

Recessive LOXHD1 Variants Cause a Milder Prelingual Hearing Loss: Genotype-Phenotype Correlation and Three Additional Patients With Novel Variants

Sha Yu

Children's Hospital of Fudan University <https://orcid.org/0000-0002-8487-974X>

Wen-xia Chen

Department of Otolaryngology-Head and Neck Surgery, Children's Hospital of Fudan University, Shanghai, China

Yun-Fei Zhang

Department of Otolaryngology-Head and Neck Surgery, Children's Hospital of Fudan University, Shanghai, China

Chao Chen

Department of Otolaryngology-Head and Neck Surgery, Children's Hospital of Fudan University, Shanghai, China

Yihua Ni

Department of Otolaryngology-Head and Neck Surgery, Children's Hospital of Fudan University, Shanghai, China

Bo Duan

Department of Otolaryngology-Head and Neck Surgery, Children's Hospital of Fudan University, Shanghai, China

Huijun Wang

Center for Molecular Medicine, Pediatrics Research Institute, Children's Hospital of Fudan University, Shanghai, China

Zheng-min Xu (✉ 13916320945@163.com)

Department of Otolaryngology-Head and Neck Surgery, Children's Hospital of Fudan University, Shanghai, China

Research

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Abstract

Background

Biallelic mutations in *LOXHD1* have been identified as the cause of DFNB77 (deafness, autosomal recessive 77). It is a novel, progressive, severe-profound, and late-onset non-syndromic hearing loss, and is genetically and phenotypically highly heterogeneous. This study aimed to provide an additional three cases of DFNB77 to analyze this complex disease.

Methods

We presented three cases of pediatric patients with prelingual milder form of the DFNB77 with residual hearing at low frequencies. Trio whole-exome sequencing (WES) was conducted to identify the pathogenic variants. Additionally, we reviewed the literature to further analyze the relationships between the genotype and audiology phenotype of *LOXHD1* worldwide.

Results

Six novel possible pathogenic *LOXHD1* variants in three patients were identified by WES, including three missense, one nonsense, and two splicing variants. The literature review showed that 68.5% of DFNB77 patient onset before five years old; Most variants (60%) were associated with a milder phenotype, particularly variants in the protein domain of PLAT 7 and PLAT 9. We found that compared with homozygous *LOXHD1* variants, individuals with heterozygous compound variants had a significantly milder phenotype, especially individuals carrying one missense and one splicing or bi-allelic missense variants ($P < 0.05$). Audiometric analysis at different ages showed that the hearing loss degree was aggravated at all frequencies in adulthood and more severe in elderhood.

Conclusions

We report three children with hearing loss carrying six novel *LOXHD1* variants identified by WES. Furthermore, our work indicates that DFNB77 may be milder than previously reported, and recommends considering the genotype combination and mutation location of *LOXHD1* and race-specificity in DFNB77 molecular diagnoses and management.

1. Background

Hearing loss (HL) is the most common sensory impairment, affecting around 466 million people worldwide, of which 7% are children (World Health Organization (WHO); <https://www.who.int/pbd/deafness/estimates/en/>), accessed June 2020). The incidence of HL is 1 to 2 per 1000 newborns [1]. At least half of the congenital HL cases account for genetic factors [2]. More than 120 genes have been reported associated with non-syndromic hearing loss (NSHL); about 65% related to autosomal recessive inherited non-syndromic hearing loss (ARNSHL) (Hereditary Hearing Loss Homepage; <https://hereditaryhearingloss.org>), accessed June 2020). The most common genes associated with ARNSHL, such as *GJB2*, *SLC26A4*, *MYO15A*, and *CDH23*, have been well published worldwide [3] [4]. Relatively, *LOXHD1* was a novel gene described by Grillet et al. in 2009 [5]. Knowledge of the genotype-phenotype correlations is limited; this hampers the diagnosis, genetic counseling, and treatment for ARNSHL patients.

LOXHD1 is the causative gene of autosomal recessive deafness 77 (DFNB77, OMIM #613079). DFNB77 is a rare, progressive type of ARNSHL. *LOXHD1* is located on chromosome 18q12-21 and encodes the protein lipoxxygenase homology domain 1 (LOXHD1), which is highly conserved. LOXHD1 contains 15 PLAT (polycystin-1, lipoxxygenase, alpha-toxin) domains. Studies have shown that PLAT domains help proteins target the cell membrane and mediate the interactions of proteins [6] [7]. Mice with a homozygous missense of *Loxhd1* demonstrated that the *Loxhd1* is mainly expressed in mature hair cells and stereocilia membrane, which is required in maintaining the normal function of cochlea hair cells [5].

To date, about 100 cases with DFNB77 have been reported [8] [9] [10] [11] [5]. However, the acoustic characteristics are heterogeneous. The onset age of HL varied from neonate to adulthood; the severity of HL varied from mild to profound; the progression of HL varied from stable to progressive. Furthermore, it is presently unclear for the correlation between *LOXHD1* mutations and the audiology phenotype.

This study delineates the genetic and clinical characteristics of three Chinese patients with prelingual HL, harboring novel pathogenic variants in *LOXHD1*. Additionally, we summarized the clinical and genetic characteristics of reported patients with DFNB77 and explored the genotype-phenotype correlation.

2. Materials And Methods

2.1 Subjects

Three Chinese children (two boys and one girl) with prelingual HL from three unrelated families were recruited from our otolaryngology department of Children's Hospital of Fudan University. We obtained the written informed consent from participants' parents before analysis. The study protocol was approved by the Ethics Committee of Children's Hospital, Fudan University (2019-094 and 2019-214).

2.2 Clinical evaluations

Medical records and family history using a questionnaire focusing on the onset age and progression of HL, pedigree, tinnitus, and vertigo was obtained from all subjects. All participants received hearing tests, including otoscopy, tympanometry, distortion product otoacoustic emission (DPOAE), and pure tone

average (PTA). Auditory brainstem response (ABR) and steady-state auditory response (ASSR) were performed on individuals under five years old or could not complete PTA. Further speech audiometry was performed on patients over five years old using standard Chinese single syllable word chart cards. In patients #1 and #3, a temporal bone computed tomography (CT) and magnetic resonance imaging (MRI) scan was performed.

