THE REGISTRY

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

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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.

A 3-Dimensional Analysis of the Endolymph Drainage System in Meniere's Disease

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Introduction

Meniere's disease is an inner ear disorder characterized by episodic vertigo, tinnitus, and aural fullness.[1] Yamakawa[2] and Hallpike and Cairns[3] were the first authors to report hydrops in Meniere's. Since then, the assumption that endolymphatic hydrops is the direct cause of symptoms in patients with Meniere's has been questioned.[1,4–12]

The purpose of this study was to compare anatomic findings in three groups: Meniere's disease (MD), endolymphatic hydrops without vestibular symptoms (ELH), and normal controls. We measured the volume of the vestibular aqueduct, endolymphatic sinus and duct, and intratemporal portion of the endolymphatic sac; the size of the internal and external aperture of the vestibular aqueduct; and the opening (if present) of the utriculo-endolymphatic valve (aka Bast's valve).

Materials and Methods

The MD group included 16 temporal bones from 16 donors (10 men and 6 women; mean 70.18±13.24 years; range: 45–89). The ELH group included 16 temporal bones from 16 donors (13 men and 3 women; mean age 66.43±10.99 years; range: 45–85) who had histologic signs of endolymphatic hydrops but did not meet the diagnostic criteria for Meniere's disease. Then, finally, we included 16 non-diseased temporal bones from 14 donors (11 men and 5 women; mean 63.11±10.83 years; range: 41–80).

Using a scanner (PathScan IV, Meyer Instruments, TX), the slides were scanned and the following areas were labeled: (1) the bony limits of the vestibular aqueduct, (2) the lumen of the endolymphatic sinus and duct, and (3) the bony limits of the intratemporal

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endolymphatic sac. To generate the 3-D reconstruction model for measuring volume, we used Amira software (Amira 3-D FEI, OR) (Figure 1). Measuring the extratemporal portion of the endolymphatic sac was not feasible using the study's methodology.

We then measured the internal and external apertures of the vestibular aqueduct, as well as the opening of Bast's valve (if present). This opening was only considered for analysis in temporal bones with intact utricular membranes.

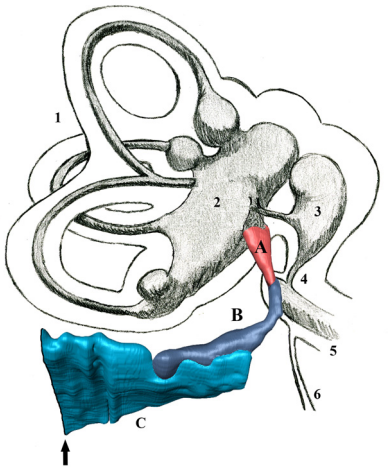

Figure 1: A 3-D model generated to calculate the volume of the endolymph drainage system compartments, with a schematic representation of the vestibular system. A = endolymphatic sinus; B = vestibular aqueduct and endolymphatic duct; C = intratemporal portion of the endolymphatic sac; Arrow = external aperture of the vestibular aqueduct; 1 = semicircular canals; 2 = utricle; 3 = saccule; 4 = ductus reuniens; 5 = basal turn of the cochlea; 6 = cochlear aqueduct.

Results

In the MD group, the volume of the vestibular aqueduct, endolymphatic duct, and intratemporal portion of the endolymphatic sac was significantly lower, as compared to both the ELH (Figures 2 and 3) and non-diseased groups (Table 1). In the ELH group, we found no differences when comparing them with the non-diseased group in terms of the volume of any of those structures. Between the three groups, the difference in the volume of the endolymphatic sinus (Figure 4) was not significant ($P>0.05$).

In the MD group, the mean size of the external aperture of the vestibular aqueduct was smaller than both of the other groups ($P=0.001$) (Table 1). No difference was observed in the size of the internal aperture of the vestibular aqueduct among the three groups ($P>0.05$).

Of the 11 measurable specimens in the MD group, the valve was closed in six (54.5 percent) and in the other five specimens, the mean width of the opening was 0.21±0.17 mm (range: 0.053 to 0.42 mm) (Table 1 and Figure 4). In all eight of the measurable specimens in the ELH group and in all seven of the measurable specimens in the non-diseased group, the valve was closed.

