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the REGISTRY

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

CONTENT

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.

CAN MODERN MOLECULAR AND IMMUNOSTAINING TECHNOLOGIES LEAD TO DISCOVERIES IN HUMAN TEMPORAL BONE RESEARCH?

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Introduction

The hearing and balance organs of the inner ear are relatively inaccessible and a biopsy of these tissues would result in irreparable damage and organ dysfunction. Therefore, insight into the pathology of ear diseases can be obtained only through postmortem studies of temporal bones. The specimens in most archival collections of human temporal bones have been fixed in formalin and embedded in celloidin. These treatments have created obstacles to the use of modern molecular techniques that have been developed for the evaluation of fresh and frozen tissues. Obtaining DNA and other molecules from these archival specimens has proven to be challenging, however, adaptations of innovative methodologies have produced exciting results and demonstrate incredible opportunities for researchers to explore. Three and half years ago, we initiated molecular studies on these archival materials to gain a better understanding of the mechanisms involved in age-related loss, referred to as presbycusis. A brief description of our studies is presented below.

Mitochondrial DNA deletions in presbycusis

Somatic mitochondrial DNA (mtDNA) deletions have been associated with age-related diseases in many post-mitotic tissues. The cochlear tissues are known to contain an abundance of mitochondria and this observation has prompted a search for mtDNA deletions in the cochlea. The presence of the mtDNA common deletion (CD) has been reported in cochlear tissues from individuals with presbycusis; however, the significance of this finding was initially unclear (1). Subsequent studies utilizing nested polymerase chain reaction (PCR) assays, a technique developed to identify deletions in minute samples, have demonstrated the presence of multiple deletions in the major arc of the mtDNA genome in addition to the CD in cochlear tissue from individuals with presbycusis (2). Long range PCR, a technique which can survey DNA sequences over 10 kilobase pairs (kb) in length, has also been utilized to detect the presence of previously unreported large scale mtDNA deletions.

Prior to our recent report (3), quantitative methods of measuring mtDNA deletions had not been employed in the study of presbycusis. As a result, a clear association between mtDNA deletion levels and the development of hearing loss with aging in humans had not been established. In this study, real time PCR assays were utilized to quantify the mtDNA CD level in cochlear tissue from individuals with presbycusis and individuals with normal hearing. **(Fig. 1)**

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The results of this investigation demonstrated a statistically significant difference in the CD level between the presbycusis group and the age-matched control group. In addition, a statistically significant correlation between the CD level and the severity of hearing loss was present. These findings suggest that an accumulation of the CD may play a role in the development of presbycusis.

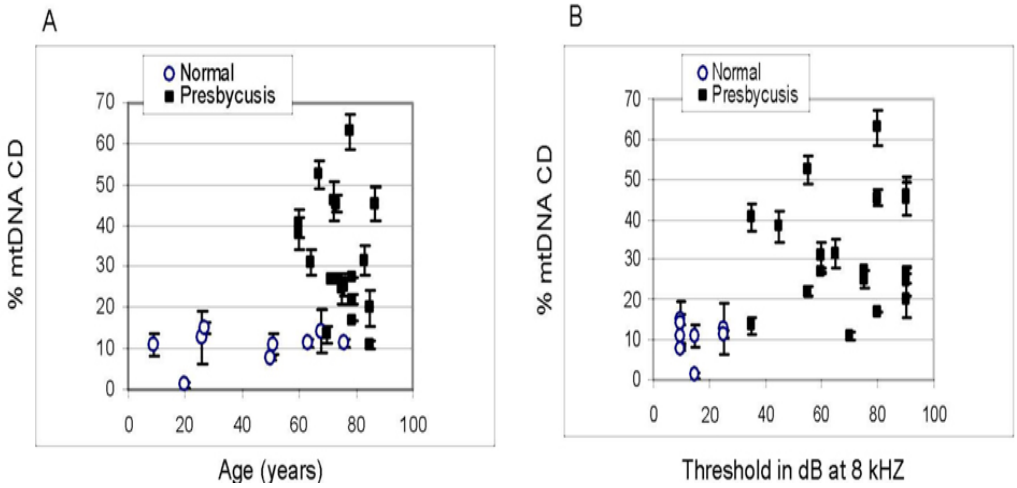

Figure 1. *Quantification of the mtDNA CD level in cochlear tissue samples from 19 individuals with presbycusis and 9 controls with normal hearing. (A) MtDNA CD level versus age. A significant correlation between CD level and age was observed. (B) MtDNA CD level versus audiometric threshold. A significant correlation between CD level and the severity of hearing loss was observed in the age matched control groups. Error bars represent standard deviation with respect to repeated measurements of the same tissue sample. Laryngoscope 2009, In Press.*

