

selected genes using massively parallel DNA sequencing (MPS) technology have enabled successful identification of causative mutations in large genes such as *MYO15A*. Accordingly, an increasing number of reports on *MYO15A* mutations in hearing loss populations have been published.⁴⁻⁷ These reports suggest that MPS screening of candidate genes is an effective and useful strategy. The present study uses a large nonbiased cohort and MPS to clarify the contribution of *MYO15A* mutations in hearing loss populations.

Subjects and Methods

Subjects

A total of 1120 Japanese hearing loss patients (autosomal dominant SNHL, 266; ARNSHL, 600; unknown, 254) from 53 otorhinolaryngology departments nationwide participated in this study. Written informed consent was obtained from all subjects (or from their next of kin, caretaker, or guardian on the behalf of minors/children) prior to enrollment. This study was approved by the Shinshu University Ethical Committee as well as the respective institutional review boards of the other participating institutions.

Amplicon Library Preparation

Amplicon libraries were prepared according to the manufacturer's instructions using an Ion AmpliSeq Custom Panel (Applied Biosystems, Life Technologies, Carlsbad, California, USA) for 63 genes that reportedly cause non-syndromic hearing loss. The detailed protocol is described elsewhere.⁸ The amplicon libraries were diluted to 20 pM, and equal amounts of 6 libraries for 6 patients were pooled for one sequence reaction.

Emulsion Polymerase Chain Reaction and Sequencing

Emulsion polymerase chain reaction (PCR) and sequencing were performed according to the manufacturer's instructions. The detailed protocol is described elsewhere.⁸ MPS was performed with an Ion Torrent Personal Genome Machine (PGM) using an Ion PGM 200 Sequencing Kit and an Ion 318 Chip (Life Technologies).

Base Call and Data Analysis

Sequence data were mapped against the human genome sequence (build GRCh37/hg19) with the Torrent Mapping Alignment Program. After sequence mapping, variant regions were piled up with Torrent Variant Caller plug-in software. After variant detection, effects were analyzed using ANNOVAR software.^{9,10} Missense, nonsense, insertion/deletion, and splicing variants were then selected from the identified variants. Variants were further selected if they

were less than 1% of (1) the 1000 Genome database,¹¹ (2) the 6500 exome variants in the Exome Variant Server,¹² (3) the data set of 1208 Japanese exome variants in the Human Genetic Variation Database,¹³ and (4) 269 in-house Japanese normal hearing controls.

To predict the pathogenicity of missense variants, the functional prediction software included on the wANNOVAR website was used: Sorting Intolerant from Tolerant (SIFT),¹⁴ Polymorphism Phenotyping (PolyPhen2),¹⁵ MutationTaster,¹⁶ Mutation Assessor,¹⁷ Functional Analysis through Hidden Markov Models,¹⁸ RadialSVM, and LR.¹⁹ Candidate mutations were confirmed by Sanger sequencing, and the responsible mutations were identified by segregation analysis using samples from the patients' family members.

Results

Detected Mutations

One nonsense mutation, 9 missense mutations, and 3 frame shift mutations were identified (Table 1). Nine mutations were novel causative mutations.

All detected mutations were confirmed by Sanger sequencing, and segregation analysis was consistent with them being plausible disease-causing mutations. Mutations were predicted to be pathologic using the aforementioned software programs. None, except for the missense mutation c.9478C>T (p.L3160F), were found in the control subjects.

Clinical Findings

Eleven patients in 10 families were identified in this study. All families were compatible with autosomal recessive inheritance. Clinical features and genotypes of the patients are summarized in Table 2 and Figure 1.

Two cases of biallelic mutation, c.[9478C>T];[1185 dupC] (p.[E396fsX431];[L3160F]) (patient ID 4404) and c.[9413T>A];[9413T>A] (p.[L3138Q];[L3138Q]) (patient ID 1852), were reported previously.^{3,8} Age at onset varied from 0 to 14 years. Seven patients displayed prelingual sensorineural hearing loss ranging from severe to profound. Of these, 4 children had undergone bilateral cochlear implantation. The remaining 4 patients were diagnosed with late-onset progressive hearing loss ranging from moderate to severe.

Some patients also had tinnitus, but none had vertigo. One patient (ID 4404) with *MYO15A* mutations presented with normal vestibular function, as evaluated by a caloric test and vestibular evoked myogenic potential, and showed no symptoms other than hearing loss.

