



The

Registry

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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 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.

Otopathology in a Patient with Syndromic Stapes Ankylosis due to NOG Mutation

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Introduction

The NOG gene, which encodes the protein noggin, is critical in normal skeletal and joint development, and antagonizes the actions of bone morphogenetic proteins (BMPs).^{1,2} Mutations in the NOG gene are responsible for multiple rare, autosomal dominant syndromes known as NOG-related Symphalangism Spectrum Disorders (NOG-SSD) that include congenital conductive hearing loss and skeletal abnormalities among the features. Three of the NOG-SSD feature hearing loss: (1) proximal symphalangism (SYM1), (2) multiple synostoses syndrome (SYNS1), and (3) stapes ankylosis with broad thumbs and toes (SABTT) (also known as Teunissen-Cremers Syndrome).^{3,4} Hearing loss is predominantly conductive in these syndromes due to stapes fixation. Evidence for stapes fixation is based on clinical findings of a normal tympanic membrane, conductive hearing loss, absent stapedial reflexes, and intraoperative findings.⁴ Not surprisingly, many of these patients are initially diagnosed with otosclerosis, especially when the onset of hearing loss is unclear, and skeletal abnormalities are subtle. We describe here the temporal bone histopathology, clinical course, and audiometry in a patient with a heterozygous nonsense mutation in NOG and Stapes Ankylosis with Broad Thumbs and Toes Syndrome.

Case History

This patient reported bilateral congenital hearing loss that was progressive. He began wearing hearing aids at age 22. He was diagnosed with otosclerosis and underwent a left fenestration procedure at age 34, and then a left stapedectomy at age 37. Clinic notes report a slight improvement after left stapedectomy, but he subsequently wore a right

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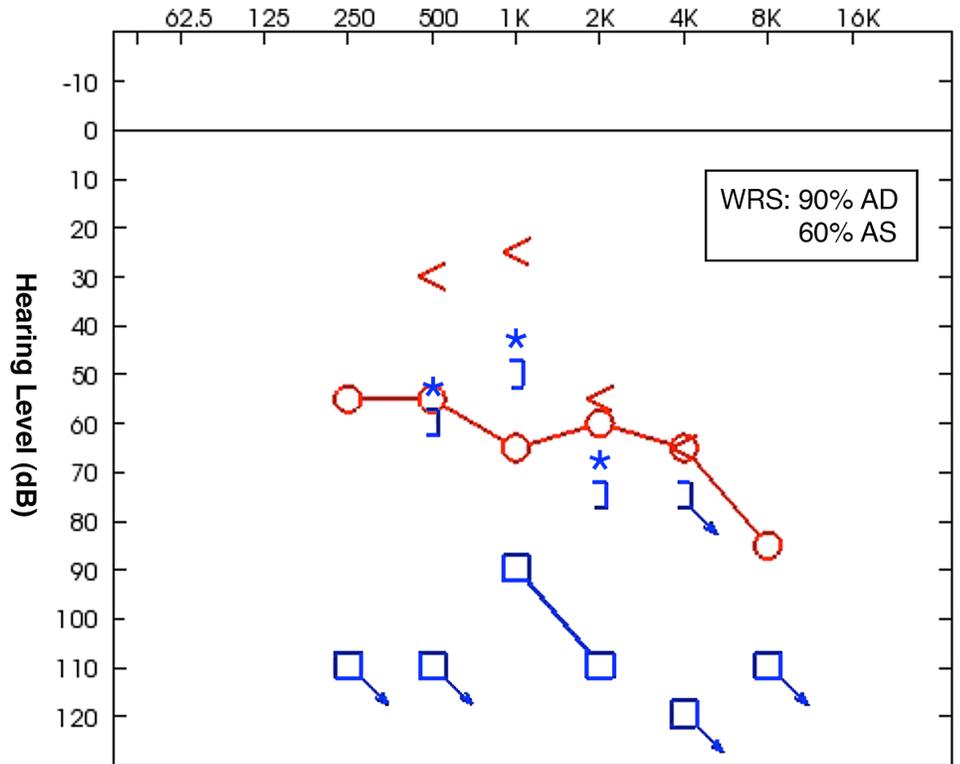
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Fig. 1 **FREQUENCY (Hz)**



