THE REGISTRY

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

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CONTENTS

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.

NIDCD National Temporal Bone Laboratory at UCLA

A report on the reorganized laboratory: Past, present, and future challenges

Article contributed by Fred H. Linthicum, Jr., MD, and Akira Ishiyama, MD,
NIDCD National Temporal Bone Laboratory at UCLA.

The past

Los Angeles Foundation of Otology/The House Ear Institute

The idea of asking patients to pledge their temporal bones (bones containing middle and inner portions of the ear) was conceived by Howard P. House, MD, the founder of the Los Angeles Foundation of Otology (later named the House Ear Institute), in 1952. Soon after, a pledge form co-signed by the next of kin was developed, leading to the first pair of donated temporal bones being acquired in 1957. These bones were sent to the laboratory of John Lindsay, MD, at the University of Chicago for processing, as were the next 18 pairs of pledged bones in the ensuing three years.

In 1960, a temporal bone processing laboratory was formally established at the Los Angeles Foundation of Otology under the part-time direction of Fred H. Linthicum, Jr., MD, a founding member of the Otologic Medical Group (now known as the House Clinic). At the suggestion of Arum Glorig, MD, former Director to the Foundation, George Kelemen, MD, who first described necrotizing external otitis in diabetics, was appointed as the full-time director of the laboratory. Dr. Kelemen hired two technicians and remained the director until his death in 1984. Dr. Linthicum was then appointed the new full-time director, a position in which he still holds today.

UCLA Temporal Bone Laboratory

In the mid-sixties, the University of California, Los Angeles (UCLA) Temporal Bone Laboratory was established from the support of the first UCLA otologist, Victor Goodhill, MD. At this time, the laboratory was headed by Ruth Gussen, MD, a board-certified pathologist, and under her direction, the laboratory began to establish modern protocols to study human temporal bones, including immunohistochemistry in microdissected temporal bones. With grant support from the National Institutes of Health (NIH) and the National Institute on Deafness and Other Communication Disorders (NIDCD), Akira Ishiyama, MD, joined the team and has since become a

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pioneer in the application of unbiased stereological quantitative techniques to reliably estimate the number of vestibular hair cells and spiral and vestibular ganglia neurons in microdissected endorgans and celloidin embedded sections stained with hematoxylin and eosin (H&E).

The Neurotology and House Histologic Temporal Bone Laboratory of UCLA

In 2014, the House Ear Institute laboratory moved its operations to the UCLA campus, merging with the existing UCLA temporal bone laboratory and operating under a new name, the **Neurotology and House Histologic Temporal Bone Laboratory of UCLA**. With remaining ties to the House Clinic, this move added 300 pairs of temporal bones to the UCLA inventory, making it one of the largest temporal bone banks in the world.

Together, the laboratories have received a total of 4,874 pledges. There are now 3,500 outstanding and 1,267 temporal bones have been obtained and processed. Some of the bones have been received from outside sources such as those pledged to the National Temporal Bone Balance and Hearing Resource Registry at Massachusetts Eye and Ear and patients of the co-investigators of the original cochlear implant program. Eight hundred fifty-nine pairs of bones (including 32 cochlear implant bones) have been processed while 87 are in various stages of processing, a procedure that takes up to a year. Data from the laboratories has also been used in 285 scientific publications authored by the lab's personnel and a host of fellows, both domestic and foreign, which have rotated through the laboratory.

The present

The Neurotology and House Histologic Temporal Bone Laboratory of UCLA focuses on the microscopic study of the ears of individuals who have suffered from a hearing or balance problem and have willed their temporal bones for scientific analysis. By comparing abnormal microscopic findings in a large number of temporal bones from individuals with similar clinical findings, scientists can learn the underlying anatomical abnormality responsible for a particular disability. Among the goals of the lab is to apply rapid tissue collection and expedited fixation and tissue processing protocols aimed to preserve antigenic sites for the identification of inner ear specific proteins by immunocytochemical techniques and morphological preservation to apply design-based quantitative stereological methods to estimate the number of hair cells, supporting cells, and primary afferent neurons in the human cochlea and vestibule.