Outcome of Cochlear Implantation

Four children with *MYO15A* compound heterozygous mutations (JHLB0494, 4404, 4852, JHLB1139) underwent

Table 1. Possible Pathogenic Variants Found in This Study.

Nucleotide Change	Amino Acid Change	SIFT ^a	PolyPhen2_HDIV	Polyphen2_HVAR	Mut_Taster ^a	Mut_Assessor ^a	FATHMM ^a	RadialSVM ^a	LR	Allele Frequency in Control (n = 269)	Reference
c.535G>T	p.E179X	0.93								0	This study
c.1185dupC	p.E396fsX431									0	8
c.4072G>A	p.G1358S					0.86	0.6	0.664	0.987	0	This study
c.5978G>A	p.R1993Q	0.95		0.703		0.681	0.49	0.575	0.658	0	This study
c.6487delG	p.A2153fs									0	This study
c.6703T>C	p.S2235P	0.91	0.93	0.368		0.697	0.488	0.51	0.614	0	This study
c.6731G>A	p.G2244E	0.98		0.999		0.71	0.491	0.638	0.825	0	21
c.8198A>C	p.E2733A	0.96		0.996		0.698	0.505	0.642	0.846	0	This study
c.9413T>A	p.L3138Q					0.789	0.553	0.681	0.953	0	3, 21
c.9478C>T ^b	p.L3160F ^b	0.99	0.977	0.914		0.658	0.515	0.583	0.768	0.0074	8, 21
c.9517G>A	p.G3173R					0.688	0.509	0.656	0.871	0	This study
c.10249_10251delTCC	p.F3417del									0	This study
c.10263C>G	p.I3421M		0.987	0.828	0.76	0.626	0.44	0.588	0.856	0	This study

^aThe prediction scores of each algorithm included on the wANNOVAR website were converted from the original scoring system. Scores closer to 1.0 indicated the mutation was more damaging, and those closer to 0 indicated they were more tolerant.

^bControversial variant (see Discussion).

Table 2. Details of the Phenotype and Genotype of 11 Patients From 10 Families With Biallelic Mutations.

Family No.	Patient ID	Nucleotide Change	Amino Acid Change	Age	Age at Onset	Intervention	Age at Surgery	Hearing Level (dB) ^a	Progression	Tinnitus	Vertigo
1	JHLB-0878	c.[535G>T];[9413T>A]	p.[E179X];[L3138Q]	3	0	Hearing aid		60	-	-	-
2	JHLB-0494	c.[1185dupC];[9478C>T] ^b	p.[E396fsX43I];[L3160F] ^b	3	0	Bilateral cochlear implant	1 y/3 y	106.3	-	-	-
2	4404	c.[1185dupC];[9478C>T] ^b	p.[E396fsX43I];[L3160F] ^b	6	0	Bilateral cochlear implant	4 y/7 y	102.5	+	-	-
3	4852	c.[8198A>C];[10249_10251delTCC]	p.[E2733A];[3417del]	8	0	Bilateral cochlear implant	1 y/4 y	110	-	-	-
4	1852	c.[9413T>A];[9413T>A]	p.[L3138Q];[L3138Q]	17	0	Hearing aid		103.8	-	-	-
5	4644	c.[9413T>A];[9478C>T] ^b	p.[L3138Q];[L3160F] ^b	33	0	Hearing aid		91.3	+	-	-
6	JHLB-1139	c.[6487delG];[6731G>A]	p.[A2153fs];[G2244E]	6	1	Bilateral cochlear implant	2 y/3 y	115	-	-	-
7	1832	c.[5978G>A];[9517G>A]	p.[R1993Q];[G3173R]	25	8	Hearing aid		78.8	+	-	-
8	JHLB-0578	c.[5978G>A];[6487delG]	p.[R1993Q];[A2153fs]	20	10	Hearing aid		72.5	+	+	-
9	4673	c.[6703T>C];[10263C>G]	p.[S2235P];[I3421M]	30	12	Hearing aid		75	+	+	-
10	JHLB-0408	c.[4072G>A];[10263C>G]	p.[G1358S];[I3421M]	21	14	None		42.5	+	-	-

Abbreviations: +, existing symptoms; -, without symptoms.

^aAverage of 500, 1000, 2000, and 4000 Hz.

^bControversial variant (see Discussion).

