pace of aging in contrast to the cochlea, suggesting distinctive age-related cellular and molecular mechanisms between the two systems. Thus, in the current study, we investigated the age-related cellular and molecular alterations in the vestibular system particularly focusing on how it differs from cochlear aging.

Our vestibular sensory evoked potential, auditory brainstem response, and distortion product otoacoustic emissions and the endolymphatic potential measurements revealed age-related vestibular and auditory functional decline and the different paces of aging of the two sensory systems in the same mice. Morphological analysis using histology, super-resolution confocal-microscopy, and scanning electron microscopy revealed degeneration of stereocilia and alterations in hair and supporting cell soma. Using single-cell RNA sequencing of hair cells and supporting cells from the auditory and vestibular sensory epithelia from adult and aging CBA/J mice, we were able to identify shared and unique genes and molecular processes associated with vestibular aging and the disparity of aging trajectories of the two systems contributing to differential onset of ARVL and ARHL.

Our findings delineated the relationship between the onset of age-induced vestibular dysfunction and cellular and molecular degeneration of the vestibular sensory epithelium, uncovering novel insights into mechanisms governing vestibular aging leading to ARVL. Moreover, the comparative analysis between vestibular and cochlear aging revealed mechanism(s) contributing to the delayed onset of vestibular aging, in contrast to cochlea. Our findings pave the way to developing selective therapeutic interventions to prevent ARVL.

**Validation of a newly developed SPL Chirp for intracochlear ECochG measurement**

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**Introduction:** Intracochlear electrocochleography (ECochG) records electrical potentials generated in the inner ear in response to acoustic stimuli. Previous studies have demonstrated that ECochG recordings are related to the remaining inner ear function. Recently intracochlear ECochG measurement tool was applied during CI surgery to gain a better understanding of the impact of the implant on the inner ear function. For the stimulation, a newly developed SPL chirp will be applied.

**Aim:** The aims of this study were to validate SPL chirp and secondly, to perform real time intracochlear EcochG recordings during the electrode advancement and maneuvering during the cochlear implantation

**Material and methods:** Ten patients implanted with the Flex electrodes, with various degree of hearing preservation were postoperatively tested for SPL chirps and tone bursts of 250, 500, 1000, 2000 and 4000 Hz. The recordings was performed for each active electrode in alternating mode. The frequency specific response amplitudes of tone bursts were compared with those of SPL chirp1 and SPL chirp2.

**Results:** In every subject we obtained response to tone bursts and SPL chirp responses. Generally, SPL chirp frequency specific amplitudes were equal or lower than those for tone bursts obtained at the same stimuli level. The frequency specific amplitudes varied from more than1μV (noise flor) to about 100uV.

**Conclusions:** SPL chirps are useful stimuli to be used during the intraoperative monitoring of hearing preservation cochlear implant surgery as a time-reduction paradigm comparing to burst stimulation. Such stimuli may provide additional information of cochlea specific information related to hearing preservation.

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**Whole organ imaging of the mature and aged mammalian vestibular system**

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Aging is associated with an increased risk of falling, which is contributed to by diminished balance. However, the precise anatomical changes occurring within the vestibular system as it ages are debated. In this study, we used

immunofluorescence, tissue clearing, and 2-photon microscopy to visualize the anatomical changes that occur as the vestibular system ages. The entire temporal bone was dissected and decalcified, instead of dissecting individual vestibular organs, to minimize tissue damage and distortions during dissection. We compared tested multiple clearing methods, both aqueous and solvent based methods to find which works best for the vestibular organs and settled on using the ethyl cinnamate method. These methods allow imaging of the entire vestibular system sensory cells in their native orientations. Using a combination of antibodies, we can label all hair cells as well as the type II hair cells. Using automated analyses, we delineate the region of the sensory epithelia and automate the counting and mapping of the locations of each cell. Using this protocol, we quantify the number of hair cells in both mature (1–2 months) and aged (36–40 months) epithelia to determine how hair cell numbers change with age. We also correlate these changes with vestibular function using vestibular evoked potential recordings. This study lays the groundwork for determining changes in the vestibular system with age to determine the pathophysiological changes.

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**Zebrafish in vivo functional investigation of *TBC1D24* linked with autosomal dominant hearing loss reveals structural and functional defects of the inner ear**

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*TBC1D24* genetic variants are causally involved in the development of both autosomal recessive hearing loss and epilepsy syndromes, and autosomal dominant hearing loss (ADHL). So far, our group published four novel ADHL-causative *TBC1D24* probably pathogenic variants by performing high-throughput genetic testing in families with ADHL, and more variants are yet to be revealed. In the light of current discoveries, variants in *TBC1D24* emerge as a more significant cause of ADHL.

The molecular mechanism behind the *TBC1D24*-associated ADHL is unknown. Using a zebrafish model, we investigated involvement of *TBC1D24* in hearing and the functional effects of the associated ADHL-causing genetic variants. Different methodological approaches were used in the study, including (i) expression studies by whole mount in situ hybridization (WISH), qPCR on different developmental stages and cryosections, (ii) assessment of the zebrafish ear and neuromast hair cell morphology by high-resolution imaging and (iii) behavioral studies in a developed tbc1d24-deficient zebrafish models (by knock-down or knock-out of tbc1d24) and in overexpression and rescue tbc1d24 models.

We show that the morpholino-mediated knockdown of Tbc1d24 resulted in defective ear kinocilia structure and reduced locomotor activity of the embryos. The observed phenotypes were rescued by a wild-type *TBC1D24* mRNA but not by a mutant mRNA carrying the ADHL-causing variant c.553G>A (p.Asp185Asn), supporting its pathogenic potential. CRISPR-Cas9-mediated knockout of tbc1d24 led to mechanosensory deficiency of lateral line neuromasts. Overexpression of *TBC1D24* mRNA resulted in developmental abnormalities associated with ciliary dysfunction and mesendodermal mispatterning. We observed that the ADHL-causing *TBC1D24* variants: c.553G>A (p.Asp185Asn); c.1460A>T (p.His487Leu), c.1461C>G (p.His487Gln) or a novel variant c.905T>G (p.Leu302Arg) alleviated the effect of overexpression, indicating that these variants disrupt the *TBC1D24* function. Furthermore, the zebrafish phenotypes correspond to the severity of ADHL. Specific changes in ear structures upon *TBC1D24* overexpression further highlighted its tissue-specific role in ciliary function and inner ear development.

Our findings provide functional evidence for the pathogenic potential of the ADHL-causing *TBC1D24* variants and lead to new insights into the function of *TBC1D24* in cilia morphogenesis.

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## Posters

### AAV-regulated Serpine2 overexpression promotes hair cell regeneration

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Inner ear hair cell (HC) damage is irreversible in mammals, but is has been shown that supporting cells (SCs) have the potential to differentiate into HCs. Serpine2, a serine protease inhibitor, encodes protease nexin 1, and this has been suggested to be a factor that promotes HC regeneration. In this study, we overexpressed Serpine2 in inner ear SCs cultured in two- and three-dimensional(2D and 3D) systems using the Adeno-associated virus-inner ear (AAV-ie) vector, which promoted organoid expansion and HC differentiation. Overexpression of Serpine2 in the mouse cochlea through the round window membrane (RWM) injection promoted SC proliferation and HC regeneration, and the regenerated HCs were found to be derived from Lgr5+ SCs. In conclusion, our findings indicate that Serpine2 overexpression promotes HC regeneration and suggest that the utilization of inner ear progenitor cells in combination with AAVs might be a promising therapeutic target for hearing restoration.

### Accuracy and consistency of ChatGPT responses to questions related to physiology of hearing

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**Introduction:** ChatGPT has been tested in many disciplines, but only a few studies have involved hearing diagnosis, and none have focused on hearing physiology. The consistency of the chatbot's responses to the same questions posed multiple times has not been well investigated either. This study aimed to assess the accuracy and repeatability of ChatGPT 3.5 and 4 on test questions related to otoacoustic emissions and auditory brainstem responses. Of particular interest was the short-term repeatability of responses, which was tested over four separate days within one week.

**Material and methods:** The questions which focused on hearing physiology were posed five times to both ChatGPT 3.5 and ChatGPT 4 on each of four days (two days in one week and two days in the following week). The accuracy and the repeatability of the responses over time were evaluated.

**Results:** The overall accuracy of ChatGPT 3.5 was 48–49%, while that of ChatGPT 4 was 65–69%. ChatGPT 3.5 consistently failed to pass the threshold of 50% correct responses. Within a single day, the percent agreement was 76–79% for ChatGPT 3.5 and 87–88% for ChatGPT 4. The percent agreement between responses from different days was 75–79% for ChatGPT 3.5 and 85–88% for ChatGPT 4.

**Conclusions:** ChatGPT 4 outperforms ChatGPT 3.5 both in accuracy and repeatability over time. However, the significant variability in responses raises doubts about the potential professional applications of both versions.

### Characterizing hair bundle maturation in the mouse utricle during embryonic and postnatal development

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Sensory hair cells are mechanoreceptors required for hearing and balance functions. Stereociliary bundles play a critical role in mechanoelectrical transduction (MET), many prior studies examining developing and regenerated hair cells have assessed bundle morphology to determine cell maturity. However, only few studies have systematically assessed hair bundle dimensions during embryonic and postnatal development. In this study, utricles were collected from embryonic (E) 13.5, 15.5, *E*18.5, postnatal day (P) 0, P37 and P180 wild type mice, and immunostained with the kinocilia marker α-Tubulin and stereocilia marker phalloidin. From 3D reconstructed images of hair cells, kinocilia height, tallest and shortest cilia, and volume were measured. At E13.5, height of kinocilia, tallest and shortest cilia were relatively uniform. Starting at E15.5, wide distributions of hair bundle and kinocilia heights were observed, possibly because of maturing kinocilia and bundles in older hair cells and the emergence of hair bundles and kinocilia in new hair cells in late embryonic and early postnatal periods. Some longer kinocilia appeared curled or bent. Short bundles were still observed in adult mouse utricle. Expression of the actin-crosslinking protein FSCN2 was also examined to characterize the maturity of hair bundles. FSCN2 was absent at E13.5 and E15.5, became detectable in most hair bundles by E18.5 and *P*0, but

some short bundles still lacked FSCN2 even at 6 months. To mark newly born hair cells, we fate-mapped supporting cells in Plp1CreERT/+; Rosa26RtdTomato/+ mice by treating them with tamoxifen at P3. At 1, 2 and 6 months, most bundles of traced hair cells still appeared relatively shorter than untraced ones, and continue to display short, curled or bent kinocilia, resembling those in the early embryonic stages. By 6 months, bundles of only 46% of traced hair cells expressed FSCN2, compared to 97% of the untraced hair cells. Together, our data indicate that hair cells in the embryonic utricle display short kinocilia and stereocilia that elongate over time. Newly added hair cells display short bundle and kinocilia that resemble those during embryonic periods, with some remain detectable in the adult utricle.

**Comparing the protective effect of antioxidant and anti-inflammatory drugs, anakinra and rosmarinic acid, against styrene-induced ototoxicity**

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Among various solvents used in industries, the aromatic hydrocarbon styrene is strongly associated with ototoxic effects in workers. Our research group previously demonstrated that the primary ototoxic effect of styrene is mediated by the interplay between oxidative stress and inflammation. Indeed, as reactive oxygen species accumulate, the cochlea's natural antioxidant defenses become inadequate, leading to oxidative status imbalance and enhanced inflammatory markers, responsible for hair cell death and hearing loss. Nowadays effective therapeutic interventions for styrene-induced ototoxicity are still lacking. In this study, we compared the protective effects of an antioxidant molecule, rosmarinic acid (RA), and an anti-inflammatory agent, anakinra (Ana), an antagonist of the IL-1β receptor, to evaluate their potential as novel pharmacological treatments against styrene-induced ototoxicity. To this aim, adult male Wistar rats were exposed to styrene (400 mg/kg) by gavage for 3 weeks, 5 consecutive days/week. Before each styrene administration, a subgroup of animals was treated with RA at a dosage of 10 mg/kg intraperitoneally injected, whereas a second subgroup received a dosage of 40 mg/kg of Ana by intramuscular injection. To assess the efficacy of the two different treatments, we performed the auditory brainstem responses (ABRs) at 7, 14, and 21 days after styrene and antioxidant or anti-inflammatory treatment onset. Our results showed a protective effect of both RA and Ana against styrene-induced cochlear damage, with a significant decrease in hearing thresholds in treated animals, compared to styrene-exposed animals. At the end of treatment, we conducted immunofluorescence and molecular biology analyses on cochlear specimens to evaluate changes in molecular markers linked to oxidative stress and inflammation, thus assessing the treatment efficacy. We observed a decrease of oxidative stress markers, as well as of inflammatory agents, indicating that both the antioxidant and the anti-inflammatory treatment can potentiate endogenous responses counteracting oxidative stress and inflammation, thus reducing hearing loss. Collectively, our data show that the treatment with an antioxidant or an anti-inflammatory drug can be effective against styrene-induced ototoxicity, with potential clinical applications to prevent worker health and reduce hearing loss.

**Computational model of the peripheral auditory system: ion channel distribution in inner hair cell synapses**

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This poster presents a comprehensive computational model of the mammalian peripheral auditory system, encompassing the outer and middle ears, cochlea, and auditory nerve. While the model integrates various physiological aspects across the entire auditory system, this study primarily examines the distribution of CaV1.3 channels in inner hair cell ribbon synapses and their impact on synaptic behavior. The model's holistic approach allows for the examination of individual hearing components and their interactions, providing valuable insights into normal hearing processes and the impacts of various defects. These findings have potential applications in studying hearing impairments and developing auditory prosthetics. Our simulations show that different spatial distributions of CaV1.3 channels result in varying spontaneous rates, thresholds, and sensitivities of the ribbon synapse, thereby affecting auditory signal processing. At low stimulus levels, single CaV1.3 channel openings significantly contribute to vesicle release events, highlighting nanodomain control. As stimulus levels increase, vesicle releases are predominantly influenced by multiple channels, indicating a shift towards microdomain control. This dual mechanism ensures high sensitivity and a wide dynamic range of the ribbon synapse.