**Audiogram age 69
Died age 78**

*** Masking dilemma**

hearing aid only and was dependent on this ear for hearing. An audiogram done at age 69 demonstrates right mixed hearing loss, and profound hearing loss on the left (Figure 1). At age 67, he was evaluated by a geneticist and diagnosed with Stapes Ankylosis with Broad Thumbs and Toes, a congenital stapes ankylosis syndrome. He underwent skeletal x-rays, which identified short, broad middle and distal phalanges of the thumbs bilaterally, and symphalangism (fusion of the distal and middle phalanges) of the right foot only. He was also noted to have hyperopia, a broad sloping forehead, bulbous tip of the nose, and limited cervical range of motion. His family history was consistent with autosomal dominant syndromic hearing loss, with 8 affected family members. The family was studied by Dr. Marci Lesperance and colleagues, and a heterozygous nonsense mutation in the NOG gene was identified that segregated with hearing loss.⁵

Histopathology

Both ears are notable for stapes fixation due to obliteration of the stapedovestibular joint space with ossified cartilage (Figures 2, 3, 4). This accounts for his conductive hearing loss in life. The ossified cartilage has the typical appearance of cartilage with chondrocytes, but the surrounding matrix is deeply basophilic consistent with ossification. The footplate is fixed anteriorly and posteriorly in both ears. There is normal cartilage on the vestibular surface of the footplate and very thin bone on the tympanic surface. In the left (operated ear), a central fenestration of the stapes footplate can be seen, although there is no stapes prosthesis remaining (Figure 4).

In the right ear (unoperated ear), the malleus and incus are normal, with no fixation or incudomalleal joint abnormality. In the left ear, only a remnant of the manubrium

