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Novel *PTPRQ* mutations identified in three congenital hearing loss patients with various types of hearing loss

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Abstract

Objective—We present three patients with congenital sensorineural hearing loss (SNHL) caused by the novel *PTPRQ* mutations, including clinical manifestations and phenotypic features.

Methods—Two hundred and twenty (220) Japanese subjects with sensorineural hearing loss from unrelated and non-consanguineous families were enrolled in the study. Targeted genomic enrichment with massively parallel sequencing of all known non-syndromic hearing loss genes was performed to identify the genetic cause of hearing loss.

Results—Four novel causative *PTPRQ* mutations were identified in three cases. Case 1 had progressive profound SNHL with homozygous nonsense mutation. Case 2 had non-progressive profound SNHL with compound heterozygous mutation (nonsense and missense mutation). Case 3 had non-progressive moderate SNHL with compound heterozygous mutation (missense and splice site mutation). Caloric test and vestibular evoked myogenic potential (VEMP) test showed vestibular dysfunction in Case 1.

Conclusion—Hearing loss levels and progression among the present cases were varied, and there seem to be no obvious correlation between genotypes and the phenotypic features of their hearing loss. The *PTPRQ* mutation appeared to be responsible for the vestibular dysfunction.

Keywords

Hearing loss; PTPRQ; DFNB84; massively parallel sequencing

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DECLARATION OF CONFLICTING INTERESTS

All authors have declared no competing financial interests.

INTRODUCTION

Hearing loss is the most common sensory impairment in humans. Genetic causes account for the largest proportion of congenital sensorineural hearing loss (SNHL). Hearing loss is an extremely heterogeneous disorder, and approximately 75% of hereditary hearing loss is nonsyndromic. Therefore, it is difficult to predict the clinical course on the basis of clinical findings. Genetic test is one way to resolve this problem. However, due to the extreme heterogeneity of SNHL, and much labor and expense are required for analysis when using conventional Sanger sequencing.

Recent advances in targeted genomic enrichment with massively parallel sequencing (TGE +MPS) have made it possible to sequence all known causative genes simultaneously.[1,2] This technology has been reported to afford an effective approach to the diagnosis of genetic hearing loss, particularly in terms of sensitivity, specificity and reproducibility.[1]

In this study, we performed genetic testing using TGE+MPS to analyze the genetic etiology of Japanese hearing loss patients, and identified the mutations in the *PTPRQ* (protein tyrosine phosphatase receptor Q) gene. The *PTPRQ* gene is one of the latest identified as a cause of non-syndromic SNHL. The locus had been mapped on chromosome 12q21.31, and was assigned DFNB84.[3] The *PTPRQ* gene is comprised of 58 exons, and encodes the PTPRQ protein, which is one of the membrane proteins localized in the basal region of the stereocilia.[3,4,5] The PTPRQ protein has three domains: the extracellular domain (fibronectin type 3 domain), the membrane spanning domain (tansmembrane domain), and the cytoplasmic domain (phosphatase domain).[3,6,7] The PTPRQ protein is known to play key roles in the regulation of actin filaments reorganization, cell shape changes and shaft connector formation.[4,8,9] Sakaguchi et al. reported that the PTPRQ protein appears to maintain the organization of the cell surface coat and the structure of the overall stereocilia bundle through interactions with Myosin VI.[5]

Until now, only three families with *PTPRQ* mutations have been reported, and most of the phenotypic features remain unclear.[3,6] Here, we describe three Japanese patients with congenital SNHL caused by the novel *PTPRQ* mutations.

SUBJECTS and METHODS

Subjects

We recruited two groups from a Japanese hearing loss population for this study. All subjects had presumed non-syndromic SNHL. For each proband, informed consent was obtained to participate in this study, which was approved by the human subjects ethical committee associated with each clinic. Clinical information and blood samples were obtained for each proband and for all consenting affected and unaffected relatives. This study was approved by the Ethical Committee of Shinshu University and Yokohama City University.

The first group – Yokohama samples—Twenty-six (26) Japanese subjects from unrelated and nonconsanguineous families were enrolled. These subjects visited to

Yokohama City University hospital for examination of hearing loss and participated in this study.

The second group – Shinshu samples—One hundred ninety four (194) Japanese subjects from unrelated and non-consanguineous families were ascertained through 33 otolaryngology clinics in 28 prefectures across Japan.

