Published in final edited form as:

Otol Neurotol. 2008 April ; 29(3): 271–274. doi:10.1097/MAO.0b013e31816a8998.

Human Temporal Bone Consortium for Research Resource Enhancement

Saumil N. Merchant, M.D., Michael J. McKenna, M.D., Joe C. Adams, Ph.D., and Joseph B. Nadol Jr., M.D.

Massachusetts Eye and Ear, Infirmary/Harvard Medical School, Boston, Massachusetts, U.S.A

Jose Fayad, M.D., Robert Gellibolian, Ph.D., and Fred H. Linthicum Jr., M.D.

House Ear Institute, Los Angeles, California, U.S.A

Akira Ishiyama, M.D., Ivan Lopez, Ph.D., Gail Ishiyama, M.D., and Robert Baloh, M.D.

University of California at Los Angeles, Los Angeles, California, U.S.A

Christopher Platt, Ph.D.

National Institute on Deafness and Other Communication Disorders, Bethesda, Maryland, U.S.A

Knowledge of the pathologic basis of disease is central to the study of medicine. Otolaryngology is unique because the inner ear is inaccessible during life so that conventional techniques of pathologic studies such as biopsy and surgical excision are not feasible. Hence, insight into the pathologic basis of ear disease within the 3-dimensional framework of the inner ear and its surroundings can be obtained only by postmortem study of temporal bones and by developing better animal models. Improved understanding of the pathology and pathogenesis of auditory and vestibular system disorders will lead to more rational diagnosis and management of these disorders. The procurement, processing, and study of human temporal bones are time consuming and costly and is a research endeavor performed in the few existing temporal bone laboratories.

There are several reasons why continued study of human temporal bones is warranted: 1) to determine the still unknown pathologic bases of a large number of auditory and vestibular disorders, including genetic defects; 2) to compare human otopathology with animal models to determine which models provide valid information on the cellular and molecular bases of human inner ear disorders; 3) to benefit the practice of otologic surgery by providing postoperative information otherwise unobtainable; and 4) to obtain and study specimens from individuals across the human lifespan, with well-documented normal or age-appropriate levels of hearing and balance function.

Historically, methods for temporal bone study can be viewed as having passed through several technologic periods (1). The period of light microscopic study began at the turn of the last century and encompassed the celloidin embedding and serial sectioning method. Autopsy specimens were often flawed by postmortem autolysis and preparation artifact, and these early studies concentrated on gross correlations of pathologic change with clinical manifestations of disease. The period of cytologic description began when Guild (2) initiated the method of graphic reconstruction of the cochlea and placed more emphasis on technical quality and specific disordered cytology. Meanwhile, standard audiometric tests became available, and more meaningful correlations of morphologic change with functional disorders could be made. The period of cytologic quantification began in the 1950s and 1960s with Schuknecht's (3,4) descriptions of correlations of hearing loss with losses of various cytologic elements in the sensory and neural systems of the cochlea. Such cytologic quantification was possible because the histologic preparations were technically excellent, and temporal bones were acquired soon after death to minimize postmortem autolysis. We are now entering another period that might

be characterized as methodological integration whereby the same temporal bone can be used for light microscopic study and for electron microscopy, immunostaining, and molecular studies involving genomic and proteomic assays. The use of such technologies in combination may provide new information that could revolutionize our understanding of otologic disorders.

