Characterizing individual differences: Audiometric phenotypes of age-related hearing loss

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Metabolic presbyacusis, or the degeneration of the cochlear lateral wall and decline of the endocochlear potential, largely accounts for age-related threshold elevations observed in laboratory animals raised in quiet and may underlie the characteristic audiogram of older humans. The "audiometric phenotype" associated with metabolic presbyacusis differs from audiograms associated with sensory losses resulting from ototoxic drug and noise exposures. Evidence supporting metabolic and sensory phenotypes in audiograms from older adults can be derived from demographic information (age, gender), environmental exposures (noise and ototoxic drug histories), and stability or changes in audiometric phenotypes as individuals age. When confirmed with biological markers and longitudinal analyses, well-defined audiometric phenotypes of human age-related hearing loss can contribute to explanations of individual differences in auditory function for older adults.

INTRODUCTION

Naturally occurring age-related changes to the auditory periphery in older adults combine with damaging effects of a lifetime of environmental exposures and disease processes. Subsequent anatomic, physiologic, and neurochemical deficits result in reduced detection for low-level signals (hearing loss) and impaired suprathreshold auditory function, including complex signal processing and speech understanding. As such, the aging auditory periphery delivers degraded signal representations for processing by the central auditory pathways and cortex. At the same time, older adults may be increasingly affected by changes in cognitive abilities, including declines in working memory, executive function, attention, and processing speed; reduced ability to suppress irrelevant information; and inadequate compensation strategies. Taken together, these effects may impose increased cognitive demands on an aging brain with already limited resources and loss of inhibition. Thus, multiple risk factors (aging, noise, drugs, disease, infections, comorbid conditions) and multiple sources of pathology in the auditory system (hair cells and lateral wall of the cochlea, auditory nerve, central auditory pathways, cortex) contribute to large individual differences. These complex and interactive effects throughout the aging auditory system highlight the critical need for evidence to (1) allocate declines to each risk factor, especially aging, (2) explain individual differences, (3) identify

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Proceedings of ISAAR 2015: Individual Hearing Loss – Characterization, Modelling, Compensation Strategies. 5th symposium on Auditory and Audiological Research. August 2015, Nyborg, Denmark. Edited by S. Santurette, T. Dau, J. C. Dalsgaard, L. Tranebjærg, and T. Andersen. ISBN: 978-87-990013-5-4. The Danavox Jubilee Foundation, 2015.

promising targets for intervention, and (4) develop strategies to prevent or delay the onset of age-related changes.

SOURCES OF AGE-RELATED PATHOLOGY

Lateral wall and stria vascularis

The cochlear lateral wall is responsible for production and maintenance of the endocochlear potential (EP), which is a positive voltage of 80-100 mV present in the endolymph of the scala media and serves as the battery that provides voltage to the outer hair cells (OHCs), or cochlear amplifier. Laboratory animals raised in quiet (e.g., "quiet-aged" gerbils) demonstrate a systematic degeneration of the lateral wall and reduced EP, which deprives the cochlear amplifier of its essential power supply (Schulte and Schmiedt, 1992; Schmiedt, 1996; Gratton *et al*., 1997). These changes (1) reduce cochlear amplifier gain in the lower frequencies by as much as 20 dB and in the higher frequencies by as much as 60 dB, and (2) reduce but maintain cochlear nonlinearities, such as compression and otoacoustic emissions (OAEs). Although OHCs are preserved, age-related reduction in EP results in changes in OHC function (Schmiedt *et al*., 1990; Schmiedt, 1996). The frequency-specific neural threshold loss of quiet-aged gerbils measured with compound action potentials (CAP) is associated with EP loss and is not associated with OHC loss, and defines the gradually sloping audiogram of older gerbils (Schmiedt *et al*., 2002; Lang *et al*., 2003; Lang *et al*., 2010; Mills *et al*., 2004; Schmiedt, 2010).

Outer hair cells and cochlear amplifier

With environmental exposures from ototoxic drug or excess noise exposure, sensory and non-sensory cell loss result in threshold shifts of ~50-70 dB, loss of the cochlear amplifier, and loss of cochlear nonlinearities (such as absent OAEs). These characteristics are not seen in quiet-aged gerbils (Mills *et al*., 1990; Schmiedt *et al*., 1990; Tarnowski *et al*., 1991).

