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

Winter 2012 Volume 20, Number 2

the Registry

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 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 Silent and Imminent Threat

by Richard A. Chole, M.D., Ph.D., and Michael J. McKenna, M.D.
Co-Chairs, Otopathology Task Force

On September 12, 2012, during this year's AAO–HNSF Annual Meeting & OTO EXPO in Washington, D.C., an Otopathology Task Force was convened to address a serious and imminent threat to our specialty. This Task Force was organized because of an initiative by Michael M. Paparella, M.D. It was chaired by Richard A. Chole, M.D., Ph.D., and sanctioned by the American Academy of Otolaryngology–Head and Neck Surgery. Present were some of the preeminent leaders in our field. There was no debate regarding the gravity or seriousness of the problem at hand. The specialty of otolaryngology is on the verge of losing its ability to examine the pathology of the human ear. If this were to occur, we would no longer be able to characterize the pathology of a host of problems that we see and treat on a daily basis. It will stifle our ability to develop new and effective treatments and to evaluate the results of our clinical interventions. Without this fundamental discipline, our specialty will justifiably lose all credibility with our medical and surgical colleagues and our patients. To better understand the scope of the problem, it is essential to review how we got here in the first place.

The study of human otopathology is unlike all other pathologic endeavors. It requires a specialized laboratory and unique and intricate processing techniques that take years to master. These techniques cannot be learned from a book or instructional video, but rather take years of mentorship and practice. Similarly, the expertise required to examine and evaluate pathologic specimens takes years of dedicated study and is not a component of the formal educational process in either pathology or otolaryngology training programs. Historically, the great majority of otopathologists have been otolaryngologists.

In 1980, there were 32 active temporal bone laboratories throughout the world with 25 located in the United States. The field was thriving with a critical mass of investigators. The work performed within these facilities is largely responsible for the pathologic characterization of many of the diseases we treat on a frequent basis, including otosclerosis, Meniere's disease, chronic otitis media and many others.

the Registry

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Today, there are three remaining labs in the world, all located within the United States. Insufficient operating funds threaten two of these labs, which are on the verge of closing. This abrupt decline resulted from a significant reduction in research funding for human otopathology and departmental discretionary funds used to support these labs. Most alarming is the near extinction of the technical and pathological expertise. Despite this, there remains a multitude of otologic disorders for which the pathology has not been well characterized with poor treatment options for our patients.

Several years ago, a group of concerned leaders in the field approached the National Institute on Deafness and Other Communication Disorders (NIDCD) with their concerns. These discussions led to the formation of a human temporal bone registry and a research network, resulting in the acquisition of pathologic specimens and for funding of a limited number of labs. This funding is specifically for hypothesis driven research and does not support the ongoing processing and evaluations of new pathologic specimens that only become available when a patient with a well documented otologic problem dies. It has been this slow and steady process of investigation that has led to the greatest advancements in our understanding of human otopathology and without which our field will almost certainly begin to stagnate.

The solution to this impending problem is not entirely clear. It will likely require both financial and institutional support. To this end, Michael Paparella, M.D., has personally pledged more than $500,000 during the next 14 years and established an annual lectureship in human otopathology to be given at the AAO–HNSF meeting. Joseph Nadol, Jr., M.D., gave the inaugural lecture at this year's annual meeting where he eloquently highlighted the importance of human otopathology to the clinical practice of otology and reviewed the dilemma outlined above. The purpose of this communication is to educate the AAO–HNS membership. The task force will continue to actively explore all options to circumvent this potential disaster. There will come a time in the near future when we will call upon the AAO–HNS membership for support. This is a problem that will certainly affect the future of our specialty and will require a unified response.

This editorial originally appeared in the December 2012 issue of Bulletin, *the newsletter of the American Academy of Otolaryngology—Head and Neck Surgery. It is reproduced here with permission kindly granted by the publisher.*

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Upcoming Meetings

The NIDCD National Temporal Bone, Hearing and Balance Pathology Resource Registry plans to exhibit at 36th Annual Mid-Winter Meetings of the Association for Research in Otolaryngology.

February 16-20, 2013
Baltimore Marriott Waterfront Hotel
Baltimore, Maryland

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Cracking the Genetic Code in Archived Temporal Bones

by Joni K. Doherty, M.D., Ph.D., Jose N. Fayad, M.D., and Fred H. Linthicum, Jr., M.D.
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We have set out to investigate the causes underlying some genetic forms of hearing loss and other inner ear diseases using archived human temporal bones. Hearing impairment is the most common sensory deficit and affects a third of adults over the age of 60.[1] Congenital hearing loss affects at least three in 1,000 newborns and is the second most common birth defect after heart anomalies.[2] Well over 100 genetic loci and at least 64 genes have been identified in association with nonsyndromic sensorineural hearing loss (NSSNHL). Yet, only three of theses genetic mutations have been characterized at the otopathological level: DFNA9[3, 4], DFNA17[5], and DFNB1 (due to *GJB2* mutation). The remaining question for the majority of these hereditary hearing impairment (HHI) mutations is: what goes wrong in the inner ear to produce the hearing loss?