**Diagnostic genome sequencing improves diagnostic yield in a single center study of 100 patients with non-syndromic and syndromic hearing impairment**

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**Introduction:** Hearing impairment (HI) is a common sensory disorder, which is genetically heterogeneous. Identification of causative variants underlying HI is challenging, since >100 genes for non-syndromic HI and >450 genes for syndromic HI have been reported.

**Material and methods:** In this study, 100 index patients with HI and variable additional clinical features underwent whole genome sequencing (WGS) in clinical settings. The samples were analyzed using virtual gene-panels of 174 (76% of patients) or 500 genes, respectively, for patients suspected of

having non-syndromic and syndromic HI. Nine patients had prior to WGS been prescreened for *DFNB1*, *SLC26A4*- and/or *STRC*-related HI and six using gene-panels for HI.

**Results:** A definite genetic diagnosis was made in 42/100 patients, distributed in 25 different genes. In total, 45 different likely pathogenic/pathogenic variants were detected, and 14 variants were novel. In addition, six patients had variants of uncertain significance (VUS) identified, where further work-up was recommended, which might change the classification to likely pathogenic. Finally, in an additional ten patients only one pathogenic variant was identified, so far. Variants in rare/recently identified genes causative of HI included *PLS1* and *ATOH1*.

**Conclusions:** WGS allowed detection of a definite or possible genetic diagnosis in ~48% of 100 cases (~53% excluding patients with unilateral HI and patients prescreened with previous NGS HI panel). Causative variants were found in >25 different genes, including both common and rare/recently identified HI genes, emphasizing the genetic heterogeneity of the condition. In non-solved selected families, the data will be re-analyzed with improved methods.

## Differences in petrosal bone marrow distribution between rat and mouse

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Using tissue clearing and immunofluorescence, we have recently characterized the adult rat temporal bone marrow distribution, and in particular the distribution and connections to the inner ear of petrosal bone marrow (doi: 10.3389/fneur.2024.1386654). Bone marrow was identified by its high cellular content and by the presence of cell populations belonging to the hemopoietic niche (e.g. megakaryocytes, see companion abstract from our group). In the cleared rat petrosal bone, autofluorescence allowed delineation of the otic capsule. The largest marrow island was found outside of the otic capsule, surrounding semicircular canal arms, and connecting to the dura through bone channels similar to those of calvarial bone, with only a few channels directed towards the bony labyrinth. Unexpectedly, bone marrow was also observed within the otic capsule endochondral layer, forming small clusters associated to the vestibule (VEM) and cochlear apex (CAEM). Endochondral bone marrow was connected through vascular loops to the labyrinth, and through straight channels to dural sinuses. The latter also received vascular connection from marrow located in surrounding bones, suggesting a role as immune barrier restricting pathogen spread from ear to brain. In mouse, petrosal bone marrow distribution was overall similar to the rat but displayed a few differences. The most evident difference was the volume ratio of CAEM over total petrosal bone marrow (23 ± 1% in mouse, $n = 2$; 2 ± 1% in rat, $n = 8$; $p < 10E\text{-}8$). Moreover, CAEM and VEM were connected by vascular bridges in mouse but not in rat. Given the importance of local bone marrow in the immune reactions of brain (doi: 10.1111/imr.13120) and middle ear (doi: 10.3389/fgene.2022.985214), this difference in CAEM volume and connectivity calls for attention in choosing a model for human inner ear immune reactions.

## Disrupted *GRHL2* transcriptional activity as a mechanism of autosomal dominant hearing loss development (DFNA28)

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**Introduction:** *GRHL2* is one from over 50 genes causative of autosomal dominant hearing loss (ADHL); it is also implicated in other disorders, including cancers. *GRHL2* encodes a transcription factor but up to now only a handful of ADHL-related *GRHL2* pathogenic variants have been reported. Their mode of action leading to ADHL development remains unknown. The aim of the study was to identify the genetic basis of ADHL in a multigeneration family with postlingual, progressive HL and to gain insight into the molecular mechanism of the ADHL-related (DFNA28) *GRHL2* mutations.

**Material and methods:** Genomic DNA was isolated from the peripheral blood samples of the proband and other family members ($n = 8$). Next-generation sequencing was performed using a multi-gene panel with 237 HL-related genes. Segregation analysis of the selected *GRHL2* variant with HL in the family was performed by Sanger sequencing. For four different ADHL-related *GRHL2* variants expression vectors were prepared and luciferase reporter gene assay was conducted in HEK293T cells.

**Results:** In the family a novel heterozygous *GRHL2* variant (NM_024915.4: c.1061C>T; NP_079191.2: p.(Ala354Val)) segregating with HL was identified. It localizes in the region corresponding to the DNA binding domain. The functional effect of the variant as well as of the other two *GRHL2* variants located in the DNA-binding domain (i.e. c.1258-1G>A, p.(Gly420Glufs*111) and c.1276C>T, p.(Arg426*)) was a reduction in *GRHL2* transcriptional activity. In contrast, the c.1609–1610insC (p.(Arg537Profs*11)) variant affecting the DNA dimerization domain of the *GRHL2* protein acted in a different way leading to a strong activation of the GRHL-responsive promoter.

**Conclusions:** Our data show that only truncating *GRHL2* mutations can cause ADHL. The pathogenicity of the novel missense ADHL-related *GRHL2* variant was strengthened by the results of functional assays. *GRHL2* mutations causing ADHL demonstrated both suppression and activation of *GRHL2* transcriptional activity and the effect seems to depend on where the variant is located. While the variants located in the DNA-binding domain showed haploinsufficiency, the variant located in the DNA dimerization domain presented a gain of function effect. Our study sheds new light on the mechanism of *GRHL2* mutations leading to hearing loss.

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**Early transtympanic administration of rhBDNF exerts a multifaceted neuroprotective effect against cisplatin--induced hearing loss**

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Cisplatin-induced sensorineural hearing loss is a significant clinical challenge and, currently, only one drug has been approved by Food and Drug Administration as an effective treatment. Thus, several efforts are needed to better understand cisplatin mechanism of damage and to explore new therapeutic strategies. Although the potential effects of brain-derived neurotrophic factor (BDNF) have previously been investigated in some ototoxicity models, its efficacy in cisplatin-induced hearing loss remains uncertain. This study aimed to investigate the therapeutic potential of recombinant human BDNF (rhBDNF) local delivery in counteracting cochlear damage in an in in vivo model of cisplatin-induced ototoxicity. Thus, adult Wistar rats were treated with cisplatin (12 mg/kg, intraperitoneally injected) and after one hour, they received 5 mg/kg of rhBDNF suspended in a thermogel by a transtympanic injection. Auditory brainstem responses were recorded to evaluate hearing function at 1, 3 and 7 days after treatment. Seven days after cisplatin treatment, we collected cochlear samples to perform, morphological, immunofluorescence, and molecular analyses to investigate the molecular mechanisms underlying the beneficial effects of our rhBDNF formulation. Our data showed that rhBDNF mitigates hearing loss in cisplatin-exposed rats by preserving synaptic connections in the cochlear epithelium and reducing hair cell and spiral ganglion neuron death. rhBDNF maintains the balance of its receptor levels (pTrkB and p75), boosting TrkB-CREB pro-survival signalling and reducing caspase 3-dependent apoptosis in the cochlea. Additionally, it activates antioxidant mechanisms while inhibiting inflammation and promoting vascular repair. Overall, our study demonstrates that the early transtympanic treatment with rhBDNF plays a multifaceted protective role against cisplatin-induced ototoxicity, thus holding promise as a novel potential approach to preserve hearing in adult and pediatric patients undergoing cisplatin-based chemotherapy.

**Electrical and cytotoxical examination of electrospun PVDF-TrFE fiber mats**

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Implantation of cochlear implants (CI) triggers various biological reactions in the patient's cochlea. Being unable to remove the foreign structure by itself, the body insulates the implant by surrounding it with connective tissue. Modification of the CI electrodes surfaces is one way to reduce the adhesion of connective tissue. Electrospinning is an electrohydrodynamic process used to generate thin mats. These fine micro networks can be used to alter the surface of CI electrodes. In this study, fiber meshes consisting of the hydrophobic poly(vinylidene fluoride-trifluoroethylene) (PVDF-TrFE) were used to investigate their contribution to electric conductivity and their cytotoxic potential for fibroblasts. The fiber mesh was cut into Ø 14 mm circular samples. Conventional SEM-holders were used as sample holders for the electrical measurements. Using a self-constructed chamber with four identical measuring cells, the increase in impedance due to the additional layer of fibers was investigated. In addition, cytotoxicity assays with rectangular samples as described in ISO 10993-12 were performed. For further investigations, model electrodes out of platinum-iridium wire were manufactured such that their surface area resembles that of CI electrodes. These models were embedded in silicone (Sylgard 184) and electrically analysed to test different wettability methods. Measured impedances for the fiber mesh samples show a mean increase in impedances of 225.99 ± 107.09 Ω compared to the reference samples. The only constraint in biocompatibility was found with 100% extraction solution (69 ± 4.88% cell viability). The mean surface area of the model electrodes was 0.386 ± 0.024 mm2 with impedances of about 1 kΩ (1024.19 ± 107.61 Ω). Cytotoxic and electric characterisation of PVDF-TrFE electrospun fiber meshes was successfully performed. Reliable model electrodes were manufactured and can be used in further investigations regarding the influence of the fiber mats on the impedance of CI electrodes. Additionally, cell proliferation behaviour on the fiber mats will be investigated to refine the cytotoxic influence.

**Elucidating the molecular diversity of the non-human primate cochlea**

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Unraveling the intricate composition and function of the cochlea is paramount to comprehending the mechanisms underlying sound perception and the pathogenesis of auditory disorders. The mammalian cochlea displays a highly organized structure, which contributes to the diversity and complexity of auditory processing. However, the cellular

intricacies in non-human primates remain largely unexplored. In the present study, we employed high throughput transcriptomic sequencing to profile over 11,280 nuclei across virtually all cochlear cell types in both juvenile and adult *Macaca fascicularis* at single-cell resolution. Our analysis unveiled remarkable heterogeneity both across and within cell types. Despite a largely conserved cellular composition of the cochlea, glial cells exhibited substantial species-specific diversity, while hair cells and spiral ganglion neurons with specialized transcriptional programs were well mapped onto their murine counterparts, underscoring the similarities that persist despite evolutionary divergence. Furthermore, we constructed a disease map associated with hearing loss, establishing this transcriptomic atlas of the macaque cochlea as an indispensable resource for future investigations in both human and non-human primates.

**Enhanced spiral ganglion neuron transduction for neurotrophin gene therapy with novel capsid-engineered AAV vector**

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Hearing loss (HL) affects over 460 million people worldwide, significantly affecting quality of life. Genetic analysis has identified more than 150 causative monogenic genes for non-syndromic sensorineural hearing loss, presenting attractive targets for gene therapy interventions. These approaches also have the potential to enhance conventional treatment strategies. Particularly interesting in this regard are cochlear implants (CI), used for severe to profound HL. However, their efficacy is believed to depend on spiral ganglion neuron (SGN) survival. Recognizing the potential of neurotrophins to enhance SGN survival and improve CI outcomes, we here report on the development of a novel adeno-associated virus (AAV) vector optimized for transducing SGN, even in adult mice and at low vector doses. For this purpose, the capsid was engineered to display a heptamer peptide, which was previously derived from a phage library screen.

Structure-focused modeling of our novel vector Var9 indicated a clear change in cell attachment receptor binding due to peptide insertion compared to its parental serotype AAV2. Predictions were confirmed using affinity chromatography and competition assays. Interestingly, Var9 demonstrated faster transgene expression in HEI-OC1 cells, a murine otic progenitor cell line, despite significantly lower entry efficiency, indicating enhanced intracellular processing of the vector. Subsequent IF-FISH analysis at single-cell level as well as our indirect uncoating assay revealed that Var9 vectors clearly outperform AAV2 vectors regarding kinetics and level of uncoating (3-fold), i.e. release of their genome from the capsid, a prerequisite for transgene transcription. Finally, in a neurotrophic gene therapy approach, Var9 effectively prevented SGN degeneration by overexpressing BDNF in SGN of deafened mice. Histological analysis of the cochlea revealed remarkable protective effects of SGN comparable to untreated control levels in all cochlear turns of mice treated with Var9-BDNF.

In conclusion, our novel AAV vector demonstrated superior properties crucial for efficient SGN transduction and will be further refined for clinical applications, aiming to enhance neural survival and improve outcomes for cochlear gene therapy in CI recipients.

**Exploring the link between noise-induced trauma and peripheral inflammation**

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Traditionally, the cochlea has been considered as an immunological privileged site, as the blood-labyrinth barrier provides isolation from the systemic immune system. However, the presence of immune cells in the cochlea has been recently reported, suggesting that immune-mediated processes can play a crucial role within the auditory system. In this context, both protective and detrimental T-cell functions have been linked to normal hearing and hearing loss, respectively, thereby emphasizing the dual role of T cells in cochlear health. Current knowledge also suggests that a balanced presence and activity of T cells is crucial for tissue homeostasis in the cochlea, avoiding pathologies such as age-related hearing loss and autoimmune inner ear diseases. Although cochlea-resident T cells are scarce under physiological conditions, the number of T cells appear to rise in response to acoustic trauma revealing the active recruitment of peripheral immune cells through cochlear blood vessels. In order to evaluate the induction of a peripheral inflammatory response upon noise trauma, we exposed mice to excessive noise (115 dB) for two hours. Hearing levels were determined 72 h and 1 week after noise-exposure, which revealed a substantial increase in hearing thresholds in all mice. To evaluate if the auditory stimulation would trigger a peripheral immune response we isolated splenocytes and analyzed cytokine production in different immune subsets

by high parametric flow cytometry. The analyses revealed increased activation of both CD4+ and CD8+ T cells, illustrated by significant increases in the expression of INF-γ, TNF and CD44. These data suggest a peripheral component in the elevated inflammatory response following acoustic trauma. Future research will be performed to explore the link between noise-induced hearing loss, peripheral inflammation and more specifically antigen-specificity of the observed increase in activated CD4+ and CD8+ T-cell populations.