Methods

The first group – Yokohama samples

Amplicon Library Preparation: An Amplicon library was prepared with an Ion AmpliSeq™ Custom Panel (Applied Biosystems, Life Technologies, Carlsbad, CA) for 63 genes reported to cause non-syndromic hearing loss according to the manufacturers' instructions. The detailed protocol was described elsewhere.[10] After amplicon libraries preparation, amplicon libraries were diluted to 20pM and equal amounts of the 6 libraries for 6 patients were pooled for one sequence reaction.

Emulsion PCR and Sequencing: The emulsion PCR and Sequencing were performed according to the manufacturer's instructions. The detailed protocol was described elsewhere.[10] MPS was performed with an Ion Torrent Personal Genome Machine (PGM) system using the Ion PGM™ 200 Sequencing Kit and Ion 318™ Chip (Life Technologies).

The second group – Shinshu samples

Targeted Genomic Enrichment and Massively Parallel Sequencing: TGE of all exons of all genes implicated in non-syndromic SNHL, including non-syndromic SNHL mimics, was completed as described, targeting 89 genes as part of the OtoSCOPE® v5 platform. Libraries were prepared using a modification of the solution-based Agilent SureSelect target enrichment system (Agilent Technologies, Santa Clara, CA).[11]

In brief, 3μg gDNA was randomly fragmented to an average size of 250 bp (Covaris Acoustic Solubilizer; Covaris Inc., Woburn, MA), fragment ends were repaired, A-tails were added, and sequencing adaptors were ligated before the first amplification. Solid phase reverse immobilization purifications were performed between each enzymatic reaction. Hybridization and capture with RNA baits was followed by a second amplification before pooling for sequencing. Minimal amplification was used – typically 8 cycles for the prehybridization PCR (range 8–10 cycles) using NEB Phusion HF Master Mix (New England BioLabs Inc, Ipswich, MA) and 14 cycles for the post-hybridization PCR (range 12–16 cycles) using Agilent Herculase II Fusion DNA Polymerase. All samples were barcoded and multiplexed before sequencing on either an Illumina MiSeq or HiSeq (Illumina Inc, San Diego, CA) in pools of 4-6 or 48, respectively, using 100-bp paired-end reads.

Base Call and Data Analysis

The first group – Yokohama samples: The sequence data were mapped against the human genome sequence (build GRCh37/hg19) with a Torrent Mapping Alignment Program. After sequence mapping, the DNA variant regions were piled up with Torrent Variant Caller plug-

in software. After variant detection, their effects were analyzed using ANNOVAR software.[12,13] The missense, nonsense, insertion/deletion and splicing variants were selected from among the identified variants. Variants were further selected as less than 1% of 1) the 1,000 genome database (http://www.1000genomes.org/), 2) the 6,500 exome variants (http://evs.gs.washington.edu/EVS/), 3) the Human Genetic Variation Database (dataset for 1,208 Japanese exome variants) (http://www.genome.med.kyoti-u.ac.jp/SnpDB/index.html), and 4) the 269 in-house Japanese normal hearing controls. To predict the pathogenicity of missense variants, the following functional prediction software was used; PhyloP (http://hgdownload.cse.ucsc.edu/goldenPath/hg18/phyloP44way/), Sorting Intolerant from Tolerant (SIFT; http://sift.jcvi.org/), Polymorphism Phenotyping (PolyPhen2; http://genetics.bwh.harvard.edu/pph2/), LRT (http://www.genetics.wustl.edu/jflab/lrt_query.html), MutationTaster (http:// www.mutationtaster.org/), and GERP++(http://mendel.stanford.edu/SidowLab/downloads/gerp/index.html).

The second group – Shinshu samples: Data were analyzed as described using a local installation of the open-source Galaxy software (http://galaxyproject.org) and the following open-source tools: BWA[14] for read mapping, Picard for duplicate removal, GATK[15] for local re-alignment and variant calling and NGSRich[16] for enrichment statistics.[2] We reported and annotated variants with custom software.

Variant Confirmation

Candidate mutations were confirmed by Sanger sequencing and the responsible mutations were identified by segregation analysis using samples from among the patients' family members.

RESULTS

We identified three cases that had the causative *PTPRQ* mutations in this study (220 hearing loss patients).