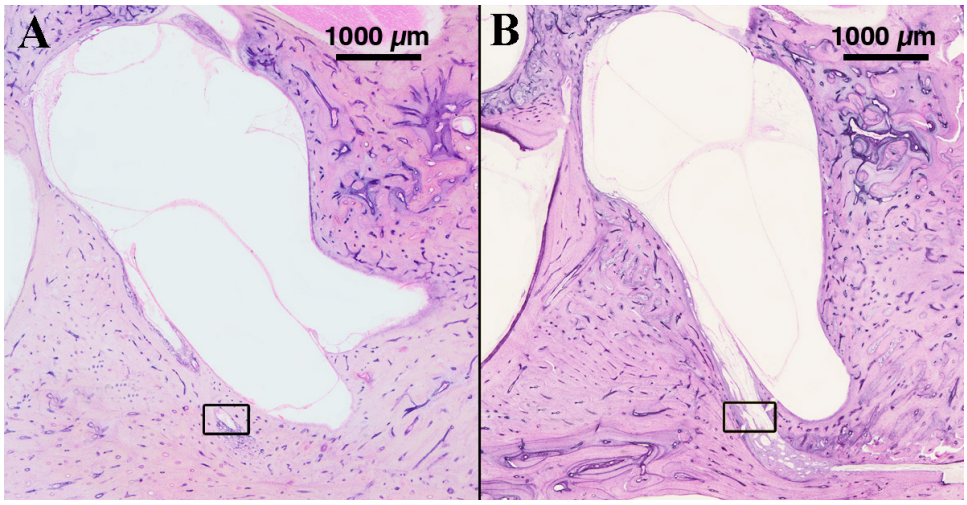

Figure 2: Two representative horizontal sections of human temporal bones, showing the vestibular aqueduct and endolymphatic duct in two of the study groups (hematoxylin and eosin; 2x). A = endolymphatic hydrops; B = Meniere's disease. Squared area = vestibular aqueduct and endolymphatic duct.

Table 1. Measurements of endolymphatic structures by group

	Structure	Meniere's disease group	Endolymphatic hydrops group	Non-diseased group
Mean	Vestibular aqueduct	2.85*	7.93	7.93
volume	Endolymphatic sinus	0.29	0.26	0.34
(mm^3)	Endolymphatic duct	0.34*	0.40	0.48
	Intratemporal portion of endolymphatic sac	1.95*	6.21	5.97
Mean	Internal aperture	0.35	0.42	0.38
length	External aperture	3.95*	8.18	7.28
(mm)	Bast's valve	0.21	–	–

*Statistically different from non-diseased group

Discussion

In the ELH and non-diseased groups, the volume and openings of the structures were similar. However, in the MD group, the external opening of the vestibular aqueduct was smaller, and the volume of the vestibular aqueduct, endolymphatic duct, and intratemporal portion of the endolymphatic sac was significantly lower—suggesting at least partial obstruction of the endolymph drainage.

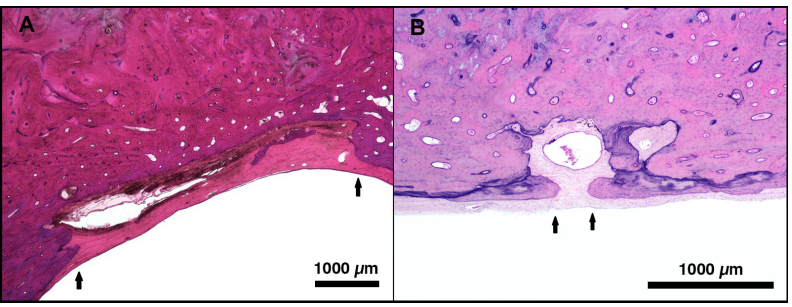

Figure 3: Two representative horizontal sections of human temporal bones, showing the endolymphatic sac and the external aperture of the vestibular aqueduct in two of the study groups (hematoxylin and eosin; 4x). A = endolymphatic hydrops; B = Meniere's disease. Arrows = bony edges of the external aperture of the vestibular aqueduct.