**Expression of P2X2, P2X4, and adenosine A1 receptors in sheep and human cochlea: a translational study**

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Purinergic receptors have been identified in virtually all mammalian tissues to regulate fundamental cellular processes, leading to increased focus on its therapeutic potential. In rodent cochlea, purinergic receptors (P2X1,2,3,4,6,7, P2Y1,2,4,6,12, adenosine A1, A2A, A3) have been identified to play roles in cochlear (patho)physiology. However, previous findings from rodent models have yet to be translated into other mammalian species. Our earlier screening showed that several purinergic receptor subtypes are also expressed in sheep and human cochlea. The aim of this study is to characterize in detail the immunolabelling of P2X2, P2X4, and adenosine A1 receptors (P2X2R, P2X4R, adenosine A1R) in the sheep and human cochlea to contribute to understanding the potential functional roles played cochlear (patho)physiology.

Fixed, decalcified adult New Zealand Romney sheep temporal bones and celloidin-embedded adult human temporal bone sections were used for immunohistochemistry. Sub-type specific rabbit polyclonal antibodies raised against P2X2, P2X4, and adenosine A1 receptors were used with cellular markers. Super-resolution confocal imaging (Zeiss LSM800 Airyscan) was used for data acquisition.

P2X2R immunolabelling was present in outer hair cells (OHCs), inner hair cells (IHC), Deiters' cells, outer sulcus cells, basal cells of the stria vascularis, and the Reissner's membrane in sheep cochleae. At high resolution, strong immunolabelling was observed along the reticular laminar. Strong immunolabelling was also observed in the stereocilia and within the cuticular plates. P2X4R immunolabelling was present in OHCs and IHC, and the Reissner's membrane sheep cochleae. However, P2X4R immunolabelling was predominantly localized to the cytoplasm of OHCs. Adenosine A1R immunolabelling was predominantly localized to the IHC and Deiter's cells in sheep cochleae. Comparative data for P2X2R, P2X4R, and adenosine A1R immunolabelling in adult human cochleae with no known history of hearing impairment will also be presented.

Our results show that the expression patterns of P2X2R, P2X4R, and adenosine A1R appear comparatively conserved across mammalian species. A future study will be conducted to test the inferred functional role of P2X2R, P2X4R, and adenosine A1R in adult sheep models. Furthermore, our results support the potential for purinergic signaling as a target for future pharmacological-based interventions aimed at mitigating hearing loss.

**GelMA promotes inner ear organoidogenesis by regulating Mmp-mediated extracellular matrix remodeling**

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Permanent damage and loss of inner ear hair cells due to genetic mutations or external factors such as drugs and noise are the main causes of irreversible hearing loss. Inner ear organoids can provide an in vitro model for studying the underlying mechanisms of injury and for developing new therapeutic approaches. However, there is lack of protocols for the rapid and efficient establishment of inner ear organoids. In this study, we established a novel method for creating inner ear organoids using a synthetic GelMA hydrogel culture system to promote the spontaneous aggregation and assembly of inner ear stem cells in order to rapidly form organoids, and we found that the extracellular matrix undergoes extensive and rapid remodeling during self-assembly. The expression and activity of the Mmp family of extracellular matrix degradation enzymes, especially matrix metalloproteinase 9 (Mmp9), were increased in inner ear organoids in the hydrogel culture system, and inhibition of Mmps significantly inhibited the formation of inner ear organoids. Our study is the first to combine analysis of the extracellular matrix with inner ear organogenesis and provides a rapid and efficient method to model inner ear organoids.

**Genetic analysis reveals novel variants in a cohort of patients affected by sensorineural hearing loss and enlarged vestibular aqueduct (EVA)**

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Biallelic pathogenic variants in the *SLC26A4* gene, coding for the anion exchanger pendrin, are responsible for Pendred syndrome and nonsyndromic recessive hearing loss DFNB4. Both are associated with an enlarged vestibular aqueduct (EVA), the most common malformation of the inner ear. We recruited the first Austrian cohort of patients with hearing loss and EVA to define the prevalence of pathogenic variants in *SLC26A4* and discover novel EVA-associated genes.

The coding region and intron-exon boundaries of known EVA genes were amplified by PCR and Sanger sequenced. The presence of the Caucasian EVA (CEVA) haplotype was determined with the rhAmp® SNP Assays (IDT). Copy number variation (CNV) in the *SLC26A4* and *STRC* genes was assessed using a TaqMan™ Assay on QuantStudio3D. For undiagnosed patients, whole exome sequencing (WES) was performed. The pathogenicity of novel *SLC26A4* and TJP2 variants was evaluated by functional and molecular assays.

Biallelic pathogenic variants in *SLC26A4* were detected in 5/33 patients. Based on the perchlorate discharge test, one had Pendred syndrome. Monoallelic variants in *SLC26A4* were detected in 5/33 patients. Two were benign based on functional and molecular tests. The CEVA haplotype was found in 6 patients, 3 carried monoallelic pathogenic *SLC26A4* variants, 2 carried biallelic pathogenic variants, and one carried a monoallelic benign variant. Pathogenic variants in *FOXI1* (1/33), *POU3F4* (2/33) and *GJB2* (2/33) were also identified. No CNV of *SLC26A4* and *STRC* was found. WES of patients negative for known causative genes (15/33) detected variants in 6 EVA-unrelated genes (*SCD5*, *REST*, *EDNRB*, *TJP2*, *TMC1*, and *CDH23*) in 5 patients. Cell-based assays showed that the novel TJP2 variant leads to an aberrantly localized protein product, supporting its pathogenicity.

Sequence alterations in *SLC26A4* and/or the CEVA haplotype, *FOXI1*, *POU3F4*, and *GJB2* genes are responsible for hearing loss and EVA in 14/33 patients of this cohort. WES led to the identification of 6 genes previously not associated with EVA and allowed for the diagnosis of additional 5 patients. The genetic causes remain unidentified in 42% (14/33) of patients. Functional and molecular studies are needed to define the pathogenicity of novel variants and establish a causal link with disease.

**Genetic diversity of hearing loss and its connection to auditory development of cochlear-implanted children**

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**Introduction:** Each year, approximately 1–6 out of every 1000 children are born with severe to profound hearing loss (HL). In the majority of them HL is genetically determined and usually two pathogenic variants are detected in the DFNB1 locus. The aim of the study was to dissect the genetic background of non-DFNB1 HL in CI patients and to analyze their auditory development.

**Material and methods:** The study group ($n = 51$) was recruited from patients with isolated profound prelingual deafness who received CI before the age of 24 months. All patients were negative for DFNB1 locus pathogenic variants. Genomic DNA was isolated from blood samples. In probands whole exome sequencing (WES) was performed. Validation of selected variants and family segregation analysis were performed using standard Sanger sequencing. Identified copy number variants were examined with aCGH and qPCR. Evaluation of patients auditory development was performed with the LittlEARS questionnaire (LEAQ) in three subsequent intervals – at the time of cochlear implant activation as well as in 5th and 9th month after CI.

**Results:** Causative variants were identified in 74.5% of patients (38/51). The majority of them are localized in the *MYO15A* ($n = 7$) and *PAX3* ($n = 5$) genes. Among the detected genetic variants, 28% (15/54) were inherited in an autosomal dominant manner and eight of them occurred de novo. A syndromic form of HL was diagnosed in 27% (14/51) of patients. The auditory development of the studied children was the most dynamic in the first 5 months after CI and slowed down between the 5 and 9 months of using the device. No differences were observed between the auditory development of patients with an identified and unknown genetic causes of HL.

**Conclusions:** Obtained results show a high heterogeneity of genetic HL causes in the population of Polish DFNB1-negative cochlear-implanted patients. All tested children were good candidates for CI as their HL causative genetic variants are localized in genes preferentially expressed in the cochlea. In a group of patients without an identified genetic cause, the tested area should be expanded and more advanced technologies enabling full genome analysis (WGS) should be used.

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**Harnessing AI for enhanced analysis of cochlear imaging data**

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The sensory epithelium of the mammalian cochlea exhibits a tightly organized pattern of sensory hair cells along the so-called tonotopic axis. High-resolution imaging now commonly generates large datasets from light and electron microscopy, but analyzing these massive datasets has become a bottleneck, exacerbated by the lack of efficient tools that can mitigate user biases and manual labor.

Recent advances in Artificial Intelligence and Machine Learning (AI/ML) are transforming our ability to analyze extensive datasets and accelerate scientific discovery, particularly in tasks related to bio-image analysis. We will present examples of AI/ML-based applications we have developed for analyzing large inner ear imaging datasets, demonstrating how these technologies can expedite traditional time-consuming analyses and help overcome barriers in the field. These tools serve as a blueprint for developing novel applications in the field of auditory neuroscience.

To develop one such tool, we first assembled a diverse, carefully annotated dataset comprising 2D images of auditory hair cells captured using fluorescence microscopy, contributed by the global auditory research community. We then developed an AI/ML-based application trained on this dataset that automates the detection, classification, and quantification of hair cells along the tonotopic axis. The tool leverages advanced deep learning libraries and architectures, resulting in robust, generalizable models. Next, we extended AI/ML models to a more complex challenge: analyzing serial 3D electron microscopy datasets. We developed a novel tool for volumetric instance segmentation of mitochondria, which significantly enhances the structural analysis of subcellular organelles in electron microscopy volumes.

Our results illustrate significant time savings and increased reproducibility, utilizing open-source technologies and free software to build tools that can be shared as standalone tools or ImageJ plugins. These developments streamline data processing across various imaging modalities commonly used in the field of auditory neuroscience and enable detailed, quantitative analysis of large datasets to aid in discoveries that may have been overlooked otherwise.

While not exhaustive, these case studies underscore the essential steps for developing and employing AI/ML-based tools to address complex biological questions, highlighting the potential of these technologies to advance studies that rely heavily on detailed imaging data analysis.

**Hearing loss as the main clinical presentation in NLRP3-associated autoinflammatory disease**

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The NLRP3 gene mutations are the cause of autosomal dominant autoinflammatory disorders (*NLRP3*-AID). Recently, hearing loss (HL) has been found to be the sole or major manifestation of *NLRP3*-AID. Here, we tested 110 autosomal dominant HL families with a custom panel of 237 HL genes and found one family carrying the *NLRP3* c.1872C>G, p.Ser624Arg mutation. Functional studies revealed that this novel variant is a gain of function mutation, leading to increased activity of caspase-1 and subsequent oversecretion of proinflammatory interleukin-1b. Clinical reanalysis of the affected individuals, together with serological evidence of inflammation and pathological cochlear enhancement on FLAIR-MRI images, guided our diagnosis to atypical *NLRP3*-AID. The study highlights the role of genetic analysis in patients with progressive postlingual HL. This can help to identify individuals with hereditary HL as a consequence of *NLRP3*-AID and allow timely and effective treatment with interleukin-1-receptor antagonist.

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**How do you define bone marrow in the petrosal bone?**

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Bone marrow is a highly cellular connective tissue, containing both fixed elements (blood vessels, adipocytes, stromal and staminal cells) and mobile cells (blood cellular elements and their precursors). Since mature immune cells are also found in other connective tissues, especially during inflammation, the only way to identify bone marrow with certainty is by labelling its unique components, i.e. blood cell precursors and stromal components. This however creates two problems: 1-mobile cells are lost, in variable measure, during tissue sectioning, and 2-most bone marrow cells do not display unique epitopes, just unique combinations of them.

Within most bones, these problems do not significantly affect marrow identification, since the latter is contained in large, well-defined bone cavities, and even after some cell loss, most of its elements are still present in sufficient quantity to be visualized by immunofluorescence. The temporal bone,

however, displays unique structural complexity, and in particular the petrosal bone surrounding the inner ear is made of extremely dense bone formed by endochondral ossification (different from calvaria, which are formed by membranous ossification) and even displays cartilage remnants in the adult. Although immune cells have been observed throughout the petrosal bone, only at the petrosal apex bone marrow has been identified as such.

Recently, however, in the human temporal bone, cavities compatible with bone marrow were found by synchrotron imaging to be located between the cochlear base and endolymphatic sac and connected with the latter through bone channels (Liu et al., 2024, doi: 10.3389/fneur.2024.1355785). In a similar position, by using tissue clearing, we observed in the rat temporal bone highly cellular cavities with a similar connection pattern (Perin et al., 2024, doi: 10.3389/fneur.2024.1386654). These cavities could be as small as only containing a few hundred cells only, and in previous work they had been addressed as perivascular connective. However, even the smallest cavities contained megakaryocytes, which are platelet precursor and are confined to marrow in other bones. Therefore, the small cavities within the petrosal bone can be identified as bone marrow, and may be involved in local inner ear immune responses.