DFNA9 is caused by a mutation in the *COCH* gene encoding the abundant inner ear protein, cochlin. DFNA9 exhibits autosomal dominant inheritance and manifests as adult-onset sensorineural hearing loss (SNHL) with variable vestibular dysfunction. Our laboratory and others previously characterized the otopathological changes noted in the human ear from histopathological investigations of temporal bones from patients with DFNA9 hereditary hearing impairment.[3] Interestingly, cartilage-like deposits were noted within the tympanic membrane and ossicular joints (Figure 1A).

One of these DFNA9 temporal bones was further investigated to identify the genetic mutation in the *COCH* gene. Using reverse proteomics on the cochlin protein extracted from this temporal bone, liquid chromatography and mass spectrometry revealed a single amino acid transition (A119T) in the LCCL domain of cochlin from this patient's temporal bone (Figure 1B). Mutations in the LCCL domain are among the most common causes of DFNA9 and have been shown to affect cochlin protein folding.[4] By tracing this amino acid change to the corresponding exon at the DNA level, a single nucleotide mutation was predicted to result in the defect. Therefore, genomic DNA analysis of the *COCH* gene was undertaken using DNA harvested from the formalin-fixed, celloidin-embedded archived temporal bone. Targeted polymerase

Figure 1. *DFNA9 temporal bone histology demonstrating cartilage deposit within incudomalleolar joint (A).[3] Cochlin protein sequence from DFNA9 case (mut) and control (w.t.), demonstrating the specific peptide sequence, identified by LC/MS, of the LCCL domain where the A119T mutation (green in w.t., red in mut) occurs in this patient (B). Sequence of LCCL domain region containing the predicted heterozygous nucleotide change (GA) from PCR amplification of genomic DNA extracted from the same patient's temporal bone.*

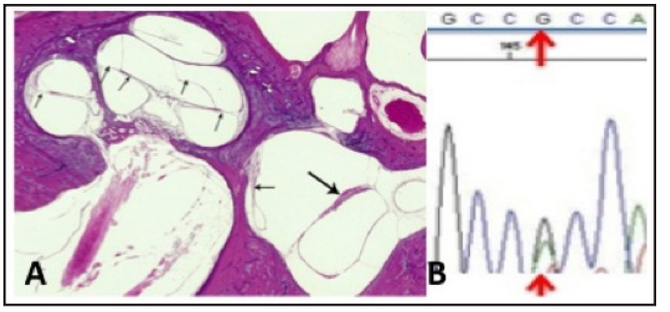

Figure 2. *Horizontal midmodiolar section through DFNA17 temporal bone (H&E-stained) demonstrating Organ of Corti degeneration (small arrows in cochlea) and saccular degeneration (small arrow in vestibule) while the utricle remains intact (large arrow) (A). Sequence of MYH9 exon 17 from temporal bone showing heterozygosity (GA) transition (red arrow) in one allele (denoted by double peak) that was originally reported by Lalwani et al (B).[5]*

chain reaction (PCR) amplification of the *COCH* gene revealed the predicted heterozygous mutation in the *COCH* gene (Figure 1C).

Another example of a genetic mutation causing HHI that has been characterized at the otopathological level is DFNA17. Like DFNA9, this HHI is autosomal dominantly inherited. DFNA17 is due to a mutation in the myosin 9 heavy chain protein (encoded by *MYH9*) and manifests as cochleosaccular degeneration, termed Scheibe deformity (Figure 2A).[5] Individuals affected by DFNA17 experience an early onset progressive SNHL by age 10. In our temporal bone collection, we have the temporal bones of one of the family members from the original family on which genetic linkage analysis was performed resulting in identification of the MYH9 polymorphism within exon 17.[5, 6]

We extracted DNA from this DFNA17 temporal bone and PCR-amplified a 222 base pair segment spanning exon 17 of the MYH9 gene. Sequencing of the PCR products revealed the heterozygous mutation in MHY9 (Figure 2B) that was previously reported from this patient's blood.

Together, these studies corroborate the merit of studying the genetic mutations and otopathology in human temporal bones related to HHI. In years past, temporal bones have been collected post-mortem without additional tissue harvested for DNA extraction or analysis. Moving forward, we are now collecting buccal swab tissue from temporal bone donors at the time of their pledge. This will enable a more thorough genetic analysis and otohistopathological association for future investigations. Understanding what these genetic mutations disrupt in the inner ear will facilitate the further elucidation of inner ear structure and function. Moreover, this understanding may enable the development of mutation-specific hearing loss therapies in the future.

