

# THE REGISTRY

Newsletter of the NIDCD National Temporal Bone, Hearing and Balance Pathology Resource Registry



Summer 2016  
Vol. 23, No. 2

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## MISSION STATEMENT

The NIDCD National Temporal Bone, Hearing and Balance Pathology Resource Registry was established in 1992 by the National Institute on Deafness and Other Communication Disorders (NIDCD) of the National Institutes of Health (NIH) to continue and expand upon the former National Temporal Bone Banks (NTBB) Program. The Registry promotes research on hearing and balance disorders and serves as a resource for the public and the scientific community about research on the pathology of the human auditory and vestibular systems.

## Histologic grade of otosclerosis correlates with computed tomography densitometry measurements in human temporal bone specimens

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### Introduction

Otosclerosis is a disease of the otic capsule, which causes abnormal bony remodeling and is a common cause of progressive, adult-onset hearing loss. It may result in a conductive hearing loss due to stapedial fixation by a focus of otosclerosis around the oval window, a mixed hearing loss when there are additional pericochlear foci of otosclerosis, or, exceedingly rarely, a purely sensorineural hearing loss. Although the accuracy for diagnosis of otosclerosis based on otoscopy and audiometry alone is quite high, multi-detector high-resolution computed tomography (CT) of the temporal bone can help confirm the diagnosis, rule out other possible causes of conductive hearing loss, determine the extent of retrofenestral disease, and inform decisions on when to use systemic treatments such as bisphosphonates.<sup>1-4</sup>

The radiologic diagnosis of otosclerosis is typically based on identification of a radiolucent area on the CT, and occasionally stapedial footplate thickening or a double ring sign. Several authors have demonstrated the utility of CT densitometry in the diagnosis of otosclerosis using clinical CTs from operated patients with otosclerosis<sup>5-7</sup>, though pathologic confirmation of disease, or histologic activity assessment was not available. Karosi et al. examined patients who underwent a total stapedectomy, such that the stapes footplate was available for histologic evaluation, and found that the sensitivity of CT for

*continued on page 2*



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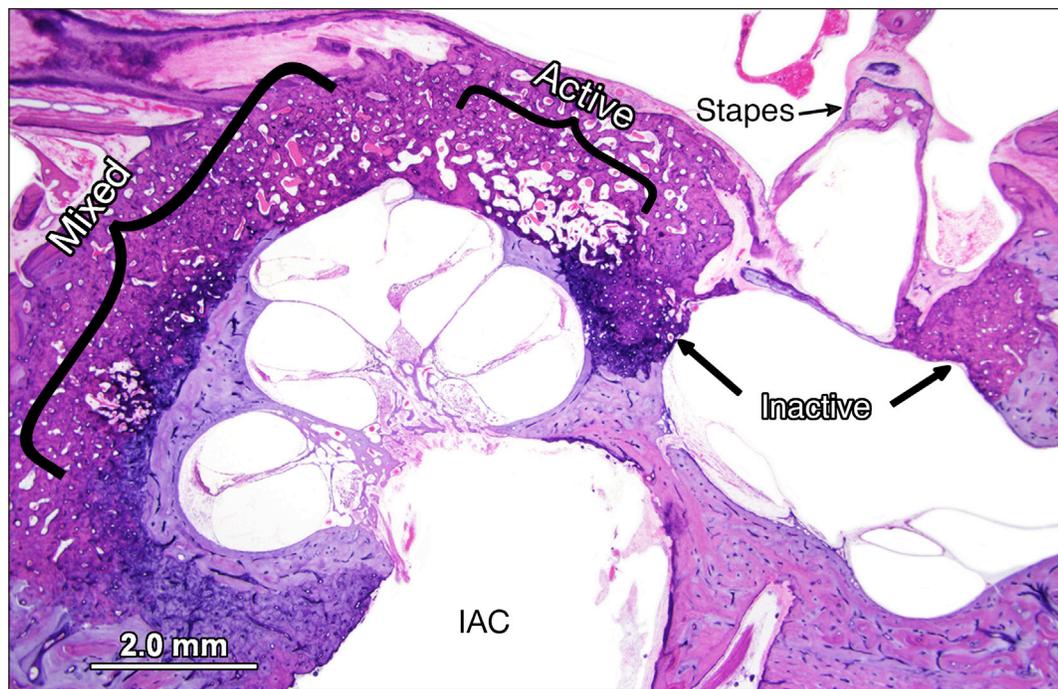
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## NIDCD National Temporal Bone, Hearing and Balance Pathology Resource Registry