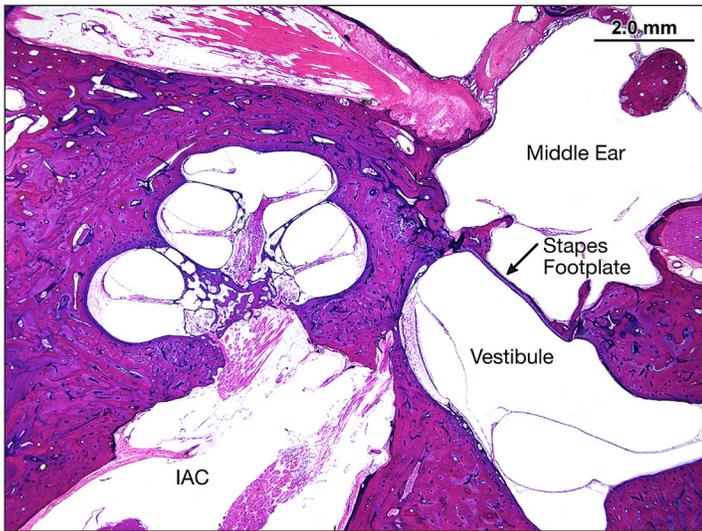


Fig. 2

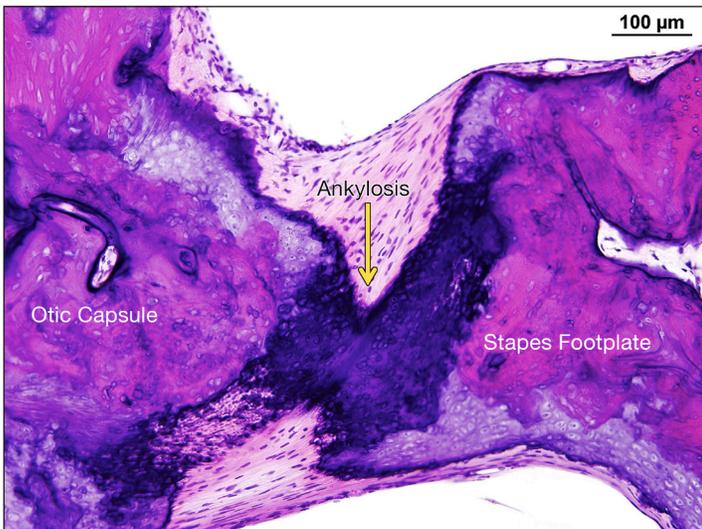


Fig. 3

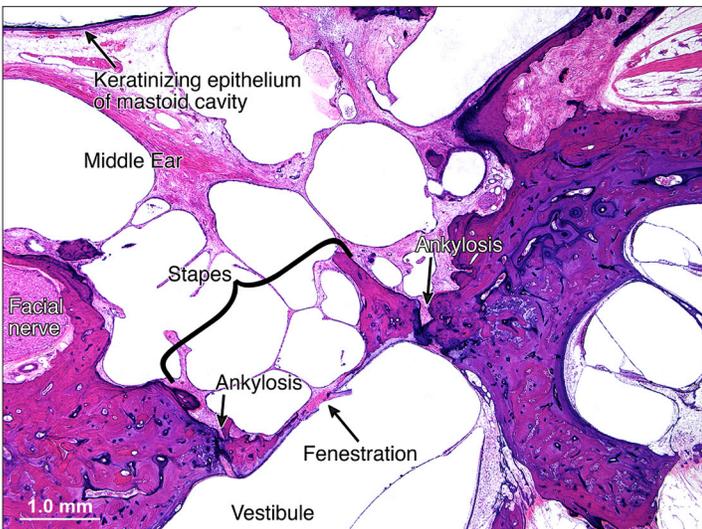


Fig. 4

of the malleus remains, and the incus has been removed. The middle ear space is filled with fibrocystic adipose tissue, likely the result of a graft placed during fenestration surgery. There is a canal wall down the mastoid cavity, which is epithelial cell lined, and has a thin layer of sloughing keratin debris (Figure 4). The lateral semicircular canal fenestration was identified, although there was a thick fibrous layer of tissue and neo-ossification covering the fenestration.

In both ears, the Organ of Corti is mostly preserved throughout the turns, and there is generally preserved stria vascularis. In the right ear, the spiral ganglion neurons are normal in number for age. In the left (operated) ear, there is a significant loss of spiral ganglion neurons throughout all turns of cochlea, which accounts for the sensorineural hearing loss.

There are no foci of otosclerosis in either ear.

Discussion

Temporal bone histopathology in patients with congenital stapes ankylosis has identified anomalies ranging from an atresia plate (an ankylotic stapes footplate with no supra-structure) to ossification of the stapedovestibular joint or cartilaginous fixation across the joint.^{6,7} Clinical descriptions of congenital stapes ankylosis based on operative findings have noted identification of a thick footplate with absence of the annular ligament or primarily posterior stapedovestibular joint fixation.^{7,8} This case reports the middle ear findings and cause of stapes ankylosis in a patient with a specific syndromic NOG related disorder. This patient with Stapes Ankylosis with Broad Thumbs and Toes had ossified cartilage extending across the stapedovestibular joints, and an otherwise normal middle ear and essentially normal cochlea and spiral ganglion cell count in the unoperated ear. It is unclear whether there is variability in the temporal bone pathology among patients with NOG related disorders, and further temporal bone histology studies are needed to elucidate this.

Clinical implications for the histopathologic findings identified in this case relate to candidacy for surgical treatment of the conductive hearing loss. In this case, in the unoperated right ear, there are no temporal bone pathology findings that would suggest an increased likelihood of sensorineural hearing loss or reason for persistent conductive hearing loss after stapedectomy. Other affected family members have undergone successful stapes surgery. However, the stapes surgeon is advised to consider alternative diagnoses to otosclerosis, when there is a strong family history of conductive hearing loss, hyperopia, and/or evidence of other skeletal anomalies.

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Conclusion

A heterozygous nonsense mutation in NOG resulted in Stapes Ankylosis with Broad Thumbs and Toes Syndrome in this patient. The temporal bone histopathologic correlate to his right sided conductive hearing loss is stapes fixation due to ossified cartilage that extends across the stapedovestibular joint. Surgical pathology after a fenestration procedure and stapedectomy is noted in the left ear, with similar findings of stapes ankylosis. ■

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FIGURE LEGENDS

Figure 1. An audiogram done at age 69, 10 years prior to his death, demonstrates right mixed hearing loss, and profound hearing loss on the left (Figure 1). On the right, there is a 30 to 40 dB low frequency conductive component with a word recognition score (WRS) of 60 dB and speech discrimination score (SDS) of 90% at 80dB. On the left, there is a 90 dB pure tone threshold for 1000 Hz, but there is no response at the higher and lower frequencies. Bone thresholds could not be determined due to masking dilemma. SRT for the left was reported as 90 dB and WRS was reported as 60%, but this may also be affected by masking dilemma.