The lab processes human temporal bones (HTB) obtained at autopsy by the traditional method of celloidin embedding and uses the microdissection technique to obtain micro-dissected endorgans to be embedded in plastic or cut using cryostat. They have two celloidin microtomes, one cryostat (frozen sections), and ultramicrotome for thick and ultrathin sections.

> ***Celloidin embedding of temporal bones:*** When bones are received they are placed in formalin for two weeks and then in ethylenediaminetetraacetic acid (EDTA) until shown by X-ray to be calcium free. They are then immersed in increasing concentrations of celloidin until suitable for sectioning that is done in 20-micron thickness. Each section is placed on a numbered tissue. Every tenth section is stained with H&E and mounted on a numbered slide. The other nine sections can be stored in 80 percent alcohol for future use, including immunohistochemistry or 3-D reconstruction, and these sections may be shared with other laboratories. The instructions for processing the bones include decalcification with EDTA over months such that fewer tissue artifacts are incurred, compared with use of the various acids that have been assessed in the past.
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> ***Microdissection technique of auditory and vestibular endorgans:*** Using the microdissection technique, they obtain auditory and vestibular endorgans that can

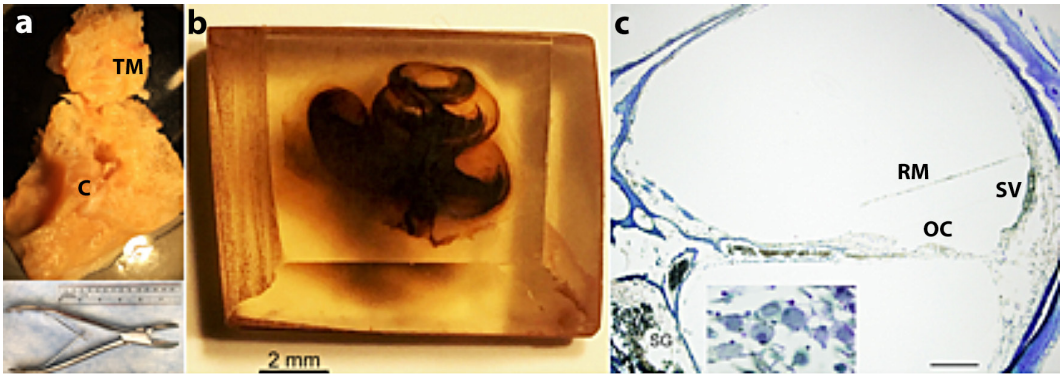

Figure 1. Alternative processing use of human inner ear tissue. a) The cochlea is being microdissected from the temporal bone. Forceps used for microdissection (bottom panel). b) The cochlea microdissected from the temporal bone was embedded in plastic. c) Five-micron thick sections obtained from the block in (b). Low magnification shows the organ of Corti. Inset is a higher magnification view of neurons of the spiral ganglia (SG), bar in c) is 200 µm.

TM: Tympanic Membrane, **C:** Cochlea, **RM:** Reissner's Membrane; **OC:** Organ of Corti, **SV:** Stria Vascularis

be subsequently embedded in plastic for histopathological analysis (Figure 1). The microdissected tissue can be further processed for transmission electron microscopy (TEM) or scanning EM (SEM). The bone is removed with large size forceps and the microdissection of the cochlea and vestibular endorgans can be achieved in five days. The endorgans are then post-fixed with osmium, dehydrated, and embedded in resin. The tissue can then be cut to five microns and stained with toluidine blue. The expeditious harvesting of the temporal bone and immediate processing allows for the study of the cochlea and vestibular endorgans in about three weeks. Additionally, they have demonstrated that the microdissection technique can be used to embed auditory and vestibular endorgans specimens in plastic (EPON) to obtain thin sections (1-5 µm) for light and transmission electron microscopy (60-90 nm) observations, frozen to obtain cryostat sections (8 to 20 µm thick) (5). Microdissected HTB tissue can also be embedded in paraffin for immunohistochemistry. Remaining tissue can be saved for sharing with other laboratories.

