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the REGISTRY

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

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The REGISTRY is published semiannually by the NIDCD National Temporal Bone, Hearing and Balance Pathology Resource Registry. The 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.

HUMAN OTOPATHOLOGY AND BASIC SCIENCE: PARTNERS IN TRANSLATIONAL RESEARCH

Symposium held at the meeting of the Association for Research in Otolaryngology, February 7, 2010, Anaheim, California

Human temporal bones provide a valuable resource for the study of the pathology and pathophysiology of disorders in hearing, balance and facial nerve function. Advances in cellular and molecular biology over the last several years have provided new opportunities for enhancing our understanding of ear disease. A 3-hour symposium sponsored by the Human Temporal Bone Consortium for Research Resource Enhancement took place at the recent meeting of the Association for Research in Otolaryngology in Anaheim, California, on February 6, 2010. The goal of the Symposium was two-fold: 1) To show how human otopathology research and basic science approaches can complement each other in advancing our understanding of and development of treatments for otologic disorders; and 2) To demonstrate the resources developed by the Temporal Bone Consortium that have been made available to the broader research community. The symposium was moderated by Saumil N. Merchant, M.D. and M. Charles Liberman, Ph.D. In this issue of the Registry's newsletter, we present a synopsis of the proceedings of the Symposium.

Introductory remarks were delivered by Christopher Platt, Ph.D., Program Director, Hearing and Balance Central Pathways/DSP, National Institute on Deafness and other Communication Disorders (NIDCD) (Figure 1). Dr. Platt pointed out that the inner ear, which contains some of the most delicate and highly tuned sensory structures in the body, is housed deep within the temporal bone where it is surrounded by the otic capsule, the densest bone in the body. The inaccessibility of the inner ear to direct examination and to surgical biopsy during life makes it particularly challenging for physicians and scientists trying to decipher the pathologic and molecular underpinnings of otologic disease. These challenges notwithstanding, Dr. Platt noted that recent advances in cellular and molecular biology have paved the way for application of innovative ideas to the study of human temporal bones. One result has been the establishment of the Human Temporal Bone Consortium for Research Resource Enhancement, a

Figure 1. Dr. Christopher Platt, Program Director, Hearing and Balance Central Pathways/DSP, National Institute on Deafness and other Communication Disorders (NIDCD) making introductory remarks.

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cooperative agreement utilizing the U24 grant mechanism between the NIDCD and the Otopathology Laboratories at the Massachusetts Eye and Ear Infirmary and Harvard Medical School, the House Ear Institute, and the University of California at Los Angeles.

A unique aspect of the Symposium was that each topic was covered by a clinician and a basic scientist who had been working collaboratively on that topic; the resulting research and discoveries helped to demonstrate the translational potential of such collaborations.

Mechanisms of Genetic Deafness: Inner Ear Phenotypes in the Human and Mouse Models

Saumil N. Merchant, M.D., and M. Charles Liberman, Ph.D.
Massachusetts Eye and Ear Infirmary and Harvard Medical School, Boston, MA

Rapid strides have been made in discovering genes responsible for hereditary hearing loss in the human. Mutations in over 50 genes have been described in various types of non-syndromic deafness. On the other hand, human otopathology has been characterized in only 3 of these conditions, DFNA9, DFNA17 and DFNB1. The paucity of human otopathology reports can be attributed to several factors: inaccessibility of the inner ear for study during life by biopsy; the fact that patients do not die from genetic deafness; the inner ear is not removed at routine autopsy; general pathologists do not study the human inner ear; and the delicate membranous labyrinth is encased in dense petrous bone necessitating special techniques of study. Some of these obstacles are being overcome by the efforts of the NIDCD National Temporal Bone Registry and the Temporal Bone Consortium.

The best characterized histopathologic phenotype of non-syndromic deafness is DFNA9, an autosomal dominant disorder characterized by adult-onset progressive deafness and disequilibrium. DFNA9 is caused by point mutations in the COCH gene which encodes the protein cochlin (Robertson et al., Nature Genetics, 1998; 20:299). The inner ear phenotype in DFNA9 is unique, unlike any other otologic disorder, and is characterized by degeneration of fibrocytes of the spiral ligament and spiral limbus within the cochlea with concomitant deposition of an acellular eosinophilic staining substance. The vestibular sense organs show atrophy of sensory cells and nerve endings, along with eosinophilic deposits. Since affected individuals have normal hearing and balance for the first few decades of life, significant potential exists for innovative therapies to prevent or treat the audiovestibular dysfunction. In order to do so, one needs to uncover the pathophysiology of the disorder which can be investigated using mouse models.