Primary auditory neurons

Quiet-aged gerbils also demonstrate primary neural degeneration, which is not related to sensory cell loss. The spiral ganglion cells are reduced in size and number along the entire cochlear duct, and there is selective loss or inactivity of low spontaneous-rate auditory nerve fibers (Hellstrom and Schmiedt, 1990; Schmiedt *et al*., 1996; Schulte *et al*., 1996; Suryadevara *et al*., 2001; Lang *et al*., 2002; Mills *et al*., 2006). These results are consistent with evidence from human archival temporal bones, which show spiral ganglion cells declining with age, even without hair cell loss (Otte *et al*., 1978; Makary *et al*., 2011). In addition to primary neural degeneration in aging animal models and humans, the early noise trauma mouse model (Kujawa and Liberman, 2009) also shows a loss of hair cell synapses and terminals, loss of spiral ganglion neurons, and selective loss of low spontaneous-rate auditory nerve fibers. Of importance is that this "neural presbyacusis" (1) can occur without threshold elevation (i.e., with a normal audiogram), (2) affects neural coding at high signal levels (i.e., shallow CAP amplitude-intensity or input-output

functions) and in noise, and affects suprathreshold auditory behavior, all of which can put older adults at greater disadvantage. Moreover, these neural declines, along with deterioration of the lateral wall and hair cell dysfunction, represent additional sources of individual differences.

ARE AGE-RELATED PATHOLOGIES SEEN IN HUMAN AUDIOGRAMS?

The observance of a characteristic audiogram of older laboratory animals raised in quiet (a mild, flat hearing loss at lower frequencies coupled with a gradually sloping hearing loss at higher frequencies) led to the question of whether metabolic presbyacusis also defines the gradually sloping audiogram of older humans. That is, we were interested in determining if age-related conditions of cochlear and neural pathology, as described earlier from animal models, can be consistently observed in human audiograms of older adults. To answer this question, we first developed schematic boundaries for 5 audiometric phenotypes, based on 5 hypothesized conditions: older-normal, pre-metabolic, metabolic, sensory, and metabolic+sensory (Schmiedt, 2010; Dubno *et al*., 2013; see Fig. 1). The combined metabolic+sensory phenotype is consistent with the notion that, in contrast to quiet-aged gerbils, audiograms of older adults likely reflect the effects of environmental exposures (noise, drug) combined with age-related declines in the auditory periphery unrelated to these exposures. Next, we searched initial audiograms stored in the MUSC longitudinal human subject database for “exemplars”, (best examples) of each phenotype. Of 1,728 initial audiograms (obtained at enrollment in the longitudinal study), 22% were identified by expert raters as exemplars with no knowledge of subject demographics.

Validation of audiometric phenotypes

To validate this approach, we predicted the phenotypes of the exemplar audiograms using three machine learning tools, Support Vector Machines, Random Forests, and nonlinear Quadratic Discriminant Analysis (QDA). Each of the machine learning tools classified the audiograms by comparing pure-tone thresholds to a prior distribution and finding the maximum probability. Nonlinear QDA was selected because covariances across frequency are not equivalent and the exemplar phenotypes (thresholds as a function of frequency) are nonlinear. Each of the three machine learning tools replicated expert judgements with a similarly high degree of accuracy (93.2% for QDA). Given that the results for the three procedures were comparable, QDA was selected as the procedure for future analyses based on its ability to capture the nonlinearities in the audiogram and because it is a widely understood method as compared to the other two methods. Also at this time, the decision was made to eliminate the pre-metabolic phenotype because only a small number of audiograms (3%) were classified. An additional concern was that pre-metabolic is not a distinct phenotype, but an early stage of the metabolic phenotype (i.e., a transition from older-normal to metabolic). An automated classifier as used here provides a means to study new samples to further replicate and validate our results, with the long-term goal of evaluating genetic and biological mechanisms of