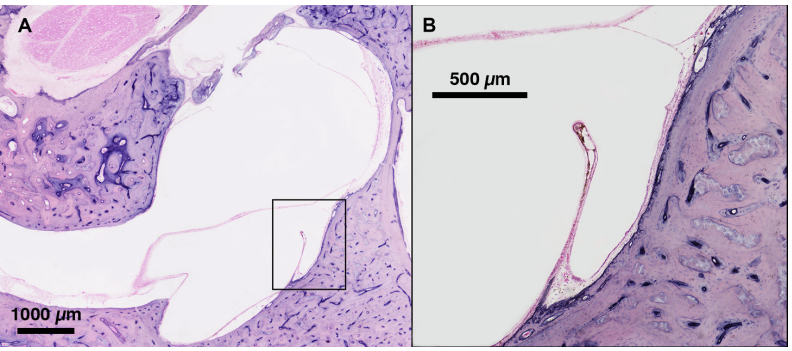

Figure 4: A representative horizontal section of a human temporal bone, from a donor who had Meniere's disease (hematoxylin and eosin). A = panoramic view of the vestibule, showing a bulging of the saccular wall onto the footplate. The squared area includes Bast's valve, which is open. B = squared area from "A," seen in higher magnification (x10).

These findings could correlate with both the degree of endolymphatic hydrops and the development of clinical symptoms in patients with Meniere's disease. Given these anatomic differences, high-resolution MRI and CT scans could become valuable diagnostic tools in atypical presentations of Meniere's disease.

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Sensorineural Hearing Loss in Otosclerosis: The Role of Cochlear Macrophages, A Potential Therapeutic Target

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Otosclerosis is a disorder of abnormal bone remodeling within the otic capsule of humans that often results in a conductive hearing loss that can be helped by surgery or amplification. However, it is estimated that in 20 to 30 percent of affected individuals, the remodeling process will penetrate the cochlear endosteum and result in a progressive sensorineural hearing loss. In some instances, cases may progress to profound hearing loss and those affected become candidates for cochlear implantation.

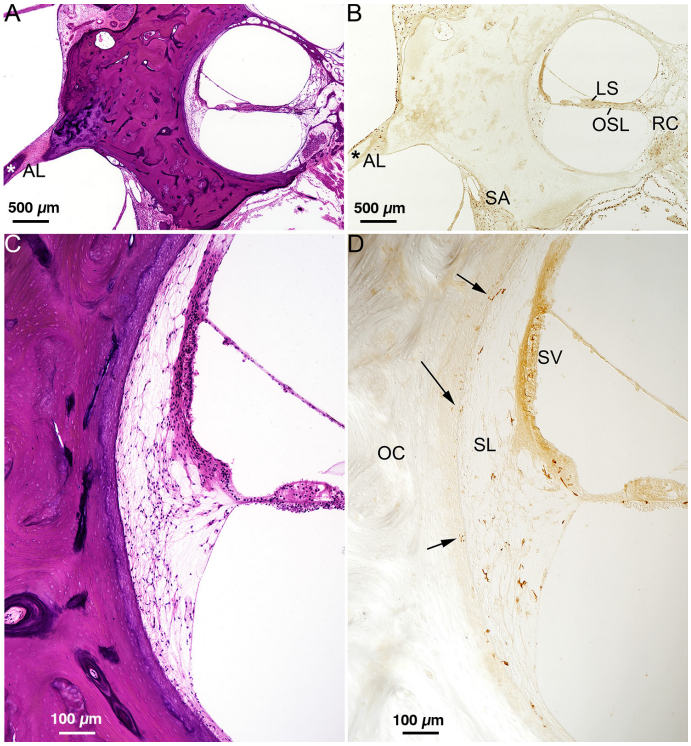


Figure 1: Microglial-like cochlear macrophages are not readily identifiable with conventional hematoxylin and eosin staining (panels A and C). Immunolabeling of a serial section with anti-Iba1 antibody, a marker known to be specific for microglia, demonstrates the abundance of these cells in the inner ear (panels B and D). They are found within the annular ligament (AL) of the stapes footplate (asterisk), within the connective tissue of the saccule (SA), within Rosenthal's canal (RC), along the osseous spiral lamina (OSL), within the limbus spiralis (LS), around the blood vessels of the stria vascularis (SV), and in the spiral ligament (SL). Note the macrophages/microglia (arrows), which reside within the spiral ligament (SL) and extend their processes into the canaliculi of the otic capsule (OC). This specimen is from an 82-year-old female with no known otologic disease other than changes due to age.