Mutation analysis

We identified novel one nonsense mutation, two missense mutations and one splicing junction mutation in the *PTPRQ* gene (NM_00145026). Case 1: AG 8960 had a homozygous mutation. This mutation corresponded to c.1261C>T, leading to p.Arg421stop (Figure 1). Case 2: SNS 2193 had a compound heterozygous mutation. This mutation corresponded to c.166C>G and 1261C>T, leading to p.Pro56Ala and Arg421Stop (Figure 2). Case 3: SNS 2912 had a compound heterozygous mutation. This mutation corresponded to c.6453+3delA, and 4640T>C which leads to p.Met1349Thr (Figure 3). As shown in figures 1, 2 and 3, the Sanger sequencing for family segregation was confirmed to each pedigree. None of these mutations were identified in the 1000 genome database, the 5400 exome variants and 1208 Japanese exome variants, in addition to the 269 in-house Japanese normal hearing controls data base.

Details of Cases

Case 1 sample; ID No. AG 8960—The affected patient is a 19-year-old male. Newborn hearing screening was not performed at his birth. He had no particular complications in the perinatal period. The parent noticed his speech delay given that he only had a few spoken words at the age of three. He had been referred to Yokohama City University Hospital, Department of Otolaryngology, for hearing examinations. A play audiometry showed bilateral moderate hearing loss that was approximately 50 dBHL in the right ear and 75 dBHL in the left ear, which occurred together with otitis media with effusion. He was promptly fitted for hearing aids bilaterally. As a result of the hearing aids, he acquired age appropriate spoken language. When he was 14 years old, he was aware of his own deterioration in hearing. A pure-tone audiometry (PTA) showed sloping high frequency SNHL that was on average 75 dBHL in both ears. Over a period of five years, his high frequency hearing gradually deteriorated. His hearing loss accelerated and his hearing aids were ineffective by the time he was 19. Bilateral congenital progressive hearing loss was diagnosed.

He suffered from tinnitus. He had no history of vertigo, but his elementary school teachers pointed out that he fell down frequently. The otoscopic examination revealed a normal tympanic membrane. The computed tomography (CT) and magnetic resonance imaging (MRI) of the temporal bone showed no abnormal malformations. The caloric test and the vestibular evoked myogenic potential (VEMP) test were performed at the age of 19 years old. These tests showed a hypo-caloric response on the right side, and no caloric response and no VEMP response on the left side. His parents, brother and other relatives had no history of hearing impairment.

He underwent cochlear implantation; MED-EL FLEX28 in his right ear at the age of 19, and obtained adequate amplification. Preoperative sound field threshold levels with hearing aids were approximately 60 dBSPL at 500 to 2000 Hz. Postoperative sound field threshold levels with cochlear implant were 40 dBSPL at 125 through 4000 Hz.

His pedigree, hearing level and vestibular test results are shown in Figure 1.

Case 2 sample; ID No. SNS 2193—The patient was a 16-year-old female with congenital SNHL. She had no particular complications in the perinatal period. However at age 1 year 5 months, her mother suspected hearing loss because of her poor response to sound. She underwent a hearing examination, and an auditory brainstem response (ABR) with click stimuli showed no response to 100 dBnHL in both ears. A conditioned orientation response (COR) audiometry showed a threshold above 90 dBHL in all frequencies bilaterally. Congenital severe-profound SNHL was suspected, and she was fitted for bilateral hearing aids at the age of 2. Over a period of fourteen years, her hearing loss was unchanged. At the age of 16, PTA showed high frequency sloping profound SNHL. She had no history of vertigo in the following years. CT showed no abnormality of the inner and middle ears. Her parents, sister, brother and other relatives had no history of hearing impairment. Her pedigree and hearing levels are shown in Figure 2.

Case 3 sample; ID No. SNS 2912—The affected patient was an 18-year-old female with SNHL. She had no particular complications in the perinatal period. Bilateral hearing loss was identified at an elementary school health check-up, and she was referred to a general hospital clinic of Otolaryngology at the age of 7. A PTA showed bilateral moderate hearing loss, approximately 60 dBHL in both ears. She started to wear hearing aids bilaterally. Over a period of eleven years, her hearing loss did not deteriorate and was stable. Bilateral moderate SNHL was diagnosed, possibly congenital or early onset was suspected. She suffered from bilateral tinnitus when she turned 11. She had no history of vertigo in the following years. CT showed no abnormality of the inner and middle ears. She had a dizygotic twin and this twin sister had the same level of SNHL. Her parents and other relatives had no history of hearing impairment. Her pedigree and hearing levels are shown in Figure 3.

DISCUSSION

We identified novel causative mutations in the *PTPRQ* gene as a cause of congenital SNHL in a Japanese population using TGE+MPS.