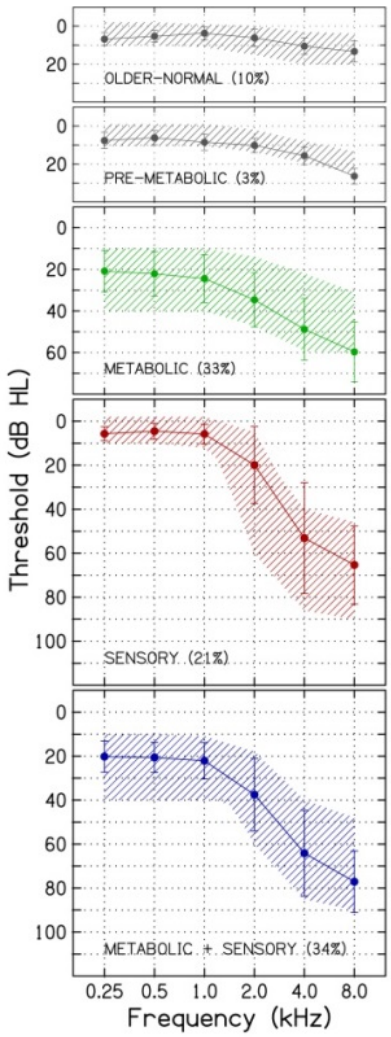

Fig. 1: Schematic boundaries of five phenotypes of age-related hearing loss (shaded regions). Symbols and error bars are mean thresholds (±1 standard error) of exemplar audiograms. See text for additional details.

age-related hearing loss in humans. Until that occurs, the phenotypic classifications, and their hypothesized underlying mechanisms based on animal models of metabolic and sensory loss (as described earlier), should be considered putative in nature (Dubno *et al.*, 2013).

How well do phenotypes correspond to predicted demographics?

We further assessed the accuracy of the classifier by determining how well the audiograms assigned to the phenotypes were consistent with the predefined schematic boundaries and corresponded to predicted demographics, such as age, gender, and noise exposure history. Individual estimates of noise history were

obtained from a 7-item self-report questionnaire on occupational and non-occupational noise exposures – see Lee *et al.* (2005) and Dubno *et al.* (2013) for additional results. Results showed that, on average, individuals with older-normal phenotypes were youngest, whereas individuals with metabolic phenotypes were oldest (consistent with EP declining with increasing age). Individuals with sensory phenotypes (sensory and metabolic+sensory) were primarily male, whereas older-normal and metabolic phenotypes were primarily female. Sensory phenotypes were more likely to have positive noise exposure histories, whereas older-normal and metabolic phenotypes were less likely to have positive noise histories. Next, we validated the approach by classifying non-exemplar initial audiograms according to the four phenotypes (N=1,379). QDA classifications showed high consistency of threshold, age, gender, and noise histories within groups (Dubno *et al.*, 2013). Thus, using cross-sectional data, classifications of audiometric phenotypes were consistent with expert judgements, and revealed that individuals with audiograms classified as metabolic phenotypes were older, predominately female, and had negative noise exposure histories, consistent threshold elevations resulting from a declining EP.

Using longitudinal data from the MUSC human subject database to assess stability of audiometric phenotypes over time

Using longitudinal data from the MUSC human subject database, we determined the likelihood of metabolic phenotypes increasing with age. The human subject database currently contains data from ~1,500 participants (~450 active participants), of which 69% are age 60 and older, 60% are female, and nearly 30% are racial/ethnic minorities. The database contains more than 20,000 audiograms (more than 10,000 lab visits × 2 ears). Participants of all ages are recruited from the Charleston area, including local audiology and otolaryngology clinics, assisted living facilities, senior centers, and health fairs. Participants must be 18 and older, in good general health to be able to visit the laboratory multiple times, and no evidence of conductive hearing loss, active otologic disease, or significant cognitive decline. There is no restriction on amount of hearing loss, but hearing abilities must be good enough to provide measurable results on a majority of the test battery. Measures are repeated yearly or every 2-3 years for longitudinal data.

Audiometric measures include hearing for pure tones, including extended high frequencies, ability to understand speech in quiet and in noise, otoacoustic emissions, upward and downward spread of masking, middle ear function, and auditory brainstem responses (e.g., Lee *et al.*, 2005; Dubno *et al.*, 1997; 2008). Study participants provide oral or written responses to self-report questionnaires on medical history, prescription and over-the-counter drugs, noise history, hearing-aid history, hearing handicap, tinnitus, smoking, and handedness. A cognitive battery includes tests of attention, working memory, processing speed, and perceived workload. Brain imaging is obtained on a subset of participants while they are listening to and understanding low-pass filtered speech or speech in background noise. Each participant has an otologic exam and provides blood for clinical chemistries and to extract DNA for whole exome sequencing. Finally, participants

are offered the opportunity to donate their temporal bones for future structural-functional analyses.

