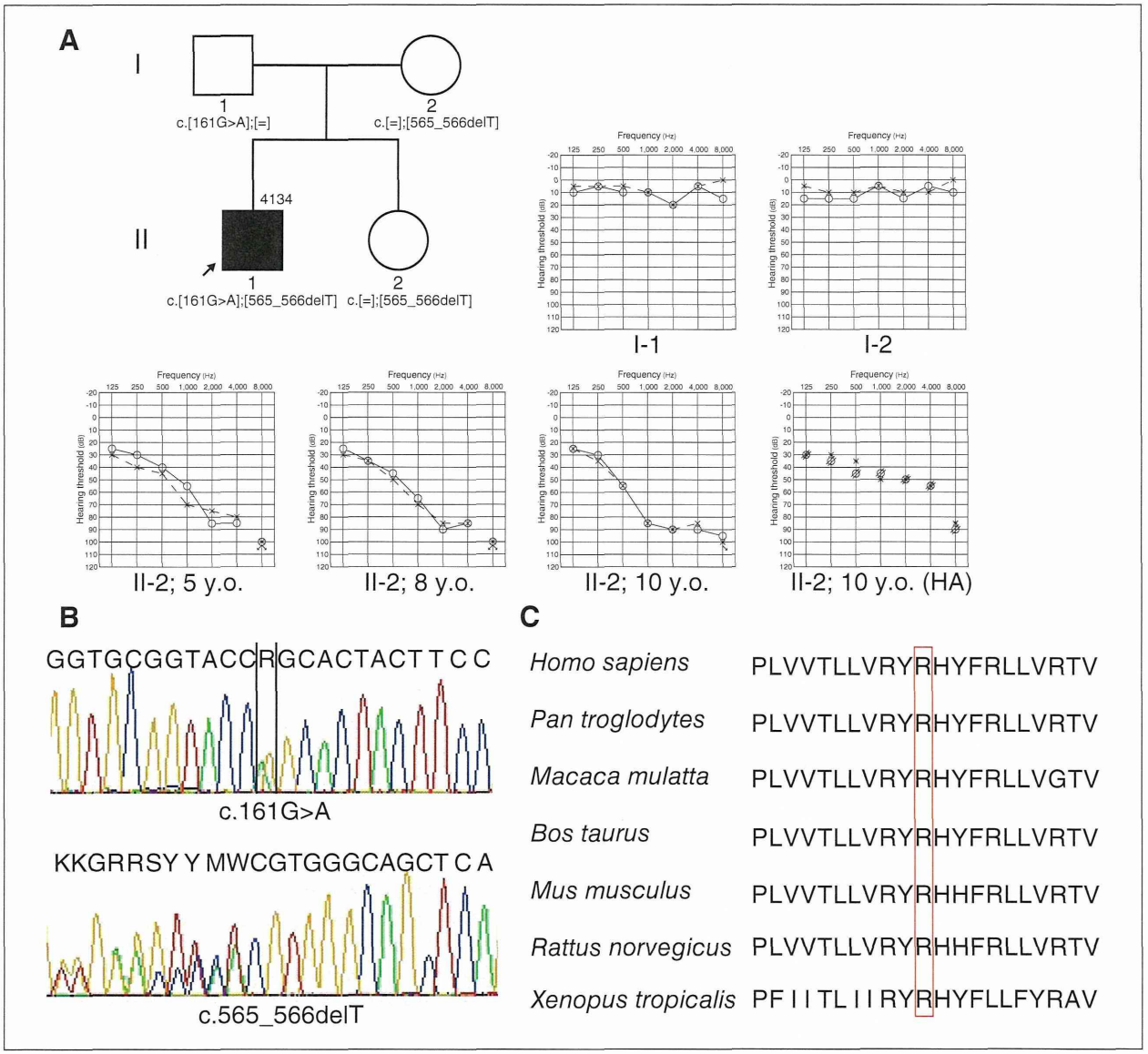

Figure 1. Pedigree and pure-tone audiograms of family members as well as the results of *LRTOMT* mutation analysis. (A) Pedigree shows that the sporadic nature of the cases and allele segregation is compatible with autosomal recessive inheritance. Pure-tone audiograms show the deterioration of the threshold from 55 to 85 dB at 1000 Hz over 5 years, suggesting progressive hearing loss. HA, hearing thresholds with hearing aids. (B) The electropherogram of mutations in case ID 4134. (C) Conservation of the region of the LRTOMT2 protein including the missense mutation (c.161G>A) site.

in both ears, and behavioral observation audiometry (BOA) showed thresholds of 30 to 50 dB at around 500 to 2000 Hz. Computed tomography (CT) findings of the middle and inner ear were normal. He was diagnosed with bilateral symmetric moderate sloping hearing loss in the high frequencies. He was promptly fitted with bilateral hearing aids. At the age of 5 years, pure-tone audiometry (PTA) was performed, showing down sloping moderate SNHL. He received followed-up auditory assessment for 6 years, and his hearing loss progressed at 1000 Hz and higher frequencies. He did not suffer from tinnitus or dizziness during this period.

His parents and younger sister had normal hearing; there was no positive family history of hearing loss or other cognitive disorders. Audiologic assessment results and pedigree are shown in Figure 1A.

Table 1. Known Mutations in the *LRTOMT* Gene and Associated Phenotypes.[a]

Nucleotide Change	Amino Acid Change	Type of Mutation	Zygosity	Hearing Loss Onset	Type of Hearing Loss	Population	Reference
c.47T>C	p.Leu16Pro	Missense	Homozygous	Prelingual	Severe-profound	Iranian	Du et al[5]
c.102G>A	p.Met34Ile	Missense	Homozygous	Prelingual	Profound	Iranian	Babanejad et al[6]
c.107delC	p.Ser35SerfsX13	Frameshift	Homozygous	Congenital	Profound	Iranian	Vanwesemael et al[7]
c.120G>T	p.Glu40Asp	Missense	Homozygous	Prelingual	Profound	Iranian	Babanejad et al[6]
c.121C>T	p.Arg41Trp	Missense	Homozygous	NA	NA	Iranian	Babanejad et al[6]
c.122G>A	p.Arg41Gln	Missense	Homozygous	Congenital	Severe	Tunisian	Ahmed et al[4]
c.122G>A	p.Arg41Gln	Missense	Homozygous	Congenital	Moderate-severe	Moroccan	Charif et al[8]
c.193T>C	p.Trp65Arg	Missense	Homozygous	NA	NA	Tunisian	Ahmed et al[4]
c.208G>A	p.Glu70Lys	Missense	Homozygous	Congenital	Profound	Pakistani	Ahmed et al[4]
c.213C>G	p.Tyr71X	Missense	Homozygous	Prelingual	Severe-profound	Iranian	Du et al[5]
c.238+4A>C	p.Ala29SerfsX54	Frameshift	Homozygous	Congenital	Severe-profound	Turkish	Ahmed et al[4]
c.161G>A	p.Arg54Gln	Missense	Compound heterozygous	Prelingual	Moderate	Japanese	This study
c.565_566delT	p.Ile188ThrfsX7	Frameshift		Prelingual	Moderate	Japanese	This study

Abbreviation: NA, not applicable or not reported.
[a]All nucleotide and amino acid changes are assigned to HGVS NM_001145309.

