Precision Diagnostics for Complex Sensorineural Hearing Loss

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Abstract. Sensorineural hearing loss is a complex pathology characterized by variable combinations of inner and outer hair cell (IHC/OHC) injury, reduced endocochlear potential (EP), and cochlear synaptopathy. An umbrella diagnosis of SNHL belies the diversity of possible underlying cochlear pathophysiology profiles and the associated divergent listening outcomes among patients with similar audiograms. Current clinical tools and guidelines do not adequately capture this diversity in hearing, hindering individualized audiological management and targeted treatments. Precision diagnostics are especially critical for determining candidacy for new pharmacological interventions that target specific peripheral dysfunctions. Our diagnostic approach addresses the complexity of underlying pathologies by using a battery of biomarkers rather than a single metric of hearing. This method helps to tease apart the relative contributions of different pathologies to hearing outcomes. In addition, our coordinated battery in multiple pre-clinical chinchilla models of SNHL and in humans with SNHL not only examines isolated pathologies but also explores potential interactions among various sources of cochlear dysfunction. Analyses show significant variability in biomarker profiles among individuals with similar audiometric thresholds. In humans, individuals with similar hearing thresholds can diverge both in their biomarker profile and their behavioral outcomes. This dataset begins to evaluate our battery of biomarkers, which provides opportunities to explore machine learning techniques geared towards precision diagnostics and is a valuable first step toward understanding the mechanisms of cochlear dysfunctions that lead to individual differences in hearing outcomes.

INTRODUCTION

Though sensorineural hearing loss (SNHL) is considered a single clinical category, numerous prior studies in both animal models and humans show that most acquired hearing losses are a composite of multiple cochlear and neural pathologies. To acknowledge this mix of mechanisms that contribute to hearing disorders, and as a step toward establishing systematic terminology for precision diagnostics in audiology, we use the term *complex SNHL* to refer to peripheral hearing loss that results from a combination of physiological factors in the cochlea and auditory nerve. In the fields of genetics and precision medicine, the term complex is used to describe disorders that have multifactorial etiologies or where response to treatment can be highly variable [1]. A patient's specific profile of cochlear pathology is likely to correlate with perceptual outcomes and benefit from a given treatment, given the differential effects of cochlear pathologies on neural processing of sound.

A precision medicine approach, which attempts to tailor treatment to an individual's specific dysfunctions, can lead to more effective treatments for hearing loss by moving beyond generic gain adjustments to compensate for loss of audibility. Current hearing aid fittings rely on audiologist expertise and patient feedback, so greater initial customization could reduce clinical time, guesswork, and cost. Furthermore, pharmaceutical interventions and gene therapies for hearing loss have the potential to revolutionize treatment for hearing loss. However, the lack of appropriate diagnostics to determine the ideal candidates for treatment remains a significant barrier. Improved diagnostic tools that are sensitive to specific pathologies, even in the context of other cochlear dysfunctions, are needed

to understand the relationship between peripheral dysfunction and perception and to effectively administer targeted treatment options.

Cross-Species, Multi-metric Profiling of SNHL

All profiling approaches in human subjects must contend with two key issues. First, pathologies cannot be confirmed *in vivo* for human subjects. Second, the complexity of SNHL means that individual factors contributing to hearing loss may interact in unpredictable ways. Thus, a single metric of hearing is unlikely to disambiguate between multiple contributing factors. Our cross-species study of physiologic precision diagnostic tools addresses these challenges. In animal models, we control hearing loss etiology and obtain pre- and post- exposure measurements to measure the effect of specific pathologies on our biomarkers. In human subjects, we can link biomarkers of sensorineural pathology to specific functional hearing outcomes. Furthermore, our approach utilizes a battery of biomarkers rather than a single metric of hearing. This multi-metric approach has already shown promise—joint-otoacoustic emission profiles, which examine the relative distortion and reflection components of otoacoustic emissions (OAEs), likely disambiguate specific aspects of cochlear pathologies that are missed by either OAE-type alone. Mixed-effects models and machine learning approaches are important to these types of data analyses as pattern classification across multiple measures of cochlear pathophysiology, multiple frequencies, and each group is a challenge using simple statistical techniques.