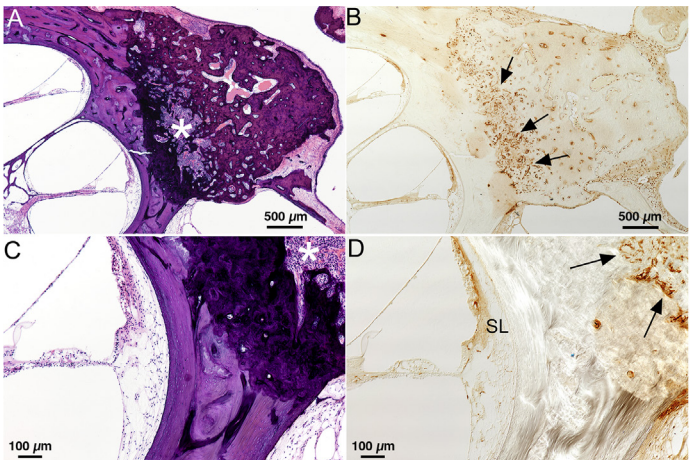
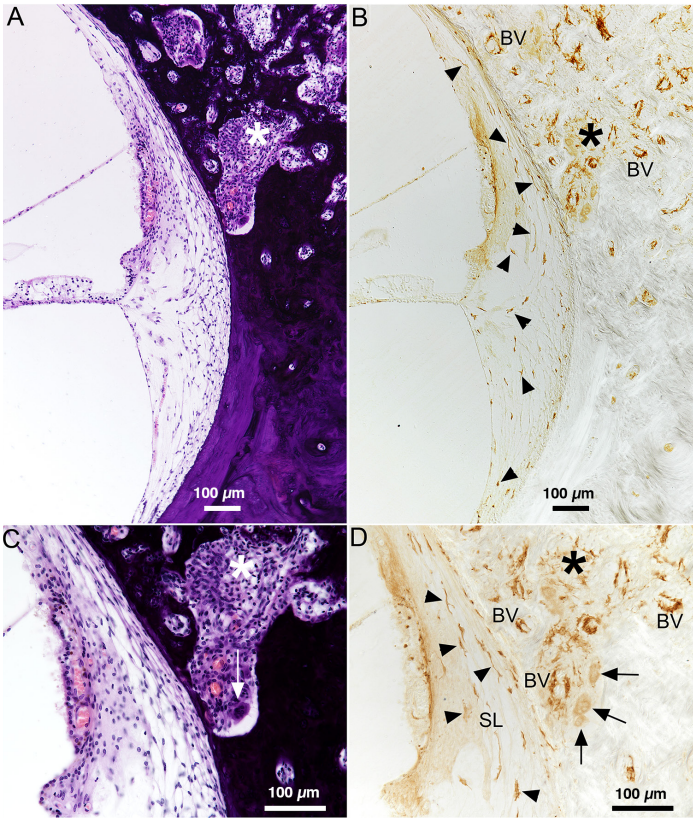


Figure 2: The otosclerotic focus (asterisk in panels A and C) has not breached the endosteum of the cochlea. There is a very active area (arrows in panel B) full of Iba1+ labeled macrophages (panel B). There is no increase in the number of Iba1+ labeling within the spiral ligament (SL in panel D). This specimen is from a 75-year-old female with otosclerosis.

Human otopathology studies from patients with clinical otosclerosis that penetrates the cochlea have revealed the site of cochlear injury is the spiral ligament[1,2,3] and, to a lesser degree the stria vascularis[4,5]. Remarkably, the organ of Corti and spiral ganglion are well preserved. When an active otosclerotic lesion penetrates the cochlear endosteum, there is a resultant degeneration of spiral ligament cells and a deposition of a hyline material. There is a strong correlation between the degree of spiral ligament damage and sensorineural hearing loss. The mechanism by which an active otosclerotic lesion results in spiral ligament cell death is not known.