Mutation Analysis

We performed MPS and identified 1 novel frame-shift mutation and 1 missense mutation. The former mutation corresponded to c.565_566delT (NM_001145309) in exon 9 and led to a frameshift mutation and truncation (p.Ile188ThrfsX7). The second mutation was c.161G>A (p.Arg54Gln) located in exon 7, which was strongly suspected to be pathogenic. In silico prediction software (SIFT, MutationTaster, and Polyphen2) indicated the mutation as damaging (0.84, 0.98, and 1.00 [the maximum scores were 1.00], respectively). We also performed Sanger sequencing for the family segregation study and a confirmation of the variant MPS outputted result. As shown in Figure 1B, Sanger sequencing results revealed that the parents had 1 of either mutation in heterozygote and his younger sister had the heterozygous c.161G>A mutation. None of these mutations were identified in the 192 Japanese normal hearing controls. The residue is conserved as arginine in all sequenced vertebrates (Figure 1C).

Discussion

In this report, we identified a novel compound heterozygous mutation in the *LRTOMT* gene among sporadic hearing loss cases that were presumably autosomal recessive inherited. This is the first case reported to be affected by the compound heterozygous mutation (Table 1). Previously, there have been only 8 families reported with hearing loss caused by mutations in *LRTOMT*, and these were all homozygous mutations due to consanguineous families. In this study, we found a patient with hearing loss caused by a compound heterozygous mutation in a nonconsanguineous family. The c.161G>A mutation (NM_001145309) corresponded to a p.Arg54Gln substitution, which changed the basicity of arginine into a neutral glutamine residue. This arginine residue in the LRTOMT protein region of the mutated site was conserved among the other species. The other mutation corresponded to c.565_566delT (NM_001145309) and led to a frameshift mutation and a subsequent truncation of the protein (p.Ile188ThrfsX7) in exon 9. The mutated residues were segregated in both alleles of *LRTOMT*, present within the LRTOMT2 protein coding region. The LRTOMT2 protein has a transmembrane catechol-O-methyltransferase (COMT) domain and is also known as COMT2. This is strongly expressed in inner and outer hair cells and also in the vestibular organ.[5] Du et al[5] generated a mouse model of *Comt2* mutation (*add* mice) and found that the mice were profoundly deaf and had vestibular defects. Degeneration of the organ of Corti and disorganization of the stereocilia were observed by 8 weeks of age. Thus, these findings support the argument that mutations in LRTOMT2 are associated with hair cell defects and lead to SNHL. As shown in Figure 2, all previously reported mutations are assigned to the LRTOMT2 (NM_001145309) region, not to the LRTOMT1 (NM_145309) region. The majority of mutations, including the missense mutation that we identified, accumulate in exon 7 of the LRTOMT2 coding region. Therefore, the mutations in *LRTOMT2* are more likely to affect hearing loss through hair cell degeneration, and the region that exon 7 encodes might be a mutational hot spot in the *LRTOMT* gene.

Previous studies have shown that affected individuals had severe to profound prelingual SNHL, whereas the case with *LRTOMT* mutations that we identified had only moderate SNHL. The frameshift mutation, c.565_566delT, is located in

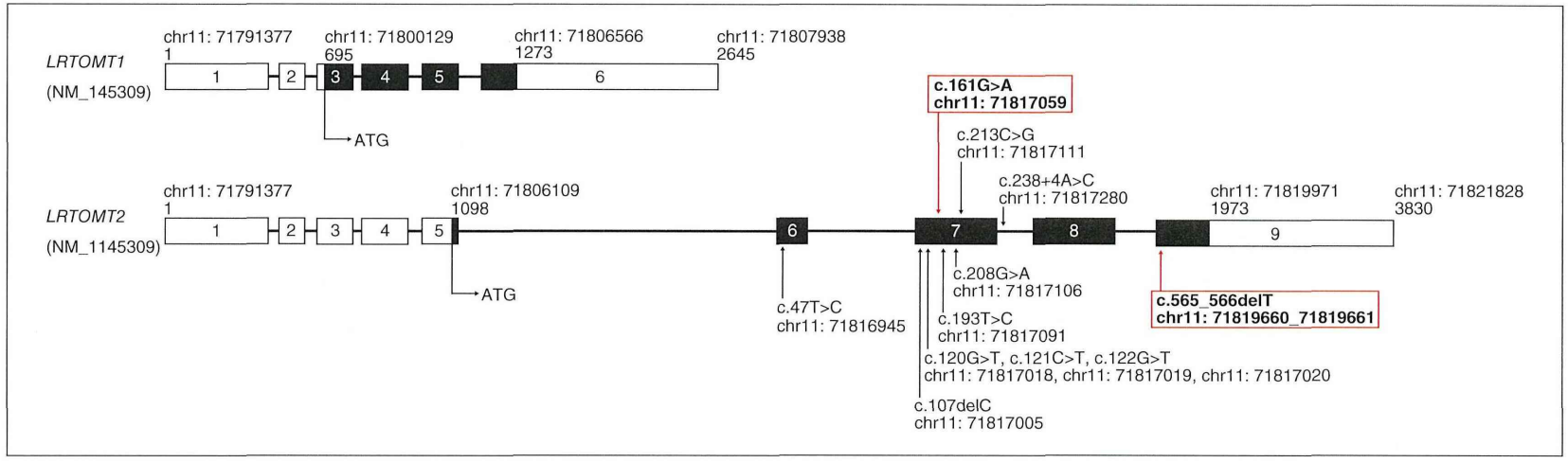

Figure 2. Two isoforms encoded by the *LRTOMT* gene. LRTOMT1 (NM_145309) and LRTOMT2 (NM_001145309) consist of 6 and 9 exons, respectively. Both are translated into 2 alternate reading frames using different exon sets; that of LRTOMT1 starts in exon 3 and that of LRTOMT2 starts in exon 5. CDS regions are colored black. Most of the previously reported mutations are located in exon 7 of the LRTOMT2 region.