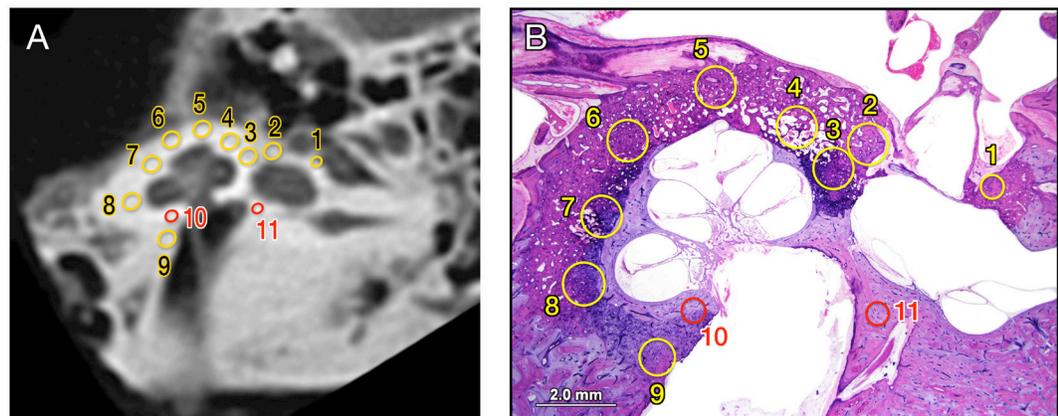
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**Figure 1.** Histologic section demonstrating the multifocal nature of otosclerosis. Within the same temporal bone, there are areas of active otosclerosis (dominated by large pseudovascular spaces), inactive otosclerosis (small and few pseudovascular spaces and lamellar bone), and mixed activity.



**Figure 2.** Maps demonstrating the location of the nine regions of interest (ROIs) and two reference regions on the (A) computed tomography image and the (B) histologic section.

the diagnosis of otosclerosis was higher in patients with active otosclerosis in the footplate (76%) compared to those with inactive otosclerosis (62%).<sup>8</sup> This suggests that CT may be able to distinguish different histologic grades or activity levels.

Histologically, active otosclerosis is characterized by large pseudovascular spaces, as well as high vascularity, high cellularity, osteoclasts, and deposition of woven bone. Inactive otosclerosis has few pseudovascular spaces, which are small if present, low vascularity, low cellularity, no osteoclasts, and predominantly lamellar bone.<sup>9-14</sup> Within a single temporal bone, there are often multiple foci of otosclerosis, which contain areas of active, inactive, and mixed otosclerosis (Figure 1).

In this study, we utilized human temporal bone specimens that underwent a multi-detector high-resolution temporal bone CT scan after fixation, and prior to decalcification,

## Histologic Grading of Otosclerosis

Grade	Definition	Histologic Activity Correlate
0	No otosclerosis	No otosclerosis
1	Otosclerosis with < 25% of area comprised of pseudovascular spaces	Inactive otosclerosis
2	Otosclerosis with 25 - 50% of area comprised of pseudovascular spaces	Mixed areas of inactive and active otosclerosis
3	Otosclerosis with > 50% of area comprised of pseudovascular spaces	Active otosclerosis

**Table 1.** Histologic Grade of Otosclerosis

sectioning, and additional histologic processing. This enabled a direct comparison of the histologic grade of otosclerosis to the CT densitometry measurement (in Hounsfield units) at multiple points around the otic capsule. The goals of this project were to determine if CT densitometry could be used to objectively distinguish otosclerosis from normal bone, and, further, to determine whether histologic grades of otosclerosis could be distinguished on CT by densitometry. Here, we present some preliminary results from this ongoing study.