We recorded PTA with an Interacoustics AC40 clinical audiometer and HAD-280 earphones. It was conducted in an audiometric test room where the ambient noise level is less than 25 dB (sound pressure level), and the patient was instructed on how to play the test. The audiometric configurations were classified into six different types: rising, mid-frequency u-shaped, high-frequency u-shaped, high-frequency gently sloping, high-frequency steeply sloping, and flat (Supplementary Table 1). Moreover, the audiograms that did not meet any of the criteria above are classified as unspecified. The grades of hearing loss were classified by the pure tone average at 0.5, 1, 2, and 4 kHz in the better ear according to the WHO classification of HL, as follows: 26-40 dB, mild HL; 41-60 dB, moderate HL; 61-80 dB, severe HL; and ≥ 81 dB, profound HL. (WHO; https://www.who.int/pbd/deafness/hearing_impairment_grades/en/), accessed June 2020).

2.3 Trio whole exome sequencing and bioinformatics analysis

Genomic DNA of peripheral blood was extracted using standard techniques with the QIAamp DNA Blood Mini Kit (QIAGEN, Germany). Whole exome sequencing (WES) was performed on all patients and their parents following the previously published method [12]. Briefly, DNA fragments were enriched for the target region of the coding sequence exons using the Agilent SureSelect XT Human All Exon 50 Mb kit. Subsequently, the sequencing was performed on Illumina's HiSeq2000 sequencer according to its instructions (San Diego, CA, USA).

The sequence data were mapped to the human reference genome (GRCh37/hg19) with a Burrows-Wheeler Aligner (BWA) software (<http://bio-bwa.sourceforge.net/>). For variant calling, single-nucleotide variant and small indels were detected by GATK best practice (<https://gatk.broadinstitute.org/hc/en-us>). The variants were annotated by ANNOVAR and VEP software [13], and filtered against 1000Genomes projects (<https://www.internationalgenome.org/>), gnomAD (<https://gnomad.broadinstitute.org/>), ExAC (<http://exac.broadinstitute.org/>). The pathogenicity of the candidate variants was evaluated by predictor software, including SIFT (<https://sift.bii.a-star.edu.sg/>), PolyPhen2 (<http://genetics.bwh.harvard.edu/pph2/>), Mutation Taster (<http://www.mutationtaster.org/>), and CADD (<https://cadd.gs.washington.edu>). The strength of the splicing domains was analyzed by Human splicing Finder v.3.1 (<http://umd.be/Redirect.html>).

The pathogenicity of a variant was classified based on the ACMG (American College of Medical Genetics and Genomics) standards and guidelines [14]. The candidate variants were confirmed by Sanger sequencing with an ABI 3730 Genetic Analyzer (Applied Biosystems). The sequence variant data have been submitted to the ClinVar database (SUB8294387).

2.4 In silico prediction of protein structure and evolutionary conservation

The 3D models of human wild-type *LOXHD1* and its mutant proteins (Gly2013Glu, Arg631Cys) were generated using Mutalyzer (<https://www.mutalyzer.nl/>) and Swiss-model servers (<https://swissmodel.expasy.org/>). The protein template SMTL IDs selected for Gly2013Glu and Arg631Cys were 3v99.1 and 3cwz.1 [15] [16]. The 3D protein structures were visualized with PyMOL 1.7.4.5. To check the evolutionary conservation, we compared the amino acid/nucleotide of human with its orthologs like rhesus, mouse, rat, dog, elephant, chicken, *x.tropicalis*, and lamprey using Weblogo (<https://weblogo.berkeley.edu/>)

2.5 Literature review

We conducted a literature search in Google, PubMed, and HGMD (<http://www.hgmd.cf.ac.uk/ac/index.php>) using the keywords "*LOXHD1*", "DFNB77", and "hearing loss" from July 2009 to Oct 2020. Inclusion criteria: 1) original articles, 2) English-language articles, 3) reports of *LOXHD1* variants with HL in humans.

3. Results

3.1 Clinical characteristics of patients with *LOXHD1* variants

In this study, three affected children from three non-consanguineous Chinese families were identified. All of these families have negative family histories of congenital or early-onset HL. The detailed clinical features of these patients are listed in Table 1.

All patients presented with a prelingual onset hearing loss, and the diagnosed age varied from 2 to 8 years old. They all had bilateral, moderate to severe sensorineural HL (Fig. 1A). DPOAEs of all patients were absent bilaterally. PTA revealed that patient #1 and #2 had severe HL. ABR and ASSR revealed that patients #3 had bilateral moderate HL, and no increases in interpeak latencies were observed. The hearing loss was found to worsen with age in patient #1, with three years' follow-up. Speech audiometry showed that the speech recognition thresholds (SRT) were 80 dB and 85 dB in the left and right ear of patient #1; 65 dB and 100 dB in the left and right ear of patient #2 (Fig. 1B). Moreover, a maximum of 95% and 100% speech recognition was attained at 110 dB sound level in the better ear of patient #1 and patient #2. Speech in the noise test showed that 50% speech recognition was attained at the signal-to-noise of 5 dB with 100 dB for patient #1, while 60% speech recognition was attained at signal-to-noise 5 dB with 100 dB for patient #2.

None of the affected individuals complained of tinnitus and vertigo. Besides, none of the family members complained of eye pain, glare, or blurred vision. Otoloscopic examination and external ear inspection were normal in all patients. The MRI and CT of the brain and temporal bones of the left and right ears for patient #1 and patient #2 demonstrated to be normal (Fig. 1C). No known causes of acquired HL were found in our patients, such as prenatal infection, TORCH (toxoplasmosis, rubella, CMV, HSV) infection, aminoglycoside exposure, otoacoustic trauma, meningitis.

None had delayed motor development or dysmorphology. Besides, the Wechsler Intelligence Scale test for children (WISC-III) was performed on patients #2, and the score was 44 of Verbal IQ, 98 of Performance IQ, 63 of full-scale IQ.

3.2 Variation detection and functional analysis

We identified six novel *LOXHD1* variants. All the variants were detected in a compound heterozygous state (Fig. 1D). The variants consisted of one nonsense, two splicing, and three missense variants. The six variants were all located in a well-conserved site (Fig. 2A). Besides, all variants are located in PLAT domains of *LOXHD1* protein, with one except (c.4814T>C, p.Met1605Thr) (Fig. 3A). Based on the ACMG guidelines, the variants were categorized into two pathogenic, three likely pathogenic, and one uncertain significance (Table 2).