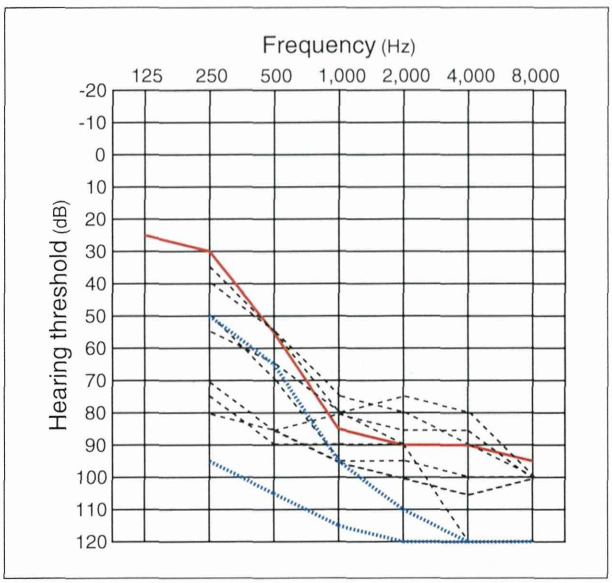

Figure 3. Overlapping audiogram of the affected individuals with *LRTOMT* mutations. Red line indicates the patient in this study. Dotted lines (in black) indicate the individuals reported by Kalay et al,[1] and dotted lines (in blue) indicate the individuals reported by Tlili et al.[3]

the region near the 3′-end and C-terminus of the LRTOMT2 region (Figure 2), in which the mutated allele might be translated partially. It can be supposed that an incomplete LRTOMT2 protein with residual activity was formed in the present case exhibiting moderate SNHL. Kalay et al[1] and Tlili et al[3] reported audiologic profiles for each individual showing high frequency sloping hearing loss. As shown in Figure 3, these overlapping PTA results were identical or similar to our case, but these studies had no longitudinal results. The serial audiologic findings for the 6 years of our study show the deterioration of hearing level in the middle frequencies. Our patient still had residual hearing in the lower frequencies, with hearing aids necessary, but hearing ability and speech perception with hearing aids are speculated to be further reduced due to the deterioration of mid- to high-frequency hearing. We suggest that careful management of hearing is necessary for *LRTOMT* hearing loss patients.

In conclusion, our results indicate that mutations in the *LRTOMT* gene lead to alterations in the LRTOMT2 (COMT2) protein and might be involved in progressive SNHL. Further studies, including a long-term follow-up and accurate characterization of phenotypic features, will afford a better understanding of the *LRTOMT* gene.

Acknowledgments

The authors thank Mr David Callaghan for his help in preparing the manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by a Health and Labour Sciences Research Grant for Research on Rare and Intractable Diseases and Comprehensive Research on Disability Health and Welfare from the Ministry of Health, Labour and Welfare of Japan (S.U.), and by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (S.U.).

References

1. Kalay E, Caylan R, Kiroglu AF, et al. A novel locus for autosomal recessive nonsyndromic hearing impairment, DFNB63, maps to chromosome 11q13.2-q13.4. *J Mol Med (Berl)*. 2007;85(4):397-404.
2. Khan SY, Riazuddin S, Tariq M, et al. Autosomal recessive nonsyndromic deafness locus DFNB63 at chromosome 11q13.2-q13.3. *Hum Genet*. 2007;120(6):789-793.
3. Tlili A, Masmoudi S, Dhouib H, et al. Localization of a novel autosomal recessive non-syndromic hearing impairment locus DFNB63 to chromosome 11q13.3-q13.4. *Ann Hum Genet*. 2007;71(pt 2):271-275.
4. Ahmed ZM, Masmoudi S, Kalay E, et al. Mutations of LRTOMT, a fusion gene with alternative reading frames, cause nonsyndromic deafness in humans. *Nat Genet*. 2008;40(11):1335-1340.
5. Du X, Schwander M, Moresco EM, et al. A catechol-O-methyltransferase that is essential for auditory function in mice and humans. *Proc Natl Acad Sci U S A*. 2008;105(38):14609-14614.
6. Babanejad M, Fattahi Z, Bazazzadegan N, et al. A comprehensive study to determine heterogeneity of autosomal recessive nonsyndromic hearing loss in Iran. *Am J Med Genet A*. 2012;158A(10):2485-2492.
7. Vanwesemael M, Schrauwen I, Ceuppens R, et al. A 1 bp deletion in the dual reading frame deafness gene LRTOMT causes a frameshift from the first into the second reading frame. *Am J Med Genet A*. 2011;155A(8):2021-2023.
8. Charif M, Bounaceur S, Abidi O, et al. The c.242G>A mutation in LRTOMT gene is responsible for a high prevalence of deafness in the Moroccan population. *Mol Biol Rep*. 2012;39(12):11011-11016.
9. Taghizadeh SH, Kazeminezhad SR, Sefidgar SA, et al. Investigation of LRTOMT gene (locus DFNB63) mutations in Iranian patients with autosomal recessive non-syndromic hearing loss. *Int J Mol Cell Med*. 2013;2(1):41-45.
10. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*. 2009;25(14):1754-1760.
11. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. *Nat Methods*. 2010;7(4):248-249.

Annals of Otology, Rhinology & Laryngology
1–12

DOI: 10.1177/0003489415575059
aor.sagepub.com
SAGE

Deafness Gene Variations in a 1120 Nonsyndromic Hearing Loss Cohort: Molecular Epidemiology and Deafness Mutation Spectrum of Patients in Japan

Shin-ya Nishio, PhD[1,2] and Shin-ichi Usami, MD, PhD[1,2]

Abstract

Objectives: To elucidate the molecular epidemiology of hearing loss in a large number of Japanese patients analyzed using massively parallel DNA sequencing (MPS) of target genes.

Methods: We performed MPS of target genes using the Ion PGM system with the Ion AmpliSeq and HiSeq 2000 systems using SureSelect in 1389 samples (1120 nonsyndromic hearing loss cases and 269 normal hearing controls). We filtered the variants identified using allele frequencies in a large number of controls and 12 predication program scores.

Results: We identified 8376 kinds of variants in the 1389 samples, and 409 835 total variants were detected. After filtering the variants, we selected 2631 kinds of candidate variants. The number of *GJB2* mutations was exceptionally high among these variants, followed by those in *CDH23, SLC26A4, MYO15A, COL11A2, MYO7A*, and *OTOF*.

Conclusions: We performed a large number of MPS analyses and clarified the genetic background of Japanese patients with hearing loss. This data set will be a powerful tool to discover rare causative gene mutations in highly heterogeneous monogenic diseases and reveal the genetic epidemiology of deafness.