**Identification of novel components of the lower tip-link complex: a proteomic and AI-based approach**

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Understanding the complex workings of auditory mechano-electrical transduction (MET) in the auditory hair cells is essential for comprehending the mechanisms underlying hearing. We present a novel approach integrating single-cell proteomics, affinity-purification mass spectrometry (AP-MS), and AI modeling to identify new components of the lower auditory MET complex, crucial for auditory function. Specifically, we seek to identify new components of the lower tip-link complex via AP-MS with specialized antibodies against the known tip-link component protocadherin 15 (Pcdh15). Protein-protein interactions between potential new and known MET components will be validated using Alphafold2. Simultaneously, we construct an exhaustive proteomic profile of inner hair cells (IHCs) and outer hair cells (OHCs) isolated from the murine cochlea, employing a suction pipette technique for single-cell isolation. Our sample preparation method yields promising results, with over 500 protein groups per single OHC. While proteomic analyses of vestibular hair bundles exist, none focus specifically on IHCs and OHCs. Ultimately, the aim is to conduct a comparative analysis between OHCs and the more difficult-to-isolate IHCs. Furthermore, we intend to correlate this dataset with established molecular structures in auditory mechanisms and have them serve as complimentary validation for AP-MS experiments with known components of the hearing machinery. These findings will offer critical insights into cochlear mechanosensation, advancing our understanding of auditory biology and providing a framework for innovative therapeutic strategies to address hearing impairments.

**Implementing swept-tone and level distortion-product and stimulus-frequency otoacoustic emission recording protocols in rats**

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Age-related hearing loss (ARHL) progression and outer hair cell (OHC) functional decline have previously been reported in Wistar rats but limited to non-linear distortion-product otoacoustic emissions (DPOAEs). Linear reflection-type stimulus-frequency otoacoustic emissions (SFOAEs) have not yet been established for this species and may provide additional information about cochlear amplification, tuning and onset of hearing loss. In human studies, inclusion of both OAE types in cochlear assessment may provide complimentary information. Here we report initial efforts to broaden the toolbox for time-efficient and objective assessment of cochlear function in rats with swept-stimulus DPOAE and SFOAE measurements.

SFOAE measurement feasibility and repeatability were first confirmed in male Wistar rats. Then, for fixed level OAEs measured across range of frequencies, we implemented a "swept-tone" paradigm, testing various frequency sweep rates. To further speed up data collection, the frequency range (~4–40 kHz) was divided into 2 or 3 subranges played simultaneously. Results of single vs. multiple frequency sweep SFOAE and DPOAE at varying rates were compared to OAEs measured with steady-state discrete tones. We also studied the effects of sweeping the stimulus level, at fixed frequency for measurements of DPOAE input-output functions at six f2 frequencies. Stimulus levels were swept at variable rates with up to three pairs of stimulus frequencies were played simultaneously. Results were compared to DPOAE input-output functions measured with discrete tones.

For the SFOAE repeatability test, a conventional discrete-tone suppression paradigm was used. The maximum average within-subject differences were 5.0 ± 7.1 dB within-day and 6.2 ± 13.5 dB between days.

Swept-tone SFOAE and DPOAE recordings showed amplitudes similar to discrete-tone with rates up to 2 octaves/sec. Whereas DPOAEs could be recorded with 3 simultaneous sweeps (<3 dB difference), SFOAE results from multiple sweeps were not as consistent. Swept-level DPOAEs produced results identical to discrete-tone up to a 80 dB/sec rate and allowed simultaneous recording of 3 frequencies without interference. These data confirm that SFOAEs can be measured in a consistent and time-efficient manner in rats, with levels comparable to other laboratory species. Further work aims

to demonstrate the evolution of SFOAEs with aging, in comparison to ABRs and DPOAEs.

**In vitro biocompatibility study of Polyvinyl difluoride piezoelectric nanofibers for cochlear implants**

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The cochlear implant (CI) is currently the gold standard in treating sensorineural hearing loss (SNHL) as it activates the cochlear nerve bypassing the hair cells, allowing the brain to hear sound waves. The goal of ongoing research is to create self-powered cochlear stimulation devices based on piezoelectric nanomaterials that can improve the quality of life for patients with SNHL and reduce the side effects of traditional CIs.

Polyvinyldifluoride (PVDF) piezoelectric nanofibers are a new type of piezoelectric nanostructure developed for biological purposes. The fiber can be coated with barium titanate (BaTiO3) nanoparticles or graphene nanosheets (GN) to enhance the piezoelectric coefficients. The purpose of the study is to evaluate the in vitro biocompatibility of PVDF nanofibers, which appear promising as alternatives for producing next-generation CIs.

Pure PVDF, BaTiO3-coated PVDF, and GN-coated PVDF were evaluated on three cell lines: HaCaT, OC-k3, and PC12. Viability, morphological changes, and neuritic outgrowth were evaluated in vitro using these cell lines as a model for the cochlear tissues.

The morphology study indicated that HaCaT, OC-k3, and PC12 cells were healthy, well-preserved, and had normal structural characteristics at all times tested. According to cell viability results, the fibers caused an increase in cell metabolism after 72 hours of incubation, especially on OC-k3 and PC12. On the HaCaT cell line, the fibers exhibited a slight but not significant reduction of cell metabolic activity starting from 48 hours of exposure. In addition, BaTiO3-coated PVDF have the most favorable results when it comes to the number of branch points and average length of neurites in PC12 cells, leading to a conclusion that BaTiO3 nanoparticles enhance the complex processes of PC12 cells.

To summarize, the investigation revealed that the tested nanofibers exhibited high biocompatibility in vitro, particularly with cochlear and neuronal cells, and the piezoelectric nanofibers with barium titanate particles be used to develop the next generation of self-powered cochlear implants. To conclude, these piezoelectric nanofibers have the ability to stimulate the cochlea, even though additional research is needed to achieve adequate mechanical and electrical performance.

**In vivo calcium imaging in the developing mouse cochlea**

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**Introduction:** Sensory-independent calcium activity regulates the development of mammalian sensory systems. Our current understanding of the origin and modulation of these calcium signals comes from ex vivo experimental work, which cannot replicate the sophisticated anatomy, innervation and physiology of the intact mammalian cochlea. We have developed surgical and microscopy approaches that, combined with transgenic animals expressing fluorescent indicators, allow us to study how mammalian sensory hair cells operate in vivo. Using this approach, we investigated the dynamics of spontaneous calcium activity in the prehearing cochlea of live mice at the cellular level.

**Material and methods:** Mice (P3–P10) expressing the genetically encoded calcium indicator GCaMP6f in either the hair cells or the supporting cells were anaesthetised using isoflurane and their body temperature maintained with a heat mat. The surgical procedure only led to a very small opening in the apical coil of the cochlear bone, leaving the cochlear canals intact and unopened. The mouse was then transferred on the stage of a two-photon microscope equipped with long working distance water immersion objectives for imaging.

**Results:** This approach allowed us to record from the same cochlear region spanning 15–30 IHCs. We found that IHCs and supporting cells displayed spontaneous calcium activity in vivo throughout the age-range investigated. IHC activity mostly appeared in bursts and some IHCs appeared to transition between quiescent periods and periods of prolonged spontaneous activity. Nearby IHCs displayed both independent and coordinated activity, which was compatible with the modulation on IHC excitability by calcium waves from the supporting cells.

**Conclusions:** Our approach provides significant insights into the nature of spontaneous cochlear activity in prehearing mice. These findings provide the first in vivo physiological recordings of spontaneous calcium activity occurring in the mouse pre-hearing cochlea. The application of two-photon imaging to study cochlear activity in vivo offers a promising avenue for future research.

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**Inner ear malformations caused by mutations in *Slc26a4* gene and it's regulative elements presented in a zebrafish model**

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Congenital hearing loss can be caused by genetic abnormalities leading to inner ear malformations (IEM). SLC26A4 (pendrin) is an anion exchanger expressed in the inner ear, thyroid and β-intercalated cells in the kidney. Mutations in *SLC26A4* cause a common form of IEMS: enlarged vestibular aqueduct (EVA), often accompanied by incomplete partition type 2 (EVA/IP2). However, only 25% of patients have confirmed mutations in *SLC26A4*. It has been reported that a group of polymorphisms upstream of the *SLC26A4* gene, the CEVA haplotype, is frequently found in patients with monoallelic *SLC26A4* mutations. Since the publication of the zebrafish genome in 2001, the zebrafish has become an increasingly popular animal model for studying human diseases. The transparency of the larvae, the large number of offspring and the accessibility of various methods of genetic, chemical and physical manipulation make it a very useful model for studying inner ear development. During early development, the inner ear undergoes dynamic changes from an otic placode and otic vesicle to a labyrinth of semicircular canals. Since data on the expression of genes associated with hearing are incomplete, we present here a detailed account of expression during early development. Secondly, we plan to create a zebrafish model for human *SLC26A4* gene expression by introducing human upstream regulatory elements into the zebrafish genome.

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**Investigation of cochlear implant impedances over time**

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**Introduction:** Structure and hearing preservation are important factors in cochlear implant (CI) surgery. To improve outcomes, impedance measurements of the CI electrode can be used to monitor these parameters after electrode insertion to detect delayed hearing losses: electrode impedance is thought to be a biomarker for inner ear inflammation after insertion of a CI electrode into the cochlea. Within the present study, impedance changes over time were analyzed using an app-based solution for daily in-vivo impedance measurements of CI recipients.

**Material and methods:** $N = 22$ participants were asked to use a research software, the Telemetry Study App, to measure the impedance of all 12 electrode contacts morning and evening for a period of at least 4 months after implantation. Depending on the start of electrical stimulation, the cohort was divided into (1) direct activation (DA) and (2) non-DA group.

**Results:** In both groups, lower impedances were observed in the evening compared to the morning with the onset of electrical stimulation. An increase in mean impedances was shown up to 10 days after surgery. Mean impedances of the DA group reached a plateau after about 30 days after surgery while the non-DA group continued to show a slight increase. Impedance values of the DA group remained mostly unaffected from the start of the first fitting week (~day 40 postoperatively) but decreased in the non-DA group. Mean impedances of the non-DA group were slightly lower than those of the DA group at this time but converged at the end of the investigation period.

**Conclusions:** The Telemetry App allowed for daily impedance measurements and hence for monitoring of the inner ear condition. With increased measurement frequency, the impedance development over time after CI surgery could be shown. In addition, daily impedance fluctuations were observed with the onset of electrical stimulation.

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**Is there a relationship between voice and hearing?**

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**Introduction:** Nowadays, hearing impairment is one of the key challenges at the forefront of paediatric otorhinolaryngology. In the absence of auditory feedback, children with impaired hearing suffer from voice and speech disturbances due to inability to control their own voices. Thus, the purpose of this study was to describe the nature of voice abnormalities in children with hearing loss.

**Material and methods:** The study involved 100 aged 4–12 years with a diagnosis of bilateral chronic sensorineural hearing loss. The children underwent subjective and objective voice research methods.

**Results:** The most prevalent complaint in children (72.94%) was a change in voice quality. Endoscopy of the larynx showed no pathological findings in 87.0%. Acoustic analysis of the voice in children with hearing loss showed the following voice disorders: in Grades 3 to 4 hearing loss, a shift in F0 towards low frequencies of 239.78 Hz is seen (95% CI 228.6–250.95) ($p < 0.05$). Children with hearing loss had high Jitter of 1.82 (95% CI 1.22–2.43) that was nearly three times over the normal limits ($p < 0.05$). MPT in children with Grades 3 to 4 hearing loss was lower than that in children with normal voice and hearing and measured 5.41 s (95% CI 1.22–2.43) ($p < 0.05$).

**Conclusions:** The study found that hearing impairment is associated with the development of voice abnormalities. Medical care for children with Grades 3 to 4 hearing loss should include measures to identify and treat those voice abnormalities.

### *KDM5B* controls sensory neuron subtype diversity

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**Introduction:** The transformation of initial auditory stimuli is fundamentally upon the synaptic connections originating from hair cells and projecting to the spiral ganglion neurons (SGNs) within the cochlea. During stages of neuronal development, epigenetic modulations coordinated synergistically with transcription regulators play a pivotal role in the determination and preservation of neuron subtypes. Mature murine cochlear SGNs comprise four distinct subtypes, specifically designated as Type Ia, Ib, Ic, and II; however, the definitive regulatory factors conferring specification and maintenance of these subtypes are yet to be comprehensively elucidated.

**Methods:** 1. Single-cell transcriptomic analysis: Seurat, Harmony, SCENIC, Metascape. 2. Adono-associated virus (AAV)-based delivery system to murine cochlea. 3. Single-molecular in situ hybridization (RNASCOPE). 4. Electrophysiological patch-clamp recording. 5. Immunofluorescence staining.

**Results:** In this study, we initially constructed a single-cell transcriptomic landscape of SGNs from embryonic, neonatal, and adult developmental stages. Utilizing the SCENIC algorithm designed to predict transcription factor (TF) activity, we were able to identify a sequence of TFs exhibiting time-specific expression patterns. Interestingly, our data revealed a substantial association between the epigenetic cofactor KDM5B (Lysine-specific demethylase 5B) and the timing of initial subtype specification which typically occurs at birth or just prior. Altering the gene expression of Kdm5b in the mouse cochlea at birth through an AAV-based round window injection revealed bidirectional effects on subtype markers (Calb1, Calb2, and Lypd1). Specifically, a significant downregulation or even complete ablation was observed subsequent to shRNA--mediated knockdown of *Kdm5b*. Conversely, overexpression of Kdm5b resulted in a reinforcement of mixed SGN identity. Notably, both genetic manipulations consequently induced auditory impairments in the mice, as determined via the auditory brainstem response (ABR) test. Additionally, bulk RNA sequencing data derived from profiling cochlear tissue echoed these findings, reinforcing the importance of Kdm5b in the specification process of SGN subtypes. In sum, our results indicate that the epigenetic regulator *Kdm5b* is indispensable in effectively determining SGN subtype specification.

**Conclusions:** 1. *Kdm5b* expression is restricted to early SGN development. 2. *Kdm5b* specifies and maintains SGN subtype identity.

