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The natural history of *OTOF*-related auditory neuropathy spectrum disorders: a multicenter study

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CONFLICT OF INTEREST STATEMENT:

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Abstract

OBJECTIVE: To refine the natural history and genotype-phenotype correlations of *OTOF*-related auditory neuropathy spectrum disorders (ANSND) through the analysis of audiograms and distortion product otoacoustic emissions (DPOAEs) in a diverse cohort of individuals with hearing loss.

METHODS: Audiograms and DPOAEs were collected from individuals diagnosed with *OTOF*-related ANSD by comprehensive genetic testing and from the published literature. Comparative analysis was undertaken to determine genotype-phenotype relationships using a Monte Carlo algorithm.

RESULTS: 67 audiograms and 25 DPOAEs from 49 unique individuals positive for *OTOF*-related ANSD were collected. 51 unique *OTOF* pathogenic variants were identified of which 21 were missense and 30 were loss of function (LoF; nonsense, splice-site, copy number variants, and indels). There was a statistically significant difference in low, middle, and high frequency pure tone average hearing loss thresholds with missense/missense and LoF/missense genotypes (average: 70.9, 76.0, and 73.4 dB hearing loss) compared with LoF/LoF genotypes (average: 88.5, 95.6, and 94.7 dB hearing loss) via Tukey's test with age as a co-variate ($P = 0.0180, 0.0327,$ and 0.0347 respectively). Hearing declined during adolescence with missense/missense and LoF/missense genotypes with an annual mid-frequency threshold deterioration of 0.87 dB/year and 1.87 dB/year, respectively. 8.5% of measured frequencies via DPOAE were lost per year in individuals with serial tests.

CONCLUSIONS: Audioprofiling of *OTOF*-related ANSD suggests significantly worse hearing with LoF/LoF genotypes. *OTOF*-related ANSD causes a unique pattern of variably progressive autosomal recessive auditory neuropathy that may be amenable to gene therapy in selected clinical scenarios.

Keywords

OTOF; auditory neuropathy spectrum disorders; natural history; Audioprofiling

INTRODUCTION:

Permanent hearing loss is common in children and is most often attributable to a genetic etiology (Smith, Bale and White, 2005). Phenotypes range from nonsyndromic hearing loss (NSHL) to deafness in the context of multi-system organ involvement. To date, more than 150 genes and over 8,000 variants have been implicated in the pathogenesis of hearing loss (Azaiez *et al.*, 2018). This heterogeneity underscores the need for gene-specific phenotype studies.

OTOF encodes for the protein otoferlin, which is a member of the ferlin family of large transmembrane proteins essential for cell membrane fusion and vesicle formation. Expression of otoferlin has been reported only within the organ of Corti, vestibular hair

cells, and central nervous system. Otoferlin is an essential component of Ca^{2+} -dependent inner ear hair cell neurotransmitter exocytosis (Strenzke *et al.*, 2016; Hams *et al.*, 2017; Michalski *et al.*, 2017). Pathogenic variants in *OTOF* are linked to autosomal recessive nonsyndromic auditory neuropathy spectrum disorders (ANSD) and temperature-sensitive ANSD (Azaiez, Thorpe and Smith, 2021). Among affected patients with nonsyndromic ANSD, severity of hearing loss ranges from mild-to-moderate (Yildirim-Baylan *et al.*, 2014) to profound (Iwasa *et al.*, 2013).

To date, no relationship between *OTOF* genotype and the severity of associated hearing loss has been reported. The objective of this paper was to analyze audiograms in a diverse cohort of individuals with *OTOF*-related ANSD, and to correlate these data with patient age and genotype. As a proxy for outer hair cell function, otoacoustic emission testing was also analyzed. Defining the natural history of *OTOF*-related deafness is essential for counseling affected individuals and in the design of gene-therapy based clinical trials.

METHODS

DATA SOURCE –

Audiograms and DPOAEs were collected from individuals with *OTOF*-related ANSD after comprehensive genetic testing either in the Molecular Otolaryngology and Renal Research Laboratories (MORL), Iowa City, IA, USA or the Chinese Deafness Genetics Consortium (CDGC), Center for Medical Genetics, Southwest Hospital, Army Medical University, Chongqing, China. To collect additional clinical data, a literature review of *OTOF*-related deafness was completed and reported audiograms and DPOAEs were included. The literature search was conducted by reviewing the references for all variants annotated as likely pathogenic or pathogenic in the Deafness Variation Database (Azaiez *et al.*, 2018) and in ClinVar. Individuals with temperature-sensitive ANSD or only a single variant in *OTOF* were excluded. This study was approved by the Institutional Review Boards of both institutions. The data for variant interpretation was reported to the Deafness Variation Database (<http://deafnessvariationdatabase.org/>).

AUDIOMETRY –

The standard audiogram was recorded between 250 and 8000 Hz. Depending on age, hearing was tested by behavioral observation audiometry, visual reinforcement audiometry, or conventional audiometry. For inclusion in this study, at least 4 separate frequencies must have been tested. Only air conduction thresholds were included. Audiograms marked as “poor” reliability were excluded. To generate a single audiogram, the better hearing ear was used at each frequency, as previously described (Taylor *et al.*, 2013). Linear interpolation and extrapolation were used to approximate missing values between 250 and 8000 Hz. Frequency averages were defined as low (250, 500 Hz), middle (1000, 2000 Hz) and high (4000, 8000 Hz). In addition, we collected qualitative descriptions of audiometry for affected individuals reported in the literature. This included severity (e.g., “mild” to “profound”) and shape (e.g., “flat” or “upsloping”). Frequency-specific hearing loss severity was defined as mild (26–40 dB), moderate (41–55 dB), moderately severe (56–70 dB), severe (71–90 dB), or profound (>90 dB).