Keywords

hearing loss, massively parallel DNA sequencing, next-generation DNA sequencer, molecular epidemiology

Introduction

Congenital hearing loss is one of the most common sensory disorders, occurring in 1 of 700 to 1000 newborns. Approximately 50% to 70% of cases are attributable to genetic causes,[1] and 10% to 25% of cases are attributable to congenital cytomegalovirus infection. More than 80 genes have been identified as a cause of hearing loss and an estimated 100 genes are involved in hearing loss.[2]

Despite such advances in gene identification, clinicians and/or geneticists sometimes encounter difficulties related to molecular diagnosis in a clinical setting; for example, the family size is not large enough to allow linkage analysis, meaning that only limited familial information for predicting the causative gene is available. In such cases, targeted exon sequencing of selected genes using massively parallel DNA sequencing (MPS) technology will potentially enable us to systematically tackle previously intractable monogenic disorders and improve molecular diagnosis.

An increasing number of articles regarding gene discovery and successful clinical application for the identification of genes responsible for deafness using MPS have recently been published.[3-16] We applied MPS technology to (1) discover causative mutations in relatively rare causative genes[12,13] and (2) clarify the molecular epidemiology.[12] Our results demonstrated that MPS-based screening is powerful in terms of identifying mutations in rare causative genes, and from an epidemiological view point, *GJB2* mutations are involved in 30% to 40% cases of deafness, while the remaining cases of hearing loss arise from various rare genes/mutations that were not easy to identify using the conventional one-by-one screening approach.

For clinical application to genetic heterogeneous diseases, systemic screening of known genes in a cost-effective manner is required. Hybridization-based capture is commonly used for genomic target enrichment, but for clinical application,

[1]Department of Otorhinolaryngology, Shinshu University School of Medicine, Matsumoto, Japan
[2]Department of Hearing Implant Sciences, Shinshu University School of Medicine, Matsumoto, Japan

Corresponding Author:
Shin-ichi Usami, Department of Otorhinolaryngology, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto 390-8621, Japan.
Email: usami@shinshu-u.ac.jp

polymerase chain reaction (PCR)–based technologies in combination with MPS have also been proposed.[11,13,16,17]

In the current study, on the basis of our PCR-based technologies in combination with MPS,[13,17] we increased the number of patients (1120 cases of nonsyndromic hearing loss) to establish a database for clinical molecular diagnosis and to confirm the molecular epidemiology of deafness. Data analysis concerning diagnostic sensitivity and specificity, which is important for clinical application, was also performed.

Subjects and Methods

Subjects

A total of 1120 Japanese patients (266 autosomal dominant or mitochondrial inheritance cases, 600 autosomal recessive inheritance or sporadic cases, and 253 unknown family history cases) with bilateral nonsyndromic sensorineural hearing loss from 53 ear, nose, and throat departments nationwide participated in this study. In addition, 269 normal hearing controls, confirmed by pure-tone audiometry, were also enrolled. Informed written consent was obtained from all subjects, their next of kin, caretakers, or guardians (in the case of minors) prior to participation. This study was approved by the Shinshu University Ethical Committee and the ethics committees of all other participating institutions listed in the Acknowledgments.

Genetic Analysis

We performed the MPS analysis using an Ion PGM with Ion AmpliSeq for 1174 samples (905 hearing loss cases and 269 normal hearing controls) and using HiSeq 2000 with SureSelect in 215 cases.

Amplicon Library Preparation and Ion PGM Platform Sequencing

Amplicon libraries of the target gene exons from 63 genes reported to cause nonsyndromic hearing loss[2] were prepared with an Ion AmpliSeq Custom Panel (Life Technologies, Foster City, California, USA). These libraries were designed with an Ion AmpliSeq Designer (Life Techologies), and amplicon libraries were prepared using an Ion AmpliSeq Library Kit 2.0 and an Ion Xpress Barcode Adapter 1-96 Kit (Life Technologies) according to the manufacturer's instructions. After the amplicon libraries were prepared, they were diluted to 20 pM, and the same amount of libraries from the 6 libraries of 6 patients were pooled for 1 sequence reaction. The emulsion polymerase chain reaction and sequencing were performed with an Ion Torrent Personal Genome Machine (PGM) system using an Ion PGM 200 Sequencing Kit and an Ion 318 Chip (Life Technologies) according to the manufacturer's instructions. The detailed protocol has been described elsewhere.[13,17] The sequence data were mapped to the human genome sequence (build GRCh37/hg19) with the Torrent Mapping Alignment Program. After sequence mapping, the DNA variant regions were piled up with the Torrent Variant Caller plug-in software version 4.0 (Life Technologies).

Targeted Enrichment and HiSeq Platform Sequencing

The SureSelect target enrichment kit, designed for the 112 potentially deaf-causing genes, including the 63 genes reported to cause nonsyndromic hearing loss, the 22 genes reported to cause syndromic hearing loss, and the 36 genes highly expressed in the adult human inner ear by microarray analysis, was used in this study.[18] The detailed gene list is described in our previous report.[12] A 3-μg DNA aliquot was fragmented using the Covaris S2 System (Covaris, Woburn, Massachusetts, USA) to a fragment length of about 200 bp. Furthermore, the target regions were enriched using the SureSelect Target DNA Enrichment kit with a barcode adapter (Agilent Technologies, Santa Clara, California, USA) according to the manufacturer's protocol. The same amount of libraries from each of 12 patients was pooled into 1 tube and analyzed in 1 lane of the Illumina HiSeq 2000 sequencer (Illumina, San Diego, California, USA) according to the manufacturer's protocol. The sequence data were processed by filtering the read quality to QV = 30 as a cut-off and duplicate reads removed. After the filtering process, sequence reads were mapped to the human genome sequence (build GRCh37/hg19) using BWA software.[19] After sequence mapping, the DNA variant regions were piled up with GATK software.[20]

Filtering Detected Variants

After detecting the variants, the effects of the variants were analyzed using ANNOVAR software.[21,22] The missense, nonsense, insertion/deletion, and splicing variants were selected among the identified variants. Variants were further selected as <1% of: (1) the 1000 genome database,[23] (2) the 6500 exome variants,[24] (3) the human genetic variation database (data set for 1208 Japanese exome variants),[25] (4) the 269 in-house Japanese normal hearing controls, and (5) 1000 control data in the deafness variation database.[26] The filtering process is shown in Figure 1.