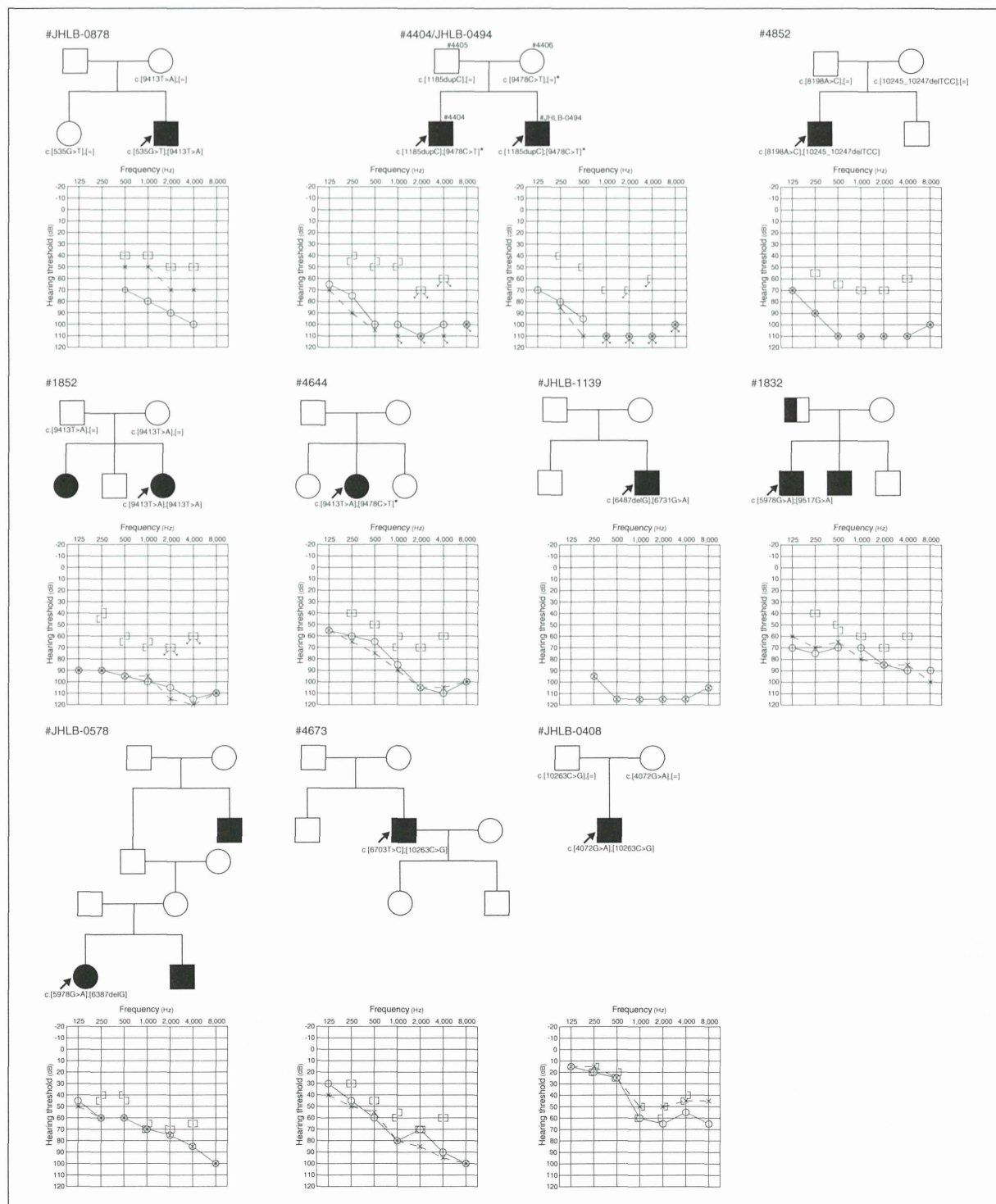


Figure I. Pedigrees, mutations, and audiograms of the patients with biallelic *MYO15A* mutations. *Controversial variant (see Discussion).

bilateral cochlear implantation for congenital hearing loss, with all showing good results. We evaluated the improvement in speech discrimination scores (using the Japanese CI2004 word test, 70dB SPL). Our results (JHLB0494, 84%; 4404, 92%; 4852, 100%) indicated that good outcomes for cochlear implantation could be achieved in patients with *MYO15A* mutations.