The fusion of both laboratories at UCLA has influenced the many cellular and molecular biological facilities available on campus. The daily interaction with researchers from the Brain Research Institute and Neurology have allowed the temporal bone lab team to implement new methods of analysis of human temporal bones, including: transmission and scanning electron microscopy to see ultrastructural changes, immunohistochemistry to identify inner ear specific proteins, *in situ* hybridization to locate a specific RNA or DNA sequence, 3-D reconstruction to explore the contours of small structures, stereology to determine volume or surface areas, use of polarized light to observe bone structure, and proteomics to characterize protein expression of inner ear tissues.

As an example of the productive interaction between both laboratories, the team highlights their recent review published in the journal *Histochemistry and Cell Biology* (Lopez et al., 2016). This review summarized the immunohistochemical protocols and methods of the human inner ear. Given the scarcity of tissue available and the high cost of processing the HTB using the traditional methods, they proposed alternative choices like microdissection, and frozen and paraffin sectioning. To optimize the use of invaluable celloidin embedded sections of the human inner ear, the UCLA laboratory as well as others, have been developing methods to remove celloidin from the sections and subsequent antigen retrieval. The best process and preserved tissue is now being shared with inner ear basic researchers to corroborate their previous finding in animal models. They are continuously developing immunofluorescence protocols that allow the identification of up to three different proteins (Lopez et al., 2016).

The future

Moving forward, UCLA researchers plan to continue recruiting temporal bone donors, updating records, implementing new protocols for the visualization of inner ear specific proteins, and training new technicians and students. Recently, they received a U24 grant from the NIH/NIDCD to continue temporal bone science research. Under this support, the researchers will able to increase collaborations with basic inner ear researchers by providing them with human inner ear tissue that is either microdissected or frozen in paraffin or celloidin embedded sections.

The researchers are hopeful that the speed and refinement of molecular biological studies on mRNA and DNA will eventually allow them to perform better genetic studies using the archival temporal bone material. The future holds promise for more methods to maximize knowledge on both normal and diseased human temporal bones in order to better treat and even prevent otologic problems, which is exactly what this new, collaborative team is working to accomplish. ●

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REFERENCES

1. Lopez IA, Ishiyama G, Hosokawa S, Hosokawa K, Acuna D, Linthicum FH, Ishiyama A. Immunohistochemical techniques for the human inner ear. Histochem Cell Biol. 2016 Oct; 146(4): 367–87.

Histopathology of the Human Inner Ear in DFNA-9

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Mutations in the novel cochlear gene COCH cause an autosomal dominantly inherited nonsyndromic sensorineural hearing loss by interfering with the production of the secreted protein cochlin. Twenty-three COCH mutations have been reported in the literature (Bae et al., 2014; Tsukada et al., 2015). The clinical phenotype includes adult onset progressive and bilaterally symmetrical sensorineural loss with reduction in word recognition scores. To date, the histopathology of the human inner ear has been described in five of these mutations including this most recent description of the histopathology in the p.L114P COCH mutation (Burgess et al., 2016). In addition, unlike the previous four cases, this most recent case was obtained from an individual with considerable residual hearing, which provided an opportunity to study the degenerative process before progression to a profound level of hearing loss had occurred.

The histopathology of the COCH mutations has been relatively uniform, as reported in the literature (Merchant et al., 2010), and includes cellular degeneration of the spiral ligament and deposition of an eosinophilic acellular material in the spiral ligament and in the distal osseous spiral lamina and limbus. The principal histologic correlate of sensorineural hearing loss is suspected to be degeneration of the dendritic processes of the spiral ganglion cells, a finding that was supported in the most recent case. Sanger sequencing was done using a muscle sample obtained at the time of temporal bone collection and preserved at -80 °C. Analysis of the resultant DNA sample demonstrated a heterozygous c.T341C missense mutation, which resulted in a p.L114P amino acid alteration in exon 5 of the COCH gene. The temporal bones were processed for light microscopy by embedment in celloidin and serial sectioning at a thickness of 20 microns and followed by 2-D reconstruction.