In 1992, the National Temporal Bone Hearing and Balance Pathology Resource Registry (the "Registry"; www.tbregistry.org) was established by the National Institute on Deafness and Other Communication Disorders (NIDCD) of the National Institutes of Health. The Registry promotes research on otopathology by serving as a resource for the public and scientific community (5). It continues and expands the former National Temporal Bone Banks program created in 1960 to encourage temporal bone donation and works closely with all temporal bone collections and laboratories in the United States. The Registry does not itself collect specimens or do research but serves many functions including enrolling temporal bone donors on a prospective basis, maintaining a 24-hour nationwide network to coordinate collection of temporal bones after a donor's death, maintaining a computerized database of all human temporal bone collections nationwide, conserving existing collections that may be at risk of being disbanded, disseminating information on the importance of temporal bone donation and research, and sponsoring professional educational activities in otopathology. Of note, more than 5,000 individuals have been recruited as temporal bone donors, and since its inception, the Registry has coordinated the retrieval of more than 700 temporal bone specimens, most from donors with well-documented otologic disorders. These specimens have been distributed to various U.S. laboratories for histopathologic processing and study. Registry activities have directly or indirectly supported more than 350 peer-reviewed articles or book chapters on human otopathology by various U.S. laboratories. The Registry is supported by a contract from the NIDCD that provides the infrastructure necessary for timely procurement of high-quality temporal bone specimens and a centralized national information source. Laboratories obtaining specimens must find their own funds and personnel to process and study the temporal bones. Despite the success of the Registry, the numbers of laboratories and investigators engaged in human temporal bone research have declined from 28 active laboratories in the United States in 1976 to fewer than 10 now. The reasons for this decline include difficulty competing for funding, escalating costs of tissue procurement, and a shortage of trained and committed physician-scientists in the field of otopathology.

Temporal bone research has played a major and significant role in enhancing the diagnosis and therapy of numerous otologic disorders, examples of which abound in standard otopathologic texts (1,6,7). A few recent examples are presented to illustrate the power of otopathologic studies in impacting both basic and clinical science:

1. DFNA9. A unique constellation of histopathologic findings of degeneration of the spiral ligament with eosinophilic deposits led to the discovery of DFNA9 (8), one of the few genetic nonsyndromic disorders involving both the auditory and vestibular systems. These initial histopathologic studies ultimately led to identification of its cause, namely, mutations in the COCH gene (9). Affected individuals exhibit significant sensorineural hearing loss despite having an intact organ of Corti (10). The histopathologic studies have supported basic science observations of the importance of the spiral ligament in inner ear physiology (11,12). Cochlin, the gene product of the COCH gene, is highly expressed within the inner ear, but its precise function and role in DFNA9 is not known. Temporal bone studies using techniques of immunostaining and proteomic analysis of archival sections have provided insight into the pathophysiology of the hearing loss (13).
2. Facial nerve paralysis. Although viruses have been implicated as etiologic agents in Bell palsy and Ramsay-Hunt syndrome, the evidence to support a viral etiology was circumstantial. Application of polymerase chain reaction amplification to archival

temporal bone sections has shown varicella-zoster viral deoxyribonucleic acid (DNA) in Ramsay-Hunt syndrome (14) and herpes simplex viral DNA in Bell palsy (15). Thus, temporal bone studies have provided support for treating these disorders with antiviral therapy.

3. Cochlear implantation. Otopathologic studies of temporal bones from individuals that received cochlear implants during life have shown no correlation between the number of surviving cochlear neuronal cells and implant performance during life (16,17). This surprising and unexpected finding refutes a long-standing assumption that cochlear implant performance would be directly correlated with the number of cochlear neurons. This finding has significant implications for design and development of cochlear implant electrode arrays.
4. Vestibular disorders. There is a paucity of knowledge regarding the pathologic basis for many forms of dizziness and vertigo. Recent studies have provided evidence of lesions at the level of vestibular hair cells and the vestibular nerve, with implications for diagnosis and therapy (18–20). The demonstration of aquaporins in the human inner ear (21) may also lead to new insights into the pathophysiology of labyrinthine disease such as Meniere's syndrome.
5. Temporal bone models. The human temporal bone contains a large number of complex structures within a small space. It can be challenging for students in the basic sciences or medical disciplines to learn this complex anatomy. Virtual models of the human temporal bone have been created from histologic sections and are available as downloadable freeware for teaching and educational purposes (22,23). These models serve as teaching tools by providing realistic, interactive, and anatomically accurate information with 3-dimensional visualization. More than 5,500 downloads (each download comprising a model plus interactive software) have occurred within the span of about a year.