Molecular models for the homeodomain of wild-type and variant-containing *LOXHD1* protein were developed and adopted a β sandwich fold containing PLAT domains. The two missense variants (c.6038G>A, p.Gly2013Glu; c.1891C>T, p.Arg631Cys) were predicted to change the polarity with surrounding active site residues and consequently destabilize the β sandwich structures (Fig. 2B).

The two splicing variants were evaluated *in silico* using the web tool HSF. The variant (c.3351-1C>A) was predicted to alter the wide-type acceptor sites and may affect splicing by HSF; variants c.4212+5G>A were predicted to alternate the original splicing donor site and then affected the splicing (Supplementary Fig. 1).

The missense variant (c.4814T>C, p.Met1605Thr) was absent in the ExAC, 1000 Genomes, and gnomAD databases and highly conserved across different species. Unfortunately, the Met1605Thr protein template cannot be obtained from Swiss-model servers, and more evidence for its pathogenicity was not obtained. Thus, the pathogenicity of this variant is uncertain.

3.3 Literature review of published cases

3.3.1 phenotype

To date, a total of 111 cases with DFNB77 were reported, including the three cases in this study (Supplementary Table 2). We found that most patients' onset age was less than ten years old (68.5% for < 5 years and 14.4% for 5-10 years). There have 16.2% of patients shown severe HL, and 50.5% shown profound HL. 28.8% of patients were described with progressive HL, and 56.2% of them occurred in adulthood (Table 3). The complete audiometric data were divided into four groups by age (<10, 10-19, 20-55, and >55 years old), and we found that the main audiogram of DFNB77 among different age groups was a high-frequency down-sloping configuration (Fig. 4). The degree of HL was observed increased in all frequencies in adulthood and more severe in elderhood. We further compared the genotype and phenotype among different populations, and we found that the genotype of *LOXHD1*, the severity of HL, the type of HL, and the audiogram had significant differences ($P < 0.05$) (Table 4). The profound HL, serious form HL, and gently sloping audiogram HL in the Middle East are obviously common than others.

3.3.2 Genotype

A total of 85 variants of *LOXHD1* were reported with the 111 reported cases, including 19 nonsense, 6 frameshifts, 2 deletions, 1 indel, 13 splice-site variants, 43 missense, and 1 synonymous variant (Fig. 3A). Among them, 67 (79%) variants were located in the PLAT domains, 13 (19%) of which were in the PLAT14 domain (Fig. 3B). The most common mutations were c.4212+1G>A; c.4714C>T, Arg1572Ter; and c.4480C>T, Arg1494Ter with a frequency of 14.8%, 8.1%, and 6.3%, respectively. The *LOXHD1* variants in 65 (59%) patients were in a compound heterozygous state (Table 5). However, in the Middle East, all *LOXHD1* variants were in the homozygous state (Table 4)

3.3.3 Genotype-phenotype correlations analysis

About 60% of variants were associated with a milder auditory phenotype, with residual hearing at low frequencies (250 Hz, 500 Hz). Moreover, 19% of the variants that cause serious phenotype were located in the PLAT14 domain, indicating that it is the most frequent domain with a serious phenotype (Fig. 3B). 55% of the variants with milder phenotype were missense variants (Fig. 3C). We further compared the different combinations of *LOXHD1* mutations. The results showed that compared with homozygous variants of *LOXHD1*, individuals with heterozygous compound variants had a significantly milder phenotype, especially individuals carrying one missense and one splicing or bi-allelic missense variants ($P < 0.05$) (Table 5).

4. Discussion

In this study, we investigated three isolated pediatric patients with six novel variants in *LOXHD1*, including three missense, one nonsense, and two rare splicing variants. So far, about 100 cases of DFNB77 have been reported in the literature. However, few data have been published with the genotype and audiology phenotype correlation.

Presently, we describe in detail the hearing loss in the three patients. All patients had a moderate-to-severe sensorineural hearing loss, which was milder in the low frequency. No increases in interpeak latencies were observed in the ABR, indicating a probably peripheral hearing loss. Speech audiometry corresponded well with pure tone audiometry, but the two patients (#1 and #2) performed poorly on speech in noise tests. Based on the above findings: HL with a reduced speech in noise performance, absent DPOAE, and normal ABR waves, we speculate that outer hair cells might be lost or that their functionality is reduced [17]. Moreover, this finding consistent with the previous murine study [5].

The six *LOXHD1* variants identified in this study were all in a compound heterozygous type. Our review also demonstrates that a compound heterozygous state of *LOXHD1* variants is the most common combination in DFNB77, except in the Middle East population. In the Middle East population, all *LOXHD1*

variants were in a homozygous state, which may be caused by the custom of consanguineous marriage. In this study, 83% (5/6) of the variants located in the PLAT domains supporting our review result that most pathogenic *LOXHD1* variants are located in the PLAT domains. It reflects that the PLAT domains are crucial for maintaining the normal function of *LOXHD1* protein.

Our review indicates that patients with *LOXHD1* variants mainly present a prelingual HL. The onset age of *LOXHD1*-related HL was initially described at 7-8 years based on the patients' own words [5]. However, recent studies suggested that the affected patients mainly had a congenital or early-onset HL [18][8][9] [19]. Nevertheless, some DFNB77 patients were reported with a late-onset HL, even onset in adulthood [20] [21]. In this study, all three patients presented with prelingual HL while they had delayed diagnosis. According to the parents' information, it was because of the pass of the neonatal hearing screening (patient #1) or inconclusive results of the screening (patients #2 and #3). Therefore, it is likely that the late-onset HL in these reported late-onset individuals was of delayed diagnosis rather than true late-onset. Besides, for mild-to-moderate HL that occurs in early childhood, the diagnosis may be delayed due to the conversational speech within this range (50-60 dB)[22].

This study suggests that *LOXHD1*-related HL in childhood is a milder form of HL, milder at low frequencies (250 Hz, 500 Hz). A murine study has demonstrated that the first damaged part of cochlear hair cells was the stereocilia in the basal cochlear turn, which respond to the highest frequencies [5], indicating low frequencies should have relative milder damage. A milder HL was also reported in some patients with *LOXHD1* variants [5] [8]. However, studies reported that some *LOXHD1* variants are associated with a more severe HL [23] [18] [9] [19]. Our review data reflected that about 60% of variants were associated with a milder auditory phenotype and the HL degree in childhood was a milder HL.

