Table 1. (continued)

Nucleotide Change	Amino Acid Change	Inheritance	CT Findings	HL Onset	Type of HL	Progression	Other Features	Perilymphatic Gusher	Year	First Author
c.686A>G	p.G229R	Inherited	IP3		SNHL				2013	Choi[27]
c.689C>T	p.T230I		Bony defect		Mixed			Gusher	1997	Friedman[6]
c.727_728insA	p.N244KfsX26	De novo (sporadic)	IP3	Congenital	SNHL	Progressive	Pervasive developmental disorder	Gusher	2014	This report
c.853-854del	p.I285Rfs43								2013	Parzefall[23]
c.862-866del	p.fs	Inherited		Early	Mixed			Gusher	1995	Binter-Glindzicz[28]
c.895delA	p.L298X				Mixed			Gusher	1995	de Kok[4]
c.910C>A	p.P303H	Inherited	IP3	Congenital					2015	Choi[18]
c.925T>C	p.S309P	Inherited	IP3	Congenital	Mixed	No			2006	Wang[29]
c.927-929del	p.S310del	Inherited	IP3	Early	Mixed				2009	Lee[16]
c.927-929del	p.S310del	Unknown (adopted)	IP3	Early	Mixed	No	Learning delay	Gusher	2010	Stankovic[8]
c.935C>T	p.A312V	Inherited	Bony defect	Congenital	SNHL		Learning difficulty		1995	Binter-Glindzicz[28]
c.950dupT	p.L317FfsX12	Inherited	IP3		Mixed				2013	Choi[27]
c.950T>G	p.L317W		Bony defect		Mixed				1995	de Kok[4]
c.967C>G	p.A323G				Mixed			Gusher	1997	de Kok[30]
c.973T>A	p.W325R	Inherited	IP3	Early	SNHL	Progressive		Gusher	2011	Schild[31]
c.985C>G	p.R329G		IP3		Mixed			Gusher	1997	Friedman[6]
c.986G>C	p.R329G	Inherited	IP3	Early	Mixed	Progressive			2009	Lee[16]
c.990A>T	p.A330S		Bony defect		SNHL	Progressive	Growth retardation	Gusher	1997	de Kok[30]
c.1000A>G	p.K334E				Mixed	Progressive			1995	de Kok[4]
c.1069delA	p.T354GfsX115	Inherited	IP3		SNHL				2013	Choi[27]
c.1084T>C	p.X362RextX113	De novo (sporadic)	IP3		SNHL				2013	Choi[27]

Abbreviations: CT, computed tomography; HL, hearing loss; IP3, incomplete partition type III; SNHL, sensorineural hearing loss.
[a]Empty rows indicate unspecified in the reports.

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Declaration of Conflicting Interests

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Mutations in the *MYO15A* Gene Are a Significant Cause of Nonsyndromic Hearing Loss: Massively Parallel DNA Sequencing–Based Analysis

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Abstract

Objectives: Screening for *MYO15A* mutations was carried out using a large cohort to clarify the frequency and clinical characteristics of patients with *MYO15A* (DFNB3) mutations in a hearing loss population.

Methods: Genetic analysis of 63 previously reported deafness genes based on massively parallel DNA sequencing (MPS) in 1120 Japanese hearing loss patients from 53 otorhinolaryngology departments was performed. Detailed clinical features of the patients with *MYO15A* mutations were then collected and analyzed.

Results: Eleven patients from 10 families were found to have compound heterozygosity for *MYO15A*. Audiograms showed profound or high frequency hearing loss, with some patients showing progressive hearing loss. Age at onset was found to vary from 0 to 14 years, which seemed to be associated with the mutation. Four children underwent bilateral cochlear implantation for congenital hearing loss, with all showing good results.

Conclusion: Mutations in the *MYO15A* gene are a notable cause of nonsyndromic hearing loss. MPS technology successfully detected mutations in relatively rare deafness genes such as *MYO15A*.

Keywords

MYO15A, DFNB3, autosomal recessive hearing loss, massively parallel DNA sequencing, next generation sequencing, cochlear implant

Introduction

Autosomal recessive nonsyndromic sensorineural hearing loss (ARNSHL) accounts for more than 80% of inherited nonsyndromic hearing loss cases. To date, more than 40 genes associated with ARNSHL have been reported.[1] The clinical features of ARNSHL (hearing level, age at onset, progressiveness, associated symptoms, etc) differ according to the causative gene/genotype. *MYO15A* (DFNB3) is one such causative gene. Comprising 66 exons and 71 kbp of DNA on chromosome 17p11.2, *MYO15A* encodes the 3530-amino acid myosin XV protein. Myosins are a large family of actin-dependent molecular motors and play a role in the hydrolysis of ATP to generate the force required for the movement of actin filaments.[2]

Screening for gene mutations in *MYO15A*, which has many exons, has progressed slowly, even though it is likely an important cause of hearing loss. We previously reported the results of Sanger sequencing of *MYO15A* mutations in a single family.[3] Recent advances in targeted exon resequencing of