Discussion

In this study, MPS-based screening successfully identified causative mutations in the relatively rare deafness gene *MYO15A*, revealing its mutation frequency, mutation spectrum, and clinical characteristics.

To date, 59 *MYO15A* mutations have been reported. Mutations and clinical features are summarized in Table 3. Most were found in a homozygous state in consanguineous families mainly located in the Middle East, Southeast Asia, and Brazil.^{4-7,20-22} The reported phenotype was severe to profound congenital hearing loss. In this study, we found an additional 9 *MYO15A* mutations detected by MPS.

According to the literature, the frequency of *MYO15A* mutations varies according to sample selection and ethnicity. We previously reported a frequency of 1.04% (1/96) in a congenital profound autosomal recessive hearing loss population.³ Most previous studies used selected samples; to our knowledge, there has been no study that used non-biased samples to determine the frequency of *MYO15A* mutations. Relatively high frequencies have been reported in samples where common genes were pre-excluded. For example, in a Chinese population, *MYO15A* mutations were found in 4% (5/125) of probands pre-excluding *GJB2*, *SLC26A4*, and mitochondrial 1555A>G mutations.⁷ Furthermore, a frequency of 3.3% (20/600) was observed in consanguineous Pakistani, Indian, and Turkish families.²¹ A high frequency (5.71%) was also reported in consanguineous Iranian families negative for mutations in *GJB2*.²³ The present study is the first to show the frequency of patients with *MYO15A* mutations in a nonbiased large cohort of hearing loss patients: 0.89% (10/1120). The frequency was 1.67% (10/600) in ARSNHL patients. These results suggest that in Japan the frequency of patients with *MYO15A* mutations is comparatively low compared to *GJB2* (27.7% = 33/119),²⁴ *SLC26A4* (15.1% = 18/119),²⁴ and *CDH23* (7.8% = 5/64)²⁵ mutations, but indicate that *MYO15A* mutations make a notable contribution in hearing loss populations.

Concerning the mutation spectrum, our findings suggest that most of the mutations are unique to the Japanese population, but it should be noted that c.6731G>A (p.G2244E) and c.9478C>T (p.L3160F) were observed across different populations; namely, Pakistani and Japanese (Table 3).

It is unknown whether this was due to a common ancestor phenomenon or mutational hot spot. The most frequent mutation was c.9413T>A (p.L3138Q), which accounts for 18.2% (4/22) of all *MYO15A* mutated families. The pathogenicity of c.9478C>T (p.L3160F), which was found in 0.74% (4/538) of control subjects, was somewhat ambiguous. In a recent study, Shearer et al²⁶ recategorized the c.9478C>T (p.L3160F) mutation as benign because of its high frequency in the control population. Conversely, all previously affected families as well as those in this study revealed co-segregation in their family history, and there was no evidence of this mutation being merely polymorphic. Accordingly, corresponding table notes were included in the figure and table. Further study is required to clarify the pathogenicity of this mutation.

Mutations were found in all domains, with no preferential distribution within the gene (Table 3).

Most of the patients included in this study showed congenital, severe to profound hearing loss. Eleven patients showed biallelic mutation with a *MYO15A* mutation. Of these, 7 patients had prelingual onset; however, the remaining 4 showed postlingual onset and progressive hearing loss. This is the first report to document patients with postlingual onset of hearing loss associated with *MYO15A* mutations.

Analysis of genotype-phenotype correlations revealed that patients with c.9413T>A (p.L3138Q) or c.9478C>T (p.L3160F) tended to have congenital and severe or profound hearing loss. In contrast, patients with c.5978G>A (p.R1993Q) or c.10263C>G (p.I3421M) tended to have postlingual onset. Four patients underwent cochlear implantation, the standard therapy for severe to profound SNHL,²⁷ and all showed good results after implantation.

No study has yet documented vestibular symptoms in patients with *MYO15A* mutations. None of the 11 patients in this study showed vestibular symptoms, supporting the previously reported phenotype. Vestibular testing was performed in 1 patient (4404), who was confirmed to have normal vestibular function. It should be noted, however, that there is a discrepancy between the human and mouse phenotypes. *MYO15A* mutations in mice cause vestibular symptoms (shaker 2 [sh2]) as well as profound hearing loss.²⁸

In conclusion, the present study provides the first evidence that patients with *MYO15A* mutations present 1 of 2 types of hearing impairment phenotype: (1) prelingual onset and severe or profound hearing loss or (2) a milder phenotype with postlingual onset and progressive hearing loss. These findings suggest possible genotype-phenotype correlations in *MYO15A* mutations. Genetic testing based on next-generation sequencing will facilitate candidate selection for cochlear implantation and personalized intervention.