Figure 1 describes the overarching goals of this work. In chinchillas, we compare biomarker profiles in multiple pre-clinical SNHL models, and in human subjects we will see how biomarker profiles correlate with speech-in-noise perception. Given the use of coordinated biomarkers across species, we will then label SNHL subtype clusters in humans with predicted underlying pathologies. This paper describes our initial approach toward a cross-species framework for physiological profiling of SNHL subtypes with a focus on the animal data.

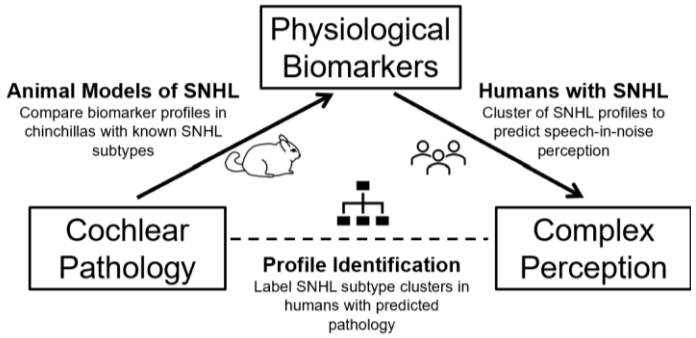

FIGURE 1. Schematic of the goals for this project. Pre-clinical chinchilla models of SNHL demonstrate how physiological biomarker profiles depend on underlying cochlear pathologies. The same biomarker battery in addition to speech-in-noise measures are collected in human subjects. Models that link biomarker profiles in chinchillas with known cochlear pathologies will be developed to predict SNHL pathology in human subjects.

METHODS

Biomarkers of Sensorineural Hearing Loss

The current biomarkers were chosen based on their sensitivity to specific cochlear pathologies and their translational potential. All human and chinchilla subjects were assessed with the same core battery of measures. Hearing thresholds were measured behaviorally via audiometry in humans and via auditory brainstem response (ABR) testing in chinchillas. Distortion product otoacoustic emissions (DPOAEs) and stimulus frequency otoacoustic emissions (SFOAEs) were measured, representing both distortion and reflection type emissions. Stimuli were swept across frequency to capture the fine structure of the response and amplitudes shown are based on an SNR-weighted average at half-octave steps from 500-16000 Hz. Wideband middle ear muscle reflexes (MEMRs) were measured using a wideband noise elicitor and a click probe. The envelope following response (EFR) to a rectangular amplitude modulated (RAM) tonal carrier was measured with either subdermal needle electrodes in chinchillas or a 32-channel EEG system in humans. Chinchillas were sedated for electrophysiological assessments with 20-40 mg/kg ketamine and 4 mg/kg xylazine. For the remaining physiologic assessments, chinchillas remained awake and were positioned comfortably in a restraint device [2].

To improve diagnostic precision for SNHL, extraneous sources of variance must be minimized so that the measure is maximally sensitive to the pathology it is designed to identify [3]. Sources of variance can include individual factors,

such as anatomical differences that affect in-ear acoustics or electrode contact, as well as methodological issues. For example, clinical otoacoustic emission systems calibrate stimulus levels at the microphone in the probe rather than adjusting the level to be stable at the eardrum. This calibration technique results in greater variability of sound pressure level reaching the eardrum and is highly sensitive to probe placement. Small variations in the depth of insertion alter the resonant frequency of the ear canal, leading to significant changes in stimulus and OAE levels. Additionally, our pilot testing revealed that sedation affects OAEs: we found that DPOAEs in sedated chinchillas were higher than when the same animals were assessed while awake. If differences in calibration and other non-hearing factors significantly impact a measurement, the often-subtle effects of a given cochlear pathology may be masked. Although the measurements used in this study have common clinical correlates, we have optimized each measure in the biomarker battery to reduce extraneous variance where possible (e.g., FPL/EPL calibrations for OAEs and MEMR).

All data were collected in sound-treated and electrically shielded booths. All procedures were approved by Purdue's IACUC (#1111000123) and IRB (#1609018209).