Our study implies that the *LOXHD1*-related HL is progressive and deteriorated at all frequencies during adulthood. A mice experiment demonstrated that the *LOXHD1* mutation could lead to degenerative changes in the hair cells of the inner ear's stereociliary [5], indicating that the *LOXHD1*-related HL should be progressive. However, to date, 42 of 111 patients have been reported with a non-progressive HL. Nevertheless, these results were questioned because only 8 (19%) of the patients received hearing tests in adulthood, and none was conducted with a long-time follow-up [8] [23][24][9]. Noteworthy, our review showed that 37.5% of DFNB77 patients were reported with progressive HL during childhood, and 6.3% had progressive HL during adolescence. Besides, the degree of HL increased in all frequencies in adulthood and more severe in elderhood. Therefore, we guess that the *LOXHD1*-related HL aggravates gently during childhood and adolescence and rapidly during adulthood. Moreover, comparing the HL progression among different *LOXHD1* variants, we found it was significantly different. Thus, except for age, the *LOXHD1* variant combinations should also be considered in evaluating the HL progression for DFNB77 patients.

Previous studies have investigated the genotype-phenotype relationships in DFNB77, but no correlation was found due to the limited cases [8] [9]. We explored the genotype characteristics associated with the severity of *LOXHD1*-related HL to explain the heterogeneity in phenotype. First, variants in the homozygous state seem to cause a more severe HL than variants in the heterozygous state (Table 5). However, most homozygous variants were reported in patients from the Middle East; and they had more severe HL than patients from East Asia, America, and Europe, where heterozygous variants are common. Thus, we suspect the difference is caused by a race-specific effect. Second, the heterozygote combination of one missense and one splicing or bi-allelic missense variants are more likely to lead to a milder HL than other combinations (Table 5). In both situations, a certain amount of wide-type protein may remain expressed [25]. Third, the type of missense and splicing variant is more likely to cause a milder HL than other variant types (Fig. 3C). The homozygous missense and splicing variant also had milder HL than the homozygous frameshift, nonsense, and indel variants (Table 5). Fourth, the variants, which are located in the PLAT 7 and PLAT 9 domain, or may only affect 2 of the 5 *LOXHD1* isoforms (transcript variant 2, NM_001145472.3; transcript variant 5, NM_001308013.1), are more likely to cause a milder HL (Fig. 3B) [8]. Moreover, this result fits different populations (Supplementary Fig. 2). Sixth, variants in the PLAT 14 domain seems more likely to lead to a more severe HL (Fig. 3B). Nevertheless, 71% (5/7) of missense variants in this domain were milder HL, and in East Asia, it reaches 80% (4/5). Thus, the HL severity in the PLAT 14 domain is ambiguous.

5. Conclusions

We identified six novel variants in the *LOXHD1* gene in three Chinese children with prelingual sensorineural HL. Our review indicates that recessive *LOXHD1* variants mainly lead to a prelingual and down-sloping HL, which is milder in childhood and aggravates rapidly in adulthood. Our work extends the spectrum of *LOXHD1* pathogenic variants, enriched our knowledge of auditory phenotypes, and provided potential genotype-phenotype correlations.

Abbreviations

ABR: auditory brainstem response; ACMG: American College of Medical Genetics and Genomics; ARNSHL: autosomal recessive inherited non-syndromic hearing loss; ASSR: steady-state auditory response; BWA: Burrows-Wheeler Aligner; CT: computed tomography; DFNB77: deafness, autosomal recessive 77; DPOAE: distortion product otoacoustic emission; HL: hearing loss; *LOXHD1*: lipoxygenase homology domain 1; MRI: magnetic resonance imaging; NSHL: non-syndromic hearing loss; PLAT: polycystin-1, lipoxygenase, alpha-toxin; PTA: pure tone average; WHO: World Health Organization; WES: whole exome sequencing.

Declarations

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Ethics approval and consent to participate

This study protocol was approved by the Ethics Committee of Children's Hospital, Fudan University (2019-094 and 2019-214). Informed consents for participation in this study were obtained and could be available by requesting the corresponding author.

Author contributions

Zheng-min Xu. and Sha Yu. designed the study. Huijun Wang. supervised the work and completed the genetic data analysis. Other co-authors collected the clinical data. Sha Yu. and Huijun Wang. drafted the manuscript together. All authors joined in the editing and review of the manuscript. All authors approved the final manuscript.

Availability of data and materials

Some of our data supporting this study's findings are not publicly available because of privacy or ethical restrictions, but the data are available by requesting the corresponding author.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

All authors agreed to publish this article.

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Tables

Table 1 Clinical features of patients with *LOXHD1* variants identified in this study

Patient	#1	#2	#3
Sex	Male	Male	Female
Ethnicity	Chinese	Chinese	Chinese
Main complaint	Speech indistinct	Speech indistinct	Hearing loss
Neonatal hearing screening	Pass (bilateral)	Failed (right) Pass (left)	Failed (right) Pass (left)
Age of HL onset	Prelingual	Prelingual	Prelingual
Age of HL diagnosis	8 years	6 years	2 years
Age of recent HL detection	11 years	7 years	2 years
Characteristics of HL			
Type	SNHL	SNHL	SNHL
Bilateral	+	+	+
Progressive	+	NA	NA
Severity	Severe	Severe	Moderate
Configuration	U-shaped	Gently sloping	NA
Method of detection	PTA	PTA	ABR, ASSR
Tinnitus	–	–	–
Vertigo	–	–	–
Motor developmental delay	–	–	–
Physical examination	Normal	Normal	Normal
Method of hearing rehabilitation	Hearing aid (bilateral)	Hearing aid (bilateral)	Nothing

Note: + indicates the feature is present. – means the feature is absent.

Abbreviations: ABR, auditory brainstem response; ASSR, steady-state auditory response; HL, hearing loss; NA, not available; PTA, pure tone average; SNHL, sensorineural hearing loss.