## Methods

### Histology

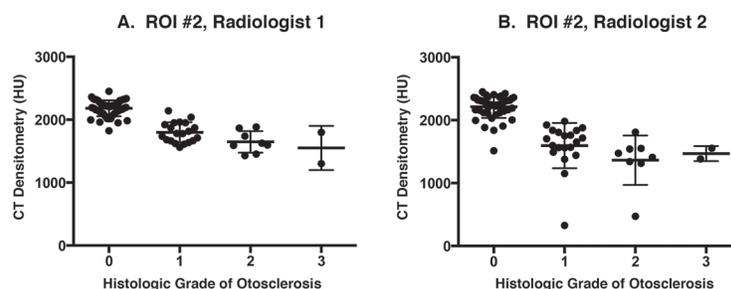
A simplified scale for grading otosclerosis was created (Table 1), based on prior scales<sup>9,10,12</sup>, as there is no uniformly used scale. The simplified scale was designed to capture the essence of active versus inactive otosclerosis, but yet be simple enough to achieve good inter-rater reliability. Two otopathologists, blinded to the radiology measurements, independently reviewed the selected slide, and recorded a grade for each of the nine regions of interest (ROIs) and two reference regions (Figure 2). A 0.5 mm or 1.0 mm region of interest (ROI) circle was used, as indicated on Figure 2 (the smaller size was used for some points to avoid overlap with structures of different density on the CT and when the designated 1.0 mm circle overlapped a structure with different density, a 0.5 mm concentric circle was used). When there was disagreement, the slide was reviewed together and a consensus grade was determined.

### Radiology

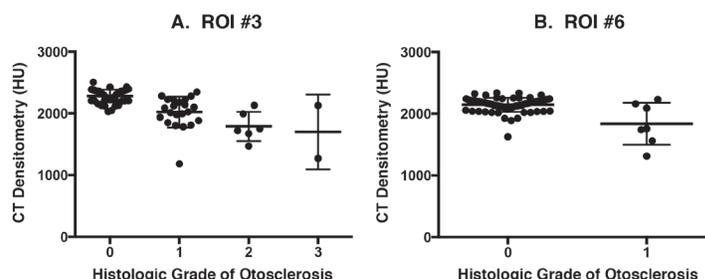
A high-resolution multi-detector CT scan was performed on all temporal bone specimens, using standard clinical parameters, including 0.5 mm collimation. The CT was reformatted to the exact plane of the selected histologic slide and a single image that best matched the histologic slide was selected. Two radiologists, who were blinded to the pathology results, independently measured the radiodensity in Hounsfield units on the selected CT image at all nine ROIs and both reference regions (Figure 2).

## Results

A total of 78 human temporal bone specimens (TBs) were reviewed, including 32 TBs with otosclerosis and 46 TBs without otosclerosis (controls). At ROI#2, which is located anterior to the oval window and is the area most often involved with otosclerosis, the density measurement mean (Hounsfield units)  $\pm$  standard deviation was  $2198 \pm 154$  for control temporal bones with no otosclerosis (grade 0),  $1697 \pm 294$  for grade 1 otosclerosis,  $1543 \pm 351$  for grades 2 and 3 combined. Thus, there was inverse correlation of density to histologic grade (Figure 3). The blinded, independent measurements by both radiologists demonstrated the same trend (Figure 3). The same inverse correlation was seen in all ROIs (with ROI#3 and #6 shown in Figure 4), although there were fewer foci of inactive or active otosclerosis at ROI#4–#9. In ROI#6 for example, there were no specimens with grade 2 or 3 otosclerosis (Figure 4).



**Figure 3.** Histologic grade of otosclerosis versus computed tomography densitometry for ROI#2. Independent, blinded measurements by two radiologists demonstrate an inverse relationship between density and grade of otosclerosis.



**Figure 4.** Histologic grade of otosclerosis versus computed tomography densitometry at ROI#3 and #6. Density measurements are lower for otosclerotic foci (grade 1, and when present grades 2 and 3) than for normal bone (grade 0) at these retrofenestral locations, similar to ROI#2 shown in Figure 3.