Many different mouse models of non-syndromic deafness have been developed. Broadly, they may be classified as resulting from random mutations (spontaneous or ENU generated), gene deletions (knockout models), or due to directed mutations (knockin models). Initially, a cochlin knockout mouse was developed which lacked immunostaining for cochlin, but the model did not demonstrate a hearing or histopathologic phenotype (Makashima et al., Hum Genet 2005;118:29). This provided evidence that DFNA9 hearing loss is not a COCH haplo-insufficiency disorder. Subsequently, a knockin mouse model for DFNA9 was developed using a targeted COCH G88E missense mutation (Robertson et al., Hum Mol Genet 2008;17:3426). In this mouse model, similar to the human condition, there was age-related progressive hearing loss and vestibular dysfunction. On the other hand, the COCH knockin mouse did not show any inner ear abnormalities on histopathology. Possible reasons for the discrepancy include differences in genetic background, and the issue of lifetime verses elapsed time, in that the effects of the protein anomaly may be probabilistic requiring extensive elapsed time to appear.

In summary, mouse and human data are complementary; both are needed to make progress in understanding deafness. An integrative approach has great potential to improve the diagnosis and treatment of genetic deafness.

Supporting Cells in Organ of Corti Regeneration and Otopathology

Neil Segil, Ph.D., Michael Hoa, M.D., and Fred Linthicum, M.D.
House Ear Institute, Los Angeles, California

In mammals, loss of sensory hair cells in the organ of Corti leads to permanent deafness due to the failure of these cells to regenerate. In contrast, regeneration occurs normally in non-mammalian vertebrates through a combination of supporting cell proliferation and re-differentiation, as well as through a process of direct transdifferentiation of supporting cells into hair cells. The discovery of these processes in non-mammalian vertebrates suggests the possibility that a better understanding of the basic biology of supporting cells may lead to the means of therapeutically manipulating mammalian supporting cells to take part in hair cell regeneration in the damaged organ of Corti.

The first part of this talk focused on advances in our understanding of organ of Corti development and regeneration in the mouse, with specific emphasis on the regulation of proliferation, and the maintenance of cell fate in postnatal supporting cell populations. The second part discussed new data analyzing and categorizing the survival of supporting cell populations in archival human temporal bones, from the temporal bone laboratories of the House Ear Institute and the Massachusetts Eye and Ear Infirmary, from deaf individuals. These supporting cell populations remain largely uncharacterized, but will, of necessity, form a part of any therapeutic strategy for hair cell regeneration and hearing restoration.

The review showed that the survival of supporting cell population varies with a variety of factors, most importantly, with the etiology of deafness. Many types of deafness are characterized by atrophy and degeneration of supporting cells which would be a challenge for successful deployment of therapies concerning hair cell regeneration. The study also showed that deafness from aminoglycoside ototoxicity is characterized by selective loss of hair cell, while supporting cells are generally preserved. Therefore, this particular etiology may be an attractive target for future deployment of hair cell regeneration technologies.

Using Human Temporal Bones, Combined with Histological and Proteomic Methods to Study the Underlying Molecular Changes associated with DFNA9.

Jose N. Fayad, M.D. and Robert Gellibolian, Ph.D.
House Ear Institute, Los Angeles, California

Formalin-fixed, celloidin-embedded (FFCE) or paraffin-embedded (FFPE) tissues are a largely unexplored archive in mass spectrometry (MS)-based proteomics, mainly due to the fact that it is extremely difficult to identify the unpredictable nature of the chemical modifications that take place as a result of fixation. Current methods such as immunohistochemistry (IHC) suffer from a lack of sensitivity and scalability. Exploring capabilities to conduct large-scale analyses of tissues using proteomic approaches becomes of paramount importance and could have far reaching implications on the morphological, histopathological as well as molecular characterization of temporal bones associated with hearing loss or balance disturbance.