The NIDCD sponsored a workshop, "Temporal Bone Histopathology Research: Laboratories and Research Training," in 2003 to assess the need to maintain active laboratories and encourage new researchers in the field. The workshop participants recognized the potential for modern molecular, genetic, and imaging technologies to help human temporal bone research make discoveries that can translate into valuable clinical advances. They also noted that individuals considering a career in human otopathology research are dissuaded by a number of misconceptions (e.g., that otopathology is a field of historic interest only, with little relevance to modern otology, and that federal funding agencies have little interest in supporting otopathologic studies). Workshop participants concluded that it is critically important to support basic processing and study of human temporal bones and training of future generations of otopathologists. It was felt that protocol-driven acquisition and processing of specimens by a consortium of laboratories would promote methodological improvements, data sharing, and recruitment and training of future researchers. To this end, NIDCD announced a funding opportunity for a Human Temporal Bone Consortium for Research Resource Enhancement (the "Consortium"), and after peer review, the Consortium was established in late 2006 with 3 member laboratories: the Massachusetts Eye and Ear Infirmary (Boston, MA), the House Ear Institute (Los Angeles, CA), and the University of California at Los Angeles (Los Angeles, CA).

The Consortium is supported as a cooperative agreement from NIDCD using the U24 funding mechanism and, in consultation with the NIDCD Program Officer, is governed by an internal Steering Committee and an external Advisory Committee. The goals of the Consortium are to improve and enhance methodologies for processing human temporal bones, to promote sharing of tissues and technologies, and to promote the recruitment and training of new investigators. The U24 mechanism includes funding of the member laboratories for processing and study of

temporal bones. The Consortium promotes multidisciplinary collaborations between laboratories to address research questions that are too difficult for single laboratories to solve and maximizes the use of specimens that are difficult to obtain. The Consortium will optimize protocols for assessing prospectively acquired temporal bones using decision trees that allow processing methods to differ depending on the donor's medical history, premortem auditory and vestibular test data, and results of imaging studies. The Consortium also will develop protocols for analysis that apply modern molecular biological techniques such as the use of immunoassays, DNA and ribonucleic acid assays, and mass spectrometry-based proteomic analysis, including techniques that may be possible on archival temporal bones. Sharing of tissues, data, and technologies will be promoted among the member laboratories and the wider scientific and research communities. Another goal is to archive information in digital format from temporal bones that have been studied and to make the information available to clinicians and researchers in the form of web-based freeware for teaching purposes.

The activities of the Consortium and the Registry are different in focus and funding, but they are complementary. The Registry is an administrative organization that provides the infrastructure for the acquisition of high-quality temporal bone specimens, whereas the Consortium concentrates on research in human otopathology and training the next generation of temporal bone researchers. The activities of the Consortium should lead to technical innovations in the study of temporal bones, sustain existing temporal bone laboratories, and become a resource for sharing specimens and data with the wider scientific and clinical communities.