Concerns have been raised regarding the integrity of the DNA in archival temporal bone specimens due to the potential for degradation or modification by the reagents used for tissue processing. The presence of an intact 10.4 kb native mtDNA fragment in temporal bone tissue samples has been demonstrated by long range PCR and product sequencing. This observation suggests that the mtDNA in these specimens is a suitable source for analysis.

Immunofluorescence and TUNEL staining
Formalin alters the configuration of proteins and can obscure antigens by modifying the epitopes recognized by antibodies. Celloidin embedding provides superior support of the delicate membranous structures of the inner ear to maintain tissue integrity during sectioning; however, inadequate removal of celloidin may limit tissue permeability resulting in poor penetration of large molecules. We reported a method of celloidin removal and antigen retrieval for immunofluorescence staining of type I collagen (4). Using confocal microscopy, alterations in the distribution f this protein were demonstrated. We have also used this immunofluorescence methodology to quantify reductions in cytochrome c oxidase (COX) expression in spiral ganglion cells.

A similar approach has been used to identify apoptotic cells with TUNEL (terminaldeoxynucleotidyl transferase mediated dUTP nick end labeling) staining.

Laser microdissection of the cochlea
Laser microdissection (LMD) has been used to isolate groups of cells and single cells from numerous tissues. We reported a LMD technique for isolating cochlear structures and individual spiral ganglion cells **(Fig. 2)** from archival celloidin embedded human temporal bone sections (5).

The specimens isolated were suitable for quantifying the mtDNA CD within these tissues using a duplex real time PCR assay and demonstrate the feasibility of using this approach to study the accumulation of mtDNA deletions in diseases of the ear. Currently, the techniques described in this article are being combined to isolate single COX deficient spiral ganglion cells and determine the total mtDNA deletion burden present. Elevated deletion levels have been shown to trigger apoptosis in other tissues and the involvement of this mechanism in the observed ganglion cell loss in presbycusis will be explored in future studies.

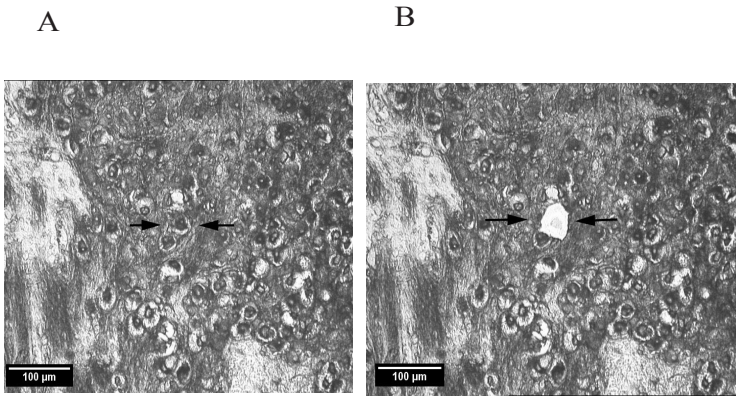

Figure 2. *Light micrograph of an unstained human temporal bone section with celloidin removed. A- Spiral ganglion prior to laser microdissection (LMD). B- Spiral ganglion following removal of a single spiral ganglion cell by LMD. Hear Res. 2008;244:1-6.*

Conclusion:

A comprehensive understanding of human disease requires investigations utilizing human specimens and cannot be achieved with the study of animal tissue alone. Human temporal bone collections have historically played an essential role in providing tissue for histopathologic studies, which has subsequently led to improved knowledge of otologic diseases and the development of improved treatments for these disorders. The progress described in this manuscript illustrates that the application of modern technologies to these archival specimens has significant potential for elucidating molecular mechanisms involved in presbycusis. With these considerations, the expansion and utilization of human temporal bone collections should remain a priority in the field of otologic research.