DFNA9 is a form of sensorineural hearing loss, clinically characterized by onset in the fourth or fifth decade of life and initially involves the high frequencies. Deafness is progressive and usually complete by the sixth decade. In addition to cochlear involvement, DFNA9 patients also exhibit a spectrum of vestibular dysfunctions. Affected individuals have mucopolysaccharide depositions in the inner ear (spiral ligament, limbus and the dendrites under the limbus, and supporting tissues of the vestibular system), in the middle ear (incudo-malleolar joint) and cartilage formation in the tympanic membrane. The abnormalities in the middle ear in the tympanic membrane and ossicular joints have not been previously described. Thus, DFNA9 is a unique type of non-syndromic genetic deafness where a gene defect has been reported to affect both the inner and middle ear structures.

The authors showed how they have successfully leveraged the application of powerful mass spectrometry-based proteomic approaches towards the molecular investigation of archival temporal bone specimens associated with the non-syndromic sensorineural deafness autosomal dominant type 9 (DFNA9), the ideal test case whose underlying molecular defect has already been linked at the genetic level to three different base mutations in the COCH gene.

Molecular and Cellular Approaches to the Human Temporal Bone

Akira Ishiyama, M.D., Ivan Lopez, Ph.D., and Gail Ishiyama, M.D.
University of California at Los Angeles School of Medicine

The purpose of the study was to present the advances in applying immunocytochemical localization in the human temporal bone using auditory and vestibular endorgans microdissected from temporal bones acquired post-mortem. With this technique there are minimal or no decalcification steps and immunohistochemical staining can be conducted and evaluated within one week. Microdissected vestibular endorgans from temporal bones were sectioned with a cryostat, or used as whole mount surface preparations. Specimens were analyzed using light and fluorescent microscopy. The resulting specimens demonstrated

excellent morphology with minimum tissue alteration and good immunoreactive signal-to-noise ratio. Immunohistochemical staining has been successfully performed in auditory and vestibular endorgans using antibodies against aquaporins, basement membrane (BM) proteins and connexins 26 and 30 (Lopez et al. 2007, Ishiyama et al. 2009; McCall et al. 2008). Using this methodology, we have provided the first comprehensive immunolocalization of aquaporins and BM proteins in the human inner ear. This method can be used to increase the resolution of unbiased stereology on nerve fiber counts in the human vestibular endorgans using immunocytochemistry (Lopez et al. 2005). Using microdissection and immunohistochemistry of human temporal bone, we have recently detected the von Willebrand A domain-related protein (WARP), an extracellular matrix molecule with restricted expression in a subset of BMs in the vasculature (Trac et al. ARO Abst 2010). WARP-immunoreactivity was located in the internal (luminal) portion of blood vessels and in calyx like structures that surround type I hair cells. The combination of microdissection and immunohistochemistry can potentially open up a new field for future human temporal bone research. Pathological changes in the sensory epithelia can be documented with the use of these techniques.

Temporal Bone Surgical Simulator I: A Resource for Clinicians and Trainees – from Cardboard Models to the Visible Ear Simulator and beyond.

Mads Sølvsten Sørensen, M.D., Ph.D., Peter Trier, and Jesper Mosegaard
Rigshospitalet, University of Copenhagen, Denmark (Mads Sørensen), and The Alexandra Institute, Aarhus, Denmark (Peter Trier and Jesper Mosegaard)

From anatomical cardboard models over simple pc-based wire frames and surface models, sophisticated volumetric 3-D representations with haptic user interaction have evolved. Most existing virtual simulators for middle ear surgery are based on CT or MRI data in which image quality is limited by the lack of natural colour, texture and detail (max. 50 voxels/mm3) of the source material. Interaction requires the purchase of software, a customized computer, and expensive peripherals dedicated exclusively to this purpose. This has limited the benefit and dissemination of virtual simulators for ear surgery.

The VES Visible Ear Simulator is based on the "Visible Ear" freeware library of digital images from a fresh frozen human temporal bone, which was segmented and volume rendered into a 3-D model of high-fidelity, true color and great anatomical realism and detail (125voxels/mm3) of the surgically relevant structures. Figure 2 shows the members of the audience using 3D glasses to visualize the models. A real-time rendering was achieved by combining advanced GPU raycasting and standard rasterization. A haptic drilling model was

Figure 2. Members of the audience using 3D glasses to view the Visible Ear Simulator presented by Dr. Mads Sørensen from Rigshospitalet and University of Copenhagen, Denmark.

calculated by using volumetric techniques.