### Long term expansion of *Lgr5* positive supporting cells and differentiation into a hair cell-like phenotype from adult mouse derived cochlear organoids

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**Introduction:** Over 400 million people worldwide suffer from hearing loss, which requires medical intervention. The current treatments available to these patients are the use of hearing aids or cochlear implants. Although they improve the patients quality of life, 'normal' hearing is not completely restored. A primary cause of hearing loss results from damage to hair cells (HCs) due to excessive noise exposure, ageing, or the exposure to ototoxic drugs. These damaged HCs are not regenerated in humans and can continue to deteriorate with time. In non-mammalian vertebrates, supporting cells (SCs) in the cochlea have regenerative capacities and give rise to new HCs after damage, even in adulthood. Research with neonatal mice has also shown that SCs can give rise to new HCs after damage in vivo. Furthermore, neonatal SCs can be expanded and differentiated into hair cells in vitro. However, for translational purposes, it is important to evaluate if this is also possible with adult-derived tissue.

**Material and methods:** Young adult and mature adult Lgr5GFP transgenic and C57CB/Bl6 mice were used for isolating cochleas. Cochleas were harvested and, after digestion with thermolysin and accumax, single cells were mixed with Matrigel, plated in 3D and grown in a high growth factor medium containing Wnt, R-spondin, and Noggin conditioned mediums. After passaging with trypsin and mechanical trituration, we evaluated proliferation and differentiation by immunofluorescence microscopy and qPCR or bulk sequencing.

**Results:** Lgr5-expressing SCs derived from adult (p30-p200) Lgr5GFP transgenic and C57CB/Bl6 mice cochleas can be expanded to at least 9 passages without losing differentiation capacity. *Lgr5* is highly expressed in expansion medium and lost after differentiation. *Atoh1* expression increases during expansion and it is lost after differentiation. Myosin7 is not expressed in expansion medium and it is highly enhanced after differentiation. In parallel, we aim to setup a co-culture system where we will culture differentiated cochlear organoids with spiral ganglion neurons (SGN), to further mimic the in vivo physiology. This research will be the first step to building a functional primary cochlear organoid model to get a better understanding of the regenerative capacities of adult derived tissue.

**Loud noise exposure is unlikely to cause DNA damage within the organ of Corti**

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**Introduction:** Noise-induced hearing loss (NIHL) affects a large proportion (Daniel, 2007) of the estimated 1.5 billion people currently living with hearing loss (WHO, 2021). NIHL, just like all other forms of hearing loss, can lead to depression and anxiety (Shukla et al., 2020), and it increases the risk of dementia (Livingston et al., 2020). However, we know little about the pathogenesis of NIHL, which is important for helping us identify prevention methods and therapies. Based on previous work showing that oxidative stress is liekly to be part of the pathogenesis of NIHL (Kishimoto-Urata et al., 2022), we hypothesise that cells within the cochlea experience DNA damage following loud noise exposure, leading to cell death seen in NIHL.

**Material and methods:** We exposed 1 month-old C57Bl/6N mice to 120dB SPL of sound (1–16 kHz) for 2 hours, before sacrificing them and fixing their cochleae. Following fixation and dissection, organs of Corti were immunolabelled for the DNA damage markers γH2AX and 53BP1. To test how the different cell types within the cochlea respond to oxidative stress, we treated the organs of Corti explanted from 1 month-old mice with the oxidising agent hydrogen peroxide, before fixing and labelling them for γH2AX and 53BP1.

**Results:** One hour post-noise exposure, γH2AX and 53BP1 immunofluorescence labelling in the cochlear cells appeared comparable to that observed in non-exposed mice. We also found that following peroxide treatment, adult hair cells show relatively little change in γH2AX labelling when compared to the supporting cells.

**Conclusions:** Our preliminary study shows that DNA damage is unlikely to mediate the cell death seen in NIHL, and that there is a difference in how adult hair cells and adult supporting cells respond to oxidative stress. One explanation for the different cellular responses is that adult hair cells are better protected against DNA damage than supporting cells. The other explanation is that adult hair cells are less able to recognise and repair DNA damage. Identifying which explanation is correct will help us understand why adult hair cells degenerate and die following an increase in oxidative stress.

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**Mammalian TMC1 or 2 are necessary for scramblase activity in cochlear hair cells**

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Auditory sensory transduction converts sound information into electrical signals through opening of mechanosensitive ion channels located at the tips of hair cell stereocilia (reviewed by Zheng and Holt, 2021). Among the proteins of the ion channel complex, we find the transmembrane channel-like (TMC) proteins 1 and 2 form the pore of hair cell transduction channels (Pan et al., 2013, 2018). The structure of TMC proteins in C. elegans worms (Jeong et al., 2022; Clark et al., 2024) and predicted mammalian TMC structures (Hahn et al. 2009; Ballesteros et al., 2018; Pan et al., 2018) are reminiscent of TMEM16 proteins, which function as $Ca^{2+}$-activated ion channels and lipid scramblases. For the current study, we confirmed lipid scramblase activity in auditory hair cells with genetic or pharmacologic disruption of *TMC1*, consistent with a previous report (Ballesteros and Swartz, 2022). We used the Annexin-V marker coupled with a fluorophore emitting at 647nm to label the phosphatidyl serine (PS) localized in the membrane at the tips of hair cell stereocilia. PS externalization was triggered by disruption of sensory transduction using the established non-ototoxic blocker, benzamil, or by genetic mutations that affect permeation properties of *TMC1*. Following application of 5 μM FM1-43 to label hair cells, we compared externalization of PS before and after benzamil treatment. We found that expression of either *TMC1* or *TMC2*, were essential for PS externalization. Tmc1/Tmc2 double knockout mice lacked PS externalization completely. We also determined that expression of exogenous human TMCs (hTMC1 or hTMC2) can induce PS externalization. Finally, we demonstrated that hair cells expressing two different human mutations in Tmc1 can constitutively evoke PS externalization. In conclusion, here we show that not only *TMC1* is essential for the lipid scramblase function in hair cells but *TMC2* can also promote scramblase activity in the absence of *TMC1*. The PS externalization can be triggered by human TMC proteins. Our data suggest that human TMC dysfunction, like mouse, may lead to dysregulation of membrane homeostasis at the tips of hair cells stereocilia and thus may contribute to auditory dysfunction due to *TMC1* mutations.

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**Minigene assay as important tool in determining the pathogenicity of genetic variants in hereditary hearing loss**

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**Introduction:** Next generation sequencing (NGS) is a method which is becoming increasingly available in clinical setting, especially in genetically heterogenous diseases as hereditary hearing loss. For each patient there are thousands of variants, including benign polymorphisms, pathogenic variants and variants of unknown clinical significance. The last ones are difficult to interpret, especially in case of silent variants and variants in non-coding parts of the gene – their mode of pathogenicity might be more elusive, i.e. alteration of splicing. Such variants require functional studies to properly assess their impact and pathogenic potential.

**Aim:** To assess the pathogenicity of 11 novel variants with possible effect on splicing using minigene assay.

**Material and methods:** Selected variants were: *ATP2B2* c.941-7C>G, EYA1 c.1475+1G>T, *EYA4* c.1282-12T>A, *GSDME* c.991delT, *GSDME* c.1127A>G, *MYO6* c.816+1G>A, *MYO6* c.1984-1G>A, *MYO6* c.3281-13A>G, *MYO7A* c.2829G>A, *MYO15A* c.9230-4 A>T, *SLC26A4* c.1001+1G>A. Each variant was detected in the custom HL gene panel performed for patient of Department of Genetics, Institute of Physiology and Pathology of Hearing. Fragments of genes of interest encompassing closest introns and exons were introduced into expression vector pDEST pCI-Neo RHO using Gateway cloning system. Cell cultures of HEK293T line were transfected with expression vectors for each gene, containing either wild type sequence or sequence with studied variant. After 48 h of incubation cell lysis and RNA isolation were performed. Transcripts were analyzed by subsequent RT-PCR, gel electrophoresis and Sanger sequencing.

**Results:** The majority of studied variants displayed their effect on splicing (9 out of 11, 82%). The most common aberrations were exon skipping and incorporation of intron fragment to the transcript, which usually resulted in frameshift and introduction of premature stop codon. In case of variants *ATP2B2* c.941-7C>G and *MYO15A* c.9230-4 A>T there were no observable signs of splicing alteration.

**Conclusions:** Genetic variants affecting splicing emerge as an important contributor to HL. The performed minigene assays allowed for better variant interpretation, which in turn allowed for the correct genetic diagnosis. The study demonstrates the significance of functional testing especially when it comes to the silent variants and intronic variants.

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**Modeling genetic inner ear hearing loss: development of hiPSC-derived inner ear organoids harboring *GJB2* mutations**

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Hearing loss is the foremost common sensory disorder globally, impacting approximately 5% of the population. The current treatment options are limited to hearing aids or cochlear implants, which mitigate symptoms but are not a permanent solution. In the context of genetic hearing loss, gene therapy could be the ultimate solution.

In Europe, the most common mutations occur in the *GJB2* gene, which encodes Connexin 26 (Cx26), a component of gap junctions, which facilitate ion transport between cells. The exchange of ions is an essential process for hearing as it functions as a message between sound detection by the hair cells and transmission of this message to the brain. Disease-causing mutations in *GJB2* lead to aberrant or non-functional protein, probably leading to improper exchange of ions between cells and thereby reduced hearing.

The only available human model for inner ear research is the inner ear organoid model, generated from human induced pluripotent stem cells (hiPSCs). This model recapitulates the diverse cell types found within the inner ear, providing insights into hearing-related mechanisms. We are employing this model system to study how *GJB2* mutations result in hearing loss.

To select the most relevant *GJB2* mutations, we performed a literature search to identify mutations associated with hearing disorders. Multiple sequence alignment and AlphaFold 2 predictions were performed to assess mutations at conserved protein sequence positions. Two selected mutations, c.35delG and c.269T>C/p.L90P, commonly found in European patients, induce a shortened Cx26 protein and a missense mutation with a structural change, respectively. Guide RNAs for these mutations are undergoing efficiency testing.

We outline selection criteria for mutations that can be studied in hiPSC-derived inner ear organoid models. This will facilitate the study of *GJB2* mutations on essential hearing cells, enhancing understanding of the disease and guiding future therapy development.

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**Monitoring the negative effects of music listening on otoacoustic emissions: a preliminary report**

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**Introduction:** Many people consider music an essential aspect of daily life, often listening to it for extended periods, surpassing safe sound levels. Prolonged exposure to loud sounds (>85 dBA) can harm the hearing organ, leading to irreversible changes in the inner ear and noise-induced hearing loss. Regular hearing tests, such as otoacoustic emission testing, are crucial for early detection of cochlear changes, particularly in cases of noise-induced hearing loss. Fortunately, advancements in technology grant us nearly limitless access to tools for monitoring hearing health.

**Purpose:** The purpose of this study was to see how exceeding recommended sound doses affects the magnitude of different types of otoacoustic emissions (OAEs).

**Material and methods:** Measurements were made on 1 person with normal hearing listening to music on an Android mobile device. Three types of OAEs were measured: click-evoked OAEs (CEOAE), distortion product OAEs (DPOAE) and spontaneous emissions (SOAE). The application's (HearAngel)

which monitors musical multimedia internal measure, the daily sound allowance (DSA), was used to estimate music exposure time. OAE measurements were made for: 100% DSA, 100% DSA + 10 minutes, 100% DSA + 30 minutes, 7 hours.

**Results:** Slightly exceeding the daily limit calculated with the app does not cause significant changes in OAE response levels. Some differences are noticeable only after several hours of musical exposure. The biggest changes occurred in the SOAEs. The amplitude of all SOAEs decreased for DSA + 30 minutes and for 7 h.

**Conclusions:** SOAEs appear to be the most sensitive type of OAE to changes in the auditory system. Unfortunately, SOAEs are currently recorded in about 60–70% of the population with normal hearing, which is the biggest limitation in the use of these measurements. However, the results obtained are promising and encourage further research.

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**Multiplexed TMT-based quantitative proteomics identified essential players involved in the mechanism of action of SENS-401 observed under normal or ototoxic conditions in intact cochlear organ cultures**

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**Introduction:** SENS-401, known as R-azasetron besylate, is a first-in-class drug candidate to treat Sudden Sensorineural Hearing Loss (SSNHL) and in clinical development for inner ear protection against Cisplatin-Induced Ototoxicity (CIO) and hearing preservation after cochlear implantation.In all three indications SENS-401 demonstrates a promising potential to improve hearing (https: //www.sensorion.com/en/). The goal of this study is to conduct a systematic analysis of proteins and pathways involved in the protective action of SENS-401.

**Material and methods:** P3-P5 Wistar rat organotypic explant cultures, including both spiral ganglion and organ of Corti intact tissue, were exposed or not to 20 h-cisplatin (Cis) and co-treated or not with SENS-401. We used unbiased tandem mass tag multiplexed quantitative proteomics coupled with high performance liquid chromatography and mass spectrometry (FPP Montpellier). Bioinformatics data processing was done with MaxQuant & Perseus software. Differentially expressed proteins (DEPs) obtained by comparing conditions were analysed using the Panther Classification System, the Database for Annotation, Visualization and Integrated Discovery (DAVID), and KEGG/Reactome/Wikipathways database resources. Differential analysis with corrected t-test (FDR 5% and s0 = 1) was considered significant when the $p$-value was < 0.05.