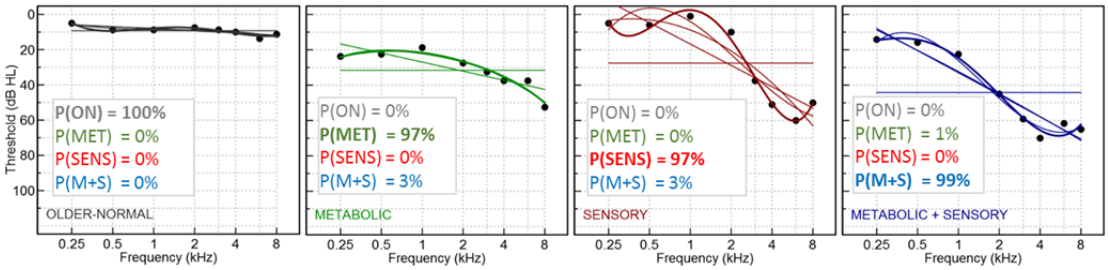

Fig. 2: Examples of audiometric phenotypes classified based on fitted curve parameters. Each filled circle is a pure-tone threshold and lines represent fits using 1-5 parameters. The legend in each panel includes the probabilities assigned to each classification. *Notes*: ON=older-normal; MET=metabolic; SENS=sensory; M+S=metabolic+sensory.

For this next phase of phenotype classifications, a new procedure was introduced, whereby phenotypes were classified based on fitted curve parameters, and then selected as before based on the maximum probability (Fig. 2). With 5 parameters, a cross-validated accuracy of 94.4% was obtained using the curve-based approach and smoothed audiograms from “clustered” time points. Laboratory visits (and pure-tone thresholds and other measurements) occur in clusters of several visits within a short time-frame (less than one year), which are then repeated every 2-3 years. Therefore, longitudinal data were defined as 2 or more clustered time points, where a cluster is 3 or more audiograms within one year. This resulted in ~7,700 audiograms averaged into 1,826 clusters from 686 ears (ranging in age from 50-93). Participants with missing data were excluded.

Phenotypes were found to be stable over time for a majority of ears (64%). In addition, a majority of right/left ears had the same phenotype (71%) and most ears matched across all time points (89%). Nevertheless, although a majority of individual ears maintained the same phenotype with increasing age, pure-tone thresholds increased (as demonstrated by longitudinal changes in thresholds obtained from serial audiograms). These increases in thresholds varied with phenotype and gender, with thresholds for metabolic phenotypes showing greater declines with increasing age.

For the 36% of ears with phenotypes that changed with increasing age, unique patterns were observed. Older-normal phenotypes transitioned to each of the other three phenotypes with approximately equal probability. Metabolic phenotypes transitioned primarily to metabolic + sensory phenotype, as did sensory phenotypes (Fig. 3). Nearly all metabolic+sensory phenotypes transitioned to the metabolic phenotype; probabilities for the initial and final phenotypes were typically less than 1.0, indicating that these audiograms may have been in an intermediate stage.

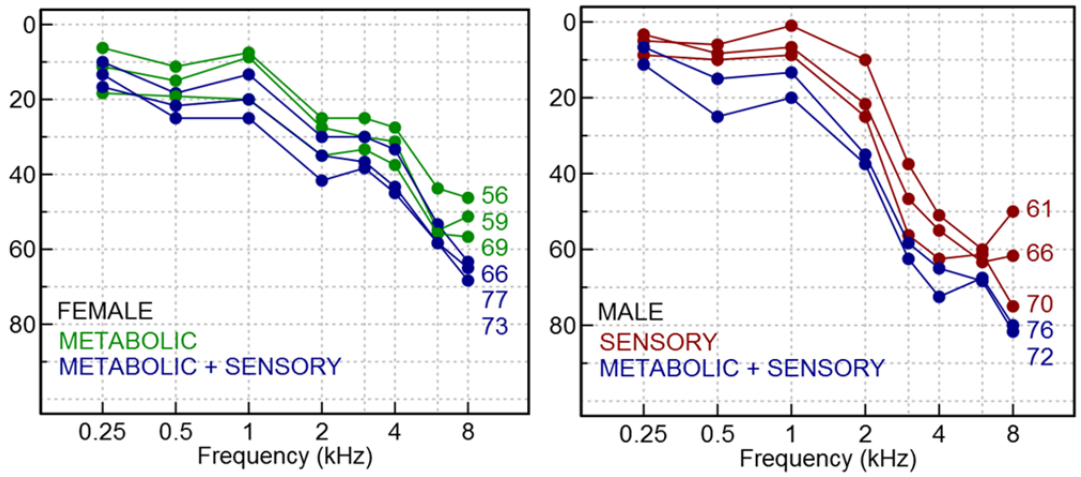

Fig. 3: Serial audiograms from two study participants illustrating phenotypes changing with age. In both cases, the transition was to the metabolic+sensory phenotype, but the initial phenotype was metabolic in one case (female, left panel) and sensory in the other case (male, right panel). Ages at the times of the measured audiograms are indicated on the right side of each panel.