Table 2 Six novel variants of *LOXHD1* gene identified in this study (NM_144612.6)

Patient	Nucleotide Change (protein change)	Type of variant	Pathogenicity	ExAC	gnomAD	1000 gene	PhyloP ^a	Polyphen-2	SIFT	MutTaster	CADD ^b
# 1	c.4212+5G>A	splice	Likely Pathogenic PM2, PM3, PP3, PP4	0	0	0	3.331	NA	NA	DC	17.15
	c.6038G>A p.(Gly2013Glu)	missense	Likely Pathogenic PM2, PM3, PM5, PP3, PP4	0	0	0	6.226	D (1.000)	D (0.005)	DC	31.00
# 2	c.3351-1G>A	splice	Pathogenic PVS1, PM2, PM3, PP4	0	0	0	5.776	NA	NA	DC	33.00
	c.4247G>A p. (Trp1416*)	nonsense	Pathogenic PVS1, PM2, PM3, PP4	0	0	0	5.652	NA	NA	DC	40.00
# 3	c.1891C>T p. (Arg631Cys) ^c NM_001145472.3	missense	Likely pathogenic PM3, PM2, PP3, PP4	0	0	0	1.110	D (0.928)	D (0.000)	DC	24.90
	c.4814T>C p. (Met1605Thr)	missense	Uncertain Significance PM2, PM3, PP4	0	0	0	1.558	P (0.884)	T (0.320)	N	22.70

Abbreviations: D, deleterious; DC, disease causing; N, polymorphism; NA, data were not available; P, possibly damaging; PVS, evidence of pathogenicity–very strong; PS, evidence of pathogenicity–strong; PM, evidence of pathogenicity–moderate; PP, evidence of pathogenicity–supporting; T, tolerated.

^a PhyloP score represents the evolutionary conservation of the nucleotide; a positive score means that nucleotide is conserved. Higher scores are considered more deleterious.

^b CADD score is a pathogenicity score based on functional and evolutionary data. Higher scores are considered more deleterious.

^c This variant affects the shorter *LOXHD1* isoform (NM_001145472.3).

Table 3 Clinical characteristics of patients with DFNB77 (N=111)

Clinical features	Number	%
Sex		
Male	37	33.3
Female	48	43.2
NA	26	23.4
Age of HL diagnosis		
<5 years	76	68.5
5-10 years	16	14.4
11-19 years	0	0.0
≥20 years	7	6.3
NA	12	10.8
Severity of HL		
Mild	11	9.9
Moderate	15	13.5
Severe	18	16.2
Profound	56	50.5
NA	11	9.9
Progression of HL		
Progressive	32	28.8
Progressive during childhood	12	37.5
Progressive during adolescence	2	6.3
Progressive during adulthood	18	56.2
Non-progressive	42	37.8
NA	37	33.3
Laterality		
Bilateral	96	86.5
Unilateral	0	0.0
NA	15	13.5

Note: Detail data of these 111 patients in literature are available in Table S2.

Abbreviations: NA, data were not available; HL, hearing loss.

Table 4 The comparison of audiological and genetic characteristics among different populations in the literature

	East Aisa (n=50)	Middle East (n=27)	Europe (n=21)	America (n=10)	Africa (n=2)	South Aisa (n=1)	<i>p</i> -Value ^a
Sex							
Male	18 (38%)	8 (47%)	9 (56%)	1 (50%)	1 (50%)	0 (0%)	0.821
Female	29 (62%)	9 (53%)	7 (44%)	1 (50)	1 (50%)	1 (100)	
Age of HL diagnosis							
<5 years	35 (73)	16 (76)	15 (79)	7 (88)	2 (100)	1 (100)	0.518
5-10 years	7 (15)	5 (24)	4 (21)	0 (0)	0 (0)	0 (0)	
≥20 years	6 (13)	0 (0)	0 (0)	1 (12)	0 (0)	0 (0)	
Genotype of <i>LOXHD1</i>							
Homozygous	17 (34)	27 (100)	2 (10)	0 (0)	0 (0)	0 (0)	0.000*
Heterozygous	33 (66)	0 (0)	19 (90)	10 (100)	2 (100)	1 (100)	
Severity of HL							
Mild	5 (10)	1 (5)	5 (25)	0 (0)	0 (0)	0 (0)	0.002*
Moderate	6 (13)	1 (5)	4 (20)	4 (50)	0 (0)	0 (0)	
Severe	15 (31)	0 (0)	3 (15)	0 (0)	0 (0)	0 (0)	
Profound	22 (46)	19 (90)	8 (40)	4 (50)	2 (100)	1 (100)	
Type of HL							
Milder ^b	24 (50)	4 (19)	12 (60)	4 (50)	0 (0)	0 (0)	0.004*
serious ^c	24 (50)	17 (81)	8 (40)	4 (50)	2 (100)	1 (100)	
Progression of HL							
Progressive	8 (36%)	4 (31%)	5 (45%)	1 (100%)	0 (0%)	0 (0%)	0.709
Non-progressive	14 (64%)	9 (69%)	6 (55%)	0 (0%)	2 (100%)	1 (100%)	
Configuration of HL							
Rising	0 (0)	0 (0)	2 (15)	NA	NA	0 (0)	0.000*
U-shaped	2 (5)	0 (0)	0 (0)	NA	NA	0 (0)	
Gently sloping	11 (25)	11 (92)	3 (23)	NA	NA	0 (0)	
Steeply sloping	15 (34)	0 (0)	5 (39)	NA	NA	0 (0)	
Flat	11 (25)	0 (0)	0 (0)	NA	NA	0 (0)	
Unspecified	5 (11)	1 (8)	3 (23)	NA	NA	1 (100)	

Note: Detail data of these 111 patients in literature are available in Table S2.

Data are presented with n (%).

Abbreviations: HL, hearing loss; NA, data were not available.

^a *p*-Value was calculated using Fisher's exact test. *p*-Value <0.05 is statistically significant*.

^b Milder hearing loss means milder at low frequencies (250 Hz, 500 Hz).

^c Serious hearing loss means no report of hearing loss milder at low frequencies.