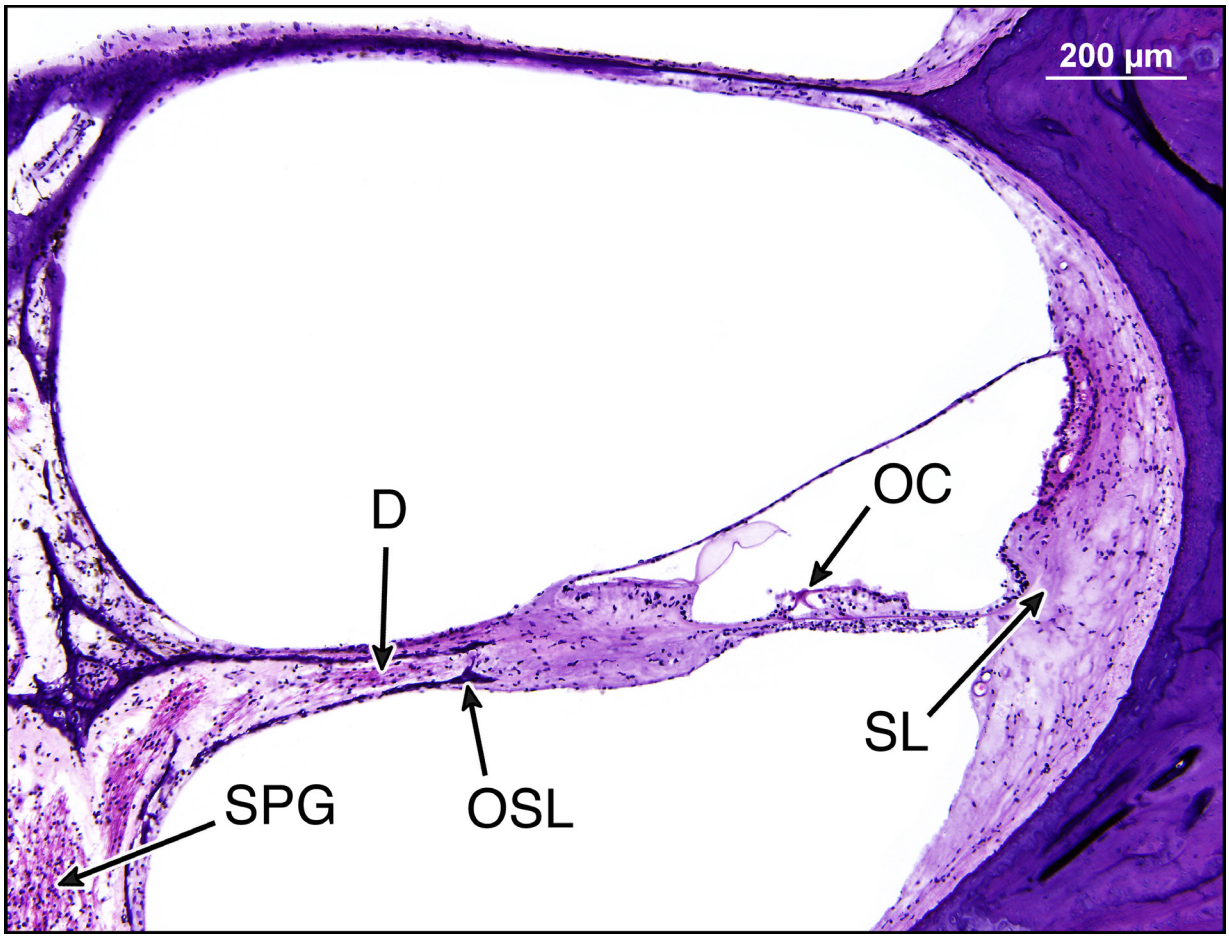

Figure 1. Histologic section of the organ of Corti in the middle turn of the left ear stained with H&E. Inner and outer hair cells were seen in the organ of Corti (OC). There was a deposition of eosinophilic material in the spiral ligament (SL) and in the distal end of the osseous spiral lamina (OSL) and limbus. Surviving dendritic processes (D) and spiral ganglion cells (SPG) were seen.

There was scattered loss of both inner and outer hair cells and degeneration of the stria vascularis. Approximately 50% of the spiral ganglion cells had degenerated. However, severe loss of the dendritic processes between the spiral ganglion cells and the organ of Corti was seen, suggesting a retrograde degeneration of the cochlear dendrites rather than a primary degeneration of the spiral ganglion cell bodies and their processes.