References

1. Schuknecht, HF. Pathology of the Ear. 2. Philadelphia, PA: Lea & Febiger; 1993.
2. Guild SR. A graphic reconstruction method for the study of the organ of Corti. *Anat Rec* 1921;22:141–57.
3. Schuknecht HF. Techniques for study of cochlear function and pathology in experimental animals. Development of the anatomical frequency scale for the cat. *Arch Otolaryngol* 1953;58:377–97.
4. Schuknecht HF. Further observations on the pathology of presbycusis. *Arch Otolaryngol* 1964;80:369–82. [PubMed: 14198699]
5. Merchant SN, Schuknecht HF, Rauch SD, et al. The National Temporal Bone, Hearing, and Balance Pathology Resource Registry. *Arch Otolaryngol Head Neck Surg* 1993;119:846–53. [PubMed: 8343246]
6. Michaels, L. Ear, Nose and Throat Histopathology. London, UK: Springer-Verlag; 1987.
7. Nager, GT. Pathology of the Ear and Temporal Bone. Baltimore, MD: Williams & Wilkins; 1993.
8. Khetarpal U, Schuknecht HF, Gacek RR, Holmes LB. Autosomal dominant sensorineural hearing loss. Pedigrees, audiologic findings, and temporal bone findings in two kindreds. *Arch Otolaryngol Head Neck Surg* 1991;117:1032–42. [PubMed: 1910721]
9. Robertson NG, Lu L, Heller S, et al. Mutations in a novel cochlear gene cause DFNA9, a human nonsyndromic deafness with vestibular dysfunction. *Nat Genet* 1998;20:299–303. [PubMed: 9806553]
10. Merchant SN, Linthicum FH, Nadol JB Jr. Histopathology of the inner ear in DFNA9. *Adv Otorhinolaryngol* 2000;56:212–7. [PubMed: 10868238]
11. Spicer SS, Schulte BA. Differentiation of inner ear fibrocytes according to their ion transport related activity. *Hear Res* 1991;56:53–64. [PubMed: 1663106]
12. Kikuchi T, Kimura RS, Paul DL, Adams JC. Gap junctions in the rat cochlea: immunohistochemical and ultrastructural analysis. *Anat Embryol (Berl)* 1995;191:101–18. [PubMed: 7726389]
13. Robertson NG, Cremers CW, Huygen PL, et al. Cochlin immunostaining of inner ear pathologic deposits and proteomic analysis in DFNA9 deafness and vestibular dysfunction. *Hum Mol Genet* 2006;15:1071–85. [PubMed: 16481359]
14. Wackym PA, Popper P, Kerner MM, Grody WW. Varicella-zoster DNA in temporal bones of patients with Ramsay Hunt syndrome. *Lancet* 1993;342:1555. [PubMed: 7902926]

15. Burgess RC, Michaels L, Bale JF Jr, Smith RJ. Polymerase chain reaction amplification of herpes simplex viral DNA from the geniculate ganglion of a patient with Bell's palsy. *Ann Otol Rhinol Laryngol* 1994;103:775–9. [PubMed: 7944168]
16. Nadol JB Jr, Shiao JY, Burgess BJ, et al. Histopathology of cochlear implants in humans. *Ann Otol Rhinol Laryngol* 2001;110:883–91. [PubMed: 11558767]
17. Fayad JN, Linthicum FH Jr. Multichannel cochlear implants: relation of histopathology to performance. *Laryngoscope* 2006;116:1310–20. [PubMed: 16885730]
18. Ishiyama A, Ishiyama GP, Lopez I, Eversole LR, Honrubia V, Baloh RW. Histopathology of idiopathic chronic recurrent vertigo. *Laryngoscope* 1996;106:1340–6. [PubMed: 8914898]
19. Baloh RW, Lopez I, Beykirch K, Ishiyama A, Honrubia V. Clinical-pathologic correlation in a patient with selective loss of hair cells in the vestibular endorgans. *Neurology* 1997;49:1377–82. [PubMed: 9371925]
20. Rauch SD. Vestibular histopathology of the human temporal bone. What can we learn? *Ann N Y Acad Sci* 2001;942:25–33. [PubMed: 11710467]
21. Lopez I, Ishiyama G, Lee M, Baloh RW, Ishiyama A. Immunohistochemical localization of aquaporins in the human inner ear. *Cell Tissue Res* 2007;328:453–60. [PubMed: 17318586]
22. Wang H, Northrop C, Burgess B, Liberman MC, Merchant SN. Three-dimensional virtual model of the human temporal bone: a stand-alone, downloadable teaching tool. *Otol Neurotol* 2006;27:452–7. [PubMed: 16791035]
23. Wang H, Merchant SN, Sorensen MS. A downloadable three-dimensional virtual model of the visible ear. *ORL J Otorhinolaryngol Relat Spec* 2007;69:63–7. [PubMed: 17124433]