Program solutions were constantly selected to provide the highest quality of images and haptics possible in an application designed for real time interaction on any standard pc platform with GPU acceleration. Realistic visualization in 2-D or optional anaglyph stereoscopic 3-D was achieved on a Core 2 Duo® pc with a GeForce 8800 GTX® graphics card (or better), and real-time surgical interaction was provided through a Phantom Omni® haptic 3-D pointing device. A range of controls allows the user to adjust the virtual environment, to save, restore and even "undo" surgical work, to collect screenshots for external use and calibrate visual and haptic performance to match the default pc equipment.

At:http://temporalboneconsortium.org/educational-resources/simulator/ the VES is offered as freeware to meet a worldwide oto-surgical demand.

Future developments may include tutor/censor functions and haptic tutorials. Moreover, the source material may support increased resolution up to 8000 voxels/mm3 and interaction with deformable soft tissue components such as skin, tympanic membrane, dura and cholesteatomas developed at random by the program. New technology may provide enhanced stereoscopic and haptic quality even with low budget commercial equipment.

Temporal Bone Surgical Simulator II: The Ohio State Experience, Towards Validation

Gregory Wiet, M.D., and Don Stredney, Ph.D.
Nationwide Children's Hospital and Ohio State University (Gregory Wiet); the Ohio Supercomputer Center (Don Stredney)

The potential impact of interactive, multimodal simulation technology to teach technical skill in surgical endeavors is undeniable. Simulation technology has become a mainstay of training in the airline and other industries and recently has been applied to technical skills training in many types of surgery. Otologic surgery presents a unique challenge of this technology in that it requires an integration of

Continued on page 6

MEETINGS

The Registry is planning to exhibit at:

DONOR DASH
a run for life

11th Annual
Sunday, July 18th, 2010
Washington Park in
Denver, Colorado

AAO-HNSF Annual Meeting & OTO EXPO
2010
September 26–29
Boston, MA

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.* The fellowships are available to:

1) U.S. hospital departments who aspire to start a new temporal bone laboratory

2) Inactive U.S. temporal bone laboratories that wish to reactivate their collections or

3) 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:

1) A 1-2 page outline of the educational or training aspect of the proposed fellowship

2) Applicant's curriculum vitae

3) Letter of support from temporal bone laboratory director or department chairman

4) Letter from the host temporal bone laboratory, indicating willingness to receive the traveling fellow

Applications should be sent to:

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the spatial comprehension of the exacting anatomy with the application of fine motor skills to develop expertise. In order to provide an advantage to Otologic surgical training, systems must be developed following an iterative design. Development must be followed by assessment followed by modification and reassessment. The process must be repeated to achieve validity in training and skill assessment. Systems must have core features including sufficient structural and visual realism to engage the user, the ability to provide an opportunity for structured repetition of skill, and the provision for active feedback to the user supplying continuous and quantified assessment. These components must be integrated within the midst of a metrics driven curriculum. This presentation outlined our approach with a focused overview of objectives, followed by progress emphasizing data acquisition, integration, dissemination, and validation within the resident curriculum. Additionally, we presented our future plans and recommendations to those involved in temporal bone research to provide data that can be integrated into more complex simulations.

Technical and Educational Resources developed by the Temporal Bone Consortium

Saumil N. Merchant, M.D.
Massachusetts Eye and Ear Infirmary and Harvard Medical School, Boston, Massachusetts

The inner ear is inaccessible for examination during life by biopsy or other techniques. Post-mortem examination of the inner ear is challenging because the delicate membranous labyrinth is encased within the dense petrous bone. There currently exist only a handful of laboratories in the world that conduct systematic investigations of human inner ear specimens.

The Human Temporal Bone Consortium for Research Resource Enhancement was recently established as a cooperative agreement using the U24 funding mechanism between NIDCD and three member laboratories: the Massachusetts Eye the Ear Infirmary, the House Ear Institute, and the University of California at Los Angeles. The goals of the Consortium are to improve and enhance methodologies for studying human temporal bones, promote sharing of tissues and technologies, and promote the recruitment and training of new investigators.

The Consortium has assembled a variety of resources of value to the research and clinical communities to facilitate research and teaching, as well as the diagnosis and treatment of otologic disorders. These resources are available as freeware, using interactive, web-based formats (www.temporalboneconsortium.org; see Figure 3).