## Discussion

These preliminary data demonstrate (1) a significant difference between mean density measurements in Hounsfield units for normal bone compared to an otosclerotic focus, and (2) demonstrate a correlation of decreasing density with increasing histologic grade of otosclerosis. Due to small numbers of specimens with grades 2 and 3 otosclerosis, these grades were combined. Ongoing statistical evaluation will seek to determine

*continued on page 4*

a “cutoff” value with probability estimates for inactive (grade 1) vs. mixed or active (grades 2 or 3) otosclerosis. One limitation of this study was some presumed error in exactly matching the 0.5 mm or 1.0 mm ROI on the CT with the same 0.5 mm or 1.0 mm area on the histologic section. We addressed this by reformatting the CT image to match the exact plane of the histologic section, and using a reference image with a map of the ROIs to optimize placement of the ROI on the CT and determination of the area for assessment on the histologic slide. Exact matching of the area of assessment is important because of the multifocal nature of otosclerosis and the inherent mixture of various grades of otosclerosis throughout even a single otosclerotic focus. We found another source of error was the variability in densitometry measurements when the perimeter of the ROI approached a structure of different density (e.g. in some specimens the promontory thickness was less than 1.0 mm) and attempted to minimize this by using a concentric 0.5 mm ROI when appropriate.

## Conclusion

Utilizing human temporal bone specimens, we demonstrated that densitometry measurements (in Hounsfield units) on a high-resolution multi-detector temporal bone CT scan can be used to objectively distinguish normal bone from otosclerosis. Furthermore, increasing histologic grade (e.g. indicating a more active otosclerotic focus) was correlated with decreasing density measurements. Further work is needed to develop a statistical model to predict histologic grade based on the density measurement, and then to translate these findings for clinical use using reference measurements. A radiologic measure of disease activity in otosclerosis may enable more informed use of medical treatments for otosclerosis, such as bisphosphonates, and may provide a measurement of treatment efficacy. ●

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## FUNDING

Research supported by NIH/NIDCD U24 DC 011943 (PI: McKenna), “Otopathology by Light Microscopy and Molecular Techniques.”

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## UPCOMING EVENTS

# CONVENTION

Hearing Loss Association of America **2016**

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## ANNUAL MEETING & OTO EXPO<sup>SM</sup>

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# Correlation between audiometric thresholds and cochlear cellular damage

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In our recently published paper (Hear Res. 2016 Feb 27; 335: 83–93), we analyzed 131 human temporal bones from 85 adult subjects (ages 19–92 years, median 69 years) and correlated the integrity of hair cells, neurons, and stria vascularis with the latest available hearing test prior to death (time range: 5 hours to 22 years, median 24 months). The patients suffered from sensorineural hearing loss (SNHL) due to various underlying diseases, e.g. presbycusis (22 ears), presbycusis together with acoustic trauma (11 ears), kanamycin ototoxicity (7 ears), sudden SNHL (6 ears), “isolated” SNHL (6 ears), otosclerosis (5 ears), and several rare diseases and syndromes.

