2pPPa2. Understanding subtle changes in auditory function with otoacoustic emissions

Linda J. Hood*, Shanda Brashears, Glenis R. Long and Carrick L. Talmadge

*Corresponding author's address: Vanderbilt University, Nashville, TN 37232, linda.j.hood@vanderbilt.edu

Otoacoustic emissions (OAEs), a sensitive measure of cochlear processing, may be altered by subtle changes in auditory function that are not measureable by usual clinical methods. We studied auditory function in carriers of genetic mutations related to recessive hereditary hearing loss where we hypothesized that carrying a single mutated copy may compromise auditory function and be reflected when sensitive assays are used. Parents who were confirmed carriers of recessive mutations associated with GJB2 (connexin 26) and obligate carriers of mutations related to recessive hearing loss not related to GJB2 mutations were compared to age and gender matched control subjects. All participants had normal pure tone and middle ear responses. Metrics included transient evoked OAEs and distortion product OAE fine structure. DPOAE fine structure was specifically explored based on the ability to isolate components that could be differentially affected by genetic mutations. The results support the hypothesis that carriers of gene mutations related to hearing loss display subtle auditory abnormalities that can be observed in OAEs. These findings will be related to other studies of subtle changes in OAEs in disorders affecting auditory function. [Supported by NIH NIDCD R01-DC03679 and VU Development Funds]
INTRODUCTION AND BACKGROUND

Hearing loss can be the consequence of a broad range of medical, environmental and hereditary factors. Understanding the characteristics of hereditary hearing loss can be helpful in defining specific mechanisms and functions of genes underlying various forms of hearing loss. Study of both individuals with hearing loss and those who are carriers of genes related to hearing loss may contribute to the understanding of the functional characteristics of genes and their phenotypic expression.

Individuals who carry one normal copy and one abnormal (mutated) copy of a gene related to recessive hearing loss typically display no apparent clinical hearing deficit. Some, but not all, past studies suggest subtle auditory abnormalities in obligate carriers of genes related to hearing loss (Anderson and Wedenberg, 1968; Marres and Cremers, 1989; Meredith et al., 1992; Stephens et al., 1995; Berlin et al., 1999; Franze et al., 2005). These reports were based upon studies of auditory threshold contours and middle-ear muscle reflex thresholds. Other studies have found no differences (Konigsmark et al., 1970; Cohen et al., 1996; Wagenaar et al., 1996). Recent reports using otoacoustic emissions (OAE) provide support for subtle auditory abnormalities in the auditory function of carriers of genes for hearing loss (Morell et al., 1998; Engel-Yeger et al., 2002).

Otoacoustic emissions provide a sensitive measure of cochlear processes associated with cochlear outer hair cell system function. Since the majority of inherited (and other) forms of hearing loss affect cochlear function, OAEs are a particularly sensitive research and clinical tool. This report focuses on measures of cochlear function with OAEs in persons who are carriers of genes related to hereditary hearing loss and the comparison of their results to a control group. The particular focus of the current analysis is on fine structure of distortion product otoacoustic emissions (DPOAE) that allows separation of contributing mechanisms that underlie the origins of OAEs (e.g., Talmadge et al., 1999). Responses to transient OAEs are also described for comparison.

METHODS

Three groups of subjects were carefully matched for auditory sensitivity and age, two factors that can influence OAE amplitude. The primary inclusion criterion beyond auditory sensitivity and age was the completion of measures of DPOAE fine structure. Only 5 subjects in the GJB2 mutation (Cx26) group met all criteria and thus all three groups were restricted to 5 subjects for the present analyses. In addition to auditory testing, all subjects in all groups had full sequencing of the GJB2 gene. Subjects with GJB2 mutations (Cx26 group, n=5) all had 35delG mutations. Two additional groups showed no mutations in GJB2 that have been related to hearing loss. One group in this category consists of obligate carriers of non-syndromic recessive (NSR) hereditary hearing loss based on the presence of two or more offspring with hearing loss (NSR group, n=5). The third group is a control group of subjects with no history of familial hearing loss and no known GJB2 mutations (control group, n=5).

Subjects were all females of the following ages: Cx26 group mean age 35.2 years (range 22-47 years); NSR group mean age 34.4 years (range 30-39 years) and control group mean age 35.4 years (range 22-47 years). The male subjects who completed the DPOAE fine structure testing all had slight hearing loss at one or more frequencies and thus were excluded. Subjects were individually matched for age as closely as possible. Pure-tone thresholds also were closely matched across groups. No threshold for octave and inter-octave frequencies from .25-8kHz was poorer than 15 dB HL (Hearing Level). Pure-tone averages (.5, 1, 2kHz) were left 4.7 and right 4.3 dB HL for the Cx26 group, left 3.7 and right 3.2 dB HL for the NSR group, and left 3.3 and right 3.8 dB HL for the control group. Middle-ear measures were normal for all subjects.
DPOAEs were recorded with an Otodynamics ILO system using tone pairs with an f1:f2 ratio of 1.22 and test levels of L1=65 and L2=55 dB SPL. Responses to tone pairs were obtained in 12 Hz steps from 1-3kHz and in 24 Hz steps above 3kHz. DPOAE data were analyzed by separating nonlinear distortion and linear reflection sources with software developed by one of the authors (CLT). The program is based on a program NIPR, a MATLAB-based analysis program that uses an Inverse Fast Fourier Transfer (IFFT)-based algorithm to convert the frequency domain complex-valued DPOAE amplitude to the time-domain, where a time window filter is applied to separate the sources based on their phase lag. The analysis program used in other studies (e.g., Long et al., 2008) was adapted (by CLT) to accommodate the larger frequency step-size used by the Otodynamics system. Data were only included for F2 frequencies below 3kHz where the step size was 12 Hz; the larger step size (24 Hz) above 3kHz proved problematic in the analysis paradigms employed. The measures extracted from the data were the amplitude in dB SPL for the unseparated DPOAE data and separated nonlinear distortion and linear reflection sources. Amplitude was calculated across the frequency range from 1.5 to 3kHz.