DISTORTION PRODUCT OTOACOUSTIC EMISSIONS –

The standard DPOAE had an f_2 (main test frequency) between 1000 and 8000 Hz. For inclusion in this study, at least 4 separate frequencies must have been tested in each ear. The raw data were examined to measure signal-to-noise ratio (SNR) at each f_2 frequency in both ears. An SNR <3 dB was considered “absent”, 3–6 dB was considered “borderline”, and >6 dB was considered “present and normal” when scoring the otoacoustic emission. In accordance with standard newborn hearing screening, at least 75% of the total tested frequencies must have been present and normal for a DPOAE to be considered a “pass”.

PLOT GENERATION AND STATISTICAL ANALYSES –

We performed statistical analysis to evaluate the significance of age and genotype versus severity of hearing loss on audiometry. To account for sampling bias due to some affected individuals and siblings having multiple audiograms within the dataset, we utilized a Monte Carlo algorithm (Walls *et al.*, 2020). Low, middle, and high frequency groups were calculated for each audiogram. With the Monte Carlo algorithm, one audiogram was randomly selected from each family included in our cohort, then an analysis of variance (ANOVA) test was performed based on genotype versus frequency group hearing level followed by Tukey’s test, with age as a covariate. This resulted in a single audiogram being included from each unique family for each test iteration (a patient with multiple audiograms was counted as a single “family”). This process was repeated for 10,000 runs for each test, and the median P value of the runs was reported. Audioprofiles were generated for two age bins (0–10 and >10 years of age) utilizing a Monte Carlo algorithm after sampling a single audiogram from each family for each age bin, repeated for 10,000 iterations. Average hearing thresholds at each frequency between 250 and 8,000 Hz were utilized to generate the audioprofiles. To calculate the effect of age on hearing threshold, linear regression analysis was performed at each frequency for each genotype as previously described (Booth, Azaiez and Smith, 2020); these slopes were averaged to calculate the annual threshold deterioration by frequency group. Statistical analyses with the Monte Carlo algorithm were performed on RStudio v1.3.1093 software (Boston, MA). Data for the audioprofile generation with the Monte Carlo algorithm was generated using Python with the SciPy library (Virtanen *et al.*, 2020). Plot generation was performed using GraphPad Prism v9.0.0 software (San Diego, CA).

RESULTS

67 audiograms with quantitative data were identified from 49 unique individuals with *OTOF*-related ANSD, ranging from 0 to 23 years of age. Twenty-two of the 67 audiograms were obtained through a review of the literature, while the remaining 45 were obtained by the authors. 26 of 67 (38.8%) audiograms were obtained by conventional pure tone audiometry, 11 (16.4%) were obtained using behavioral observation audiometry, 8 (11.9%) were obtained using visual reinforcement audiometry, and for 22 (32.8%) audiograms, the methodology was not reported. Twenty-five DPOAEs were identified from 21 unique patients, ranging from 0 to 12 years of age. All the DPOAEs were obtained by the authors. Age at time of audiogram or DPOAE is summarized in Table 1. A further 184 cases

with *OTOF*-related deafness were identified in the literature with qualitative descriptions of audiograms. Age at time of audiogram was inconsistently reported in these cases.

VARIANTS –

For individuals with quantitative data available, there were 51 unique genetic variants (41 unique variants in the audiometry group and 35 unique variants in the DPOAE cohort, with 25 variants overlapping). Of these unique variants, 21 were missense, 11 were nonsense, 8 were frameshift deletions, 8 were splice-site mutations, 1 was complex frameshift insertion and deletion, 1 was a 12-base pair in-frame deletion, and 1 was a copy number variant involving an intragenic 5.6 kb deletion. All nonsense, frameshift, splice site, and copy number variants were grouped in a “loss of function” (LoF) category; the 12-base pair in-frame deletion was also included in the LoF group. In the audiometry cohort, 26 audiograms were associated with a missense/missense genotype, 13 were associated with a LoF/missense genotype, and 27 were associated with a LoF/LoF genotype. In the DPOAE cohort, the distribution was 4, 10, and 10, respectively. In one individual in the audiometry and DPOAE cohort, three *OTOF* variants were identified (1 nonsense, 1 missense and 1 splice-site variant); as this individual did not fit in to the missense/missense, LoF/missense, or LoF/LoF categories, they were excluded from the analysis. Type and location of the variants are listed in Figure 1. A full list of the variants in the raw audiometry and DPOAE groups are available in Supplemental Table S1.

QUANTITATIVE AUDIOMETRY –

The mean hearing loss across all frequencies in all 67 audiograms was 82.3 dB (standard deviation (SD) 22.1 dB). Mean low frequency hearing threshold was 78.1 dB (SD 21.2 dB), mean middle frequency hearing threshold was 84.2 dB (SD 22.4 dB), and mean high frequency hearing threshold was 82.3 dB (SD 21.8 dB). Hearing loss at specific frequencies is summarized in Figure 2. Audiograms and average hearing loss by age are summarized in Table 1. One audiogram was excluded from genotype-specific analysis because the associated individual had three potentially causative *OTOF* variants. Genotype-phenotype correlations are summarized in Table 2 and Figure 3. As measured by low, middle, and high frequency groups, 26 audiograms from missense/missense genotypes had a mean hearing loss of 69.6, 73.7, and 72.4 dB (SD 25.1, 25.4, and 23.3 dB), 13 audiograms from LoF/missense genotypes had a mean hearing loss of 73.5, 80.6, and 75.3 dB (SD 22.0, 22.4, 19.9 dB), and 27 audiograms from LoF/LoF genotypes had a mean hearing loss of 88.5, 95.6, and 94.7 dB (SD 11.0, 12.7, and 14.8 dB). These means were statistically significantly different using ANOVA testing with age as a co-variate with a Monte Carlo algorithm for low, middle, and high frequency groups ($P=0.0180, 0.0327, \text{ and } 0.0347$, respectively). Post-hoc analysis using two-sided Tukey’s test with age as a co-variate with a Monte Carlo algorithm showed a statistically significant difference between combined missense/missense and LoF/missense genotypes as compared with LoF/LoF at low, middle, and high frequency hearing thresholds ($P=0.0309, 0.0283, \text{ and } 0.0296$, respectively). Additional comparisons are summarized in Table 2.