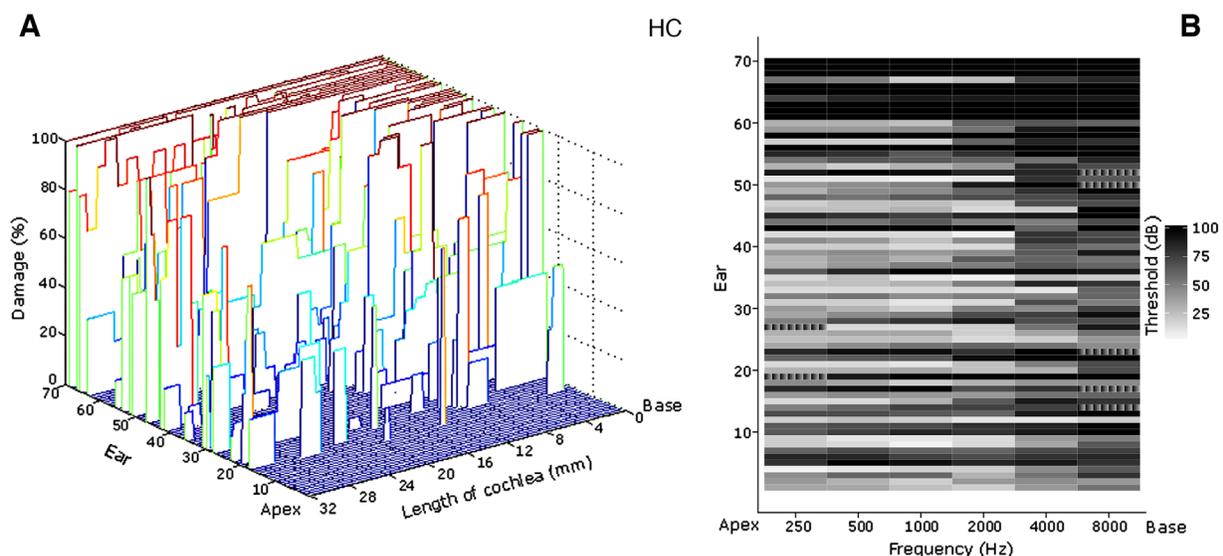
The goal of the study was to determine whether audiometric thresholds, which are the current gold standard of clinical hearing testing, predict specific cellular damage in the cochlea. Several recent animal studies questioned such a predictive value and most of the available literature on humans included a small number of temporal bones post-mortem or psychophysically determined cochlear “dead regions” *in vivo*. Quantifying the degree of correlation between audiometric thresholds and cell-specific histological changes in the cochlea is particularly

important for hearing restoration trials (including gene therapy studies) to appropriately classify patients before treatment and assess treatment effects.

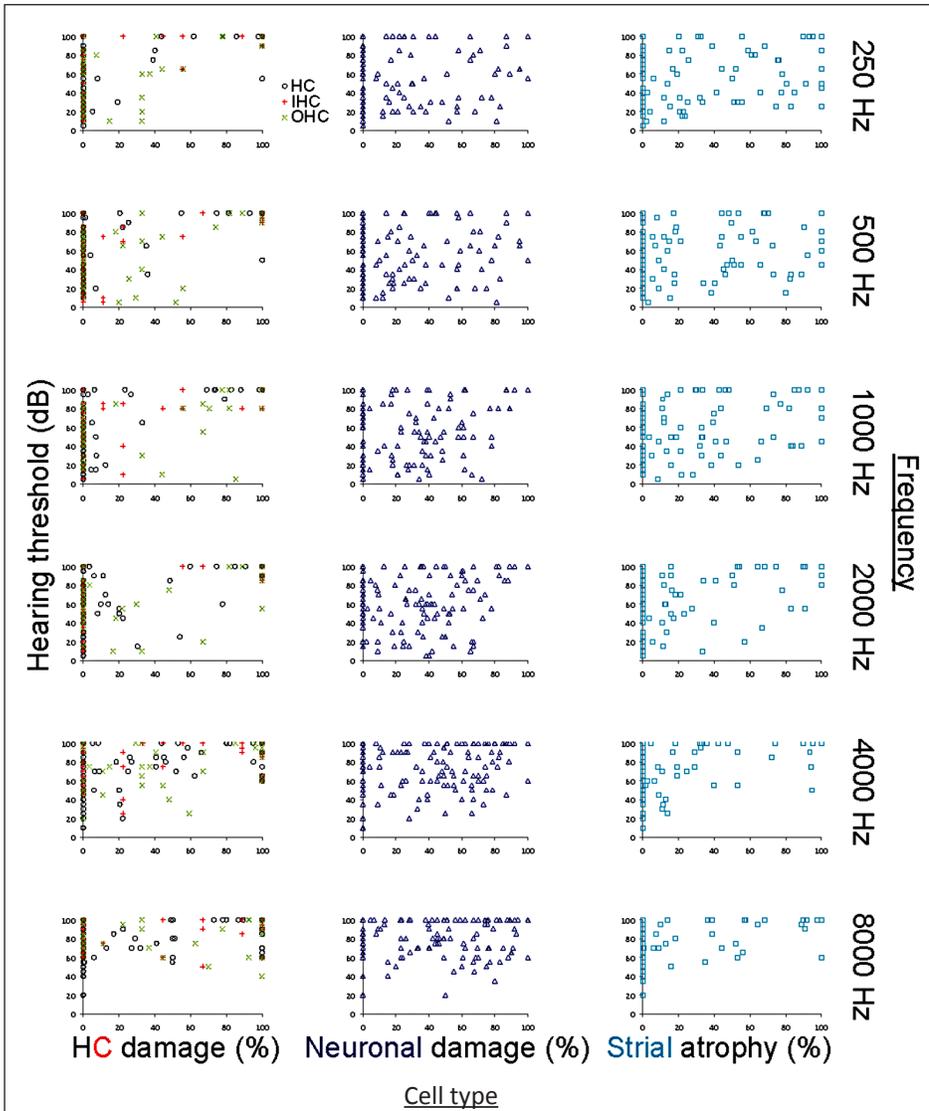
Using clinical audiograms, we extracted frequency-specific thresholds (at 250, 500, 1000, 2000, 4000, and 8000 Hertz [Hz]) and pure tone average (PTA), defined as the average decibels (dB) hearing level of the two frequencies with the lowest thresholds in the frequency range from 500 to 2000 Hz. Audiograms were plotted as linear heat maps (with increasing gray scale reflecting increasing thresholds and worse hearing [Figure 1B]) and depicted next to corresponding cytochleograms (Figure 1A). Because eye-catching correlations between audiometric and histological damage were not evident for hair cells, neurons, or stria vascularis, statistical methods were used to draw more definitive conclusions. We first calculated the position of audiometrically-tested frequencies in a cytochleograms by using a modified Greenwood function:  $f = 165.4(10^{2.1x} - 0.88)$ . We then estimated the equivalent rectangular bandwidth, a psychoacoustic measurement providing an approximation of the cochlear region responding to a certain frequency. For each frequency range, the average cellular damage was calculated.