Table 5 The comparison of audiological characteristics among different combinations of *LOXHD1* variants in the literature

Compound heterozygous state (N=65)											
	Missense &					Nonsense &				Splicing &	
	splicing (n=18)	missense (n=12)	frameshift (n=2)	deletion (n=2)	nonsense (n=14)	splicing (n=3)	deletion (n=1)	frameshift (n=5)	nonsense (n=4)	frameshift (n=1)	splicing (n=3)
Age of HL diagnosis											
<5 years	13 (72)	6 (50)	2 (100)	1 (50)	10 (84)	3 (100)	1 (100)	4 (80)	2 (50)	1 (100)	2 (67)
5-10 years	3 (17)	2 (17)	0 (0)	1 (50)	1 (8)	0 (0)	0 (0)	1 (20)	2 (50)	0 (0)	1 (33)
≥20 years	2 (11)	4 (33)	0 (0)	0 (0)	1 (8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Severity of HL											
Mild	4 (22)	2 (18)	0 (0)	0 (0)	2 (15)	0 (0)	0 (0)	0 (0)	1 (25)	0 (0)	1 (33)
Moderate	2 (11)	2 (18)	1 (50)	1 (100)	3 (23)	0 (0)	0 (0)	0 (0)	1 (25)	0 (0)	0 (0)
Severe	5 (28)	3 (27)	0 (0)	0 (0)	1 (8)	1 (33)	0 (0)	2 (40)	2 (50)	1 (100)	0 (0)
Profound	7 (39)	4 (36)	1 (50)	0 (0)	7 (54)	2 (67)	1 (100)	3 (60)	0 (0)	0 (0)	2 (67)
Type of HL											
Milder ^b	15 (83)	6 (55)	1 (50)	1 (100)	6 (46)	0 (0)	0 (0)	0 (0)	2 (50)	1 (100)	1 (33)
Serious ^c	3 (17)	5 (45)	1 (50)	0 (0)	7 (54)	3 (100)	1 (100)	5 (100)	2 (50)	0 (0)	2 (67)
Progression of HL											
Progressive	11 (73)	5 (56)	1 (50)	NA	3 (50)	1 (100)	NA	0 (0)	2 (50)	1 (100)	2 (67)
Non-progressive	4 (27)	4 (44)	1 (50)	NA	3 (50)	0 (0)	NA	4 (100)	2 (50)	0 (0)	1 (33)
Configuration of HL											
Rising	0 (0)	0 (0)	0 (0)	NA	0 (0)	1 (50)	NA	0 (0)	0 (0)	0 (0)	0 (0)
U-shaped	1 (7)	0 (0)	0 (0)	NA	0 (0)	0 (0)	NA	0 (0)	0 (0)	0 (0)	0 (0)
Gently sloping	4 (27)	4 (57)	0 (0)	NA	0 (0)	0 (0)	NA	1 (25)	2 (50)	1 (100)	1 (33)
Steeply sloping	8 (53)	2 (29)	1 (50)	NA	2 (40)	0 (0)	NA	2 (50)	0 (0)	0 (0)	1 (33)
Flat	2 (13)	1 (14)	0 (0)	NA	0 (0)	0 (0)	NA	1 (25)	1 (25)	0 (0)	1 (33)
Unspecified	0 (0)	0 (0)	1 (50)	NA	3 (60)	1 (50)	NA	0 (0)	1 (25)	0 (0)	0 (0)

Note: Detail data of these 111 patients in literature are available in Table S2.

Data are presented with n (%).

Abbreviations: HL, hearing loss; NA, data were not available.

^a p-Value was calculated using Fisher's exact test. p-Value <0.05 is statistically significant*.

^b Milder hearing loss means milder at low frequencies (250 Hz, 500 Hz).

^c Serious hearing loss means no report of hearing loss milder at low frequencies.