Table 3. MYO15A Pathogenic Mutations in Autosomal Recessive Nonsyndromic Sensorineural Hearing Loss.^a

Exon	Domain	NM_No.	Nucleotide Change	Amino Acid Change	Age at Onset	Hearing Level	Origin of Family	Reference
1	N-terminal extension	NM_016239	c.373_374delCG	p.R125VfsX101	-	-	Ashkenazi, Jewish	29
1	N-terminal extension	NM_016239	c.535G>T	p.E179X	Congenital	Moderate	Japanese	This study
1	N-terminal extension	NM_016239	c.867C>G	p.Y289X	Congenital or prelingual	Moderate to severe	Turkey	30
1	N-terminal extension	NM_016239	c.1047C>A	p.Y341X	-	-	Russian	31
1	N-terminal extension	NM_016239	c.1185dupC	p.E396fsX431	10-14 y congenital	Moderate to profound	Pakistan, Japan	8, 32
1	N-terminal extension	NM_016239	c.1387A>G	p.M463V	-	Severe to profound	Iran	23
1	N-terminal extension	NM_016239	c.3020C>A	p.P1009H	-	-	China	7
1	N-terminal extension	NM_016239	c.3313G>T	p.E1105X	-	Profound	Pakistan	8, 21
1	N-terminal extension	NM_016239	c.3336delG	p.G1112fsX1124	-	Severe to Profound	Pakistan	8, 21
2	Motor	NM_016239	c.3685C>T	p.Q1229X	Congenital	Profound	Pakistan	20
8	Motor	NM_016239	c.4072G>A	p.G1358S	Second decade	Moderate	Japan	This study
Intron 4	Motor	NM_016239	c.3756+1G>T	p.D1232fsX1241	Congenital	Profound	Pakistan	20
4	Motor	NM_016239	c.3758C>T	p.T1253I	-	Severe to profound	India	21
Intron 5	Motor	NM_016239	c.3866+1G>A	p.T1253fsX1277	-	Severe to profound	Pakistan	21
9	Motor	NM_016239	c.4176C>A	p.Y1392X	-	Severe to profound	Pakistan	21
9	Motor	NM_016239	c.4198G>A	p.V1400M	Congenital or prelingual	Severe to profound	Turkey	30
10	Motor	NM_016239	c.4240G>A	p.E1414K	-	-	Palestinian, Arab	29
10	Motor	NM_016239	c.4273C>T	p.Q1425X	-	-	Turkey	3
11	Motor	NM_016239	c.4351G>A	p.D1451N	-	Severe to profound	India	21
11	Motor	NM_016239	c.4441T>C	p.S1481P	Congenital or prelingual	Severe to profound	Turkey	4, 30
13	Motor	NM_016239	c.4652C>A	p.A1551D	-	-	Turkey	3
14	Motor	NM_016239	c.4669A>G	p.K1557E	-	Severe to profound	Pakistan	21
16	Motor	NM_016239	c.4909_4911delGAG	p.E1637del	-	Severe to profound	Iran	23
16	Motor	NM_016239	c.4998G>A	p.C1666X	-	-	Tunisia	33
18	Motor	NM_016239	c.5117_5118GC>TT	p.L1706V	-	Severe to profound	Pakistan	33

(continued)

Table 3. (continued)