Results and Discussion

DNA Sequencing Metrics and Accuracy of Each Sequencing System

MPS metrics used in this study are summarized in Supplemental Table 1 (available in the online journal). The

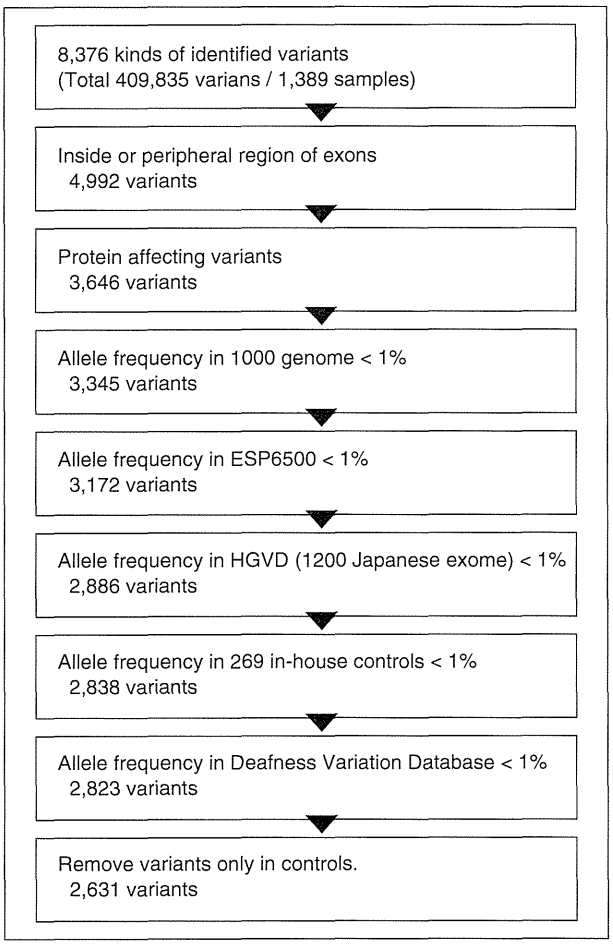

Figure 1. Algorithm applied in this study. The nonsense, splice-site, insertion-deletion, and missense variants were chosen according to this algorithm.

mean depth of coverage of the target region for 1174 samples analyzed by the Ion PGM sequencer was 284.3 ± 94.5× (range, 690.0-96.6×). The percentage of each region with more than 20× coverage (indicating the percentage of each region sequenced 20 times or more by MPS) was 97.6% ± 0.9% (range, 93.1%-99.2%). To reduce the risk of incorrect genotyping and missed *single nucleotide polymorphisms* (SNPs) in poor-coverage regions, we employed a minimum mean depth of coverage of 100 and a minimum percentage of 96% for regions with more than 20× coverage. The mean depth of coverage for 215 samples analyzed by the HiSeq 2000 sequencer was 1536.1 ± 538.4× (range, 206-5925×). The percentage of each region with more than 20× coverage (indicating the percentage of each region sequenced 20 times or more by MPS) was 98.8% ± 0.7% (range, 93.5%-99.4%).

To investigate the accuracy of the MPS used in this study, we compared the results of the Invader assay-based mutation screening[27] and MPS by blinded samples (384 samples were analyzed by both methods). As a result, 99.98% of results were identical in the Ion PGM system. Detailed information regarding this comparison was given in our recent report.[17]

DNA Variants Identified in the Large Japanese Nonsyndromic Hearing Loss Cohort

From the 1389 samples, including 1120 nonsyndromic sensorineural hearing loss cases and 269 controls, we identified 8376 kinds of variants, and 409 835 (average, 295.1 variants/sample; Figure 1) total variants were detected. Among the 8376 variants, 4992 were located in the exon region, 2 were located in exonic regions of micro-RNA MIR96, which is a causative micro RNA associated with DFNA50, and 92 were located in splicing junctions. The others were located in the 3'- untranslated region (UTR), 5'-UTR, intron, and intergenic regions. Among the exon region variants, 3646 affected proteins (2955 missense variants, 76 nonsense variants, 161 frame shift deletions, 71 frame shift insertions, 136 frame shift multibase substitutions, 89 non–frame shift deletions, 4 non–frame shift insertions, 149 non–frame shift multibase substitutions, 2 exonic splice junction substitutions, and 3 stop loss mutations). Together with the splicing junction and noncoding RNA mutations, 3742 variants remained for further analysis.

We filtered these variants using allele frequency <1% of (1) the 1000 genome project; (2) the exome variant server; (3) the human genetic variation database, which contains 1200 Japanese exome data; (4) the 269 in-house Japanese normal hearing controls; and the (5) 1000 controls in the deafness variation database. For this filtering step, we employed <1% frequency as a cutoff line because the most frequent pathogenic variants observed in the Japanese population were *GJB2*: c.235delC and *GJB2*: c.109C>G, and the allele frequencies in the Japanese control population were estimated as 0.4% and 0.6%.[28] However, there were some possibilities to filter out the frequent autosomal recessive (AR)-pathogenic variants; therefore, we did not filter out the pathogenic or likely pathogenic variants previously reported in the deafness variation database[26] and ClinVar.[29]

After filtering the many ethnic controls, 2823 variants remained (Figure 1). Among them, we removed variants only found in controls as probable polymorphisms. Finally, 2631 variants were selected as candidates (Figure 1; 2017 missense mutations, 72 nonsense mutations, 2 stop loss mutations, 131 frame shift deletions, 30 frame shift insertions, 129 frame shift multibase substitutions, 21 non–frame shift deletions, 4 non–frame shift insertions, 138 non–frame shift multibase substitutions, 2 exon split junction substitutions, 77 splicing junction regions, and 2 micro-RNA MIR96 exonic regions).

Table 1. Previously Reported Pathogenic Variants Detected in This Study.