Based on linear regression analysis, missense/missense and LoF/missense genotypes had a combined annual threshold deterioration of 1.09 dB/year, 1.25 dB/year and 0.58 dB/year at

low, middle, and high frequency groups, respectively; these rates of deterioration were not statistically significant ($P=0.0897$, 0.0566 , and 0.334 respectively). LoF/LoF genotypes had an annual threshold deterioration of 0.21 dB/year, 0.00 dB/year, and -0.75 dB/year at low, middle, and high PTAs, respectively; again, these rates of deterioration were not statistically significant ($P=0.522$, 0.993 , and 0.0833 respectively). These data are summarized in Table 3.

DISTORTION PRODUCT OTOACOUSTIC EMISSIONS –

Across all 25 DPOAEs, a median of 12 frequencies were measured in each DPOAE across both ears at an average age of 29.0 months (SD 30.8 months). In total, 294 frequencies were measured; of these, 209 (71.1%) were present and normal, 17 (5.8%) were present and abnormal (borderline), and 68 (23.1%) were absent.

Eight of 25 individuals (32.0%) had at least 75% of frequencies present and normal bilaterally. The average age at time of DPOAE was 21.1 months (SD 15.6 months) for these individuals and 31.3 months (SD 36.0) for those with less than 75% of frequencies present and normal bilaterally. These means were not statistically significantly different by unpaired t test ($P=0.454$). Sixteen of 25 individuals (64.0%) had at least 75% of frequencies present in one or both ears. The average age at time of DPOAE was 19.5 months (SD 13.5 months) for these individuals and 45.9 months (SD 44.7 months) for those with less than 75% of frequencies present and normal in at least one ear. These means were statistically significantly different by unpaired t test ($P=0.0365$).

Percentage of frequencies present by age group are summarized in Table 1. Genotype-phenotype correlations are summarized in Table 4. Of those patients with biallelic missense or LoF/missense genotypes, 2 of 14 (14.3%) had at least 75% of frequencies present bilaterally and 8/28 (28.6%) unilaterally, as compared with 5 of 10 (50.0%) bilaterally and 14 of 20 (70.0%) unilaterally with biallelic LoF genotypes. Likelihood of bilateral “pass” was not statistically significant between these groups, while likelihood of unilateral “pass” was ($P=0.0850$ and 0.0078 , respectively). There were no statistically significant differences for likelihood of having at least 75% of frequencies present and normal unilaterally or bilaterally between 0–3 and >3-year-old age groups for any genotype.

Serial DPOAEs were available in 4 patients, with an average of 17.2 months (SD 17.0 months) between the first and second DPOAE. In 5 of 8 ears (62.5%), there was a decline in frequencies present, while 1 of 8 (12.5%) showed no change and 2 of 8 (25.0%) showed an improvement (Figure 4). The ears that showed improvement were from the same patient, who had biallelic LoF mutations. In aggregate, the change in DPOAEs amounted to a loss of 12.2% of frequencies in all ears, and 8.5% of frequencies per ear per year over the 17.2-month period.

QUALITATIVE AUDIOMETRY –

45 articles associated with pathogenic and likely pathogenic *OTOF* variants from the Deafness Variation Database were reviewed, 18 of which contained qualitative descriptions of audiometry in *OTOF*-related nonsyndromic hearing loss (Yasunaga *et al.*, 1999, 2000; Adato *et al.*, 2000; Varga *et al.*, 2003, 2006; Rodríguez-Ballesteros *et al.*, 2003, 2008;

Rouillon *et al.*, 2006; Choi *et al.*, 2009; Santarelli *et al.*, 2009; Romanos *et al.*, 2009; Zadro *et al.*, 2010; Mahdieh *et al.*, 2012; Matsunaga *et al.*, 2012; Iwasa *et al.*, 2013; Yildirim-Baylan *et al.*, 2014; Jin *et al.*, 2014; Tang *et al.*, 2015). These data comprised 167 unique patients. At least 9 of the articles utilized *OTOFS* Sanger sequencing for diagnosis of the hearing loss and required the patients to have at least a severe hearing loss prior to undergoing Sanger sequencing. Of the genotypes for the 167 patients, 43 were missense/missense, 22 were LoF/missense, and 102 were LoF/LoF. Audiograms reported as less than severe (i.e., mild, moderate, or moderately severe) included 3 of 43 (6.98%) in patients with missense/missense genotypes, 1 of 22 (4.54%) in patients with LoF/missense genotypes, and 1 of 102 (0.98%) in patients with LoF/LoF genotypes.

When converting the previously described quantitative audiometry data to qualitative data based on speech/middle frequency PTA, 12 of 26 (46.2%) missense/missense patients were less than severe, 4 of 13 (30.77%) LoF/missense were less than severe, and 2 of 27 (7.4%) of the LoF/LoF were less than severe. These data were combined and analyzed by genotype using Fisher's exact test, which is summarized in Table 5. There was a statistically significant difference between missense/missense and LoF/LoF ($P = <0.0001$) as well as LoF/missense and LoF/LoF ($P = 0.0117$), but not between missense/missense and LoF/missense ($P = 0.438$).