Figure 2. Low power hematoxylin and eosin stain light microscopy of right ear demonstrating normal incus, stapes suprastructure, cochlea, and vestibule. The anterior stapes ankylosis is visible even at this low power.

Figure 3. High power hematoxylin and eosin stain light microscopy of right ear depicting ankylosis of the anterior stapes footplate to the otic capsule due to ossified cartilage in the stapedovestibular joint.

Figure 4. Low power hematoxylin and eosin stain light microscopy of left ear demonstrating the stapes footplate, with central fenestration, and anterior and posterior ankylosis.

Upcoming Events >

The NIDCD National Temporal Bone, Hearing and Balance Pathology Resource Registry will exhibit at the following event:

I'M EXHIBITING at

AAO-HNSF
ANNUAL MEETING
& OTO EXPOSM

2014 SEPTEMBER
21-24
ORLANDO, FL

Modern Studies on the Human Temporal Bone: Transmission Electron Microscopy and Immunocytochemistry

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Introduction

Recently the Temporal bone bank at the House Institute (formerly House Ear Institute) has become part of the Department of Head and Neck surgery at UCLA. Together with the UCLA Temporal bone bank, there are now 1200 temporal bone pairs available to investigate different types of diseases that affect the human inner ear. The histological, cellular and molecular biological methodologies that have been developed in both laboratories are now used to advance in the otology field. We are continuing to use classical histological techniques, immunohistochemistry, design-based stereology, 3D imaging, proteomics and genomics on inner ear sections obtained from temporal bones embedded in celloidin. We also apply the microdissection technique on temporal bones to obtain individual auditory and vestibular endorgans. Cryosections are obtained from these endorgans used for immunohistochemistry, or the microdissected endorgans are embedded in plastic to obtain ultrathin sections for light and transmission electron microscopy (TEM).

In this article we will make emphasis on the application of TEM techniques to study the human temporal bone. This topic becomes contemporary given the continuous development of imaging techniques and its application on relatively new transmission electron microscopes coupled to computer interfaces that allow the acquisition of digitized images at high resolution.

As mentioned above, in the past few years there has been resurgence in the use of TEM in biology due to the improvements of the new electron microscopes that allow the acquisition of images with very high resolution up to limits that were not possible before. For example, using high voltage electron microscopes (300-400 Kv), TEM researchers have been able to obtain three dimensional (3D) images using electron tomography at 0.24 new resolution.¹

TEM techniques have been used for decades to investigate the ultrastructural organization of the human inner. For example, Friedman et al., in 1963, used TEM to investigate the ultrastructure of the human membranous labyrinth in

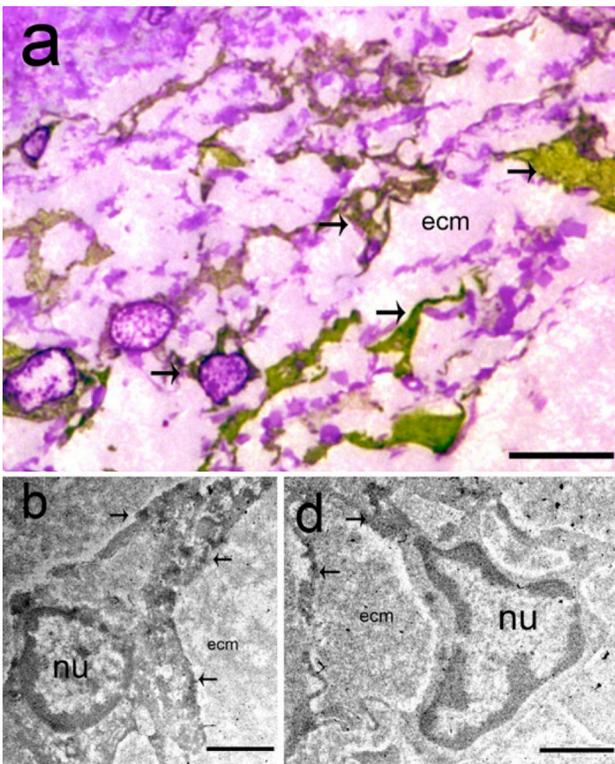
patients diagnosed with Meniere's disease.² TEM research provides ultrastructural details of the human inner ear not seen before. A recent study by Rask-Andersen et al., described the microanatomy of the human cochlea using TEM and STEM techniques.³ We have been using the TEM to investigate pathological changes in the human inner ear for several years.^{4,5} In these studies we microdissected vestibular endorgans from temporal bones obtained at autopsy.⁶ We also use vestibular endorgans obtained at surgery.⁵ Both types of tissues have been proven to be useful for immunofluorescence and molecular biology.⁷