Exposure Paradigms

Following baseline data collection, chinchillas underwent one of four treatments to induce a specific profile of SNHL. Eight chinchillas were exposed to 100 dB SPL octave-band noise (OBN) centered at 1 kHz for two hours to induce a temporary threshold shift (TTS group). Otoacoustic emissions and MEMR measured 1-2 days after exposure confirmed the noise exposure induced TTS. This exposure results in ~60% loss of IHC ribbon synapses throughout the cochlea, with little to no outer hair cell loss or elevation in ABR thresholds [4]. Six chinchillas were given 38 mg/kg (i.p.) carboplatin (CA group). At the right dosage in chinchillas, carboplatin selectively damages IHCs, sparing OHCs. This dose has been shown to result in a 5-10% loss of IHC with widespread stereocilial damage [5]. Four chinchillas received 10 mg/kg (i.m.) gentamicin (GE) followed by 40 mg/kg (i.v.) ethacrynic acid 1.5-2 hours after the first injection (GE group) [6]. Though histological reports suggest this causes a gradient of OHC loss, increasing from the apex to the base without IHC damage, we have found this exposure to result in variable degrees of more severe hearing loss than anticipated. Lastly, eight chinchillas underwent a 116 dBC 500 Hz OBN exposure to induce a permanent threshold shift (PTS group) [7]. Animals were sedated with 4 mg/kg xylazine and 20-40 mg/kg ketamine for this noise exposure procedure. Histological analyses have shown that this exposure results in complex SNHL characterized by a loss of OHCs, IHC stereocilia damage, and synaptopathy. The TTS, CA, and PTS groups were balanced with an equal number of male and female chinchillas. The GE cohort were all males. These four experimental paradigms allow for multiple comparisons of different types of underlying cochlear pathology across a range of audiometric thresholds. Post-exposure testing was completed two weeks after the exposure for the TTS, CA, and PTS groups, and 1-2 weeks after exposure for the GE group.

RESULTS

Pre-Clinical Chinchilla Models of SNHL

Baseline responses were generally identical across the groups for each of the measures, but responses varied both across and within exposure paradigms. As expected, neither the synaptopathic noise exposure (TTS) nor the carboplatin exposure significantly elevated audiometric thresholds (Fig. 2a). In the CA group, one chinchilla showed a significant threshold elevation after exposure (mean increase of 31.1 dB SPL; Fig.2a dotted blue), but the average change in threshold for the remaining chinchillas was less than 10 dB (Fig. 2a, solid blue). Both GE and PTS showed elevated thresholds, but ABR thresholds were much higher in the GE group with this exposure paradigm. The PTS exposure resulted in a moderate increase in ABR thresholds across the frequencies tested on average, but the degree of hearing loss varied across individuals; some chinchillas showed little to no change in thresholds. In this small sample, the severity of hearing loss induced appeared to be related to the dosage of ketamine the animal received during exposure. Animals that received 20-30 mg/kg ketamine sustained more hearing loss than those that received 40 mg/kg ketamine.

Increases in ABR thresholds were generally associated with decreases in DPOAE amplitudes after exposure (Fig. 2b). Despite having equivalent post-exposure ABR thresholds, TTS and CA exposed animals show small but consistent differences in DPOAE amplitudes. On average, DPOAE amplitudes were reduced slightly across the frequency range for the TTS group (Fig. 2b, red) but are elevated in the CA animals (Fig 2b, blue). PTS and GE animals showed a significant reduction in DPOAE amplitudes. The variability within the PTS group was evident in

the DPOAE amplitudes, where some chinchillas showed little to no change in DPOAE amplitude while others showed a ~20 dB decrease. At baseline, chinchillas have very robust DPOAEs with amplitudes in the range of 20-40 dB; SFOAE amplitudes are often much smaller. The average change in SFOAE amplitude (Fig. 2c) was generally less than that for DPOAEs across all exposure groups and more variable across frequency.

WB-MEMR and EFR-RAM have both been proposed as diagnostics of cochlear synaptopathy. Consistent with this expectation, the TTS group had higher MEMR thresholds (Fig. 2d) after exposure and reduced EFR magnitudes (Fig. 2e). Though the CA group also showed a decrease in EFR magnitude, it did not show an increase in MEMR threshold, highlighting the need for a multi-metric approach to differentiate these groups. PTS resulted in elevated MEMR thresholds (Fig. 2d) but again the degree of change varied across individual chinchillas. We also found that some animals in the PTS group showed an enhancement of the EFR, while others were unaffected (Fig. 2e). The GE group showed a significant increase in MEMR thresholds and a significant reduction in EFR magnitude, consistent with the degree of hearing loss.