References:

1. Bai U, Seidman MD, Hinojosa R, Quirk WS. Mitochondrial DNA deletions associated with aging and possibly presbycusis: A human archival temporal bone study. Am J Otol 1997;18:449-53.
2. Markaryan A, Nelson EG, Hinojosa R. Detection of mitochondrial DNA deletions in the cochlea and its structural elements from archival human temporal bone tisue.Mutat Res. 2008;640:38-45.
3. Markaryan A, Nelson EG, Hinojosa R. Quantification of the Mitochondrial DNA Common Deletion in Presbycusis. Laryngoscope 2009;119:1184-1189.
4. Markaryan A, Nelson EG, Tretiakova M, Hinojosa R. Immunofluorescence and TUNEL Staining of Celloidin Embedded Human Temporal Bone Tissues. Hear Res. 2008;241:1-6.
5. Markaryan A, Nelson EG, Tretiakova M, Hinojosa R. Laser Microdissection of Cochlear Structures from Celloidin Human Temporal Bone Tissues and Detection of the Mitochondrial DNA Common Deletion using Real Time PCR. Hear Res. 2008;244:1-6.

NIDCD
National Institute on Deafness and Other Communication Disorders

It's a Noisy Planet. Protect Their Hearing

Noise-induced hearing loss (NIHL) is 100 percent preventable. Yet approximately 26 million Americans between the ages of 20 and 69 have high-frequency hearing loss from overexposure to loud noises at work or during leisure activities. More than 30 million Americans are exposed to dangerous levels of noise on a regular basis (1). Children also are frequently exposed to noise levels that could permanently damage their hearing. Noise levels generated by activities as common as doing yard work, playing a band instrument, and attending sports events can result in NIHL. Research suggests that NIHL experienced at an early age may accelerate age-related hearing loss later in life (2).

In October 2008, the National Institute on Deafness and Other Communication Disorders (NIDCD), part of the National Institutes of Health (NIH), launched It's a Noisy Planet. Protect Their Hearing. The Noisy Planet campaign is designed to increase awareness among parents of children ages 8 to 12 ("tweens") about the causes and prevention of NIHL. With this information, parents and other caring adults can encourage children to adopt healthy habits that will help them protect their hearing for life.

NIDCD is focusing its campaign on the parents of tweens because children at this age are becoming more independent and developing their own attitudes and habits related to their health. They also are beginning to develop their own listening, leisure, and work habits—or soon will do so. Consequently, the tween years present an open window of opportunity to educate children about their hearing and how to protect it.

Parents still have a great deal of influence over their tween's behavior, and the Noisy Planet campaign provides them with resources that they can use to educate their children about the causes and prevention of NIHL. The campaign Web site at noisyplanet.nidcd.nih.gov provides parents with facts about NIHL, tips on how to encourage their tween to adopt healthy hearing habits, and other steps they can take to protect their tween's hearing. The site also offers information specifically for tweens, such as interactive games about noise and hearing.

1. National Institute on Deafness and Other Communication Disorders (n.d.). National Institute on Deafness and Other Communication Disorders health disparities strategic plan fiscal years 2004–2008. Available online at www.nidcd.nih.gov/about/plans/strategic/health_disp.asp.
2. Kujawa, S.G. and Liberman, M.C. (2006). Acceleration of age-related hearing loss by early noise exposure: Evidence of a misspent youth. Journal of Neuroscience, 26(7), 2115–2123. Available online at www.jneurosci.org/cgi/content/abstract/26/7/2115.

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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.* 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:

Saumil N. Merchant, M.D.
NIDCD National Temporal Bone Registry
Massachusetts Eye and Ear Infirmary
243 Charles Street
Boston, MA 02114

MEETINGS

The Registry is planning to exhibit at these upcoming meetings

American Academy of Otolaryngology—Head and Neck Surgery
Working for the Best Ear, Nose, and Throat Care

Annual Meeting & OTO EXPO
San Diego, CA , USA
October 4-7, 2009

Association for Research in Otolaryngology
Founded in 1973

33rd ARO MidWinter Meeting
Disneyland Hotel, Anaheim, CA, USA
February 6-11, 2010