The level of damage ranged from none to complete annihilation for hair cells and neurons, while the highest observed damage was approximately 90% for the stria vascularis. The degree of ipsilateral versus contralateral hair cell loss and stria atrophy was similar (within 10%) in approximately two-thirds of patients. Furthermore, the degree of inner versus outer hair cell loss was

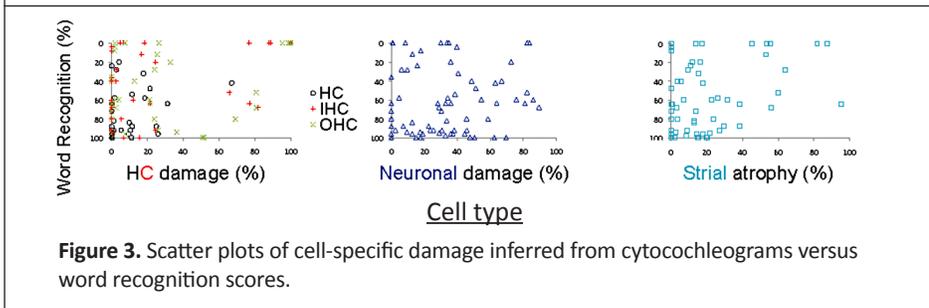
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**Figure 1.** Combined hair cell analysis. Overview of an analysis of 70 human temporal bones sorted according to the degree of histological damage at the level of inner and outer hair cells (A) and the corresponding most recent audiogram prior to death (B) listed in the same order. Color in the heat map (B) reflects dB of hearing level (HL), with white indicating normal hearing (0 dB HL) and black indicating profound deafness (100 dB HL); stripes depict responses that were not measured.



**Figure 2.** Scatter plots of cell-specific damage versus audiometric thresholds at different frequencies.



**Figure 3.** Scatter plots of cell-specific damage inferred from cytochleograms versus word recognition scores.

comparable in about two-thirds of ears. However, for spiral ganglion neurons, only approximately half of the patients showed a similar degree of damage in both ears.