**Results:** We provide for the first time proteomics data that revealed an overview of proteins/pathways involved in the SENS-401 effects, identifying 186 DEPs associated with 16 pathways, by comparing the SENS-401 to the Control condition. While 38% of the DEPs were significantly upregulated by SENS-401, 62% of DEPs were downregulated, highlighting the involvement of several combined protective pathways. In the CIO context, our proteomics data revealed that SENS-401 treatment affects up to 417 DEPs (with 52% DEPs upregulated and 48% downregulated) involving 38 pathways (SENS-401+Cis versus Cis) over the 799 DEPs and 62 pathways characterizing the ototoxic effects of Cis (Cis versus CTL). Finally, the co-treatment of SENS-401+Cis showed only 84 DEPs with 5 associated pathways when compared to Control, suggesting that SENS-401 is strongly shifting protein profiles and biological pathways towards normal condition.

**Conclusions:** We present for the first time a systematic analysis of proteins/pathways involved in SENS-401 effects under normal or ototoxic conditions. Replication and extension of these exploratory studies, including in vivo, will provide valuable insights into potential molecular targets.

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***Net1* overexpression promotes the trans-differentiation of Lgr5-positive progenitor cells into hair cells**

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**Introduction:** Sensorineural hearing loss is mainly caused by loss of sensory hair cells (HCs) due to ototoxic drugs, aging, and environmental noise. Unfortunately, hair cells are non-regenerative in the cochlea of adult mammals. Recent studies have shown that Lgr5-positive progenitors have the properties to regenerate hair cells. Net1 is a guanine nucleotide exchange factor of RhoA GTPase, which is involved in a variety of biological processes in cancer cells, including cell proliferation and differentiation. However, its roles in the cochlea have not been widely reported in vivo.

**Material and methods:** We inserted a *Net1* gene expression sequence at Hipp11 (H11) to construct Net1loxp/+ mice using CRISPR/Cas9 technology. After injecting Tamoxifen at P0-P1 to activate Cre recombinase, *Net1* was specifically overexpressed in Lgr5+ progenitor cells. We observed ectopic HCs by immunofluorescence, and determined whether the HCs were proliferated from Lgr5+ progenitor cells by EdU assay and lineage tracing, respectively, and the mechanism of Net1 enhancing HC regeneration was explored by real-time fluorescence quantitative PCR.

**Results:** By immunofluorescence, we found a large numbers of ectopic HCs in cochlea of Net1 conditionally overexpress(cOE) mice, and the EdU assay failed to detect any EdU+/Sox2+ cells. The lineage tracing results showed that more tdTomato+HCs significantly derived from Lgr5+ progenitors of *Net1* cOE mice than control. More importantly, real-time qPCR results showed that the hair cell-associated transcription factor Atoh1, was significantly increased, Wnt/β-catenin pathways was activated and TGFβ pathway was up-regulated in cochlear basilar membranes (BMs) of Net1 cOE mice. All results indicated that the Net1 cOE promoted

HCs regeneration, and these regenerated ectopic HCs may be directly transdifferentiated from Lgr5+ progenitors.

**Conclusions:** In summary, we specifically overexpressed *Net1* in neonatal mouse cochlear Lgr5+ progenitor cells and found a remarkably increased in the number of ectopic HCs compared to control mice. This study provides new evidence for the regulation of *Net1* on the regeneration of neonatal mouse cochlear HCs.

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**Neural health assessments in cochlear implant recipients using electrically evoked compound action potentials**

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**Introduction:** Hearing performance of cochlear implant (CI) users relies on the condition of the auditory nerve. Animal studies have shown that electrically evoked compound action potentials (eCAPs; responses of the nerve to current pulses) can be applied to assess this condition. In particular, relative eCAP measures, obtained by comparing eCAPs to different stimuli, are strongly correlated with neural survival (Ramekers et al., 2014). Studies in humans demonstrated the value of such relative measures in their correlations with hearing performances (Zamaninezhad et al., 2023). Here, we recorded eCAPs to various stimuli in CI recipients to examine the predictive value for hearing performance using both linguistic and non-linguistic perception tasks.

**Material and methods:** Ten subjects with severe sensorineural hearing loss, aged 60 to 80 years, received a CI (Flex28 arrays of Med-El GmbH). Intraoperatively and approximately 4 months postoperatively, eCAPs were recorded to biphasic current pulses with varying interphase gaps (IPGs; 2.1 to 30 μs) and varying current levels up to saturation level. Outcome measures include amplitude and latency at maximum current levels, and the current level halfway the amplitude growth function, level 50%. Relative eCAP measures were obtained by the difference between measures at IPG of 30 and 2.1 μs. Hearing performance was assessed by CVC in noise (+5 and +10 dB signal-to-noise ratio) perception and spectral ripple discrimination using the spectral-temporally modulated ripple test (SMRT; Aronoff and Landsberger, 2013).

**Results:** In all patients eCAPs could be recorded with amplitudes between 200 and 1000 μV. The absolute eCAP measures for IPG of 30 μs were similar for postoperative and intraoperative recordings. The IPG-difference measures substantially differed between postoperative and intraoperative recordings with postoperative measures aligning more with outcomes from animal studies. One notable correlation between hearing performance and eCAP outcome was observed: the ripple discrimination score increased with decreasing eCAP latency ($R2 = 0.5$, $p < 0.05$). Speech perception scores did not significantly vary with absolute or relative eCAP measures.

**Conclusions:** The eCAP latency appeared to have predictive value for a non-linguistic hearing outcome. We argue that speech perception is harder to predict from eCAP measures because of cognitive factors involved.

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**Non-invasive monitoring of intracranial pressure changes: utilizing otoacoustic emissions**

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**Introduction:** Invasive techniques for objectively measuring intracranial pressure (ICP) pose significant risks including infection, intracerebral hemorrhage, and brain injury (Maniker et al., 2006; Wolfe and Torbey, 2009; Scheithauer et al., 2009). Otoacoustic emissions (OAEs) provide non-invasive indicators of ICP changes, as the OAE phase reflects middle ear transmission, which is influenced by ICP via its connection to intracochlear pressure. A calibration of the technique is based on the ICP and OAE phase data collected by Buki et al. (1996) in patients undergoing controlled ICP changes during neurosurgery. Forward pressure level (FPL) calibration provides additional insight into middle ear transmission, complementing and validating OAE phase measurements.

**Material and methods:** Five International Space Station (ISS) normal-hearing astronauts were tested for distortion product otoacoustic emissions (DPOAEs) pre-flight, in microgravity in-flight conditions, and post-flight. Ground experiments were conducted involving 20 young volunteers, who were DPOAE tested in different body postures. The DPOAE response was time-frequency filtered (Moleti et al., 2012) to unmix the distortion and reflection components. The FPL calibration data provided, as a byproduct, a direct estimate of the load impedance measured in the ear canal, which is related to the middle ear reflectance.

**Results:** Systematic changes associated with microgravity and postural changes were observed for all considered physical quantities, with the DPOAE phase generally yielding the best results. Although physical quantities such as reflectance and load impedance are more directly related to ICP changes than the OAE phase, their numerical evaluation may be more difficult, because it involves ratios between complex quantities that are very sensitive to phase uncertainties.

**Conclusions:** DPOAE measurements with FPL calibration and component unmixing provide effective non-invasive indicators of ICP changes. Future research directions include applications in Glaucoma patients and absolute calibration in neural surgery environments for several types of clinical population such as hydrocephalus and intracranial tumor.

**Overexpression of Serpine2 promotes trans differentiation of Lgr5+ progenitors into hair cells in the neonatal mouse cochlea**

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Lgr5+ progenitors in neonatal mouse cochlea have the ability to regenerate hair cells (HCs) by directly transdifferentiation or mitotic regeneration, which could be induced by several genes and pathways. However, the regeneration ability of Lgr5+ progenitors is still limited in neonatal mice and is almost lost in adults. Considering HC regeneration is a complicated process involving lots of genes and pathways, it is necessary to find more key genes which could induce the proliferation and differentiation of Lgr5+ progenitors to promote HC regeneration. Here, we conjoint analysis our three previous RNA-seq data: Lgr5+ progenitors in the apical (ALPs) and basal of mouse cochlea (BLPs), neomycin-treated Lgr5+ progenitors (NLPs) and untreated Lgr5+ progenitors (ULPs), Lgr5+ progenitors and Lgr5-supporting cells (SCs), screened novel genes which we further explored their effects on the proliferation ability of Lgr5+ progenitors by sphere assay in vitro. We found that knockdown of Serpine2 inhibit only the proliferation of Lgr5+ progenitors. Serpine2, a member of the Serpins family, is involved in proliferation of various tumor cells in breast, pancreas and other organs. Our in vitro experiment data showed that Serpine2 may be also involved in the regulation of proliferation and differentiation of Lgr5+ progenitors. Here we studied the roles of Serpine2 in HC regeneration in vivo. We found that Serpine2 conditional overexpression (cOE) in Lgr5+ progenitors induced the number of ectopic HCs, especially inner HCs (IHCs) at postnatal day (P)7. Lineage tracing assay showed that these ectopic HCs are probably originated from Lgr5+ progenitors through direct trans-differentiation. Together, our data suggest that Serpine2 exerts a functional effect during the development of mouse cochlea, and may participate in the regulation of HC regeneration from supporting cells (SCs) and Lgr5+ progenitors in the neonatal mouse cochlea.

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**Refining rodent cochlear explant models for screening therapeutic drugs against ototoxicity**

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Ototoxicity is defined as damage to the inner ear, targeting cochlear and vestibular structures and sensory function, due to exposure to certain pharmaceuticals or chemicals. Drug classes most associated with ototoxicity include antibiotics, such as aminoglycosides and platinum-based chemotherapeutic agents (cisplatin). Although ototoxicity mechanisms of action are not fully elucidated, much progress has been made in identifying otoprotective solutions and/or drug replacement with reduced or no ototoxicity. There are ongoing efforts to get alternative tests and techniques to the in vivo tests, to predict early in the development the ototoxicity risk, in a rapid manner and reduce the number of animals used for in vivo tests.

Cochlear explants in neonatal rodents are an organotypic culture of the immature cochlea, facilitating the presentation of organized cellular structures within the inner ear, which are otherwise hard-to-access.The objective of this study was to develop the most accurate ototoxic ex vivo model, using rat and mice cochlear explants. The technical challenges are presented and discussed.

We worked on the different components of the explant to provide the most relevant and reliable method to analyze drug ototoxic effects on the rodent cochlear explants: (1) the parameters characterizing the explant model: age of the pups, composition of culture medium, dissection method, ototoxic reference drug dose, duration of drug exposure and culture period. (2) the markers to visualize the various structures of the cochlea, such as hair cells, supporting cells, fibers, and neurons. (3) the method of image acquisition using a laser scanning confocal microscope and the histological analysis methods based on a qualitative and quantitative assessment of hair cells (scoring of hair cell organization and counting of hair cell numbers).

The development of reliable and consistent rodent explant cultures provides significant advantages for investigating drug mechanisms of ototoxicity and developing novel therapies. Preclinical testing is a critical phase in new drug development, making it essential to continually refine and expand tools, including in vivo and in vitro models, to advance the progress of new treatments. Ultimately, these efforts contribute to reducing the burden of drug-induced hearing loss in clinical practice.

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**Sex-dependent expression of glutamate receptors in the developing murine organ of Corti**

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**Introduction:** In rodents, synaptic connections between inner hair cells (IHC) and type I spiral ganglion neurons (SGN) undergo extensive functional and structural changes before the onset of hearing (around postnatal day P12). Each synapse comprises the presynaptic ribbons containing the structural cytomatrix protein Ribeye and the postsynaptic glutamate receptors on the peripheral afferent fibers. The major excitatory neurotransmitter glutamate signals primarily via AMPA-type receptors. AMPARs are heterotetramers made up of GluA1–4 subunits. It has been shown that in the developing cochlea, the GluR3 subunit is essential for the right assembly of AMPAR GluR2 and GluR2 subunits on cochlear afferent synapses and for presynaptic ribbon morphology. Interestingly, only adult female GluR3-KO mice present early-onset hearing loss (1,2). Also, studies in rats showed that changes in AMPA receptor subunits due to neonatal handling differ for males and females (3). Based on that, the objective was to determine whether sex influences the expression or

localization of AMPA receptor subunits (GluR1-4) in cochlear synapses in young animals.

**Material and methods:** Cochlear explants were prepared from young (P4-5) C57BL/6 mice of both sexes. An enzyme-linked immunosorbent assay (ELISA) was used to measure the concentration of glutamate receptors (GluR1-GluR4) in tissue lysates. Semiquantitative RT-PCR was used to compare GluR2 gene expression. Immunofluorescence and fluorescence or confocal microscopy were used to determine GluR2 protein localization and morphology. Statistical analyses were performed using IBM SPSS.

**Results:** Glutamate receptor 2 (GluR2) protein levels in the organ of Corti lysates differed between male and female mice, with males having higher GluR2 protein levels than females ($p < 0.05$) and at the gene expression level ($p < 0.01$). Interestingly, other AMPA subunits (GluR1, GluR3, and GluR4) did not differ between the sexes. In addition, sex-specific differences in synapse morphology were detected by immunofluorescence.

**Conclusions:** Our study demonstrates that sex-related differences in GluR2 exist in the developing cochlea. It remains to be established whether sex-dependent differences in AMPA composition can also be detected in adult animals and how they affect hearing. The results obtained confirm that sex should be considered a biological variable in ex vivo studies.

## Success of targeted sequencing in the search for genetic causes of Usher syndrome type 2

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**Introduction:** Usher syndrome is one of the most common rare diseases in which both hearing impairment and retinitis pigmentosa coexist. Currently, four types of Usher syndrome are known. They are genetically heterogenous and clinically characterized based on the age of hearing loss and retinitis pigmentosa diagnosis, the degree of hearing loss and the presence of vestibular dysfunction. The aim of the study was to characterize the genetic background of Usher syndrome type 2 (USH2) in a group of Polish patients.