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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 submited to:
Michael J. McKenna, M.D.
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In Memoriam

Saumil N. Merchant, M.D., co-principal investigator of the NIDCD Temporal Bone, Hearing and Balance Pathology Resource Registry, passed away on June 29, 2012. The Gudrun Larsen Eliasen and Nels Kristian Eliasen Professor of Otology and Laryngology at the Harvard Medical School and at the Massachusetts Eye and Ear Infirmary, Dr. Merchant was a world-renowned clinical otologist, otopathologist, teacher, and research scholar.

Born in Bombay, India on Sept. 29, 1960, Dr. Merchant received his undergraduate degree at the Ramnarain Ruia College and his medical degree from the Seth GS Medical College at the University of Bombay. He went on to train in otolaryngology at the College of Physicians and Surgeons of Bombay and earned his masters of science in otolaryngology at the University of Bombay.

Dr. Merchant came to the Massachusetts Eye and Ear Infirmary in 1986 to train as a research fellow in otolaryngology. Shortly thereafter, he decided to repeat his residency training in otolaryngology in the United States, and was accepted to the Harvard Otolaryngology residency program at the Infirmary.

After completing his training, Dr. Merchant was recruited to the Otology division in the Department of Otolaryngology at the Massachusetts Eye and Ear Infirmary in 1992, where he quickly gained recognition as an exceptional member of the Department. He was appointed as the first Eliasen Professor in the Department of Otology and Laryngology at Harvard Medical School in 2007.

In addition to his clinical practice of otology, Dr. Merchant was devoted to research and teaching. He served as co-director of the Wallace Middle Ear Research Unit with his colleague and friend, John Rosowski, Ph.D. In this effort, they systematically established the scientific basis for much of the reconstructive surgery that is done in chronic ear disease and stapes surgery.

His other research passion was the study of the pathology of the human temporal bone. He served as Director of the Otopathology Laboratory and did much to enhance the methodology by which human temporal bone pathology is studied, including the application of genomic and proteomic analysis. He was highly innovative in developing a computerized database to store, analyze, and retrieve data that includes images that he made available to the scientific community on an international basis. He served as a co-principal investigator on the NIDCD Temporal Bone Hearing and Balance Pathology Resource Registry, and his research has been consistently supported by NIH funding over the years, a clear testament to the high regard in which his research work was held. His outstanding work earned him the Politzer prize for the best paper, awarded by the Politzer Society in 1999. In 2004, Dr. Merchant was elected as a member of the Collegium Oto-Rhino-Laryngologicum Amicitiae Sacrum as part of the U.S. delegation. He has given numerous named lectureships, including to the Royal Society of Medicine in London, annual memorial lectures at McGill University, the University of Chicago, and the Ben Senturia Lecture at Washington University.

Dr. Merchant was an outstanding teacher at all levels of training, including medical students, graduate students, residents in the Harvard Otolaryngology program, and clinical and research fellows. He received the William W. Montgomery Award for Excellence in Teaching in 2000, as voted on by that year's graduating resident class.

In his clinical practice, he successfully applied the principles of mechanics studied in the laboratory to middle ear reconstruction. Through his temporal bone histopathology studies, he added clarity and seminal information to the pathogenesis, diagnosis, and management of several otologic disorders.

He served as President of the Medical Staff of the Infirmary from 2006-2007. He also served as both secretary/treasurer and as a member of the Nominating Committee of the International Otopathology Society (Schuknecht Society). He was a member of the American Academy of Otolaryngology-Head and Neck Surgery, Politzer Society, American Otological Society, Executive Council of the American Neurotology Society, the Association for Research in Otolaryngology, and the American Laryngological, Rhinological, and Otological Society (Triological Society). Dr. Merchant served on the editorial board of a number of distinguished journals in our specialty. He was the recipient of the Presidential Citation from the American Otologic Society in 2009 and the recipient of the Honor Award from the American Academy of Otolaryngology-Head and Neck Surgery in 2009 for his exceptional service in its scientific programs, exhibits, continuing education and instructional courses. He was a member of the Advisory Council of the National Institute on Deafness and Other Communication Disorders.

Dr. Merchant's scholarly contributions included more than 140 articles in the peer-reviewed literature, most of which were seminal contributions based on his work on middle ear mechanics and the pathology of the ear. One of his greatest contributions to the literature was as editor of the third edition of *Schuknecht's Pathology of the Ear*, published in 2010.

Dr. Merchant was a remarkable colleague, physician, and friend who willingly gave his time and expertise to all. The Department has recently commissioned an artist to paint a portrait of Dr. Merchant to hang in the Infirmary hallways in his memory. ■

Michael J. McKenna, M.D., and Joseph B. Nadol, Jr., M.D. contributed to this story.

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