DISCUSSION:

We report the first genotype-phenotype correlations from a large, multi-family dataset for *OTOF*. Biallelic missense and LoF/missense genotypes were associated with better hearing thresholds than biallelic LoF variants, which is similar to the correlations described for *GJB2* genotypes, the most common cause of autosomal recessive hearing loss (Azaiez *et al.*, 2004; Snoeckx *et al.*, 2005). In contrast to *GJB2*-related deafness, however, there was no statistically significant difference between biallelic missense and LoF/missense genotypes, which may reflect inadequate power as LoF/missense genotypes comprised the lowest number of individuals in this study. We also could not complete a domain specific-analysis, as variants were distributed throughout the entire otoferlin protein with the exception of the C2A domain, which had no pathogenic variants (Figure 1). In aggregate, we found that biallelic missense variants and LoF/missense genotypes cause severe hearing loss, while biallelic LoF variants cause profound hearing loss.

Audioprofiling of *OTOF*-related ANSD in this study and in familial studies by others (Chiu *et al.*, 2010; Yildirim-Baylan *et al.*, 2014; Webb *et al.*, 2016) suggests progression of hearing loss with age, though linear regression did not reach statistical significance from our cohort. Our study refines the likelihood of progression – we found that biallelic missense and LoF/missense genotypes show progression of hearing loss, while biallelic LoF variants, which present as profound hearing loss, do not. However, genotype is a stronger driver of outcome than age: in aggregate, individuals over the age of 10 with biallelic missense or LoF/missense genotypes had better hearing thresholds than individuals less than the age of 10 with biallelic LoF genotypes. Nonetheless, there is a wide standard deviation in the degree of hearing loss amongst patients even with similar genotypes suggesting that there

may be environmental factors or genetic modifiers that contribute to the observed differences (Walls *et al.*, 2020).

DPOAEs in genetic forms of ANSD, including *OTOF*, have been reported to decline with age (Kitao *et al.*, 2019). This outcome is hypothesized to reflect loss of outer hair cell function. In our dataset, there did not appear to be a large loss of frequencies with age – loss was limited by the small number of individuals with serial tests and the overall young age of our DPOAE cohort, with the oldest individual being 12 years old. For children who had DPOAEs performed after age 3, over 50% of tested frequencies remained present and normal. Only one child (at the age of 2 years) had complete absence of DPOAEs at all frequencies tested. Amongst patients with serial DPOAEs, an aggregate loss of approximately 8.5% of frequencies per year per ear was noted, which is a more moderate decline than noted by Kitao et al (Kitao *et al.*, 2019) in a cohort of DPOAEs in *OTOF*-related deafness. Our cohort demonstrates a large difference in a theoretical DPOAE screening result by genotype; 14.3% of children with biallelic missense or LoF/missense genotypes would “pass” a standard newborn hearing screening bilaterally (i.e., having at least 75% of tested frequencies present and normal) compared with 50.0% of children with biallelic LoF genotypes. Patients with LoF/missense genotypes were least likely to have a passing result, with zero out of 10 patients having a bilateral “pass”. The reasons for these differences are unclear, as the average hearing loss of all patients with *OTOF*-related hearing loss are well above the threshold of detection for standard DPOAE testing.

OTOF-related ANSD causes a unique pattern of variably progressive autosomal recessive deafness. A variety of founder mutations have been described in *OTOF*, including p.Gln829Ter in the Spanish population (Migliosi *et al.*, 2002), p.Val1778Phe in the Ashkenazi Jewish population (Webb *et al.*, 2016), p.Arg1939Gln in the Japanese population (Matsunaga *et al.*, 2012) and p.Glu1700Gln in the Taiwanese population (Chiu *et al.*, 2010). In our cohort, other than p.Glu1700Gln (MAF = 0.67% in the East Asian population in gnomAD), most of the variants are rare with an MAF less than 0.05%. We included 16 audiograms from six individuals from five unique families with biallelic p.Glu1700Gln mutations. Although hearing loss was progressive in all individuals, there was phenotypic variability associated with this genotype. At 1 to 2 years of age, most individuals had a mild-to-moderate hearing loss, while one had a profound hearing loss. Further studies of intra-familial phenotypic variability for individuals with biallelic *OTOF* variants will be helpful identify environmental or other factors that contribute to these markedly different outcomes, although compiling large datasets of audiograms from individuals with exactly the same genotype will be considerably more difficult than with autosomal dominant genes.

The current standard of care for patients with *OTOF*-related ANSD is cochlear implantation. As recently reviewed by Zheng and Liu (Zheng and Liu, 2020), patients with *OTOF* mutations generally have excellent outcomes for sound perception and speech recognition. No relationships between genotype and cochlear implantation response have been noted to date. These individuals in particular benefitted from cochlear implantation before 2 years of age. Abnormal transmission at the auditory synapse caused by genetic mutations, such as in *OTOF*, are bypassed by cochlear implantation, in contrast to other forms of ANSD that

affect primarily the auditory nerve and demonstrate mixed results after cochlear implantation (Teagle *et al.*, 2010; Shearer and Hansen, 2019).

Gene therapy for *OTOF*-related ANSD is an exciting new treatment possibility, with clinical trials set to begin in humans in 2021. Gene therapies for hearing loss may be protective against future decline, or ideally, reverse damage that has already occurred in the cochlea (Omichi *et al.*, 2019). Our data suggest that individuals with at least one missense variant experience a decline in their hearing over time compared to those with biallelic LoF variants, who generally have a profound hearing loss at birth. Those with 0% residual hearing prelingually will have limited efficacy of hearing restoration secondary to inadequate stimulation of the auditory cortex (Butler and Lomber, 2013) unless intervention is performed early in life. In contrast, a child born with a moderate-to-severe hearing loss that eventually progresses to a profound hearing loss may be more likely to derive benefit from cochlear implantation or gene therapy later in life due to their matured auditory system, although speech perception is currently the gold standard audiometric parameter to test before intervention. Individuals with biallelic *OTOF* variants, particularly biallelic LoF variants, must be promptly identified and referred for specialty care to derive the greatest benefit from treatment, including cochlear implantation and gene therapy.