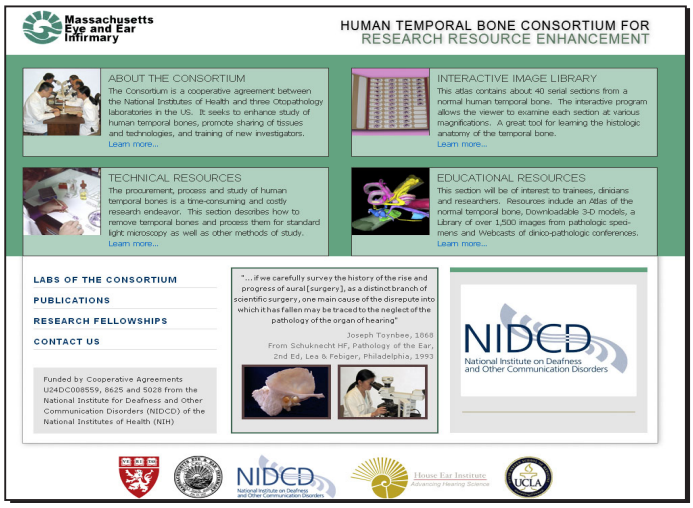

Figure 3. Home page of the website of the Temporal Bone Consortium, www.temporalbone consortium.org

These resources, which include the following, were demonstrated in real time:

1. Techniques of removal of the human temporal bone.
2. Methods of study, including microscopy, immuno-, genomic- and proteomic- assays.
3. An interactive image library of normal 2-D morphology, allowing the user to perform virtual remote microscopy at magnifications ranging from 10x to 400x.
4. Interactive image libraries correlating radiology and histology of the temporal bone.
5. Interactive, downloadable 3-D models of the inner ear and temporal bone.
6. A library of searchable and downloadable images of morphology and pathology (1,500+ images).
7. A database of archived specimens, fully searchable by text, similar to PubMed.
8. Webcast recordings of seminars in otopathology.

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Balance Program

NIDCD supports research on balance and the vestibular system. Balance disorders affect a large proportion of the population, particularly the elderly. The vestibular system, with its receptor organs located in the inner ear, plays an important role in the maintenance of one's orientation in space, the control of balance while the body is immobile and in motion, and visual fixation of objects during head movement. Vestibular disorders can therefore yield symptoms of imbalance, vertigo (the illusion of motion), disorientation, instability, falling and visual blurring (particularly during motion). Deficits in vestibular function result from diverse disease processes, including infection, trauma, toxicity, impaired blood supply, autoimmune disease, impaired metabolic function and tumors.

In addition to its role in the stabilization of gaze and balance, recent findings from NIDCD-supported studies suggest that the vestibular system plays an important role in regulating blood pressure. The information emerging from these studies holds potential clinical relevance for the understanding and management of orthostatic hypotension (lowered blood pressure related to a change in body posture).

The linear acceleration detectors of the vestibular system, the otolithic organs, detect the forces produced by head tilt and by linear (forward-to-aft, side-to-side) head movements. How the vestibular apparatus and the nervous system resolve gravitational from linear accelerations in order to accurately perceive motion and control balance is currently under active study by NIDCD-supported investigators.

Investigations supported by the NIDCD are characterizing the genes essential to normal development and function in the vestibular system. The genetic bases of several inherited cerebellar syndromes of imbalance and incoordination are currently being investigated.

The institute supports research to develop and refine tests of balance and vestibular function. Computer-controlled systems measuring eye movement and body postural responses activated by stimulating specific parts of the vestibular sense organ and nerve have been developed and validated for clinical use. Also, tests of functional disability and physical rehabilitative strategies currently being applied in clinical and research settings will have important implications for refining the rehabilitation of patients with balance and vestibular disorders.

Recently, a prototype vestibular neural prosthesis has been developed by a team of NIDCD-funded investigators. Early-stage studies with this device demonstrates that the function of the inner ear balance system can be partially restored through electrostimulation of the vestibular nerve. Research is progressing in earnest to refine the vestibular prosthesis and to determine its viability for application to vestibular-deficient humans.

For More information, please contact: Christopher Platt, Ph.D., (301) 402-3458

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FREE BROCHURES FOR YOUR OFFICE OR CLINIC ABOUT TEMPORAL BONE RESEARCH AND DONATION

That Others May Hear is a short brochure that briefly describes the functions of the Registry, and answers commonly asked questions regarding the temporal bone donation process. (Dimensions: 9" x 4")

The Gift of Hearing and Balance: Learning about Temporal Bone Donation is a 16-page, full-color booklet which describes in more detail the benefits of temporal bone research. It also answers commonly asked questions regarding the temporal bone donation process. (Dimensions: 7" x 10")

If you are willing 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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