Every tenth section was stained with hematoxylin and eosin (H&E). Immunocytochemistry was made possible with a technique developed in the Massachusetts Eye and Ear temporal bone laboratory by O'Malley et al., 2009. The celloidin embedding material was removed from the sectioned archival specimens with subsequent immunostaining using anti-myelin protein zero and anti-neurofilament stains. Both immunostains (Figures 1–3), demonstrated a severe loss of dendritic processes in the osseous spiral lamina. Immunostaining with anti-neurofilament staining demonstrated loss of the non-myelinated neurons seen within the organ of Corti. Loss of dendrites was greater than the loss of spiral ganglion cell bodies.

Histopathology with H&E staining demonstrated a lack of cellularity of the spiral ligament and eosinophilic deposition of extracellular material in both the spiral ligament and the

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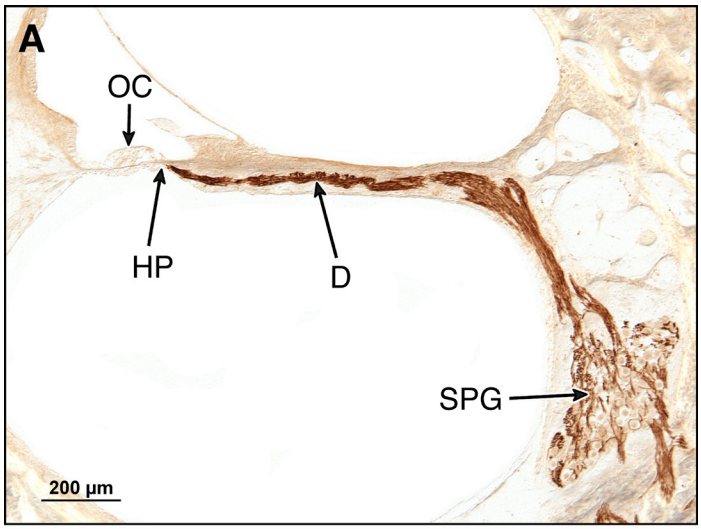


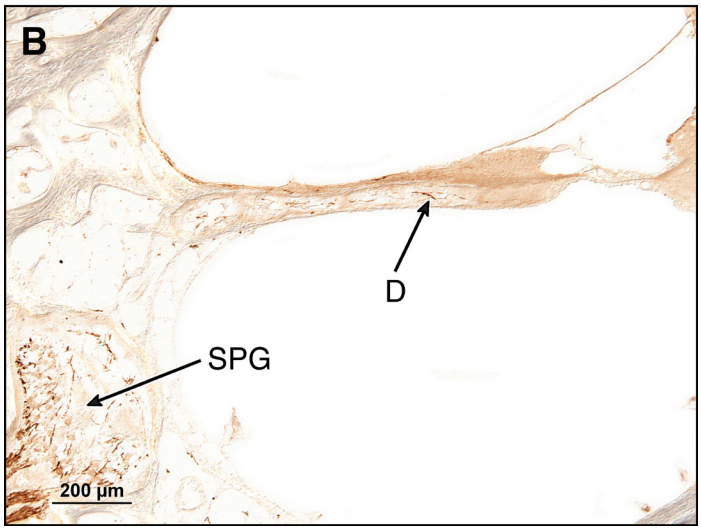
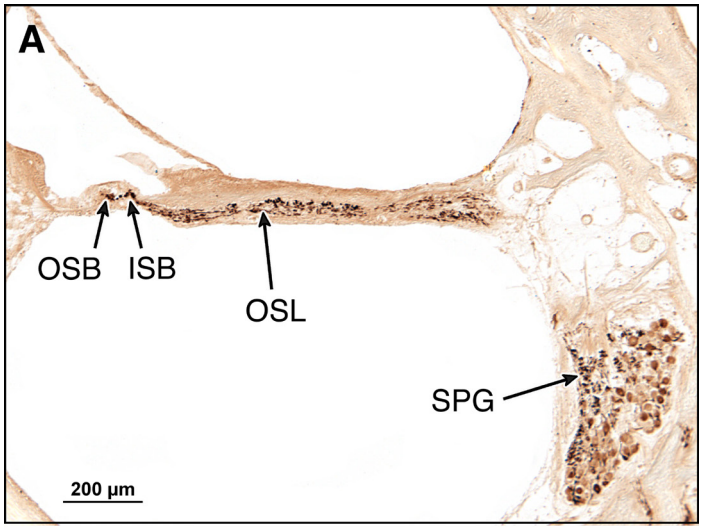