Exon	Domain	NM_No.	Nucleotide Change	Amino Acid Change	Age at Onset	Hearing Level	Origin of Family	Reference
18	Motor	NM_016239	c.5189T>C	p.G1730P	-	Severe to profound	Pakistan	21
19	Motor	NM_016239	c.5305A>G	p.T1769A	-	Severe to profound	Iran	23
21	Motor	NM_016239	c.5421delT	p.F1807LfsX6	-	Severe to profound	Iran	23
21	Motor	NM_016239	c.5492G>T	p.G1831V	-	Profound	Turkey	34
Intron 22	-	NM_016239	c.5650-1G>A	p.A1884fs	-	-	Turkey	35
23	Motor	NM_016239	c.5810G>A	p.R1937H	-	Severe to profound	Iran	23
23	Motor	NM_016239	c.5808_5814delCCGTGGC	p.R1937TfsX10	Congenital or prelingual	Severe to profound	Turkey	30
25	IQ Motif	NM_016239	c.5925G>A	p.W1975X	-	Severe to profound	Iran	23
26	IQ Motif	NM_016239	c.5978G>A	p.R1993Q	First decade	Severe	Japan	This study
27	-	NM_016239	c.6061C>T	p.Q2021X	-	Severe to profound	Pakistan	21
28	MyTH4	NM_016239	c.6217C>T	p.P2073S	Congenital	Profound	Iran	22
29	MyTH4	NM_016239	c.6331A>T	p.N2111Y	Congenital	Profound	India	36
29	MyTH4	NM_016239	c.6306_6307insG	p.A2104CfsX18	-	-	China	7
29	MyTH4	NM_016239	c.6337A>T	p.I2113F	Congenital	Profound	Indonesia	36
29	MyTH4	NM_016239	c.6340G>A	p.V2114M	-	-	China	7
29	MyTH4	NM_016239	c.6371G>A	p.R2124Q	Congenital	Profound	Iran	22
29	MyTH4	NM_016239	c.6487delG	p.A2153fs	Prelingual	Profound	Japan	This study
30	MyTH4	NM_016239	c.6614C>T	p.T2205I	Congenital	Moderate	North America	20
32	-	NM_016239	c.6703T>C	p.S2235P	Second decade	Moderate	Japan	This study
31	-	NM_016239	c.6731G>A	p.G2244E	Prelingual	Severe to profound	Pakistan, Japan	21, this study
31	-	NM_016239	c.6796G>A	p.V2266M	-	Severe to profound	Pakistan, Turkey	21
Intron 36	-	NM_016239	c.7395+3G>C	-	-	Severe to profound	Tunisia	33
39	SnAPC2 like	NM_016239	c.7801A>T	p.K2601X	Congenital	Profound	India	36
41	SnAPC2 like	NM_016239	c.7982C>A	p.S2661X	-	-	Turkey	35
46	-	NM_016239	c.8148G>T	p.Q2716H	Congenital	Profound	Pakistan	20
43	-	NM_016239	c.8158G>C	p.D2720H	-	Severe to profound	Pakistan	21
43	-	NM_016239	c.8183G>A	p.R2728H	-	-	Jewish, China	7, 29

Table 3. (continued)

Exon	Domain	NM_No.	Nucleotide Change	Amino Acid Change	Age at Onset	Hearing Level	Origin of Family	Reference
43	-	NM_016239	c.8198A>C	p.E2733A	Congenital	Profound	Japan	This study
43	-	NM_016239	c.8324G>A	p.R2775H	-	-	China	7
46	PH like	NM_016239	c.8467G>A	p.D2823N	-	Severe to profound	Iran	23
48	SH3	NM_016239	c.8767C>T	p.R2923X	-	-	China	6
Intron 49	-	NM_016239	c.8968-1G>C	-	-	Profound	Turkey	34
49	SH3	NM_016239	c.8821_8822insTG	p.V2940fsX3034	-	Severe to profound	Pakistan	21
Intron 53	-	NM_016239	c.9229+1G>A	-	-	Severe to profound	Tunisia	33
59	MyTH4	NM_016239	c.9413T>A	p.L3138Q	Congenital or prelingual	Profound	Japan	3, this study
59	MyTH4	NM_016239	c.9478C>T ^b	p.L3160F ^b	Congenital	Severe to profound	Pakistan	8, 21, This study
59	MyTH4	NM_016239	c.9517G>A	p.G3173R	First decade	Severe	Japan	This study
65	FERM central	NM_016239	c.9958_9961delGACT	p.D3320fs	First decade	Severe to profound	Brazil	37
65	FERM central		c.9995_10002dupGCCGCCCC	p.S3335AfsX121	Congenital or prelingual	Severe to profound	Turkey	30
67	FERM central	NM_016239	c.10249_10251delTCC	p.F3417del	Congenital	Profound	Japan	This study
67	FERM central	NM_016239	c.10263C>G	p.I3421M	10-19 y	Moderate to severe	Japan	This study
68	FERM central	NM_016239	c.10474C>T	p.Q3492X	-	Severe to profound	Pakistan	21
69	FERM central	NM_016239	c.10573delA	p.S3525fs	Prelingual	Severe to profound	Brazil	37

^a-, not available.

^bControversial variant (see Discussion).

Declaration of Conflicting Interests

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