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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 nonsyndromic 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			1					0	This study
c.1185dupC	p.E396fsX431									0	8
c.4072G>A	p.G1358S	1	1	1	1	0.86	0.6	0.664	0.987	0	This study
c.5978G>A	p.R1993Q	0.95	1	0.703	1	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	1	0.697	0.488	0.51	0.614	0	This study
c.6731G>A	p.G2244E	0.98	1	0.999	1	0.71	0.491	0.638	0.825	0	21
c.8198A>C	p.E2733A	0.96	1	0.996	1	0.698	0.505	0.642	0.846	0	This study
c.9413T>A	p.L3138Q	1	1	1	1	0.789	0.553	0.681	0.953	0	3, 21
c.9478C>T[b]	p.L3160F[b]	0.99	0.977	0.914	1	0.658	0.515	0.583	0.768	0.0074	8, 21
c.9517G>A	p.G3173R	1	1	1	1	0.688	0.509	0.656	0.871	0	This study
c.10249_10251delTCC	p.F3417del									0	This study
c.10263C>G	p.I3421M	1	0.987	0.828	0.76	0.626	0.44	0.588	0.856	0	This study

[a]The 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.
[b]Controversial 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.[E396fsX431];[L3160F][b]	3	0	Bilateral cochlear implant	1 y/3 y	106.3	-	-	-
2	4404	c.[1185dupC];[9478C>T][b]	p.[E396fsX431];[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.
[a]Average of 500, 1000, 2000, and 4000 Hz.
[b]Controversial variant (see Discussion).

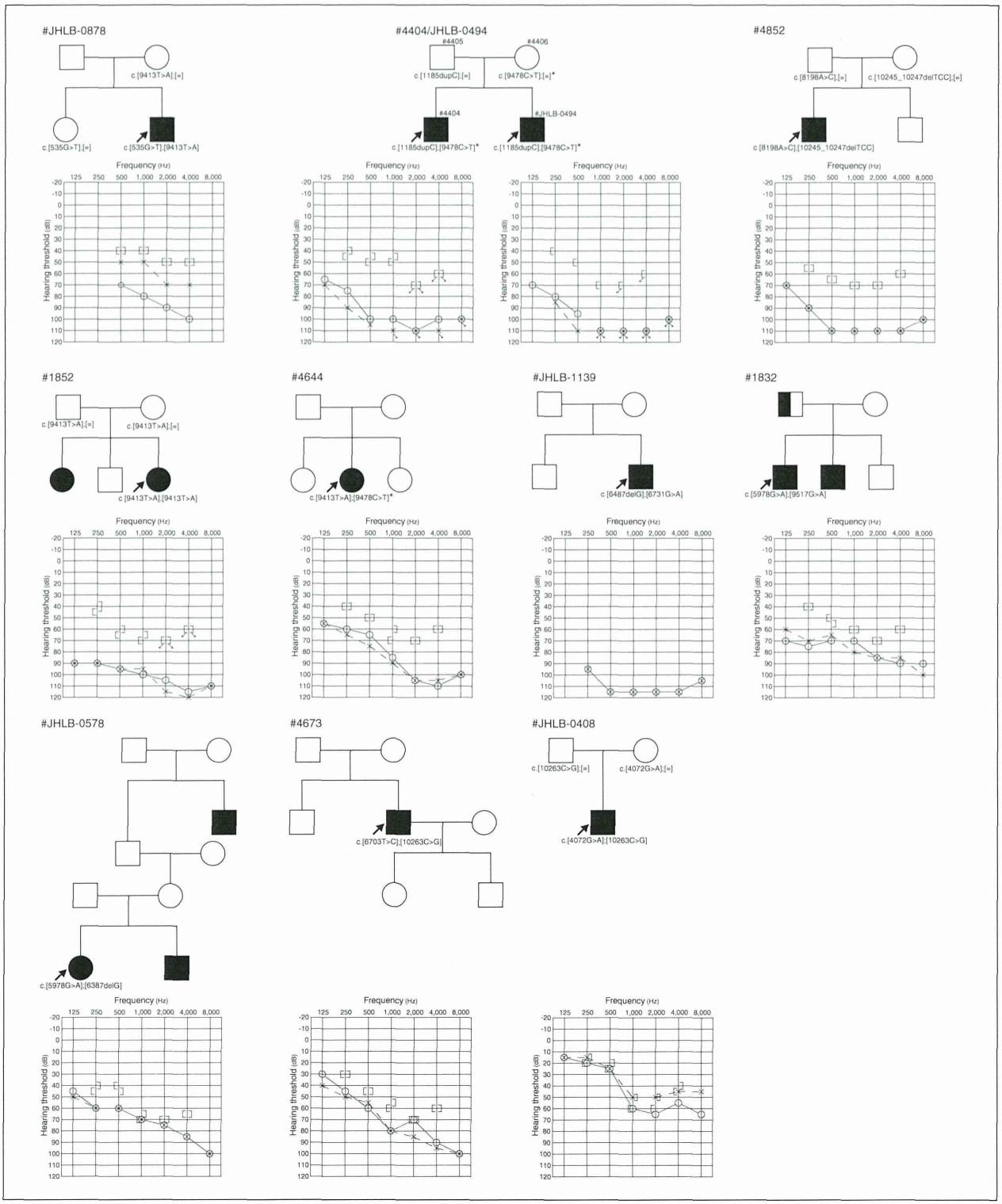

Figure 1. Pedigrees, mutations, and audiograms of the patients with biallelic *MYO15A* mutations.
*Controversial variant (see Discussion).

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, 70dBSPL). 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 nonbiased 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 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 al[26] 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

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