Figure 2. Immunostaining of the organ of Corti using anti-myelin protein zero was shown in a control specimen with no significant otologic disease (A) and in the ear affected by the COCH mutation (B). The dendrites (D) were easily seen in the normal control, extending from the spiral ganglion (SPG) to the organ of Corti (OC). The staining extended distally to the level of the habenula perforata (HP), but not into the cochlea where neurons are unmyelinated. In contrast, in the ear affected by the COCH mutation (B), only minimal staining of the dendritic processes (D) was seen in the osseous spiral lamina and spiral ganglion (SPG).



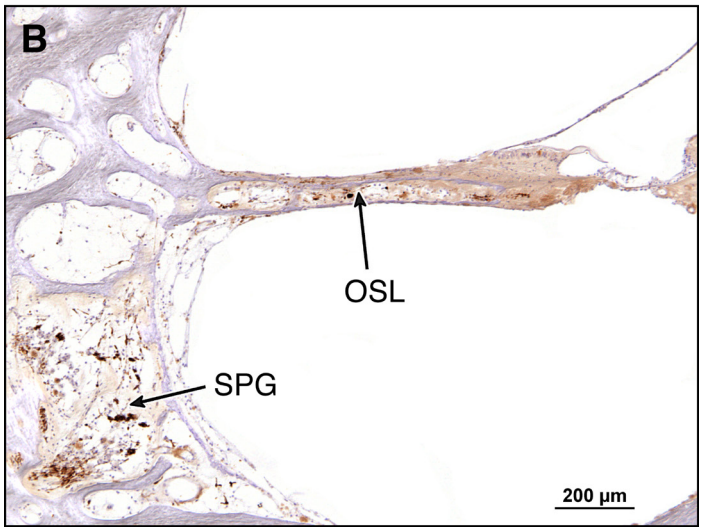


Figure 3. Anti-neurofilament immunostaining seen in a normal control (A) and in the temporal bone affected by the COCH mutation (B). The spiral ganglion cells (SPG) and the dendritic processes in the osseous spiral lamina (OSL) were deeply stained in the control ear (A). The staining extended beyond the habenula perforata to the inner spiral bundle (ISB) and outer spiral bundle (OSB) of unmyelinated fibers. In contrast, in the temporal bone carrying the COCH mutation (B), there was little staining in the spiral ganglion (SPG) and in the osseous spiral lamina (OSL).

osseous spiral lamina. This case highlights the capacity to do immunostaining on archival human temporal bones and the value of obtaining fresh frozen tissue for genetic sequencing at the time of temporal bone collection.

The role of the mutant cochlin in the pathogenesis of DFNA-9 is not fully understood. Two mechanisms have been described: alteration of extracellular matrix protein interactions (Bhattacharyya, 2006) affecting ionic homeostasis or a direct toxic effect on the dendritic processes within the osseous spiral lamina have been previously suggested (Robertson et al., 2006), and may be responsible for the apparent retrograde degeneration of distal dendritic processes.

The case report and full description of the otopathologic findings in this specimen were reported by Burgess et al., 2016. ●

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REFERENCES

1. Bae S-H, Robertson NG, Cho H-J, Morton CC, Jung DJ, Baek J-I, Choi S-Y, Lee K-Y, Kim U-K. Identification of pathogenic mechanisms of COCH mutations, abolished cochlin secretion, and intracellular aggregate formation: Genotype-phenotype correlations in DFNA9 deafness and vestibular disorders. Hum Mutat. 2014; 35: 1506–1513.

2. Tsukada K, Ichinose A, Miyagawa M, Mori K, Hattori M, Nishio S-Y, Naito Y, Kitajiri S-I, Usami S-I. Detailed hearing and vestibular profiles in patients with COCH mutations. Ann Otol Rhinol Laryngol. 2015; (Suppl 1): 100S–110S.

3. Burgess BJ, O'Malley JT, Kamakura T, Kristiansen K, Robertson NG, Morton CC, Nadol JB, Jr. Histopathology of the human inner ear in the p.L114P COCH mutation (DFNA9). Audiol Neurootol. 2016; 21(2): 88–97.