Transient otoacoustic emissions (TEOAE) were recorded to 80-microsecond clicks presented at 65 dB peak sound pressure and at a rate of 50 stimuli/second using an Otodynamics ILO system. All clicks were of the same polarity and intensity. Responses were averaged to 260 stimuli using artifact rejection. Overall rms amplitude was measured.

RESULTS

The analysis reported here focuses on DPOAE fine structure and characteristics of the separated distortion and reflection components. Mean DPOAE amplitudes for overall DPOAEs, the nonlinear distortion separated component, and the linear reflection component were all higher for the control group than for either the GJB2 mutation (Cx26) carrier group or the NSR group (see Figure 1). Mean overall DPOAE amplitude was 7.3 and 9.3 dB SPL for left and right ears, respectively, for the control group and mean overall DPOAE amplitude was 3.7 and 3.5 dB SPL for left and right ears, respectively, for the GJB2 (Cx26) group. Carriers of mutations associated with non-syndromic recessive hearing loss (NSR group), but not GJB2 mutations, also showed reduced DPOAE amplitude compared to control subjects (mean overall DPOAE amplitude of 4.3 and 4.5 dB SPL for left and right ears, respectively).
FIGURE 1. Mean (and +/- one standard error) amplitude for overall DPOAEs (DP fine overall), the separated distortion (generator: DP fine gen) and reflection (DP fine refl) components for Control, GJB2 (Cx26), and NSR subject groups.

Comparison of responses from the right and left ears suggests additional differences between groups. Overall, distortion component, and reflection component amplitudes are higher for the right than left ears for the control group. However, for the GJB2 mutation group amplitudes were similar for both ears with right ear amplitudes slightly lower than left ear amplitudes. The NSR group also displayed very little difference in amplitude between the right and left ears.

TEOAEs were lower in amplitude for carriers of the GJB2 mutation. Mean TEOAE amplitudes were 12.1 and 12.7 dB SPL for left and right ears, respectively, for control subjects and 6.8 and 8.1 dB SPL for left and right ears of carriers of single copies of GJB2 mutations. Carriers of mutations associated with non-syndromic recessive hearing loss, but not GJB2 mutations, also showed reduced amplitude (9.1 and 8.8 dB SPL for left and right ears, respectively) in TEOAEs compared to the control subjects.

DISCUSSION

The findings reported here are consistent with previous reports and our initial observations of reduced OAE amplitude in carriers of GJB2 mutations (Morell et al., 1998) and with the report of Engel-Yeger et al. (2002). The OAE characteristics observed are also consistent with the cochlear nature of the associated hearing losses, the importance of connexin 26 in maintaining normal cochlear homeostasis, and the apparent relationship between OAEs and cochlear integrity. While genetic heterogeneity in the NSR group is likely, the OAE amplitude reductions seen in this set of subjects supports potential relationships between the various genes involved and cochlear function. As more genes are identified and sequencing becomes more readily available, OAEs may be informative in other genetic types of hearing loss as well.

Observation of right-left asymmetries in transient and distortion product otoacoustic emissions has varied among studies with some reports demonstrating higher right ear amplitude in response level or signal-to-noise level (e.g., Khalfa et al., 1998; Kei et al., 1997; Keogh et al., 2001). Other studies have not observed differences in TEOAE or DPOAE amplitude between ears (e.g., Pavlovcinova et al., 2010). While there is an apparent amplitude difference between ears in the control group in the present study that is not observed in the carrier groups, more subjects are needed to fully understand possible influences of carrier status on OAE asymmetry.

Further study of DPOAE fine structure is needed in additional subjects with GJB2 mutations related to the production of connexin 26 and with other underlying genetic factors to fully understand the impact of genetic mutations on cochlear processes. We plan to continue work in this area with use of hardware, software and methods that allow smaller step sizes and refined analyses. Indeed, we have used the methods of Talmadge and Long (Talmadge et al., 1999; Long et al., 2008) in studies involving patients with Type 1 diabetes (Spankovich et al., 2010), studies of individuals in various age groups (Hatton et al., 2012), and plan to continue exploration of medial olivocochlear effects on DPOAE fine structure. In these instances DPOAE fine structure also has proved informative.

Based on the data presented in this analysis, the results found with OAE measures support the hypothesis that carriers of genetic mutations related to hearing loss display subtle changes in auditory function. Otoacoustic emissions provide a sensitive assay that allows for further understanding of auditory function when examined in combination with earlier studies that reported differences in pure-tone and middle-ear muscle reflex thresholds in carriers. Further exploration of cochlear function with
sensitive methods such as OAEs may also improve management strategies for individuals with hereditary hearing loss and their families and, in the future, be of possible use in preventing hearing loss.

ACKNOWLEDGEMENTS

This research was supported by NIH-NIDCD R01-DC03679 and Vanderbilt University Development Funds. The authors thank Bronya J. B. Keats, Ph.D., for overseeing the molecular genetics aspects of subject characterization and Charles I. Berlin, Ph.D., for scientific input into the project.

REFERENCES