**Material and methods:** A total of 55 patients with a clinical diagnosis of USH2 were recruited to the study. The DNA was isolated from peripheral blood and genetic testing was performed using three different methods: real-time genotyping with TaqMan probes, high-throughput sequencing of the *USH2A* gene, and a panel of 237 hearing-related genes. Bioinformatic and expert analysis focused on the search for single nucleotide variants (SNVs) and copy number variants (CNVs). Segregation analysis was performed using Sanger sequencing and quantitative real-time PCR. Selected novel variants probably affecting splicing were tested using minigene assay.

**Results:** The cause of *USH2* was identified in all patients. In 98% (54/55) of the individuals, causative variants were located in the *USH2A* gene. In one patient (2%; 1/55), a new homozygous terminating variant in the *ADGRV1* gene was identified. In the *USH2A* gene, 42 different genetic variants were identified (28 known and 14 novel). A total of 74% (31/42) of the variants were deleterious. The most frequently identified genetic cause of USH2 was c.11864G>A (p.Trp3955Ter), present in 29 of the studied alleles. Deletions of exons 22–24 (17 alleles) and 10–11 (8 alleles) of the *USH2A* gene also played a significant role in USH2 development.

**Conclusions:** The obtained results characterize the mutation profile responsible for USH2 development in Polish patients. Genetic testing of USH2 patients should be based on high-throughput tests that enable simultaneous identification of SNVs and CNVs. The gathered data can serve as a starting point for further genotype-phenotype association analyses and may, in the future, identify patient groups that could benefit from developing molecular and cellular therapies.

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## Tailoring AAV vectors for gene therapy of inner ear disorders by directed evolution

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Hearing loss (HL) affects approximately 20% of the global population and the treatments are currently limited to hearing aids and cochlea implants. Gene therapy offers a possibility to prevent or even cure HL. With the aim to optimize the adeno-associated virus (AAV) vector system for inner ear directed gene therapy, we generated AAV peptide display libraries based on the AAV1, AAV2 and AAV6 capsid backbones. All libraries present random unique 7-mer peptide inserts at variable region VIII of the capsid protein with diversities ranging from 80,000–622,000 (maximum likelihood estimate, MLE). We conducted high-throughput in vivo selection screens in the inner ear of adult mice, testing alternative administration routes that demand overcoming robust biological barriers. The target tissue is the organ of Corti with its crucial mechanosensory hair cells (HCs) of the inner ear, the supporting cells (SCs) and the underlying spiral ganglion neurones (SGNs). Distinct variants were found to be accumulated to up to 5% for AAV2-based variants and up to

2.5% for AAV1-derived capsids after two rounds of in vivo selection. Interestingly, some top variants were already accumulated for the AAV6 KO library after only one selection round. A total of 20 top candidates from the AAV1 and AAV2 libraries were produced as vectors. They outperform the parental serotypes and show diverse expression patterns in the adult mouse cochlea. Three promising variants had the ability to transduce outer HCs, a challenging cell type to infect, and many also targeted inner HCs. Almost half of the variants also strongly transduced all layers of the stria vascularis – a viable target tissue for the treatment of age-related HL – and the SGNs were targeted, with 4 variants being highly specific for SGNs. In addition, different intensities of fluorescent transgene expression suggest differential efficacy in delivery or vector uncoating within the cells of the inner ear. Thus, we report on a set of promising new AAV variants with distinct features developed by in vivo high throughput selection screens for improving inner ear directed gene therapy.

## The cargos and potential roles of small extracellular vesicles derived from mouse cochlear explants in a model of cisplatin-induced ototoxicity

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**Introduction:** Cisplatin, an effective chemotherapeutic agent, is clinically limited by its side effects, such as high incidence rate of ototoxicity and renal toxicity. Small extracellular vesicles (sEVs) are derived from almost all cells, and they can reflect the physiological and pathological information of parent cells and participate in intercellular communication in the progression of pathologies. However, the cargos and roles of sEVs in the progression of cisplatin-induced ototoxicity are unclear until now.

**Material and methods:** Here, we established an ex vivo model of neonatal mouse cochlea to scrutinize cisplatin-induced ototoxicity. And then, we isolated sEV from the conditional culture medium of cisplatin-induced cochlea explants and characterized it by TEM, NTA, and western blotting. Next, we used small RNA sequencing and label free LC-MS/MS to profile the miRNAs and proteins cargos of sEV, respectively.

**Results:** The small RNA sequencing of sEVs indicated that 74 microRNAs (miRNAs) were significantly upregulated and 9 miRNAs were downregulated in cisplatin-treated group (referred to as Cis-sEV) compared with control group (referred to as Ctrl-sEV). Furthermore, the targets of these differential expressed miRNAs were mainly enriched in apoptosis, inflammation, and other cell damage-associated signaling pathways, which suggested that the miRNAs of sEVs probably participate in signal communication in cisplatin-induced damage. On the other hand, LC-MS/MS analysis of sEV suggested that there is an obviously differentiation between Cis-sEV and Ctrl-sEV, with 90 proteins being upregulated and 150 proteins being downregulated, including numerous proteins that could regulate the damage response. Furthermore, we found 3 proteins (Cltc, Cct2, and Hspa8), which are potentially involved in protein homeostasis and autophagy, are verified to be up-regulated in Cis-sEV compared to Ctrl-sEV rather than in cisplatin-damaged cochlear tissue lysis, indicating that they are likely involved in important and specific intercellular communication mechanisms underlying cisplatin-induced ototoxicity via sEVs, rather than involving in intracellular roles.

**Conclusions:** Overall, this investigation offers an innovative and promising perspective on the molecular changes that occur in cochlear cells in response to cisplatin, which could lead to a better understanding of ototoxicity and potential targets for therapeutic intervention.

## The cross-rode between oxidative stress and inflammation in the auditory system damage: role of via glial cell and macrophages activation in ototoxicity

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Redox imbalance and inflammation have been proposed as the principal mechanisms of damage in the auditory system, resulting in functional alterations and hearing loss. Microglia and astrocytes play a crucial role in mediating oxidative/inflammatory injury in the central nervous system; however, the role of glial cells in the auditory damage is still elusive. In this study, we investigated glial-mediated responses to toxic injury in peripheral and central structures of the auditory pathway, i.e., the cochlea and the auditory cortex (ACx), in rats exposed to styrene, a volatile compound with well-known oto/neurotoxic properties. To this aim, male adult Wistar rats were treated with styrene (400 mg/kg daily for 3 weeks, 5/days a week). At the end of treatment (day 21) electrophysiological, morphological, immunofluorescence and molecular analyses were performed in both the cochlea and in ACx samples to evaluate the mechanisms underlying styrene-induced oto/neurotoxicity in the auditory system. We showed that the oto/neurotoxic insult induced by styrene increases oxidative stress in both cochlea and ACx. This was associated with macrophages and glial cell activation, increased expression of inflammatory markers (i.e., pro-inflammatory cytokines and chemokine receptors) and alterations in connexin (Cxs) and pannexin (Panx) expression, likely responsible for dysregulation of the microglia/astrocyte network.

Specifically, we found downregulation of Cx26 and Cx30 in the cochlea, and high level of Cx43 and Panx1 in the ACx. Collectively, our results provide novel evidence on the role of immune and glial cell activation in the oxidative/inflammatory damage induced by styrene in the auditory system at both peripheral and central levels, also involving alterations of gap junction networks. Our data suggest that targeting glial cells and connexin/pannexin expression might be useful to attenuate oxidative/inflammatory damage in the auditory system.

**The current knowledge of spiral ligament fibrocytes in cell culture: a systematic review**

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The spiral ligament in the cochlea has been suggested to play a significant role in the pathophysiology of sensorineural hearing loss (SNHL). Positioned between the stria vascularis and the bony otic capsule, this structure contains spiral ligament fibrocytes (SLFs), categorized into five main types based on their structural characteristics, immunostaining patterns and location within the spiral ligament. Together with the stria vascularis, the SLFs maintain a positive endocochlear potential (EP) in the scala media via K+ recycling, which is an essential component in the transduction mechanism of the auditory pathway. It has also been suggested that the SLFs contribute to the cochlear immune response, glutamate homeostasis and cochlear blood flow regulation. Spiral ligament damage disrupts K+ recycling, reducing the EP and subsequently causing SNHL. Despite their pivotal roles, a lot remains unknown about the SLFs. Therefore, this systematic review about SLFs in cell culture could give an overview of the current state of the art, providing a basis for future studies trying to investigate those cells in vitro.

A literature search was performed using PubMed, Web of Science and Scopus taking into account the PRISMA guidelines. Twenty-five studies were included in this review that report on SLFs in cell culture. The differences in species, sex, age, method of culturing and used antibodies for immunohistochemistry are discussed. The majority of these studies cultured spiral ligament fragments onto type I collagen-coated petri dishes; a protocol described by Gratton et al. in 1996, while some recent studies are focussing more on growing these cells in a 3D environment.

A better understanding of the currently published methods will help us to optimize fibrocyte culture techniques, thereby allowing to investigate a variety of possibilities that can significantly increase our knowledge about SLFs. Among others, these possibilities include to discover their functional characteristics, such as growth patterns, protein expression profiles and ion channel physiology, to expose the cells to hypoxia or other toxic conditions and to assess the resulting effects, to genetically modify SLFs, to explore drug repurposing possibilities, to evaluate potential therapies for SNHL and to investigate their capability as a source for transplantable cells.

**The efficacy of the chemical chaperone TUDCA in the preservation of cochlear ribbon synapses**

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The ageing process, gene mutations and noise exposure cause two types of hearing problem arising from pathology in the cochlea; the hair cell damage causing elevation of hearing thresholds and the damage of the ribbon synapses between inner hair cells (IHCs) and spiral ganglion neurons causing an auditory neuropathy syndrome, called hidden hearing loss. Tauroursodeoxycholic acid (TUDCA), a derivative of naturally occurring bile acids, can antagonize protein misfolding and endoplasmic reticulum stress as well as mitochondrial oxidative stress. As both these stress response mechanisms are thought to be involved in cochlear pathology, our aim here was to find out if TUDCA protects against progressive hearing loss and acute noise-induced hearing loss. We used the ICR (CD-1) mouse strain as a model of early-onset progressive hearing loss. We injected these mice with TUDCA (250 mg/kg sc) or PBS, twice per week, from 3 to 9 weeks of age, and then assessed the outcome. We exposed CBA/Ca mice to noise; 98 dB SPL, 8–16 kHz frequency band for 2 h. These mice received TUDCA injections on two consecutive days before the exposure and thereafter daily for 7 days, after which the outcome was analyzed. We recorded ABRs and quantified hair cell and ribbon synapse (presynaptic ribbons) numbers within the 8-to-32 kHz cochlear frequency region. CtBP2 immunostaining marked presynaptic ribbons and CtBP2/Homer 1 double-staining pairing of pre- and postsynaptic components. Compared to PBS-treated mice, systemic TUDCA administration did not prevent ABR threshold elevations or OHC loss in either trauma model. However, TUDCA conferred statistically significant protection against IHC synaptopathy in the ICR mouse model of progressive hearing loss. The concomitant robust OHC loss in these mice prevented us from assessing the physiological relevance of synapse preservation (ABR wave I amplitude). Quantification revealed a preservation of ~20% of synapses per IHC across the frequency region studied. A recent publication showed using physiological and behavioral measures that this extent of synapse preservation improves auditory temporal processing in mice (Ji et al., 2024). Together, our results suggest that TUDCA pharmacotherapy can slow down progressive ribbon synapse loss, but not synaptopathy following acute noise trauma.

**The human iPSC-derived inner ear organoid as a model for ototoxicity studies**

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Drug treatment with platinum-based chemotherapeutics or aminoglycoside antibiotics can lead to inner ear damage and subsequent hearing loss and balance disorders. Studies on ototoxicity are limited to investigations in animal models or rare and difficult to acquire human inner ear tissues. In this pioneering study, we use human induced pluripotent stem cell (hiPSC)-derived inner ear organoids (IEOs) as a model system to investigate ototoxic effects of these drugs on human inner ear cells. Here, we aim to validate the IEOs as an effective model for assessing the ototoxic effects of cisplatin and gentamicin in cultured human inner ear cells. IEOs were generated from hiPSCs and cut into 200 µm thick vibratome sections at day 75 to access the hair-cell-containing inner ear vesicles within the cultured aggregates. The ototoxic compounds were applied for 24 hours (cisplatin doses 0–100 µM; gentamicin doses 0–1000 µM) and sections were kept in culture for up to one week. Evaluation techniques included H&E and immunofluorescent staining for assessing cell morphology, protein expression and apoptosis, along with cytotoxicity assays and qPCR to analyse stress signalling and cell death pathways.

Cisplatin-treated samples showed loss of hair cells, neurons, and structural integrity of the otic vesicle in the first few days after treatment, with apoptotic nuclei in the otic epithelium and its direct surroundings. Hereafter, recovery of architecture and hair cells was observed, potentially indicating that intrinsic regenerative capacities are present in the current model. Gentamicin affected the structural integrity of the vesicle with loss of cell polarity and collapse. Also, neuronal damage and extruded cells in the lumen were observed. Together, these results validate the human inner ear organoid as a model for assessing ototoxicity. Ongoing work focusses on the time course and the ototoxic effects of both compounds by RNA expression analysis of cell stress and cell death mechanisms. This study underscores the potential translational impact of the human inner ear organoid model for ototoxicity.