Within the human spiral ligament there is a population of apparent resident macrophages[6] (Figure 1), including those that insert into the canaliculi of the otic capsule along the lateral wall of the cochlea. By standard hematoxylin and eosin staining, these macrophages are indistinguishable from other fibrocyte-like cells. They have only recently come to our attention as the result of improvements in immunolabeling of human temporal

Figure 3: Panels A and C are stained with hematoxylin and eosin. Panels B and D are labeled with anti-Iba1 antibody. There is a very active otosclerotic focus (asterisk in panels A, B, C, and D) penetrating the cochlear endosteum. Note the additional number of Iba1+ macrophages (arrowheads) within the spiral ligament (SL) compared to normal ligament in Figure 1. Many but not all of the macrophages within the active focus are associated with blood vessels (BV). Osteoclasts are visible within the active focus (arrows). This specimen is from an 86-year-old female with otosclerosis.



bones[7,8]. In fact, they are present in abundance throughout the human inner ear, including around strial vessels, along dendrites of the osseous spiral lamina, around spiral ganglion cells, and along the axons of the VIIIth cranial nerve[6].

Resident cochlear macrophages are strikingly similar in morphology to the resident macrophages of the brain known as microglia[9]. Recent advances in the understanding of the biology of microglia in the central nervous system (CNS)[10] and their role in development[11,12], synaptic pruning[11,12], synaptic stripping[13], and the development of a host of neurodegenerative diseases raise new and compelling questions about the role of cochlear macrophages in health and disease of the ear. We strongly suspect that their functional roles within the cochlea are similar to their counterparts within the CNS and, as such, are likely important participants in a host of otologic disorders including otosclerosis.

Our work in human temporal bones has shown an increased presence of macrophages in active otosclerotic lesions throughout development of the disease, implying they play a significant role

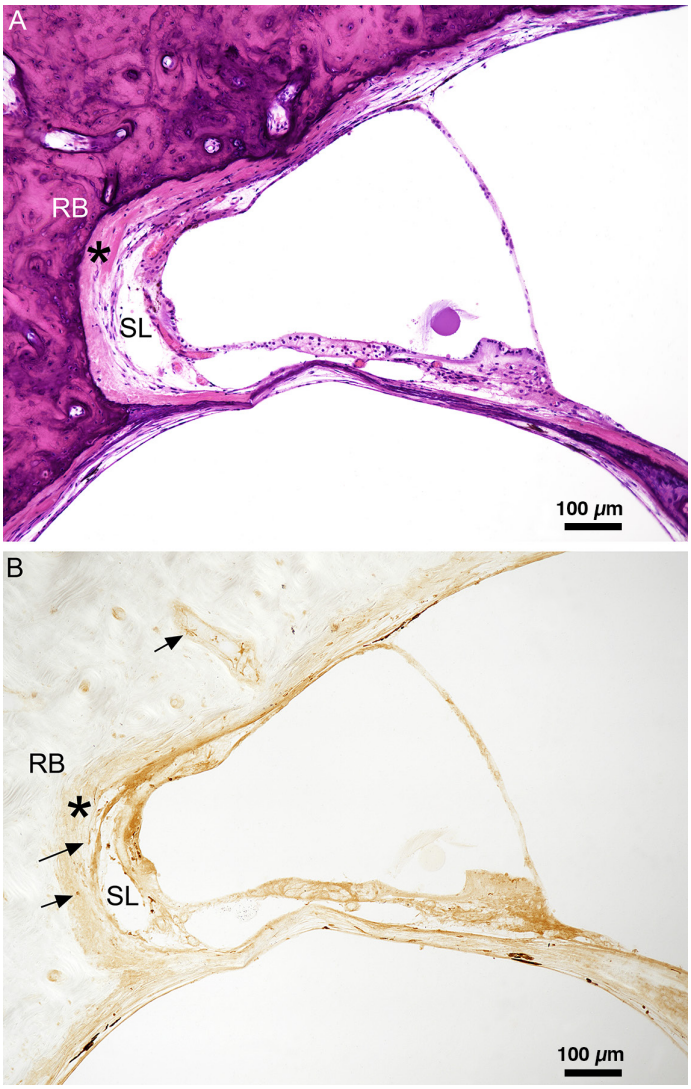


Figure 4: In panel A, the remodeled bone (RB) adjacent to the spiral ligament (SL) is no longer active. This otoscerotic focus once penetrated the endosteum of the cochlea as evidenced by the deposition of hyaline (asterisk) along the cochlear lateral wall. Panel B shows a serial section labeled with anti-Iba1 antibody and very few macrophages (arrows) are visible within the spiral ligament or elsewhere. This specimen is from a 76-year-old male with otosclerosis.