Figures

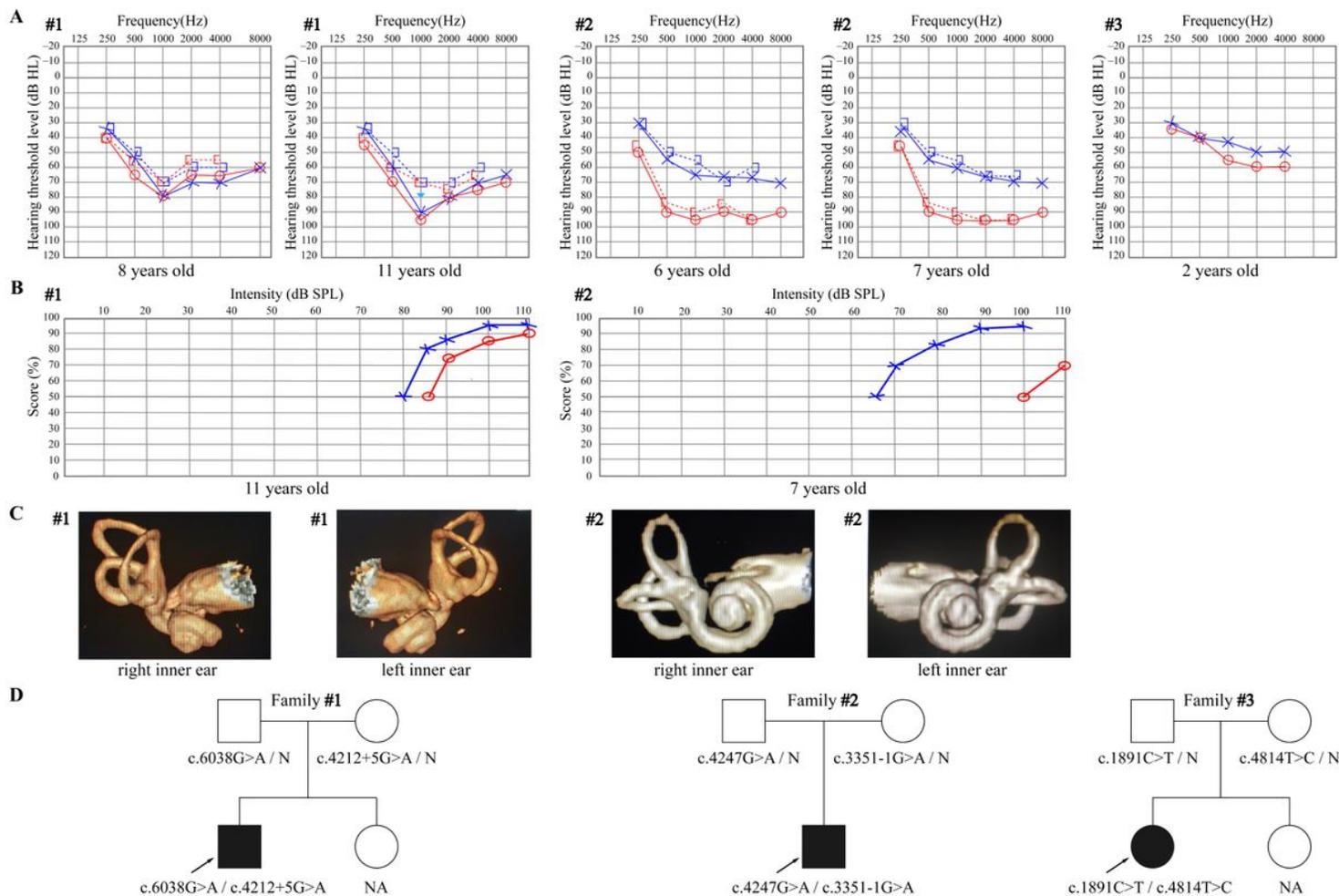


Figure 1
 Clinical characteristics and pedigree of each family with LOXHD1 variants. (A-B) Pure-tone audiogram (A; #1, #2), ASSR audiogram (A; #3), and speech audiometry (B) of the probands. Red and blue indicate hearing threshold (A) or speech recognition (B) for the right and left ears, respectively. The age at the time of audiometric testing is noted below the audiogram. (C) MRI scan with 3D reconstruction of inner ear. (D) Pedigree. Arrows show the probands in each family. Genetic findings of each individual tested are in the pedigree. N, wide type; NA, data were not available.

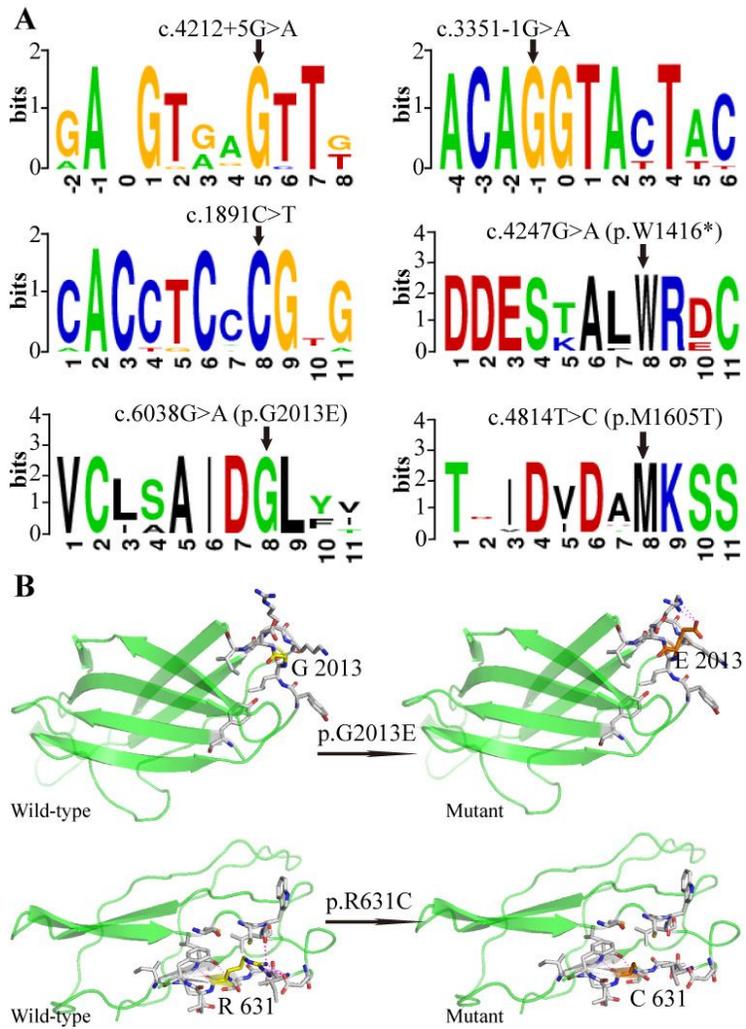


Figure 2
 Evolutionary conservation (A) and protein structural (B) predictions of LOXHD1 variants. (A) The sequence logos indicate the amino acid or nucleotide are conserved across different species. (B) Molecular models of Gly2013Glu and Arg631Cys mutants. The two variants were predicted to change the polarity with surrounding active site residues and consequently destabilize the β sandwich structures.