NEWS AND ANNOUNCEMENTS

Deafness Research Foundation

"DRF Partners with NIDCD's "It's A Noisy Planet" Campaign

Deafness Research Foundation (DRF) and the National Institute on Deafness and Other Communication Disorders (NIDCD) recognize the importance of raising awareness of the causes of, and ways to prevent, noise-induced hearing loss (NIHL). By partnering in the "It's a Noisy Planet" program, DRF and NIDCD will spread the word to protect your hearing and the hearing of your children. To learn more about NIHL and the "It's a Noisy Planet" campaign, visit www.drf.org

LAB SPOTLIGHT

The Bloom Temporal Bone Laboratory at the University of Chicago, Chicago, Illinois

Adam Markaryan, Ph.D., Erik G. Nelson, M.D., Raul Hinojosa, M.D.

As Chairman of the Section of Otolaryngology at the University of Chicago, Dr. John R. Lindsay established the Otopathology Laboratory in 1930. With the knowledge gained in this laboratory, he advocated the importance of otopathological research in improving the clinical management of patients and delivered this message to his colleagues around the world. In 1960, The National Temporal Bone Banks Program was initiated by the Deafness Research Foundation under the direction of Dr. Lindsay to facilitate the collection and distribution of temporal bones across the country. Although he retired as chairman of the department in 1965, he remained active in the laboratory until his death in 1981, providing 52 years of service to our institution.

Dr. Raul Hinojosa was recruited in 1962 by Dr. Lindsay to become the director of the Otopathology Laboratory at the University of Chicago. Prior to accepting this position, Dr. Hinojosa had completed training in general pathology in addition to training in many world renowned temporal bone laboratories, including those directed by Dr. Lindsay, Dr. George Keleman, Dr. Stacy Guild, Dr. Lücius Ruedi, Dr. Hans Engstrom, and Dr. I. Friedmann. Although he was granted emeritus status in 1995, Dr. Hinojosa has continued his role as laboratory director.

Dr. Adam Markaryan (left) and Dr. Raul Hinojosa (right)

Following his ten years of research experience in biochemistry, biophysics, and pharmacology, Dr. Erik G. Nelson initiated his work in the Otopathology Laboratory at the University of Chicago in 1984 as an otolaryngology resident. He has continued to work in the laboratory on a part time basis while maintaining a clinical practice in otology in Lake County, Illinois.

In 2001, Mrs. Margaret A. Bloom bequeathed her remaining estate to support otopathological research at the University of Chicago. This act of generosity was contributed in gratitude for the clinical otologic care provided by Dr. Lindsay to a very close family member. Through this endowment, the laboratory was revitalized and a position was created for Dr. Adam Markaryan to join the laboratory in 2006. Dr. Markaryan has a diverse background in contemporary molecular biology techniques that he acquired during his 30 year career as a scientist. His training includes a Ph.D. in Biochemistry from the Moscow State University and postdoctoral studies at the Ohio State University and the University of Illinois at Chicago. He has held appointments at the University of Illinois at Chicago as a Research Assistant Professor in the Department of Biological Sciences and the Department of Microbiology and Immunology from 1997 to 2001, and subsequently at the University of Chicago as a Research Associate (Assistant Professor) in the Department of Medicine, Section of Nephrology from 2002 to 2005. In his short tenure with the Bloom Laboratory, he has bridged a gap in our understanding between pathological findings and the molecular mechanisms involved in these processes. This opportunity to unlock the mysteries of ear disease would not have been possible without the vision and efforts of Dr. Lindsay and Dr. Hinojosa in acquiring one of the largest collections of temporal bones with complete medical histories, audiometric test results, and uniform methods of processing. Progress has been facilitated by maintaining a close relationship between scientists in other laboratories participating in the National Temporal Bone Registry program. For a more complete perspective on the research activities in the Bloom Temporal Bone Laboratory, please visit the laboratory website at: *http://surgery.uchicago.edu/specialties/otolaryngology/research/bloomlab/*

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PLEASE! Notify us of your change of address before you move. Each undelivered newsletter is returned to the Registry office at a cost of $.70. Our loss is over $1.00 per unit.
Thank you!

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.

That Others May Hear _____ 25 50 100 **The Gift of Hearing and Balance _____ 25 50 100**

NAME: _____

ADDRESS: _____

ADDRESS: _____

CITY, STATE, ZIP: _____

TELEPHONE: _____

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