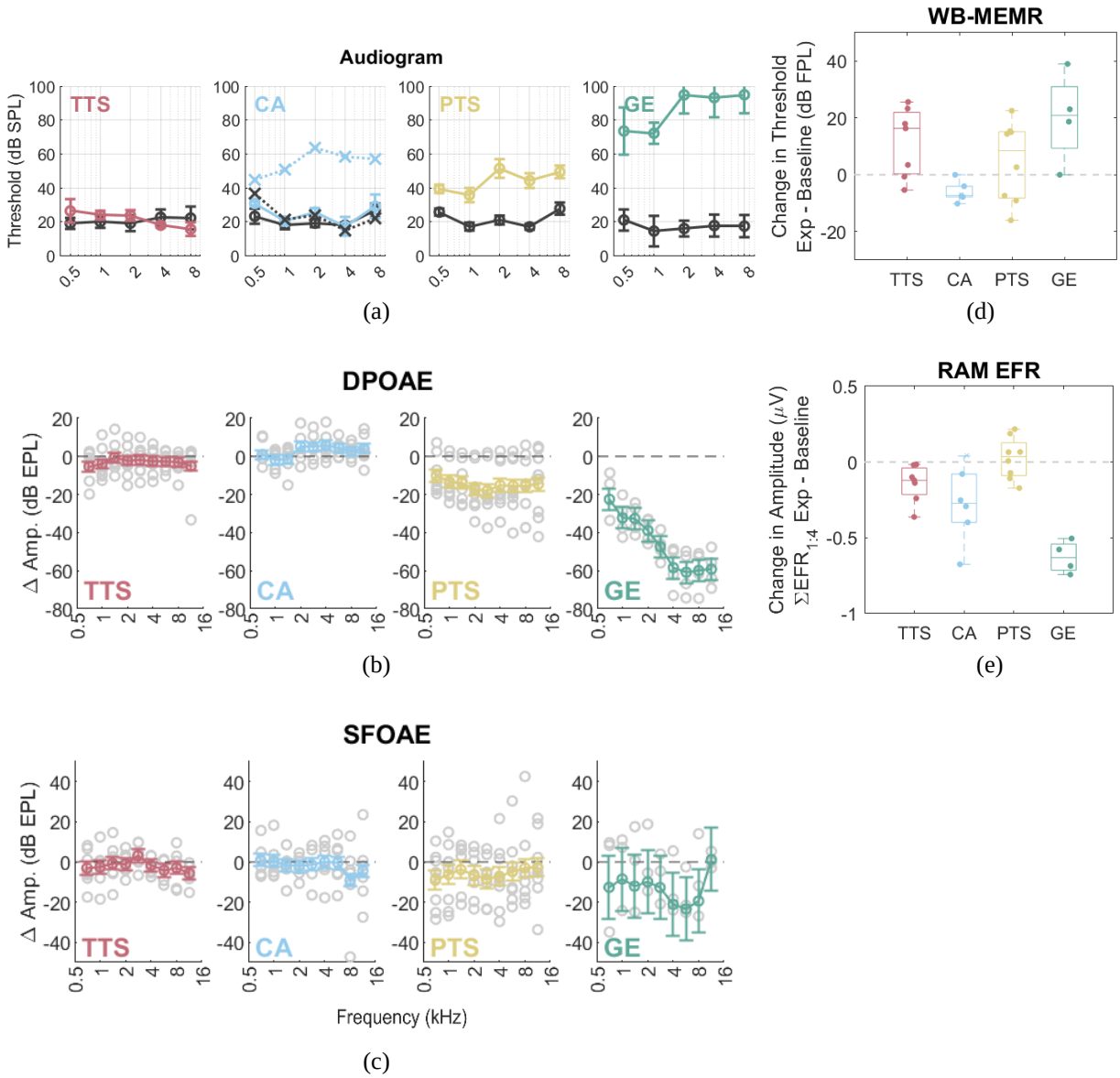

FIGURE 2. Summary results from each of the four exposure groups. **(a)** ABR audiograms with pre-exposure thresholds in black and post-exposure thresholds in color. **(b)** Change in DPOAE amplitudes and **(c)** change in SFOAE amplitudes as a function of frequency. Gray circles show individual change in amplitudes while colored lines show group averages. **(d)** Change in WB-MEMR threshold and **(e)** change EFR magnitude with RAM stimulus. The one animal with a notable change in hearing in the CA group is represented by the dotted blue line in (a) and with an x marker in (b) through (e). Error bars show standard error.