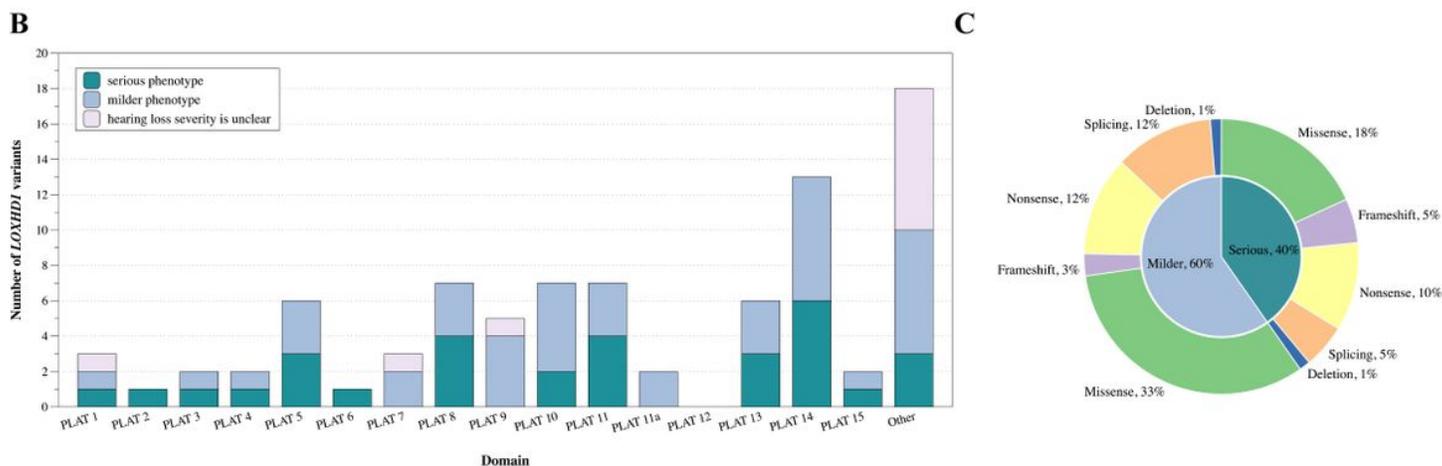
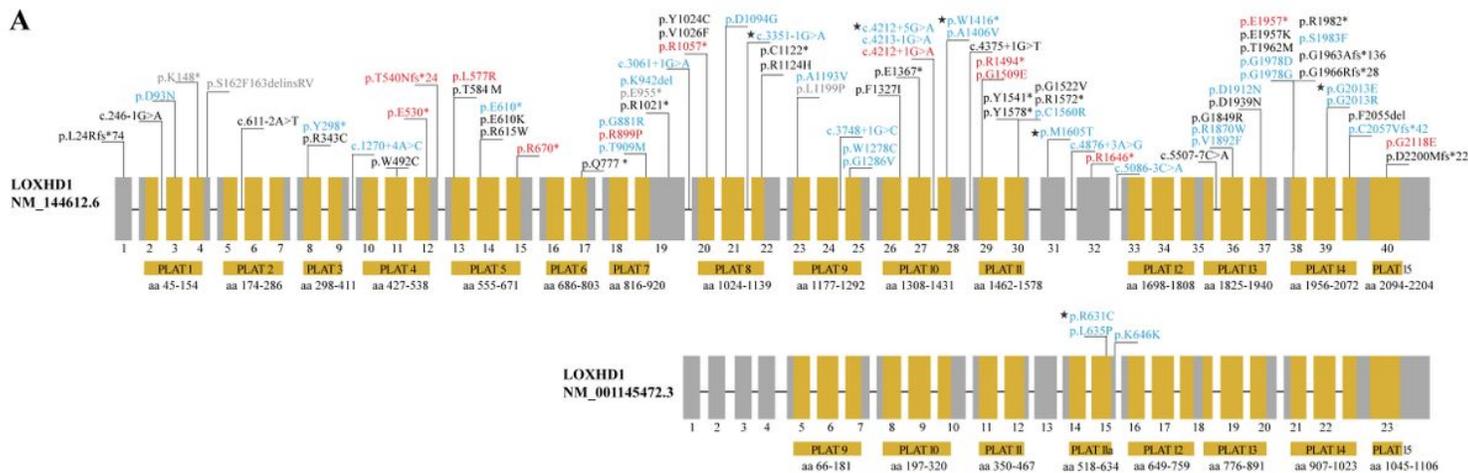


Figure 3

Overview of known LOXHD1 variants and related hearing phenotypes. (A) Schematic representations of 2 of the 5 LOXHD1 isoforms (transcript variant 1, NM_144612.6; transcript variant 2, NM_001145472.3). The blue words indicate variants with milder auditory phenotypes; the black words indicate variants with severe auditory phenotype; the red words indicate variants associated with both severe and milder phenotype; the gray words indicate variants without reported hearing phenotypes. Six black stars indicate novel variants of LOXHD1 in this study. Exons are numbered and indicated by rectangles. The PLAT (polycystin-1, lipoxygenase, alpha-toxin) protein domains in amber color are predicted with Pfam 33.1 (<http://pfam.xfam.org/>) and are numbered. (B) The number of identified LOXHD1 variants reported with three different auditory phenotypes in each PLAT domain is displayed. (C) The proportion of LOXHD1 variants with two different auditory phenotypes and the mutation types.

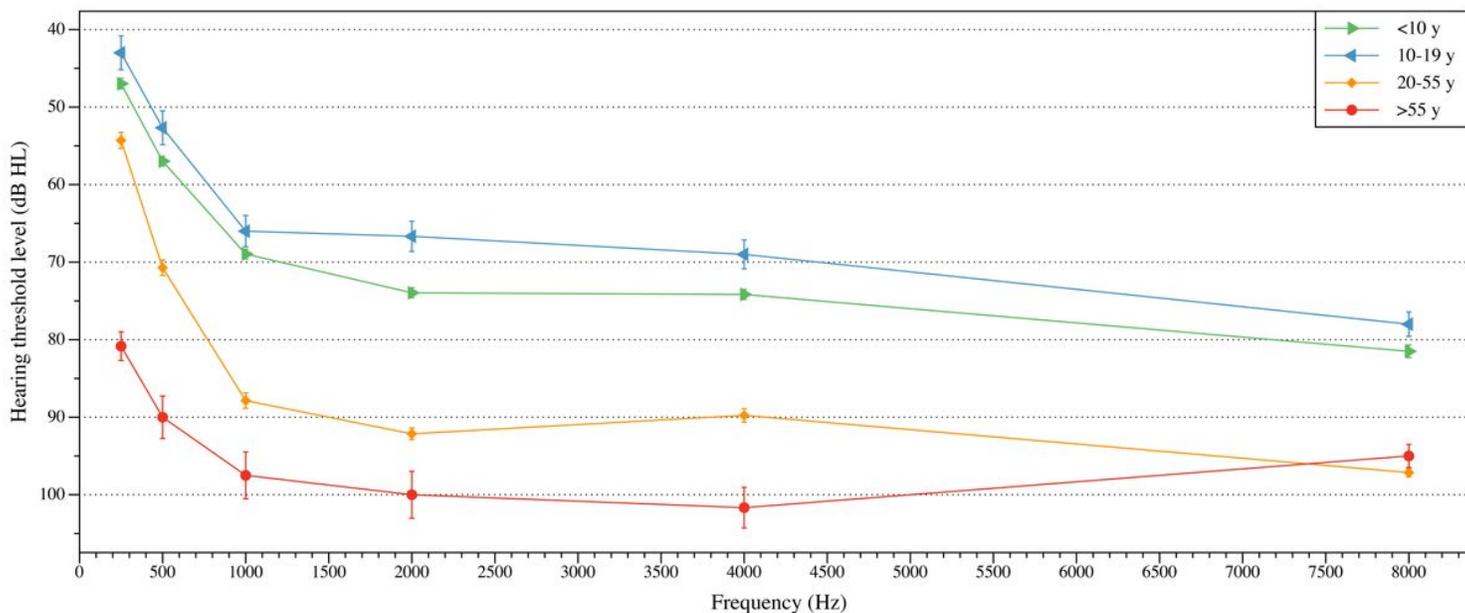


Figure 4

Audioprofiles of DFNB77 among different age groups. The audioprofiles are the average audiograms of all 111 cases reviewed in this study and are grouped in four age categories.

Supplementary Files

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