As shown in Table 1, there are a total of 12 SNHL cases with *PTPRQ* mutations, including three cases identified in this study and nine previously reported cases.[3,6] Each affected family had various degrees of hearing loss severity and progressiveness, which could correlate to the type of mutation. With respect to case 1: AG 8960, he had progressive and profound SNHL, mainly affected at high frequencies, with homozygous nonsense mutation. Four of the seven cases with homozygous nonsense mutations were described as having severe to profound SNHL; one case with a flat audiogram and three cases with a down-sloping audiogram. Another two Palestinian cases with homozygous nonsense mutation were described as having moderate SNHL, although clinical information on the deterioration of their hearing was lacking.[4] Schraders et al. also reported that hearing loss progressed over a period of 10 to 30 years, becoming profound SNHL in two Dutch cases with homozygous nonsense mutation.[3] Thus, there might be variations in hearing levels and progression even among the cases with homozygous nonsense mutations. Thus, we suspect that there is no obvious correlation between genotypes and the phenotypic features of their hearing loss.

In this study, we performed vestibular test, caloric test and vestibular evoked myogenic potential (VEMP) test, for Case 1 only. The caloric test revealed a hypo-response on the right side and no response on the left side. The VEMP test revealed no response on the left side (Figure 1D). Schraders et al. also reported that caloric test showed either no or hypo-responses on both sides in four cases with *PTPRQ* homozygous mutations.[3] A *Ptprq* knockout mice study revealed deformation of the stereocilia and hair bundles in the utricle, and defects in the hair bundles in the saccule and ampullae.[17] Vestibular evoked potentials (VsEPs) were absent in the majority of *Ptprq* knockout mice.[17] These findings suggested that the *PTPRQ* mutations might cause dysfunctions in the vestibular organs. However, Case 1 had not experience any episodes of vertigo or dizziness. The reason for this incompatibility is unclear, but the *Ptprq* knockout mice showed no obvious abnormal behavior, except when swimming.[17]

Case 1 received a cochlear implant in the right ear at the age of 19, by which time his hearing loss had gradually become severe. His sound field threshold levels were improved after implantation. We suggest that cochlear implantation could be the intervention of choice for cases with *PTPRQ* mutations.

This study was the first to identify compound heterozygous mutations. Case 2 had profound SNHL with a compound heterozygote for missense and nonsense mutations. Case 3 had moderate SNHL with a compound heterozygote for splicing site and missense mutation. Taken together with the results of previous reports (Table 1), there dose not appear to be any obvious genotype-phenotype correlation.

In summary, we performed target exon sequencing using TEG+MPS in this study, and we believe this method could be useful for identifying these rare causative genes, such as *PTPRQ* gene. The *PTPRQ* mutation also appeared to be responsible for the vestibular dysfunction. However, the vestibular symptoms might be almost unrecognizable, even though vestibular tests showed a hypo-response. The hearing loss caused by the *PTPRQ* mutation appeared to be congenital. With regard to the hearing levels and progression, we observed variations among three cases. More precise studies are necessary for better understanding the molecular basis mechanisms of the genotypes, and the hearing loss was progressive in some cases, so that the follow up of the patients needed to be lengthy to clarify their phenotypic features.

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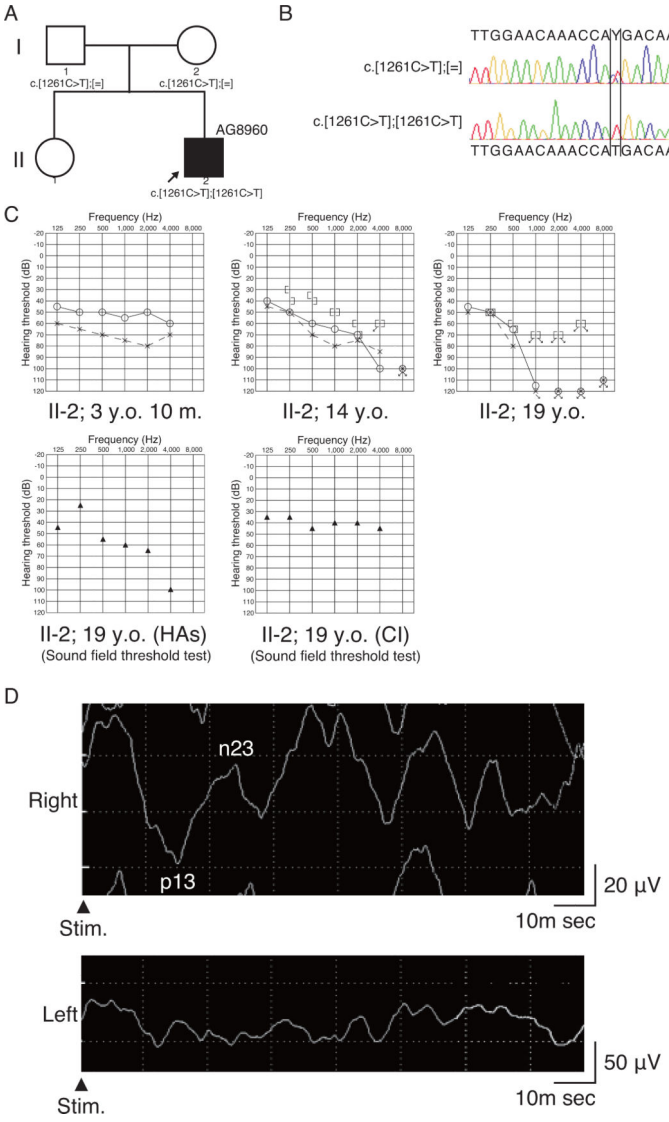