In our temporal bone laboratories at UCLA, we are continuously improving our immunohistochemical methods that allow the visualization of cellular markers that help to understand the pathophysiology of inner ear diseases using celloidin embedded sections. For example, we recently described the immunolocalization of the glutamate transporter – GLAST– in the spiral ligament using celloidin embedded sections.⁸ Although similar methods have been used in the past in the Otology field, the constant development of more sensible techniques, higher quality of biological and chemicals and high resolution TEM call for reassessment of previous methods that allow a more detailed identification of cellular components and molecular changes. Two examples are described below.

TEM and immunocytochemistry in celloidin embedded sections.

To identify specific cellular components at the subcellular level, we have been able to use celloidin embedded sections previously stained for immunohistochemistry.⁸ The use of diaminobenzidine (DAB), which is osmiophilic and electron dense, allows the identification of immunoreactive cells using TEM. Once the areas of interest are identified and digitally recorded, the coverslip is removed, the tissue osmicated, dehydrated and embedded in resin (EPON). When the resin is cured, the glass slide is removed by immersing it in liquid nitrogen, the area of interest is identified and cut in

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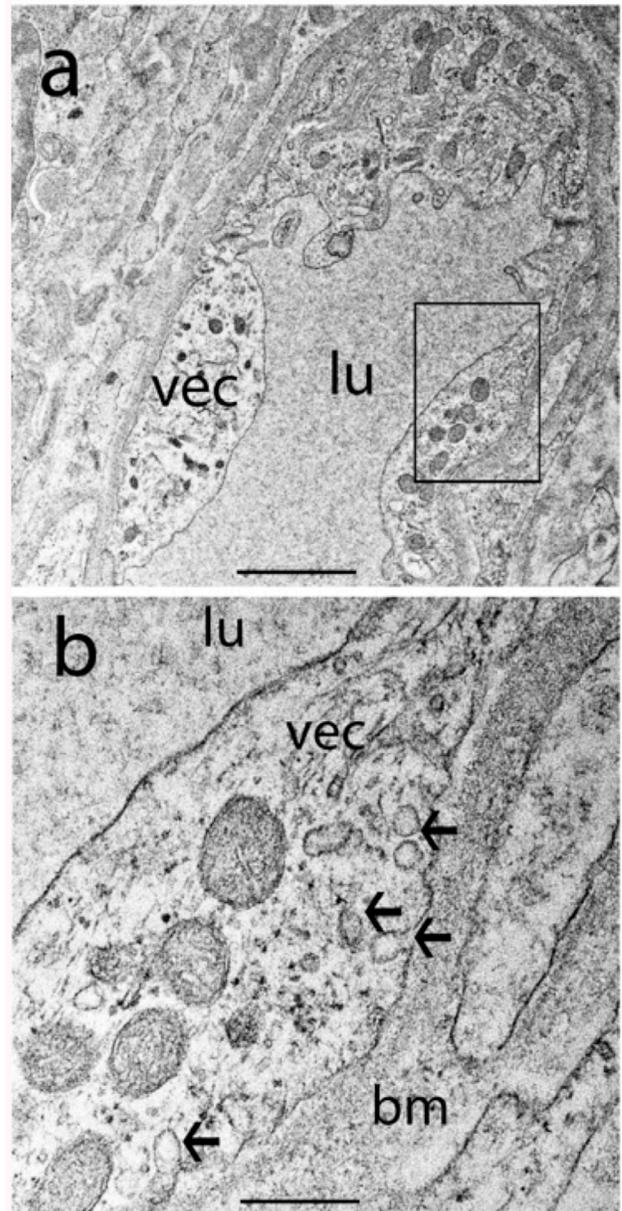


small segments (0.2 x 0.2mm) to obtain thin (0.2 micron) and ultrathin sections (0.08 micron) using a diamond knife (Diatome) and an ultramicrotome.