Profile Identification

Although there is individual variability within each group, the pattern of responses across measures for each subtype of SNHL is both distinct and not entirely predicted by the audiogram. In cases of more significant hearing loss, such as the present GE cohort, the audiogram appears correlated with each of the other measures. However, when audiometric thresholds are unchanged or only mildly elevated, there is not a consistent relationship between the audiogram and other diagnostic measures of hearing. This is especially clear in the differences between the CA and TTS groups—thresholds are not different between the groups or from baseline, but they vary in EFR magnitude, WB-MEMR thresholds, and DPOAE amplitudes. These results support our hypothesis that using these biomarkers together is more predictive of the underlying etiology than an individual measure,

Machine learning approaches are useful for combining results across metrics to predict the exposure group to which the chinchilla belongs. Our preliminary analyses are promising. Principal Component Analysis (PCA) can be used to reduce the dimensionality of the inputs (multiple values from each pre- and post-exposure measure) to fewer factors. We have begun working with models to predict the exposure condition based on the biomarker profiles, but ultimately our goal is to associate the diagnostic profile with histology, regardless of the initial exposure paradigm.

Preliminary Findings in Humans with SNHL

A coordinated study in human subjects is ongoing to measure whether our diagnostic test battery predicts speech-in-noise outcomes. Understanding in complex environments is affected by both peripheral and cognitive processes such as selective attention [8], [9]. To account for variance from cognitive factors rather than peripheral pathology, we used three speech-in-noise tests that place different relative demands on peripheral or central factors [10]. First, subjects repeated sentences embedded in a multi-talker babble using QuickSIN [11], which is widely used in audiology clinics. Second, subjects completed the Modified Rhyme Test (MRT) using monosyllabic words [12] masked by a spectrally sparse inharmonic tone complex [13]. The masker is minimally modulated to maximize peripheral interactions between speech and masker (i.e., energetic masking) while minimizing informational masking, thereby increasing the sensitivity to peripheral damage. Third, participants completed the Matrix Sentence Test (MST) in which three competing talkers say individual words staggered in time, minimizing peripheral interactions while placing high cognitive demands. MST and MRT were administered with stimuli presented through a hearing-aid simulator programmed and verified to meet individual prescriptive amplification targets (DSLv5) [14], [15] for individuals with hearing loss.

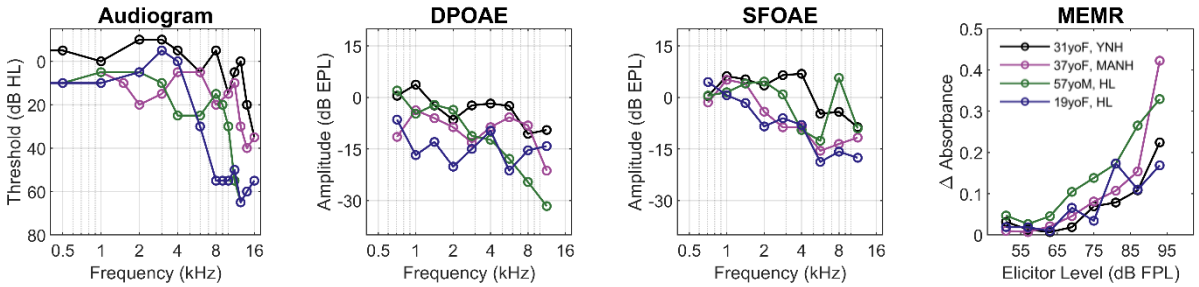

FIGURE 3. Results from four subjects, each in a different color. In all panels, black: 31-year-old female, purple: 37-year-old female, green, 57-year-old male with SNHL, blue: 19-year- old female with SNHL. **(a)** Audiometric thresholds from the right ear. Bone conduction testing confirmed all losses were sensorineural. **(b)** DPOAE amplitudes. **(c)** SFOAE amplitudes **(d)** WB-MEMR growth curves showing change in absorbance as a function of elicitor level.