in the disease process (Figure 2). We have also found that there is infiltration of large numbers of macrophages into the spiral ligament in cases where the cochlea is breached and the adjacent focus is still active (Figures 3 and 4). Hirose et al.[14,15,16,17] have conducted extensive studies on spiral ligament macrophages in acoustic trauma and inflammation in the mouse. The activity of these cells, including activation and recruitment of circulating monocytes, is clearly related to cochlear damage and sensorineural hearing loss (SNHL). We suspect a similar role in the human.

Resident macrophages have been the focus of intense investigations in the CNS, which have led to the continuous development of new and innovative microglial-targeted therapeutic approaches. It is suspected that cochlear macrophages play a fundamental role

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in spiral ligament injury and degeneration once the cochlear lateral wall has been breached by an active otosclerotic focus. The elucidation of this role holds new therapeutic potential for the prevention of sensorineural hearing loss in otosclerosis. ●

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A New Website for the Otopathology Laboratory

The Otopathology Laboratory at Massachusetts Eye and Ear/Harvard Medical School is delighted to announce the release of its new website. Now found at **OtopathologyLaboratory.org**, the website has been given a brand new look and feel.

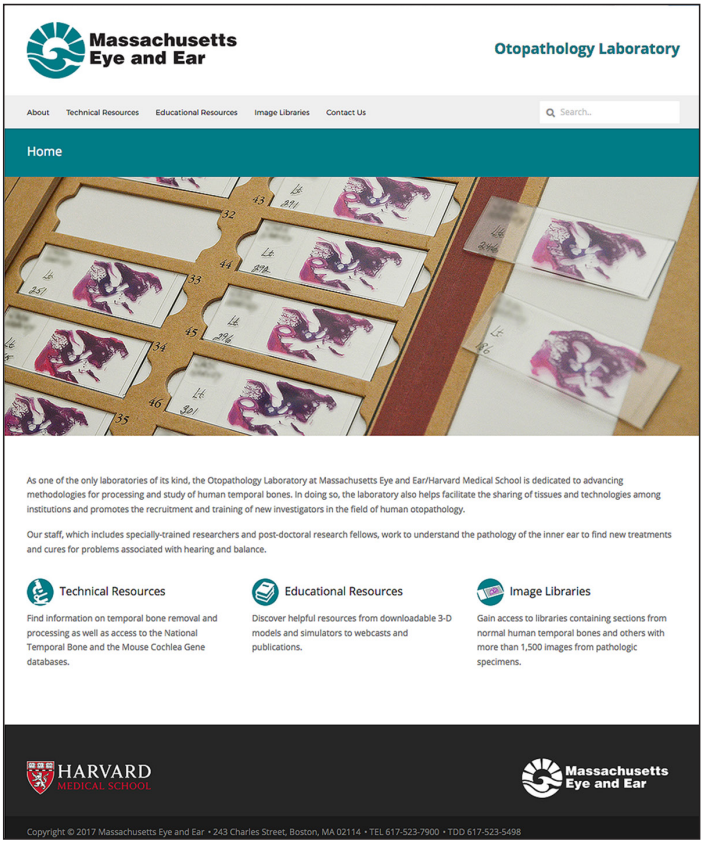
As one of the only laboratories of its kind, the Otopathology Laboratory is dedicated to advancing methodologies for the processing and study of human temporal bones. In doing so, the lab's website offers useful tools and resources, such as image libraries containing sections from normal human temporal bones, to help those interested in the field of human otopathology advance their work.

New features include:

- **New navigation:** The content is now user-friendly and organized in a manner that makes everything easy to find.
- **Mobile/tablet-friendly:** Now compatible with more devices.
- **Valuable and timely information:** The content is fresh and the resources/databases are easy to use.

We encourage everyone to visit and explore the new website!

OTOPATHOLOGY LABORATORY HOMEPAGE



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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 who wish to reactivate their collections.
- Active U.S. temporal bone laboratories who 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:

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