4. Merchant SN, Nadol JB, Jr (eds). Schuknecht's Pathology of the Ear, ed 3. Shelton CT, People's Medical Publishing House. 2010; pp 142–147.

5. O'Malley JT, Merchant SN, Burgess BJ, Jones DD, Adams JC. Effect of fixative and embedding medium on morphology and immunostaining of the cochlea. Audiol Neurotol. 2009; 14:78–87.

6. Bhattacharyya SK. Focus on molecules: Cochlin. Exp Eye Res. 2006; 82: 355–356.

7. Robertson NG, Cremers CWRJ, Huygen PLM, Ikezono T, Kartsins B, Kremer H, Kuo SF, Liberman MC, Merchant SN, Miller CE, Nadol JB, Jr, Sarracino DA, Verhagen WIM, Morton CC. Cochlin immunostaining of inner ear pathologic deposits and proteomic analysis in DFNA9 deafness and vestibular dysfunction. Hum Mol Genet. 2006; 15: 1071–1085.

UPCOMING EVENTS

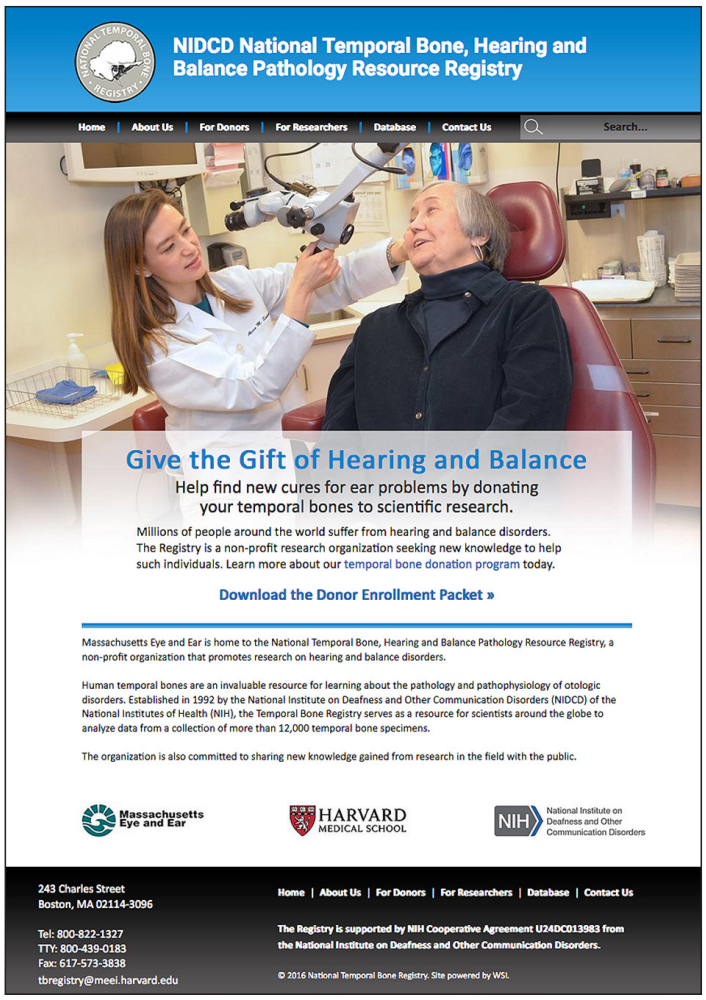
A New Look for the Registry Website

The NIDCD National Temporal Bone Registry is proud to announce the release of our newly redesigned website. The new website, which can still be found at **www.tbregistry.org**, has a fresh, modern look and a new user-friendly layout to ensure each visitor has a positive experience on our site.

Honoring our commitment to promoting research and sharing knowledge on hearing and balance disorders, the new website was designed with our audience in mind. With a more streamlined navigation system and faster loading times, each visitor, whether they are a patient, donor, physician, or researcher, can now easily access the helpful information and resources that our website provides.

We encourage everyone to visit and explore our new website, and we hope you enjoy the new interface. ●

NATIONAL TEMPORAL BONE REGISTRY HOMEPAGE



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 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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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 the amount if not listed.

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