There are several limitations to our study. The audiograms in our cohort were from multiple sources and conducted with different techniques depending on patient age. With qualitative audiograms, authors generally did not describe their cut-offs for degree of hearing loss. Test-retest threshold differences at high frequencies can be greater than ± 10 dB in approximately 1–5% of adults undergoing pure tone audiometry (Valente, Valente and Goebel, 1992; Schmuziger, Probst and Smurzynski, 2004), although we believe that the large number of audiograms in our study offsets this variability. Speech discrimination scores were rarely collected in this cohort of patients, many of whom were prelingual. Due to the importance of speech discrimination testing in auditory neuropathy, in future studies, genotype-phenotype correlations involving these tests would be helpful. For mutation type, patients were broadly grouped into missense/missense, LoF/missense, and LoF/LoF; there were insufficient numbers of studies to examine other differences in closer detail, such as comparing domains or splice site mutations versus frameshift mutations. DPOAEs are a valuable tool for assessing the function of outer hair cells but are not specific for outer hair cell function alone. It is possible that other conditions, such as otitis media, external ear anomalies, or congenital ossicular anomalies, led to false negative results in our cohort. These alternatives could not be ruled out without a thorough history and physical examination on each of the included individuals. Finally, a publication bias may exist with regards to raw audiometry in genetic deafness. For patients with a flat, profound hearing loss, researchers may simply describe the audiogram and not publish the raw data. This limitation is reflected in our Supplemental Table S2, in which most of the audiograms are qualitatively described as “profound”. Nonetheless, when these data were combined with the raw audiometry, a statistically significant difference in the qualitative severity remained. As most of our raw audiometry is comprised of patients who were diagnosed via comprehensive massively parallel sequencing without presupposition as to the diagnosis, this bias is minimized.

CONCLUSION:

Audioprofiling of *OTOF*-related ANSD suggests significantly worse hearing with LoF/LoF genotypes. *OTOF*-related ANSD causes a unique pattern of variably progressive autosomal recessive deafness that may be amenable to gene therapy in selected clinical scenarios.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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DATA AVAILABILITY:

Data supporting this study's findings are available within the article and its supplementary materials.

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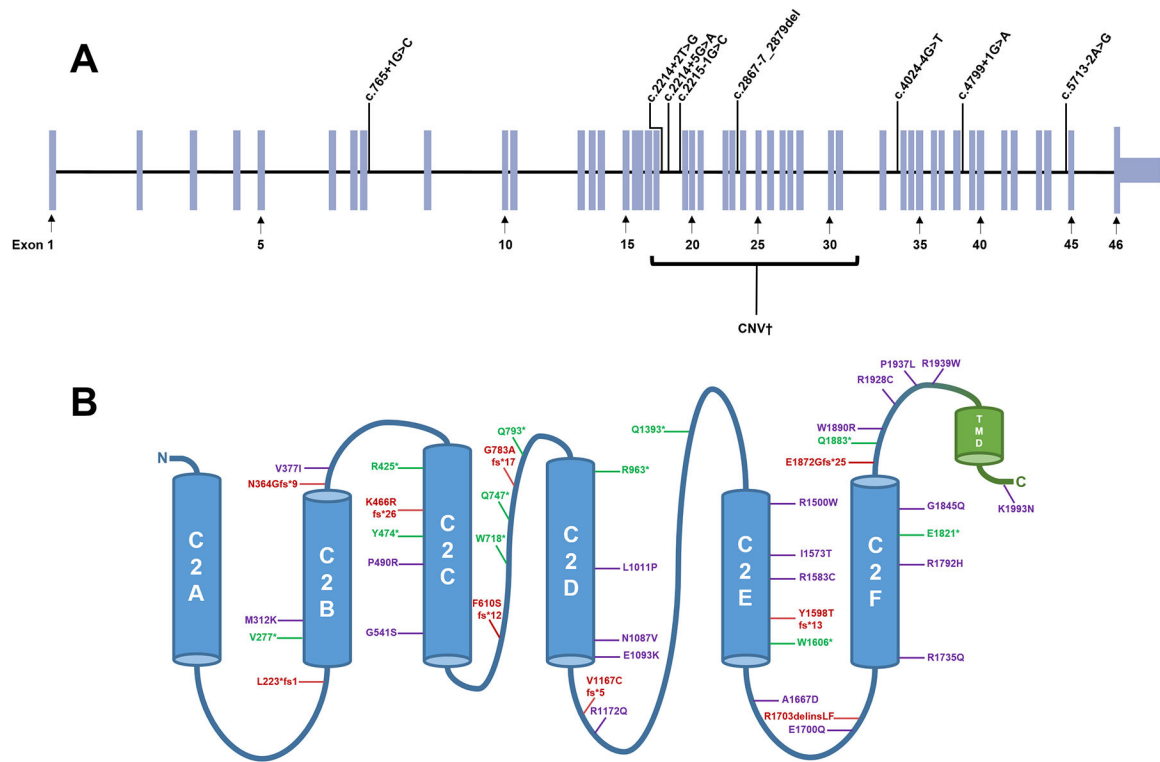


Figure 1: Map of causative *OTOF* variants included in this study, based on transcript NM_001287489.2. A, Illustration of splice site variants and CNVs. B, Illustration of coding variants; variants in red are deletions and/or insertions, variants in green are nonsense mutations, and variants in purple are missense mutations. CNV = copy number variant. † = this CNV is a deletion with breakpoints of chromosome 2:26695190–26700779.