After calculating all values for Spearman’s correlation coefficient and applying the Benjamini-Hochberg correction for multiple hypothesis testing, the highest correlation was 0.7 and many correlations were below 0.5, with 1.0 indicating perfect correlation. The three highest correlations were for inner hair cells at 250 Hz (0.67), 1000 Hz (0.67), and 2000 Hz (0.70). In contrast, spiral ganglion neurons and stria vascularis showed

poor correlations for all frequencies (Figure 2).

We also tested the correlation between the word recognition score, another essential clinical test, and cytochleogram-captured cellular damage for 70 temporal bones from 44 patients with the recorded score (Figure 3). To appropriately represent word recognition scores, the frequencies that dominantly influence word recognition scores, the frequencies were weighted based on the octave-band Speech Intelligibility Index outlined by the American National Standard. Spearman’s correlation coefficient was at most 0.38. This coefficient was not improved by performing sex- and age-specific comparisons.

Our findings help substantiate the discrepancy between audiometric thresholds and subjective hearing experience in many patients, e.g. those suffering from auditory neuropathy. While “dead regions” were described decades ago and have influenced the fitting of hearing aids, the vast majority of human trials use standardized hearing tests to examine the results of an intervention. Although there have been hints that there is not a very high correlation between cellular damage and audiometric thresholds or word recognition scores (e.g. several small studies with a focus on presbycusis), our large dataset gives further insight into this problem. Consequently, the development of novel diagnostic tools is of utmost importance, such as probes for cellular-level intracochlear imaging. The tools that have shown promise in animal models include confocal fluorescence microscopy, optical coherence tomography, and in particular two-photon micro-endoscopy, which revealed cell-specific damage in animal models in situ.

In summary, we advise against exclusively relying on audiometric thresholds or word recognition scores in clinical trials to treat deafness (e.g. gene therapy), as these metrics do not provide cell-specific information. More research to develop cell-specific diagnostic tools and adequately monitor cochlear changes at the cellular level is urgently needed. ●

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**FUNDING**

This study is supported by the Bertarelli Foundation, Wyss Center Geneva, United States Department of Defense Grant W81XWH-15-1-0472, Nancy Sayles Day Foundation, and the Lauer Tinnitus Research Center.

**REFERENCES**

- Landegger LD, Psaltis D, Stankovic KM. Human audiometric thresholds do not predict specific cellular damage in the inner ear. *Hear Res.* 2016; 335, 83-93.

# Announcing the New Temporal Bone Database

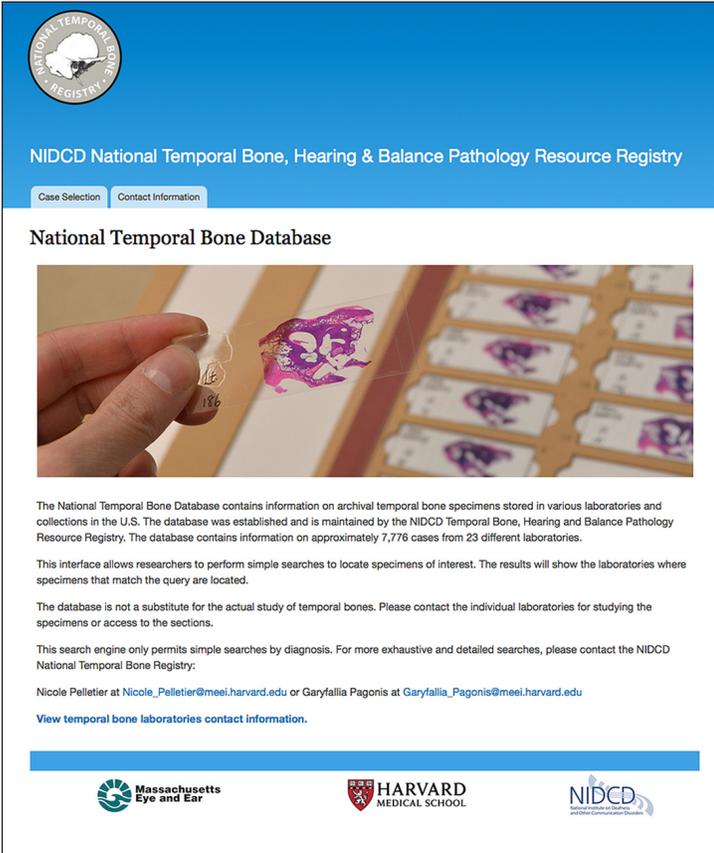
The NIDCD National Temporal Bone Registry is delighted to announce the new National Temporal Bone Database. Comprising of more than 7,700 cases from 23 different laboratories, this online database contains information on archival temporal bone specimens stored in various collections throughout the United States.