Figure 1 shows an example of this procedure. Figure 1a shows a small area of the spiral ligament (mid portion). The greenish to dark yellow color shows GLAST immunoreactive fibrocytes, cells in magenta color (toluidine blue) are non-immunoreactive cells. Once the cells of interest are identified, ultrathin sections are obtained (0.08 micron thick). Figures 1b and 1c show a GLAST immunoreactive fibrocyte (dark precipitate) and a non-reactive fibrocyte respectively. Note that in spite the long process from temporal bone harvesting to celloidin embedding, immunohistochemical staining and plastic embedding there is a very good preservation of cellular architecture.

TEM in microdissected vestibular endorgans

Using microdissected endorgans obtained from temporal bones at autopsy we have been able to investigate the anatomy of the blood labyrinthine barrier in normal and pathological conditions using TEM. Figure 2 shows a TEM micrograph of a blood vessel located at the stroma of the macula utricli microdissected from a temporal bone obtained at autopsy from an 85 year old female, with no auditory or vestibular pathology. The temporal bone was collected within 12 hour postmortem, immediately immersed in 4% paraformaldehyde (PF) for 24 hours and, thereafter the vestibular endorgans were microdissected as described previously,⁶ placed in a mixture of 4% PF/2% glutaraldehyde for 12 hours and post-fixed with a mixture of osmium tetroxide and potassium ferrocyanide (2% each). Microdissected vestibular endorgans



are immediately dehydrated and embedded in plastic (EPON). Tissue was sectioned at one micron and then at 0.08 micron, and counterstained with uranyl acetate and lead citrate. Images were obtained using a T12 FEI TEM; digital images were obtained using a digital camera coupled to the microscope. Figure 2a shows a low magnification view from a blood vessel underneath the macula utricli stroma. The micrograph shows well preserved vascular endothelial cells as well as the surrounding basement membrane. Higher magnification view allows the visualization of specific intracellular cellular components. Micrograph in Fig 2b shows the ultrastructural details of a small area of a vascular endothelial cell. Mitochondria and caveolae (cholesterol and sphingolipid-enriched invaginations of the plasma membrane),⁹ are easily identified in this area. This type of information becomes now evident by taking advantage of the TEM and the digital interface coupled to the microscope. Higher magnification observations are now possible allowing the identification of membranes details.

Conclusion

The constant improving of modern cellular biological techniques and instrumentation applied to temporal bone science will help to further understand the pathophysiology of numerous diseases that affect the inner ear. As it is happening in other organs like the brain or the eye, in the near future it will be possible to reconstruct whole cells of the human inner ear at the TEM level and to identify specific cellular markers with detail. It is important to mention that the availability of archival temporal bone sections, together with the application of alternative techniques like microdissection, would continue to provide valuable information necessary to investigate the molecular pathology of numerous diseases that affect the human inner ear. ■

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FIGURE LEGENDS

Figure 1. Immunohistochemistry and TEM in celloidin embedded material. **(a)** Light microscope image from a thin plastic section (0.2 micron thick). Toluidine blue counterstained, GLAST reaction is seen as dark amber to greenish color. **(b)** and **(c)** TEM micrographs obtained from the same specimen (ultrathin sections 0.08 micron). Arrows point to GLAST immunoreactive product. nu: nucleus, ecm: extracellular matrix. Magnification bar in a: is 10 microns, in b and c is 3 microns.

Figure 2. **(a)** TEM of a cross section of a blood vessel from the stroma of a human macula utriculi. Low magnification view. **(b)** High magnification view. vec: vascular endothelial cell, bm: basement membrane; lu: lumen. Bar in a is 1.2 microns in b is 0.22 microns.

Otopathology Mini-Travel Fellowship Program

The NIDCD National Temporal Bone Registry is pleased to announce the availability of mini-travel fellowships. The fellowships provide travel 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:

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If you would like to display either or both of these brochures, 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 not listed.

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