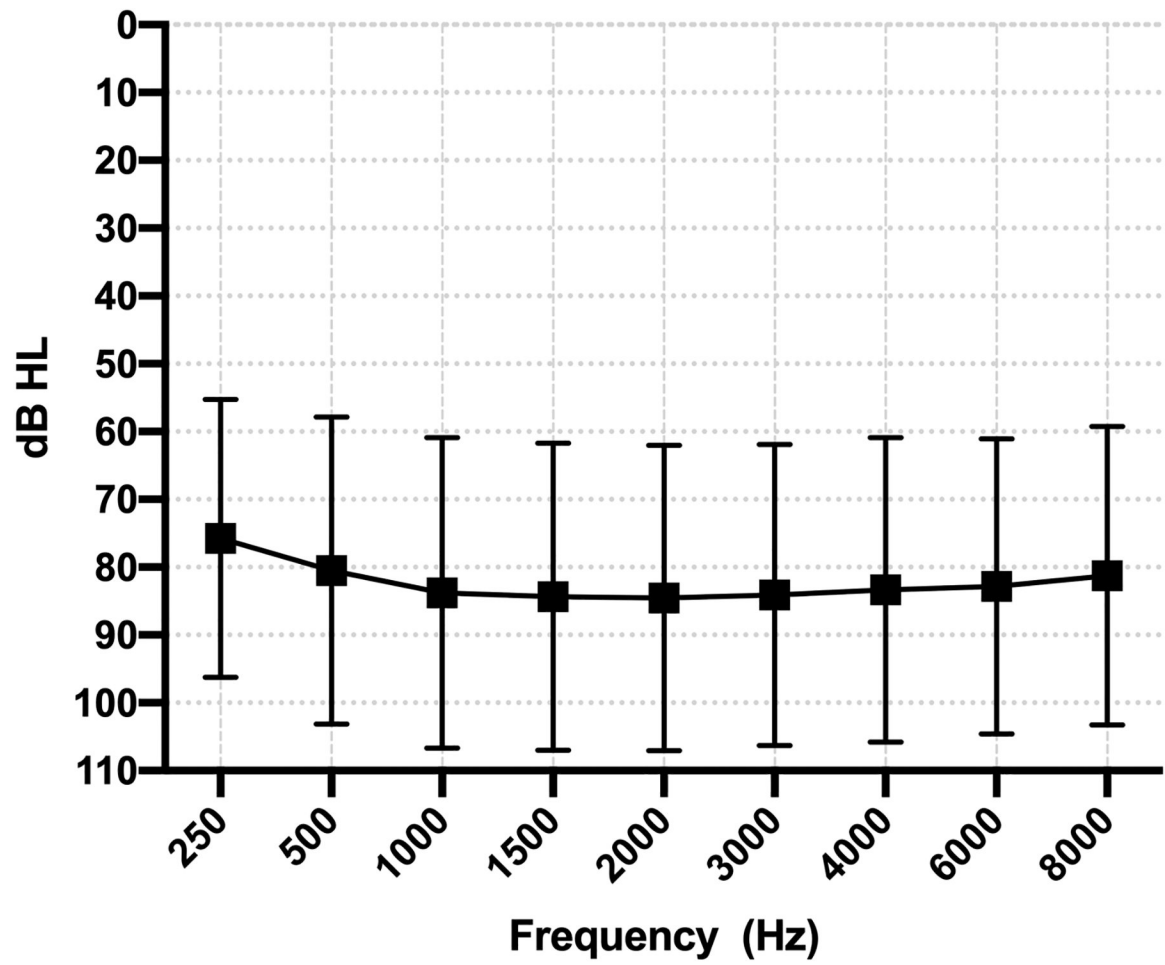


Figure 2:
Audioprofile generated by taking the average hearing level (HL) of all 67 patients with *OTOF*-related hearing loss included in this study. Error bars are the standard deviations.