Identified Variants	ESP6500	1000g	HGVD	dbSNP138	SIFT	PP2 HDVI	PP2 HVAR_	ClinVar	DVD	Disease	PMID	Pat NUM	CNT Num
ACTG1:NM_001614:c.353A>T:p.K118M				rs104894544	D	B	P	Pathogenic (DFNA20)	Pathogenic	NSHL-Dominant	13680526	3	0
ACTG1:NM_001614:c.721G>A:p.E241K				rs267606631	D	D	D	Pathogenic (DFNA20)	Pathogenic	NSHL-Dominant	19477959	1	0
CDH23:NM_022124:c.2407G>A:p.V803I			0.00141		T	B	B		Pathogenic	NSHL-Recessive	22899989	0	1
CDH23:NM_022124:c.2866G>A:p.E956K					D	D	D		Pathogenic	NSHL-Recessive	22899989	4	0
CDH23:NM_022124:c.4249C>T:p.R1417W			0.001255		D	D	P		Pathogenic	NSHL-Recessive	22899989	6	2
CDH23:NM_022124:c.5131G>A:p.V1711I	0.000079	0.000399361	0.001247	rs181611778	T	D	D		Pathogenic	NSHL-Recessive	22899989	2	0
CDH23:NM_022124:c.5147A>C:p.Q1716P					T	D	D		Pathogenic	NSHL-Recessive	17850630	4	0
CDH23:NM_022124:c.5627G>A:p.S1876N			0.003179		T	P	P		Pathogenic	NSHL-Recessive	22899989	6	0
CDH23:NM_022124:c.6085C>T:p.R2029W			0.002271		D	D	D		Pathogenic	NSHL-Recessive	17850630	19	1
CDH23:NM_022124:c.6319C>T:p.R2107X					T	.	.		Pathogenic	Usher syndrome	11090341	1	0
CDH23:NM_022124:c.6389C>T:p.A2130V					T	B	B		Pathogenic	NSHL-Recessive	22899989	2	0
CDH23:NM_022124:c.6861T>G:p.N2287K					D	D	D		Pathogenic	NSHL-Recessive	22899989	1	0
CDH23:NM_022124:c.719C>T:p.P240L		0.000199681	0.002725	rs121908354	T	D	D	Pathogenic (DFNB12)	Pathogenic	NSHL-Recessive	17850630	45	2
CDH23:NM_022124:c.902G>A:p.R301Q	0.000081			rs121908355	T	D	D	Pathogenic (Alport syndrome)	Pathogenic	NSHL-Recessive	17850630	2	0
CDH23:NM_022124:c.9127C>T:p.R3043W	0.00008				D	D	P		Pathogenic	Usher syndrome	21569298	1	0
COCH:NM_004086:c.263G>A:p.G88E				rs121908928	T	D	D	Pathogenic (DFNA9)	Pathogenic	NSHL-Dominant	9806553	1	0
COL11A2:NM_080680:c.2492C>T:p.S831L	0.000118			rs121912949	T	D	P		Pathogenic	Otospondylomegaepiphyseal dysplasia, AD	NULL	1	0
COL4A5:NM_000495:c.2215C>G:p.P739A		0.00344371	0.059811	rs104886164	T	B	B	Pathogenic (Alport syndrome)				19	0
COL4A5:NM_000495:c.2858G>T:p.G953V	0.000189	0.00794702	0.01005	rs78972735	.	.	.	Pathogenic (Alport syndrome)				2	0
CRYM:NM_001888:c.941A>C:p.K314T				rs104894512	D	P	B	Pathogenic (AD-NSHL)	Pathogenic	NSHL-Dominant	12471561	2	0
EYA1:NM_000503:c.1276G>A:p.G426S			0.00134	rs121909199	D	D	D	Pathogenic (BOR syndrome)	Pathogenic	BOR syndrome, AD	10655545	2	0
EYA1:NM_000503:c.1319G>A:p.R440Q				rs121909196	D	D	D	Pathogenic (Melnick-Fraser syndrome)	Pathogenic	BOR syndrome, AD	10464653	1	0
EYA1:NM_000503:c.724A>G:p.S242G		0.000199681	0.01083	rs191838840	T	B	B		Pathogenic	BOR syndrome, AD	12701758	4	0
GJB2:NM_004004:c.109G>A:p.V37I	0.001307	0.0153754	0.006806	rs72474224	T	D	D	Pathogenic (DFNB1A)	Pathogenic	NSHL-Recessive	10633133	47	4
GJB2:NM_004004:c.134G>A:p.G45E			0.00349	rs72561723	D	D	D	Pathogenic (DFNB1A)	Pathogenic	KID syndrome, DFNB1A	10501520	46	1
GJB2:NM_004004:c.146C>T:p.A49V			0.002494		D	P	B		Pathogenic	NSHL-Recessive	12560944	2	0
GJB2:NM_004004:c.212T>C:p.I71T			0.001166		D	D	P		Pathogenic	NSHL-Recessive	12560944	1	1
GJB2:NM_004004:c.223C>T:p.R75W				rs104894402	D	D	D	Pathogenic (DFNA3A)	Pathogenic	NSHL-Dominant	9856479	1	0
GJB2:NM_004004:c.235delC:p.L79fs		0.00159744		rs80338943				Pathogenic (DFNB1A)	Pathogenic	NSHL-Recessive	10501520	166	3
GJB2:NM_004004:c.257C>G:p.T86R					D	D	D		Pathogenic	NSHL-Recessive	12560944	11	0
GJB2:NM_004004:c.257C>T:p.T86M					D	D	D		Pathogenic	NSHL-Recessive	17041943	2	0
GJB2:NM_004004:c.29T>C:p.L10P					D	D	D		Pathogenic	NSHL-Recessive	12865758	1	0
GJB2:NM_004004:c.334_335del:p.K112fs									Pathogenic	NSHL-Recessive	9529365	1	0
GJB2:NM_004004:c.368C>A:p.T123N	0.000154	0.00179712	0.006146	rs111033188	T	B	B	Probable nonpathogenic	Pathogenic	NSHL-Recessive	10983956	4	2
GJB2:NM_004004:c.379C>T:p.R127C					D	D	B		Pathogenic	NSHL-Recessive	11587277	1	0
GJB2:NM_004004:c.389G>C:p.G130A					D	D	D		Pathogenic	NSHL-Recessive	12792423	1	0
GJB2:NM_004004:c.408C>A:p.Y136X			0.00349		T	.	.		Pathogenic	NSHL-Recessive	10501520	46	1
GJB2:NM_004004:c.427C>T:p.R143W	0.000231	0.000199681	0.002331	rs80338948	D	D	D	Pathogenic (DFNB1A)	Pathogenic	NSHL-Recessive	9471561	29	0
GJB2:NM_004004:c.511G>A:p.A171T	0.000154	0.000399361	0.001166	rs201004645	T	P	B	Probable nonpathogenic	Pathogenic	NSHL-Recessive	11438992	1	0

(continued)

Table 1. (continued)