Do ears with metabolic phenotypes increase with age?

An analysis of the numbers and percentages of ears that changed phenotypes with increasing age indicated that of those that changed, most changed to metabolic phenotypes. Specifically, 50.8% of ears transitioned to the metabolic + sensory phenotype and 23.6% of ears transitioned to the metabolic phenotype (Fig. 4). Moreover, these transitions to metabolic phenotypes occurred at older ages than transitions to other phenotypes. Finally, transitions to metabolic phenotypes were much more likely in females than males (76-85% were female), except for the transition from sensory to the metabolic+sensory phenotype, for which 70% were males.

Ongoing analyses designed to provide additional validation include assessing phenotypes with additional measures of auditory function measured longitudinally (including OAEs and speech recognition) and confirming with biological markers (genetics and otopathology from human temporal bones). For genetic analyses, audiometric phenotypes provide a framework beyond classifying older adults as either “affected” (hearing impaired) or “non-affected” (normal hearing). Currently, our approach is to search for genetic associations and structural variations in genes related to metabolic vs. non-metabolic phenotypes, which may also explain individual differences. Following that, we will initiate studies to determine the pathological and potential functional consequence of genetic variations as they relate to phenotypes of age-related hearing loss, largely through studies of human temporal bones. Such information can also drive the development of mouse models with specific mutations. Future studies will apply this phenotypic approach to understanding neural presbyacusis.

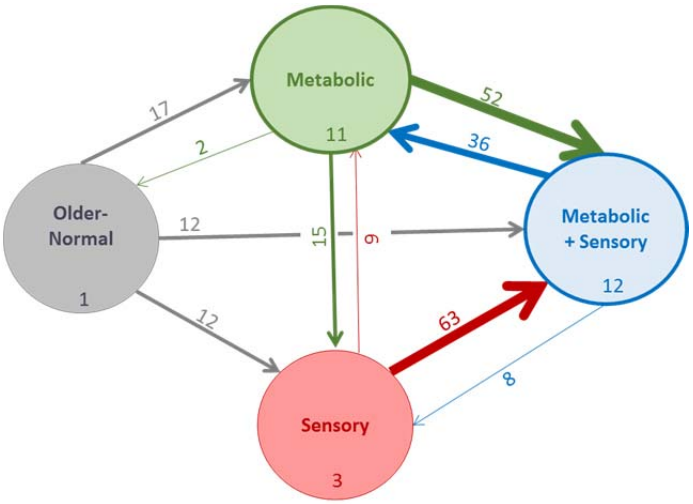

Fig. 4: Schematic illustrating transitions of ears from initial to final phenotypes. Thickness of arrows and numbers adjacent to arrows correspond to number of ears by initial and final phenotype. Thickness of borders around each circle and numbers within each circle indicate the number of ears that changed phenotype but ultimately returned to their initial phenotype. See text for additional details.

SUMMARY AND CONCLUSIONS

In summary, audiograms from middle age to older adults are consistent with predictions from animal findings associated with sensory and strial pathology. Audiograms appear to contain information about distinct presbyacusis phenotypes and also reflect large individual differences. A machine learning algorithm was trained to classify audiograms based on expert ratings (animal models) and fitted curve parameters. Classifications were consistent with phenotypic predictions based on thresholds, age, gender, and noise history. Analysis of longitudinal data showed a majority with stable phenotypes over time, even while hearing loss was increasing. The remainder showed changes in phenotypes with increasing age, with the most common change to metabolic phenotypes. Changes in phenotype differed with age and gender, also consistent with metabolic presbyacusis increasing with age. In conclusion, audiometric phenotypes are consistent with the view of age-related hearing loss as a metabolic disorder rather than a sensory disorder.

ACKNOWLEDGEMENTS

This project is supported by a grant from the National Institutes of Health/National Institute on Deafness and Other Communication Disorders (NIH/NIDCD). Significant contributions from colleagues Kenneth I. Vaden, Mark A. Eckert, Lois J. Matthews, Richard A. Schmiedt, Bradley A. Schulte, and Jayne B. Ahlstrom are gratefully acknowledged.

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