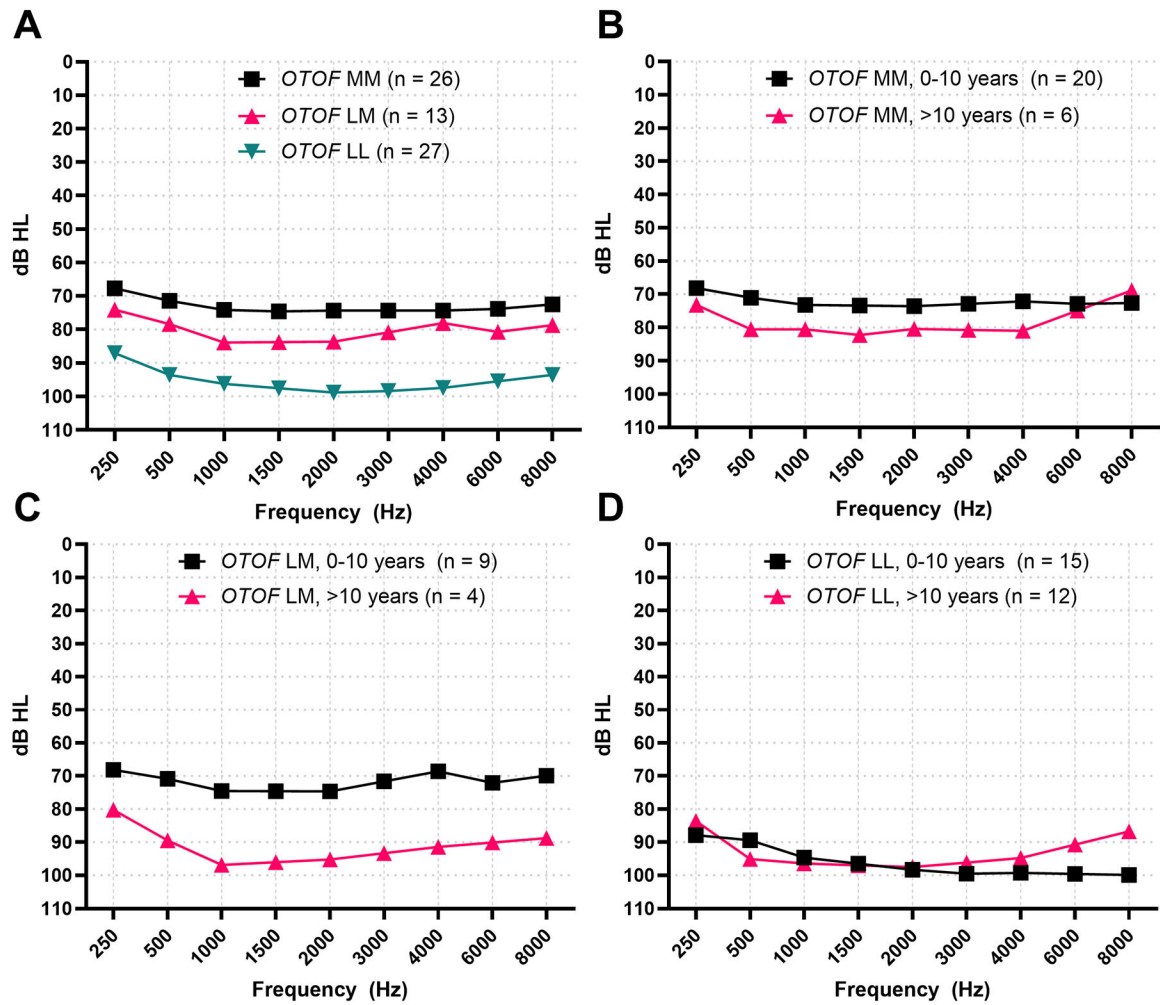


Figure 3: Stratified audioprofiles of individuals with *OTOF*-related hearing loss included in this study. A, audioprofiles based on *OTOF* genotype (MM, LM, and LL). B, audioprofiles based on individuals with an MM genotype, grouped into age bins. C, audioprofiles based on individuals with an LM genotype, grouped into age bins. D, audioprofiles based on individuals with an LL genotype, grouped into age bins. HL = hearing level, MM = missense/missense, LM = loss of function/missense, LL = loss of function/loss of function.

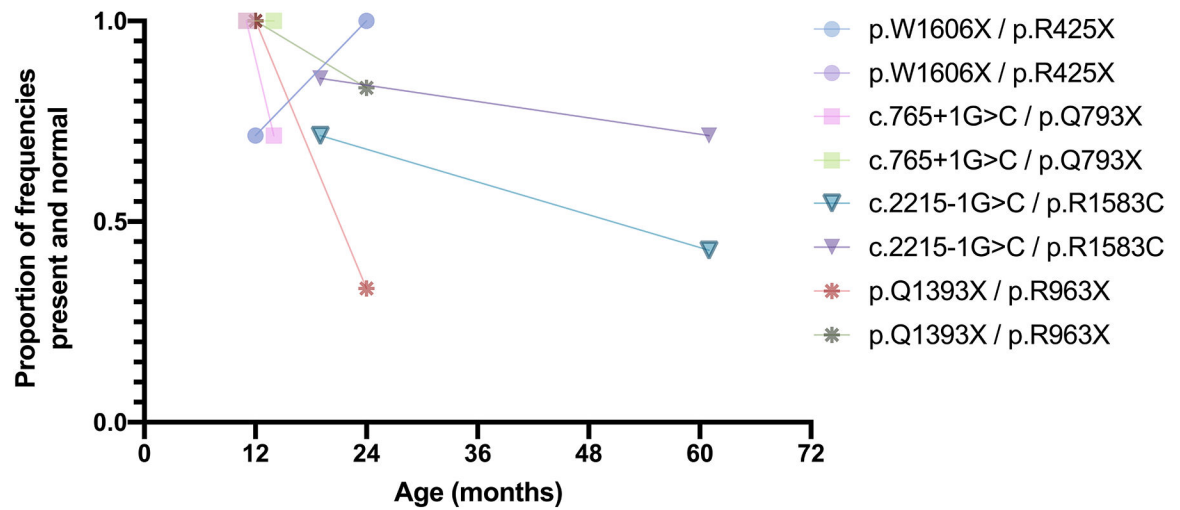


Figure 4:
DPOAE results from four individuals with *OTOF*-related hearing loss with sequential tests. Each individual had two ears tested at each time point; ears from the same individual are represented using the same shape on each graph point. Genotype is listed for each individual. DPOAE results are reported as proportion of frequencies that were present and normal compared with all frequencies tested.

Table 1.

Cohort demographic information.

Age group	Number of Audiograms	Average Hearing Level Across All Frequencies (SD)	Number of DPOAEs	Frequencies Present and Normal / Frequencies Measured (%)
0–11 months	11	77.0 dB (22.2)	4	38/48 (79.2%)
12–23 months	10	95.8 dB (21.7)	8	75/100 (75.0%)
24–35 months	12	74.8 dB (27.6)	8	68/92 (73.9%)
36–47 months	1	90 dB (N/A)	1	6/10 (60.0%)
8–59 months	1	36.4 dB (N/A)	0	N/A
60–71 months	2	74.4 dB (18.9)	2	15/22 (68.2%)
72–83 months	2	67.5 dB (21.2)	0	N/A
84–95 months	2	81.8 dB (26.5)	1	1/8 (12.5%)
96–107 months	2	69.2 dB (22.4)	0	N/A
108–119 months	1	97.8 dB (N/A)	0	N/A
120–131 months	2	69.0 dB (2.2)	0	N/A
>131 months	21	88.6 dB (11.9)	1	6/14 (42.9%)

Age distribution and characteristics of audiograms and DPOAEs from individuals with *OTOF*-related hearing loss included in this study. DPOAE = distortion product otoacoustic emission. SD = standard deviation.