Identified Variants	ESP6500	1000g	HGVD	dbSNP138	SIFT	PP2 HDVI	PP2 HVAR_	ClinVar	DVD	Disease	PMID	Pat NUM	CNT Num
GJB2:NM_004004:c.571T>C:p.F191L		0.000199681	0.004115		D	D	D	Probable nonpathogenic	Pathogenic	NSHL-Recessive	12772454	0	1
GJB2:NM_004004:c.583A>G:p.M195V			0.001166		D	D	D		Pathogenic	NSHL-Recessive	20497192	4	0
GJB2:NM_004004:c.95G>A:p.R32H				rs111033190	D	D	D	Pathogenic (DFNB1A)	Pathogenic	NSHL-Recessive	11493200	3	0
GJB2:NM_004004c.299_300del:p.H100fs				rs111033204				Pathogenic (DFNB1A)	Pathogenic	NSHL-Recessive	10633133	14	0
GJB3:NM_024009:c.538C>T:p.R180X		0.000199681		rs74315319	T	.	.	Pathogenic (DFNA2B)	Benign*	NULL	NULL	1	0
GJB3:NM_024009:c.547G>A:p.E183K	0.000077	0.000998403	0.001361	rs74315318	D	D	D	Pathogenic (DFNA2B)	Benign*	NULL	NULL	2	0
GJB3:NM_024009:c.580G>A:p.A194T		0.00139776	0.01179	rs117385606	T	B	B	Pathogenic	Benign*	NULL	NULL	16	2
GJB6:NM_006783:c.689dupA:p.N230fs	0.000639							Pathogenic				9	0
KCNQ4:NM_004700:c.546C>G:p.F182L		0.000599042	0.006579	rs80358273	T	B	B	Pathogenic (DFNA2)	Pathogenic	NSHL-Dominant	17033161	8	1
LOXHD1:NM_144612:c.4480C>T:p.R1494X	0.001314	0.000199681		rs201587138	T	.	.		Pathogenic	NSHL-Recessive	23226338	2	0
LOXHD1:NM_144612:c.469C>T:p.R157C		0.000399361			.	.	.		Pathogenic	Fuchs corneal dystrophy		1	0
LOXHD1:NM_144612:c.4714C>A:p.R1572R	0.000657	0.0181709	0.051502	rs75949023				Pathogenic (DFNB77)	Benign*	NULL	NULL	83	18
MARVELD2:NM_001244734:c.1295+1G>A					.	.	.		Pathogenic	NSHL-Recessive	18084694	1	0
MYH9:NM_002473:c.2104C>T:p.R702C				rs80338826	D	D	D	Pathogenic (Fechtner syndrome)	Pathogenic	Epstein syndrome	10973259	1	0
MYH9:NM_002473:c.2114G>A:p.R705H				rs80338828	D	D	D	Pathogenic (DFNB17)	Pathogenic	NSHL/MYH9 related diseases, AD	11023810	1	0
MYO15A:NM_016239:c.6731G>A:p.G2244E					D	D	D		Pathogenic	NSHL-Recessive	17546645	2	0
MYO15A:NM_016239:c.8467G>A:p.D2823N					D	D	D		Pathogenic	NSHL-Recessive	22736430	1	0
MYO6:NM_004999:c.3496C>T:p.R1166X				rs121912558	T	.	.	Pathogenic (DFNB37)	Pathogenic	NSHL-Recessive	12687499	1	0
MYO7A:NM_000260:c.2005C>T:p.R669X	0.000081			rs111033201	T	.	.	Pathogenic (USH1B)	Pathogenic	Usher syndrome	9718356	1	0
MYO7A:NM_000260:c.2311G>T:p.A771S			0.003129		D	P	P		Pathogenic	Usher syndrome	20844544	4	1
MYO7A:NM_000260:c.3508G>A:p.E1170K				rs111033214	D	D	D	Pathogenic (USH1B)	Pathogenic	Usher syndrome	10425080	1	0
MYO7A:NM_000260:c.3602G>C:p.C1201S		0.000798722	0.002287	rs117966637	D	D	D	Unknown	Pathogenic	Usher syndrome	23237960	3	1
MYO7A:NM_000260:c.3718C>T:p.R1240W	0.000079	0.000199681		rs371374104	D	D	D		Pathogenic	Usher syndrome	16963483	1	0
MYO7A:NM_000260:c.3979G>A:p.E1327K	0.000079			rs373169422	D	D	D		Pathogenic	Usher syndrome	12112664	1	0
MYO7A:NM_000260:c.635G>A:p.R212H				rs28934610	D	D	D	Pathogenic (USH1B)	Pathogenic	Usher syndrome	7870171	2	0
MYO7A:NM_000260:c.652G>A:p.D218N	0.00008			rs201539845	D	D	D	Pathogenic (DFNA11)	Pathogenic	NSHL-Recessive	21150918	1	0
OTOF:NM_194248:c.1236delC:p.P412fs								Pathogenic (DFNB9)				2	0
OTOF:NM_194248:c.1273C>T:p.R425X					T	.	.	Pathogenic (DFNB9)				1	0
OTOF:NM_194248c.4023+1G>A		0.00179712	0.002269	rs186810296	.	.	.	Pathogenic				5	2
PCDH15:NM_033056:c.733C>T:p.R245X	0.000384			rs111033260	T	.	.	Pathogenic (USH1F)	Benign*	NULL	NULL	2	0
SIX1:NM_005982:c.386A>G:p.Y129C				rs104894478	D	D	D	Pathogenic (BOR syndrome 3)	Pathogenic	BOR syndrome, AD	15141091	1	0
SLC26A4:NM_00441:c.1001+1G>A	0.000461			rs80338849	.	.	.	Pathogenic (DFNB4)	Pathogenic	Pendred syndrome-Recessive	9618167	1	0
SLC26A4:NM_00441:c.1115C>T:p.A372V				rs121908364	D	D	D	Pathogenic (DFNB4)	Pathogenic	NSHL; NSHL with EVA, AR	10190331	1	0
SLC26A4:NM_00441:c.1174A>T:p.N392Y		0.000199681		rs201562855	D	D	D		Pathogenic	NSHL-Recessive	12676893	1	0
SLC26A4:NM_00441:c.1229C>T:p.T410M	0.000231	0.000199681	0.00134	rs111033220	D	D	D	Pathogenic (DFNB4)	Pathogenic	NSHL with EVA/Pendred syndrome	9618167	16	1
SLC26A4:NM_00441:c.1315G>A:p.G439R					D	D	D		Pathogenic	NSHL-Recessive	17851929	2	0
SLC26A4:NM_00441:c.1489G>A:p.G497S				rs111033308	D	D	D	Pathogenic (DFNB4)	Pathogenic	NSHL with EVA/Pendred syndrome	9500541	1	0
SLC26A4:NM_00441:c.1579A>C:p.T527P			0.00134		D	D	D		Pathogenic	NSHL-Recessive	17851929	3	0
SLC26A4:NM_00441:c.165-13T>G									Pathogenic	NSHL with EVA, AR	19645628	0	1
SLC26A4:NM_00441:c.1804-6G>A		0.000599042		rs377713770					Pathogenic	NSHL-Recessive	15574297	2	0

(continued)

Table 1. (continued)