**The influence of operating point position in nonlinear undamping feedback force on amplitude minima in simulated distortion-product otoacoustic emissions**

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Furosemide has been demonstrated to reduce the endocochlear potential and influence distortion-product otoacoustic emissions (DPOAEs). Lukashkin et al. (2002) administered furosemide intraperitoneally in guinea pigs and presented cubic (2f1-f2) and quadratic (f2-f1) DPOAE input/output (I/O) functions recorded both before injecting furosemide and within the first half-hour after the injection. Over time, the amplitude of the cubic DPOAE I/O at L1 intensities up to about 50 dB SPL decreased. The cubic I/O function exhibited an amplitude minimum (notch) at L1 near 60 dB SPL, and the position of this notch shifted towards higher intensities, becoming shallower with increasing time. At the highest intensities, the cubic DPOAEs seemed to be almost unaffected by furosemide. Quadrature DPOAEs appeared to be less affected at intensities up to about 60 dB SPL, but the notch position shifted from L1 of about 50 dB SPL to about 70 dB SPL. We adjusted the operating point (OP) in the sigmoidal nonlinearity used to transform undamping feedback force in a cochlear model. The sigmoidal nonlinearity is proportional to the 2nd-order Boltzmann function, which is asymmetrical with the default position of the operating point at the inflection point. We observed that the OP position affected notches in simulated cubic DPOAE I/O functions only when shifted into the center of the sigmoidal function. In this case, the DPOAE amplitude was most reduced at the lowest intensities, and the effect of OP position was diminished at the highest intensities. Additionally, the notch shifted towards higher intensities. Simulated quadrature DPOAEs also contained a notch, and the OP point affected its position more than for the cubic DPOAEs. Direct changes to the undamping feedback force did not cause any change in the notch position. All these results indicate some agreement with the effects observed by Lukashkin et al. (2002) after the application of furosemide. However, the results should be interpreted cautiously due to several reasons. Furosemide affects the endocochlear potential, which cannot be directly simulated in our cochlear model. We can only adjust the operating point in the nonlinear function or the gain in the undamping feedback force. Additionally, the used cochlear model was designed to simulate the human cochlea, and the notch in DPOAE amplitude is located differently in L1, L2 space than in the case of the guinea pig. Lukashkin et al. measured DPOAE I/O for L1 = L2 + 10 dB SPL. We would miss the notch in simulated cubic DPOAEs for this level condition. Despite that, it is interesting that the notch position is affected only if the OP position is changed towards the center of the sigmoid. These simulations would provide a different explanation for the change in the OP position after furosemide application than that provided in recent studies applying furosemide intravenously (e.g., Strimbu et al., 2020).

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### The research of development of a miniature DNA base editor for *DFNB9* gene therapy in hereditary deafness

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OTOFERLIN (*OTOF*) mutation can lead to autosomal recessive deafness 9 (*DFNB9*), which is the main cause of auditory neuropathy. Delivering the exogenous *OTOF* gene through double AAV can restore the hearing of genetically deaf *DFNB9* mice, but the efficacy of the gene replacement therapy may diminish over time, and it cannot really solve the deafness caused by the gene mutation. In contrast, gene editing can correct mutant genes at the DNA level and fundamentally treat hereditary deafness. We screened and developed a mini-base editor and optimised AAV expression elements for constructing a single AAV delivery base editing system to cure gene therapy of genetic deafness caused by *OTOF* point mutations. Specifically, we constructed mini-ABE and sgRNA (SchABE8e-sgRNA4) on a single AAV vector and screened out small promoter and short-polyA elements for the expression of the base editing system. At the same time, we found that targeting the promoter for expression in the reverse direction achieving a more efficient cleavage efficiency. We successfully restored the hearing of *OTOF* point mutant mice to WT level and maintained it for a long time by delivering the single-base editing system to the inner ear using AAV serotype-Anc80, which is capable of efficiently transducing inner ear hair cells. Subsequently, we evaluated the safety of the SchABE8e-sgRNA4 single-base editing system in WT mice and found that both vestibular and hearing levels were maintained at the WT level, indicating the safety of our delivery system. Taken together, these findings provide new strategies for treating *DFNB9* in the clinic and lay the theoretical and experimental foundation for the clinical translation of gene editing therapy for deafness.

### The role of post-translational modifications of microtubules in the inner ear: insights from knockout mice studies

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Microtubules, an essential component of the eukaryotic cytoskeleton, perform a variety of essential functions within cells. One mechanism that regulates these diverse functions is the post-translational modification of tubulin. Although these modifications have been known for decades, research into them has only really taken off in the last few years. In particular, the impact and importance of these modifications in the sensory epithelia of the inner ear have only been marginally explored. With the discovery of tubulin-modifying enzymes and the availability of knockout mice, it is now possible to investigate the biological functions and molecular mechanisms underlying these modifications. Here, we have initiated an immunohistochemical study of the effects of post-translational modification knockout mice in the inner ear. Our results indicate that the absence or accumulation of polyglutamylated tubulin leads to different morphological changes in the mouse cochlea, whereas deacetylation results in a defective epithelium at an early age. A deeper understanding of the specific post-translational modification and the cochlea may provide new insights into the mechanisms of hearing and potential therapeutic approaches for the treatment of hearing disorders.

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### *Tmprss3* expression in the mouse cochlea

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Cochlear implants (CIs) have shown variable performance outcomes in DFNB8/10 patients carrying pathogenic mutations in *TMPRSS3*. A study by Shearer et al. (2018) which involved electrical stimulation of spiral ganglion neurons (SGNs) in CI patients revealed on average smaller electrical responses in DFNB8/10 patients compared to ones with other forms of deafness, indicating a loss of SGN function. Another study by Fasquelle et al. (2011) had previously shown a loss of more than half of SGN cell bodies in Rosenthal's canal, observed between days 90 and 180, in a mouse model with a premature stop codon in *Tmprss3* (Y206X). These observations indicate a loss of *TMPRSS3* function-mediated damage to the SGN health which could plausibly explain such variability in CI performance. However, the cell-type specific expression and function of *Tmprss3* in the cellular mosaic of Rosenthal's canal remains unclear. Combining an RNAscope assay for mRNA localization with immunohistochemistry, we semi-quantitatively assessed the cell-type specific expression levels of *Tmprss3* mRNA in the murine cochlea, with a focus on SGN subtypes. We also performed an RT-qPCR assay

with TaqMan probes on RNA isolated from mouse brainstem, and a direct few-cell RT-qPCR on the cells of the organ of Corti and SGNs. We report a strong expression of *Tmprss3* mRNA and protein in the cells of the organ of Corti with RNAscope, immunohistochemistry, and few-cell RT-qPCR. In addition, *Tmprss3* expression was detected in specific cells of the stria vascularis. Interestingly, in Rosenthal's canal, we observed *Tmprss3* mRNA enrichment in the type-II SGNs with RNAscope and immunohistochemistry in the mature cochlea. Hardly any *Tmprss3* transcripts were detected in the brainstem. Few-cell RT-qPCR revealed no abundance of *Tmprss3* mRNA transcripts in type-I SGNs. In conclusion, in contrast to our expectation, type-I SGNs do not or hardly express *Tmprss3*. Thus, the data suggest an indirect role of *TMPRSS3* for the health and function of SGNs, which will need to be studied further to understand varying CI performance in DFNB8/10 patients.

**Treatment following triple-AAV delivery in mature murine model of human *CDH23*-associated hearing loss**

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Gene therapy holds promise as a curative therapeutic method with the potential to suppress HL progression or restore hearing function, something unattainable with hearing aids or cochlear implants. To expand the scope of target genes for cochlear gene therapy, patients with progressive genetic hearing loss (HL) are considered suitable candidates in terms of the therapeutic time window for gene therapy intervention. *CDH23* is a common deafness gene that can cause either Usher syndrome type 1D or non-syndromic HL (DFNB12). The phenotype range of DFNB12 is variable from congenital to adult-onset HL. Adult-onset, *CDH23*-related HL is progressive, beginning as high-frequency HL that gradually affects low frequencies, ultimately resulting in HL across all frequencies. While this gene is an ideal target for cochlear gene therapy, the size of the *CDH23* coding sequence is 10.1 kb; therefore, the development of gene therapy using triple adeno-associated virus (AAV) vectors is necessary. In this study we aimed to investigate the transduction efficiency of triple-AAV. This study aimed to investigate the transduction efficiency of triple AAV vectors in the cochleae of adult mice, focusing on large-gene-associated HL. Additionally, we sought to evaluate the feasibility of cochlear gene therapy in a mouse model of human *CDH23*-mediated HL using the triple AAV approach. To create a reporter protein, we fused EGFP to mCherry, which was then divided into three parts, each packaged in a separate AAV2/2 vector. Four weeks after co-injecting the triple AAV vectors into 4–5-week-old mice, we assessed transduction efficiency. We found that up to 5.9% of inner hair cells were positive for both EGFP and mCherry. Subsequently, we developed triple *CDH23*-AAV vectors for therapeutic purposes. After administering these vectors to 4- to 5-week-old C57/BL6 mice, we conducted auditory tests and immunohistochemistry studies over a period of 60 weeks. Co-injecting triple *CDH23*-AAVs did not alter auditory function or lead to hair cell degeneration. In conclusion, this study confirms the feasibility of the triple-AAV approach for cochlear gene delivery. While this strategy did not produce any treatment effects, our findings suggest that large deafness genes could be potential future targets for cochlear gene therapy.

**Trichostatin A suppresses hearing loss by activating HO-1 in an Alport syndrome model**

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Alport syndrome (AS) is a genetic disorder, which is characterized by mutations in type IV collagen, leading to kidney and cochlea dysfunction and late-onset progressive hearing loss. We investigated the effect of Trichostatin A (TSA), an HDAC inhibitor, in an AS mouse model to assess its potential to inhibit hearing deterioration. Col4α3 knockout (KO) mice were treated with TSA at 3 weeks of age and hearing levels were measured using auditory brainstem response (ABR). The results demonstrate that TSA treatment significantly protects the hearing of KO mice compared to the untreated group. The TSA-treated group exhibited a reduction in the levels of oxidative stress markers 4-HNE and 3NT, along with a decrease in inflammatory cytokines, in both the mouse cochlea and in vitro cell studies. TSA treatment induced HO-1 signaling, which increased HO-1 levels and contributed to the inhibition of oxidative stress and inflammatory cytokines. These findings suggest that TSA represents a promising candidate molecule for mitigating the progression of hearing loss in AS.

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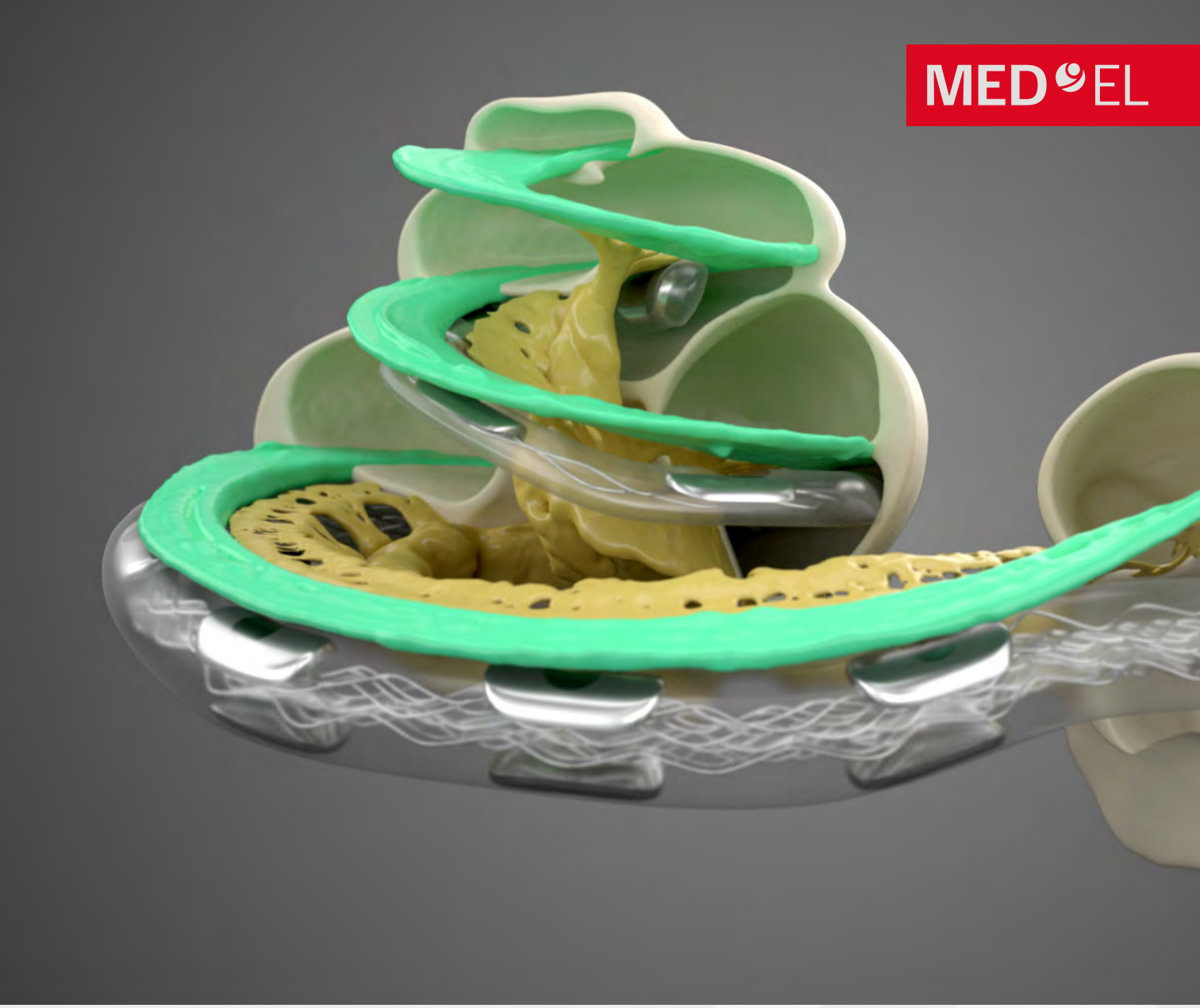

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