The new interface is full of helpful resources that allow researchers to perform searches to locate specimens of interest. Performing a search is easy: simply go to [national-tb-database.meei.harvard.edu](http://national-tb-database.meei.harvard.edu), select “Case Selection” on the top navigation, fill out the search appropriately, and then the results will show the laboratories where the desired specimens are located.

We encourage everyone to visit and explore the new, user-friendly database. Please note, this database is not a substitute for the actual study of temporal bones, therefore, we ask that you contact the individual laboratories for study of the specimens.

We hope you enjoy the new interface and discovering all the database has to offer. ●

National Temporal Bone Database homepage



NIDCD National Temporal Bone, Hearing & Balance Pathology Resource Registry

Case Selection Contact Information

## National Temporal Bone Database



The National Temporal Bone Database contains information on archival temporal bone specimens stored in various laboratories and collections in the U.S. The database was established and is maintained by the NIDCD Temporal Bone, Hearing and Balance Pathology Resource Registry. The database contains information on approximately 7,776 cases from 23 different laboratories.

This interface allows researchers to perform simple searches to locate specimens of interest. The results will show the laboratories where specimens that match the query are located.

The database is not a substitute for the actual study of temporal bones. Please contact the individual laboratories for studying the specimens or access to the sections.

This search engine only permits simple searches by diagnosis. For more exhaustive and detailed searches, please contact the NIDCD National Temporal Bone Registry:

Nicole Pelletier at [Nicole\\_Pelletier@meei.harvard.edu](mailto:Nicole_Pelletier@meei.harvard.edu) or Garyfallia Pagonis at [Garyfallia\\_Pagonis@meei.harvard.edu](mailto:Garyfallia_Pagonis@meei.harvard.edu)

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## Otopathology Mini-Travel Fellowship Program

The NIDCD National Temporal Bone Registry's mini-travel fellowships provide funds for research technicians and young investigators to visit a temporal bone laboratory for a brief educational visit, lasting approximately one week. The emphasis is on the training of research assistants, technicians, and junior faculty.

These fellowships are available to:

- U.S. hospital departments who aspire to start a new temporal bone laboratory.
- Inactive U.S. temporal bone laboratories that wish to reactivate their collections.
- Active U.S. temporal bone laboratories that wish to learn new research techniques.

Up to two fellowship awards will be made each year (\$1,000 per fellowship). The funds may be used to defray travel and lodging expenses. Applications will be decided on merit.

Interested applicants should submit the following:

- An outline of the educational or training aspect of the proposed fellowship (1-2 pages).
- Applicant's curriculum vitae.
- Letter of support from temporal bone laboratory director or department chairman.
- Letter from the host temporal bone laboratory, indicating willingness to receive the traveling fellow.

Applications should be submitted to:

**Michael J. McKenna, M.D.**  
NIDCD Temporal Bone Registry  
Massachusetts Eye and Ear  
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[michael\\_mckenna@meei.harvard.edu](mailto:michael_mckenna@meei.harvard.edu)



## Temporal Bone Removal Technicians Needed Nationwide!

For more information, please contact the Registry at:  
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## Free Brochures for your Office or Clinic about Temporal Bone Research and Donation

*The Gift of Hearing and Balance: Learning about Temporal Bone Donation* is a 16-page, full-color booklet that describes in more detail the benefits of temporal bone research. It also answers commonly asked questions regarding the temporal bone donation process. *Dimensions: 7"x10"*

If you would like to display this brochure, please complete the form below and return it to the Registry by mail or fax. The brochures will be sent to you **free of charge**. Please circle the amount requested for each brochure or write in amount if not listed.

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