Identified Variants	ESP6500	1000g	HGVD	dbSNP138	SIFT	PP2 HDVI	PP2 HVAR_	ClinVar	DVD	Disease	PMID	Pat NUM	CNT Num
SLC26A4:NM_000441:c.2162C>T:p.T721M				rs121908363	D	D	D	Pathogenic (DFNB4)	Pathogenic	NSHL with EVA/Pendred syndrome	10190331	4	0
SLC26A4:NM_000441:c.2168A>G:p.H723R		0.000399361	0.002264	rs121908362	D	D	D	Pathogenic (DFNB4)	Pathogenic	NSHL with EVA/Pendred syndrome	9618166	53	2
SLC26A4:NM_000441:c.2219G>T:p.G740V	0.000154			rs111033310	T	B	B	Unknown	Pathogenic	NSHL with EVA, AR	16570074	1	0
SLC26A4:NM_000441:c.2228T>A:p.L743X					T	.	.		Pathogenic	NSHL with EVA, AR	19954013	2	0
SLC26A4:NM_000441:c.225C>G:p.L75L	0.000231	0.000399361	0.002141	rs187447337					Pathogenic	NSHL-Recessive	23185506	1	1
SLC26A4:NM_000441:c.2283A>G:p.T761T		0.000399361	0.038462	rs202033028					Pathogenic	NSHL-Recessive	23185506	1	0
SLC26A4:NM_000441:c.367C>T:p.P123S			0.001166		T	D	D		Pathogenic	NSHL-Recessive	14508505	2	0
SLC26A4:NM_000441:c.439A>G:p.M147V			0.001667		D	D	D		Pathogenic	NSHL-Recessive	14508505	3	0
SLC26A4:NM_000441:c.601-1G>A			0.001166		.	.	.		Pathogenic	Pendred syndrome-Recessive	14508505	5	0
SLC26A4:NM_000441:c.678T>C:p.A226A									Pathogenic	NSHL-Recessive	23185506	1	0
SLC26A4:NM_000441:c.697G>C:p.V233L					T	D	D	Unknown	Pathogenic	NSHL-Recessive	17443271	1	0
SLC26A4:NM_000441:c.757A>G:p.I253V			0.001166		T	P	P		Pathogenic	NSHL-Recessive	23185506	2	0
SLC26A4:NM_000441:c.918+1G>A					.	.	.		Pathogenic	Pendred syndrome-Recessive	9618166	1	0
SLC26A4:NM_000441:c.919-18T>G									Pathogenic	NSHL-Recessive	20137612	3	0
SLC26A4:NM_000441:c.919-2A>G			0.00134	rs111033313	.	.	.	Pathogenic (DFNB4)	Pathogenic	NSHL with EVA/Pendred syndrome	10874637	8	0
SLC26A4:NM_000441:c.920C>T:p.T307M	0.000077	0.000199681	0.001166	rs144691257	D	D	D		Pathogenic	NSHL with EVA/Mondini, AR	16570074	2	0
SLC26A4:NM_000441:c.G1975G>C:p.V659L		0.000199681		rs200455203	D	P	B		Pathogenic	NSHL-Recessive	17443271	2	0
TECTA:NM_005422:c.1685C>T:p.T562M			0.00187		T	D	P		Pathogenic	NSHL-Dominant	21520338	0	1
TECTA:NM_005422:c.4198C>T:p.H1400Y		0.000199681	0.00271		T	D	P		Pathogenic	NSHL-Dominant	22718023	2	2
TECTA:NM_005422:c.5372C>G:p.P1791R					T	B	B		Pathogenic	NSHL-Dominant	21520338	1	0
TECTA:NM_005422:c.5597C>T:p.T1866M	0.000077			rs140236996	D	D	D		Pathogenic	NSHL-Dominant	20947814	1	0
TMC1:NM_138691:c.1165C>T:p.R389X	0.000077	0.000199681		rs151001642	T	.	.	Pathogenic	Pathogenic	NSHL-Recessive	15605408	1	0
TMIE:NM_147196:c.257G>A:p.R86Q					D	D	P		Pathogenic	NSHL-Recessive	20206386	1	0
TMPRSS3:NM_024022:c.916G>A:p.A306T		0.000199681	0.002058	rs181949335	.	D	D	Probable-pathogenic	Pathogenic	NSHL-Recessive	17551081	1	1
USH1C:NM_005709:c.1016G>A:p.R339Q					D	P	B		Pathogenic	Usher syndrome	22135276	0	1
USH2A:NM_206933:c.1876C>T:p.R626X					T	.	.		Pathogenic	Usher syndrome	10729113	1	0
USH2A:NM_206933:c.2802T>G:p.C934W		0.000798722	0.003333	rs201527662	D	D	D	Pathogenic (USH2A)	Benign*	NULL	NULL	3	1
USH2A:NM_206933:c.802G>A:p.G268R				rs111033280	D	D	D	Unknown	Pathogenic	Usher syndrome	18273898	1	0
USH2A:NM_206933:c.8254G>A:p.G2752R		0.000399361		rs201863550	D	D	D		Pathogenic	Usher syndrome	19737284	1	0
USH2A:NM_206933:c.8559-2A>G		0.000199681			.	.	.	Pathogenic (USH2A)	Pathogenic	Usher syndrome	19023448	6	0
WFS1:NM_006005:c.1846G>T:p.A616S		0.000199681	0.003411		T	B	B		Pathogenic	NSHL-Dominant	16408729	1	0
WFS1:NM_006005:c.1957C>T:p.R653C	0.000231	0.000199681	0.1	rs201064551	D	D	D		Pathogenic	Diabetes, AD		1	0
WFS1:NM_006005:c.2051C>T:p.A684V					D	D	D	Pathogenic (Wolfram-like syndrome, AD)	Pathogenic	Wolfram-like syndrome (deafness with optic atrophy), AD		1	0
WFS1:NM_006005:c.2146G>A:p.A716T				rs28937893	T	D	P	Pathogenic (Wolfram-like syndrome, AD)	Pathogenic	Wolfram-like syndrome (deafness with optic atrophy), AD	11709537	1	0
WFS1:NM_006005:c.2171C>T:p.P724L				rs28937890	D	D	D	Pathogenic (Wolfram-like syndrome, AD)	Pathogenic	Wolfram-like syndrome (deafness with optic atrophy), AD	9771706	0	1
WFS1:NM_006005:c.2507A>C:p.K836T					T	D	D		Pathogenic	NSHL-Dominant	19877185	1	0
WFS1:NM_006005:c.2590G>A:p.E864K				rs74315205	T	D	D	Pathogenic (Wolfram-like syndrome, AD)	Pathogenic	Wolfram-like syndrome (deafness with optic atrophy), AD		2	0
WFS1:NM_006005c.2185G>A:p.D729N		0.000399361			T	B	B		Pathogenic	Wolfram syndrome, AR	12107816	1	0

Abbreviations: 1000g, 1,000 genome database[23]*; ClinVar, Clinical variation database29; DVD: Deafness variation database*[26]*; ESP6500, 6500 exome variants*[24]*; HGVD, Human Genetic Variation Database*[25]*;* NUM, identified allele number in 269 normal hearing controls; Pat NUM, identified allele number in 1120 hearing loss cases; CNT NUM, identified allele number in 269 controls; Benign*, recently re-categorized variants (from pathogenic to benign) using a large number of many ethnic controls allele frequencies[26]; AD, autosomal dominant; AR autosomal recessive; NSHL, non-syndromic hearing loss; BOR, Branchio-oto-renal; EVA, enlarged vestibular aqueduct; PP2, PolyPhen2; PMID, PubMed ID.