Table 2:

Hearing level by genotype.

	Total no. of audiograms	Mean age (SD)	Low freq. HL (SD)	Middle freq. HL (SD)	High freq. HL (SD)
Missense/Missense and LoF/Missense	39	78.5 months (72.3)	70.9 dB (23.9)	76.0 dB (24.4)	73.4 dB (22.0)
LoF/LoF	27	87.1 months (80.1)	88.5 dB (11.0)	95.6 dB (12.7)	94.7 dB (14.8)
P-value			0.0309 * †	0.0283 * †	0.0296 * †
Missense/Missense	26	78.4 months (70.4)	69.6 dB (25.1)	73.6 dB (25.4)	72.4 dB (23.3)
LoF/LoF	27	87.1 months (80.1)	88.5 dB (11.0)	95.6 dB (12.7)	94.7 dB (14.8)
P-value			0.0647 †	0.0272 * †	0.0350 * †
Missense/Missense	26	78.4 months (70.4)	69.6 dB (25.1)	73.6 dB (25.4)	72.4 dB (23.3)
LoF/Missense	13	78.8 months (78.8)	73.5 dB (22.0)	80.6 dB (21.4)	75.3 dB (19.9)
P-value			0.693 †	0.712 †	0.401 †
LoF/Missense	13	78.8 months (78.8)	73.5 dB (22.0)	80.6 dB (21.4)	75.3 dB (19.9)
LoF/LoF	27	87.1 months (80.1)	88.5 dB (11.0)	95.6 dB (12.7)	94.7 dB (14.8)
P-value			0.534 †	0.538 †	0.166 †

Comparative analysis of hearing loss measured by audiometry by *OTOF* genotype. Low hearing frequency is the mean hearing threshold of 250 and 500 Hz. Middle hearing frequency is the mean hearing threshold of 1000 and 2000 Hz. High hearing frequency is the mean hearing threshold of 4000 and 8000 Hz.

* = statistically significant.

† = calculated via two-tailed post-hoc Tukey's test with a Monte Carlo algorithm.

Freq. = frequency. HL = hearing level. SD = standard deviation. LoF = loss of function.

Table 3.

Analysis of hearing level decline over time.

	Low freq.	Middle freq.	High freq.
MM	0.85 dB/year	0.87 dB/year	0.81 dB/year
P-value	0.286	0.326	0.958
LM	1.36 dB/year	1.87 dB/year	1.61 dB/year
P-value	0.168	0.0525	0.0613
LL	0.21 dB/year	-0.00 dB/year	-0.75 dB/year
P-value	0.522	0.993	0.0833
MM and LM	1.09 dB/year	1.25 dB/year	0.58 dB/year
P-value	0.0897	0.0566	0.3342

Linear regression analysis of hearing loss measured by audiometry over time by *OTOF* genotype. *P*-values are presented based on the slope of the curve for each line. Low hearing frequency is the mean hearing threshold of 250 and 500 Hz. Middle hearing frequency is the mean hearing threshold of 1000 and 2000 Hz. High hearing frequency is the mean hearing threshold of 4000 and 8000 Hz. Freq. = frequency. MM = missense/missense. LM = loss of function/missense. LL = loss of function/loss of function.

Table 4.

DPOAE results by genotype.

	Bilateral DPOAE Pass / Total Tests (%)	Unilateral DPOAE Pass / Total Ears Tested (%)
Missense/Missense and LoF/Missense	2/14 (14.3%)	8/28 (28.6%)
LoF/LoF	5/10 (50.0%)	14/20 (70.0%)
P-value	0.0850	0.00780*
Missense/Missense	2/4 (50.0%)	4/8 (50.0%)
LoF/LoF	5/10 (50.0%)	14/20 (70.0%)
P-value	1.000	0.400
Missense/LoF	0/10 (0.0%)	4/20 (50.0%)
LoF/LoF	5/10 (50.0%)	14/20 (70.0%)
P-value	0.0325*	0.00360*
0–3 years old, Missense/Missense	1/3 (33.3%)	2/6 (33.3%)
>3 years old, Missense/Missense	1/1 (100.0%)	2/2 (100.0%)
P-value	1.000	0.429
0–3 years old, Missense/LoF	0/6 (16.7%)	3/12 (25.0%)
>3 years old, Missense/LoF	0/4 (0.0%)	0/8 (0.0%)
P-value	1.0	0.242
0–3 years old, LoF/LoF	5/10 (50.0%)	12/20 (60.0%)
>3 years old, LoF/LoF	†	†
P-value	†	†

Comparative analysis of DPOAE results by *OTOF* genotype. A “pass” with DPOAE is defined as at least 75% of tested frequencies being present and normal. All *P*-values calculated via Fisher’s exact test.

* = statistically significant, calculated via Fisher’s exact test.

† = not applicable (no DPOAEs were from patients >3 years old with LoF/LoF genotypes).

LoF = loss of function.

Table 5:

Qualitative audiometric data by genotype.

Genotype	Total No.	No. Less than Severe	% Less than Severe	No. At Least Severe	% At Least Severe	P-value
MM	69	15	21.74%	54	78.26%	
LM	35	5	14.29%	30	85.71%	
LL	129	3	2.33%	126	97.67%	
Total	233	23	9.87%	210	90.13%	
MM vs LM						0.438 [‡]
MM vs LL						<0.0001 * [‡]
LM vs LL						0.0117 * [‡]

Comparative analysis of qualitative audiometry data from audiograms of individuals with *OTOF*-related hearing loss based on genotype. Audiograms were collected from a review of the literature and this study. Audiograms were classified in terms of severity as “less than severe” or “at least severe” based on their qualitative description. MM = missense/missense, LM = loss of function/missense. LL = loss of function/loss of function.

[‡] = calculated via Fisher’s exact test.

* = statistically significant.