Human subjects with both normal hearing thresholds and mild to moderate SNHL were recruited and classified into three groups based on audiometric thresholds from 250-8000 Hz and age as described in [20] (Young, normal hearing [YNH], middle-aged normal hearing [MANH], and hearing loss [HL]). Figure 3 shows the results from representative subjects across groups (YNH, black; MANH, purple; HL, green; HL, blue). The four subjects shown all have normal hearing sensitivity in the low frequencies but varying degrees of mild hearing loss in the high frequencies (Fig. 3a). Each of these four subjects exhibited some degree of extended high frequency hearing loss.

Comparison of the subjects across each measure reveals that although these subjects present with similar audiometric thresholds at some frequencies, their biomarker profiles differ. The subject with the best hearing thresholds (Fig. 3, black) generally shows the highest amplitude OAEs (Fig. 3b, c), but has one of the weakest MEMR responses (Fig. 3d). Similarly, the pattern of responses is not identical across DPOAE and SFOAEs (Fig. 3b, c), consistent with current understanding that the two types of OAEs provide complementary diagnostic information [16]. Differences in the EFR to RAM stimuli between individuals are also observed (data not shown). In the data collected so far, EFR magnitude appears to differentiate the YNH subjects from the MANH subjects, but both MANH and HL subjects have similarly poor EFR magnitudes. Speech-in-noise performance was best for the young, normal hearing subject in all three tests and poorer for the middle-aged and hearing-impaired subjects.

DISCUSSION

The results of this cross-species dataset provide insight into the sensitivity of individual precision biomarkers of sensorineural hearing loss and show the power of a multi-metric approach to SNHL profiling.

A challenge for the field is leveraging animal data, which can more readily establish the mechanistic basis of a given diagnostic profile, for development of sophisticated models that readily translate to human data analyses. Though the sample size of animal data may be smaller than what is possible in humans, especially if clinical diagnostic data or other database approaches can be leveraged, the data is often richer; induction of hearing loss is controlled, pre- and post-exposure measurements can be directly compared in the same animal, and histological confirmation of cochlear pathologies is possible.

While linear mixed-effects models can be utilized to evaluate the relationships between anatomical and diagnostic data in chinchillas, or between diagnostics and perception in humans, a type of machine learning known as transfer learning can be valuable for leveraging the power of coordinated cross-species studies. Transfer learning is the process of using models trained on one task (e.g., classification of chinchilla group) to perform a new task (e.g., classification of human SNHL subtypes based on diagnostic biomarkers). Transfer learning has been applied to the problem of audiogram classification to determine whether an active transfer learning process could speed audiogram measurement by using the intensity at one frequency to predict the intensity at another [17]. Transfer learning has also been applied to ABR threshold estimation [18]. Although these new methods have already shown promise in the field of audiology, transfer learning requires additional considerations about how the results of the original model will be scaled to map onto the new data. Our cross-species framework is proving to be useful for identifying relevant differences within and across species that will make this analysis more powerful.

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COMMENTS AND DISCUSSION

[Jackson Graves]: In humans, do you expect that complex SNHL is often mostly determined by one factor over others, like OHC loss over IHC loss? Or is it more likely that most cases of SNHL in humans have a mix of underlying etiologies? If the latter, would that complicate the potential clinical benefit of performing these precision diagnostics?

[Samantha Hauser]: Cases of overt hearing loss will almost always have some OHC component, but temporal bone histology seems to show that IHC loss, cochlear synaptopathy, auditory nerve degeneration, and stria vascularis atrophy are also common. This suggests that most people with acquired SNHL probably do have a mix of pathologies, but the relative proportion of each pathology may differ across the length of the cochlea and across individuals. How much subtle differences in pathophysiology profiles matter to outcomes and whether there are typical or common profiles will be explored in our human data. I'd hypothesize that there is a link between a given SNHL profile and factors like noise exposure history, medical history, and age. Ultimately, the clinical benefit of precision diagnostics is heavily connected to the available treatments—the more we know about what is causing the hearing problem, the more we can develop or apply targeted treatments, manage patient expectations for treatment, and predict or manage progression.

[Christopher Bergevin]: If you have the phase information for the DPOAEs, and there is sufficient frequency resolution, you can do the unmixing and maybe get differential sorts of information.

[Samantha Hauser]: I am unmixing the distortion and reflection components of the DPOAEs in my analysis given we are using swept tone stimuli. I am also measuring SFOAEs, and have phase information from that, so we can look more at the relationship between distortion and reflection components of OAEs and estimate tuning.