Figure 1.

Pedigree and clinical finding of case 1. (A) Pedigree showed a single case in this family. (B) Sanger sequencing and segregation analysis showed case 1: AG 8960 had the homozygous mutation, and the parents and brother had the heterozygous mutation. (C) Pure-tone audiometry showed moderate SNHL at age 3 and deterioration threshold to profound SNHL by the age 19. Preoperative sound field threshold test with HAs showed 60 dBSPL at 500 to 2000Hz. After cochlear implantation in the right ear, sound field threshold test with CI showed 40 dBSPL. HAs; hearing aids, CI; cochlear implant (D) VEMP showed no response on left side.

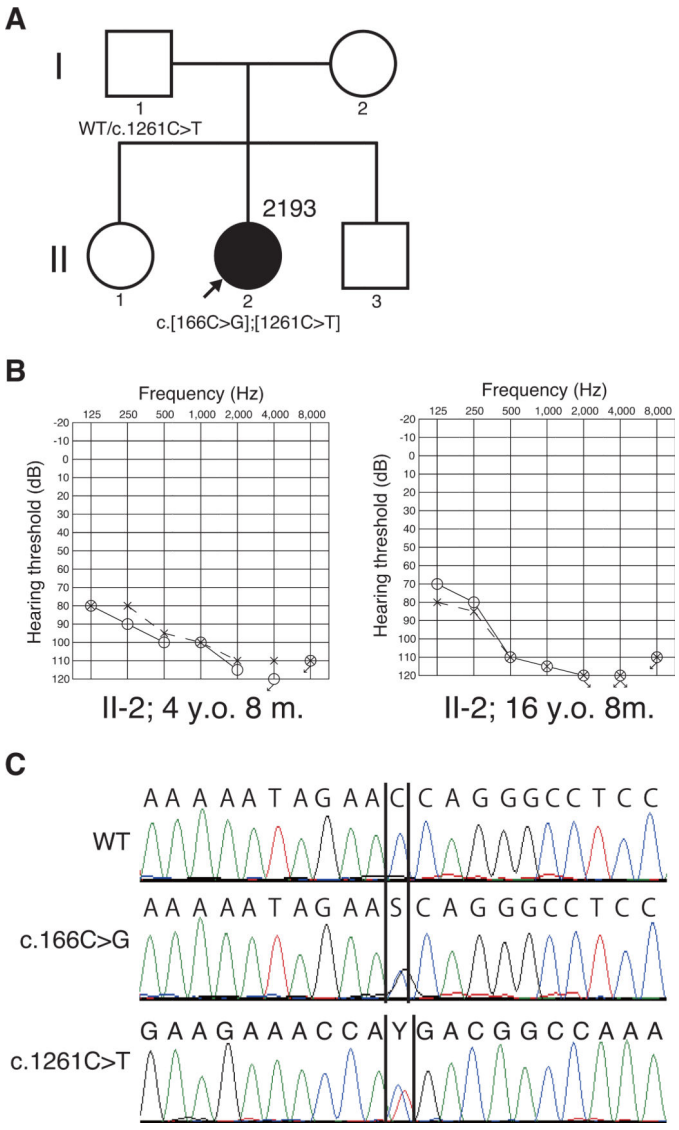

Figure 2.

Pedigree and clinical finding of case 2. (A) Pedigree showed a single case in this family. (B) Pure-tone audiometry showed non-progressive profound SNHL. (C) Sanger sequencing and segregation analysis showed case 2: ID 2193 had the compound heterozygous mutation, and her father had the heterozygous mutation.

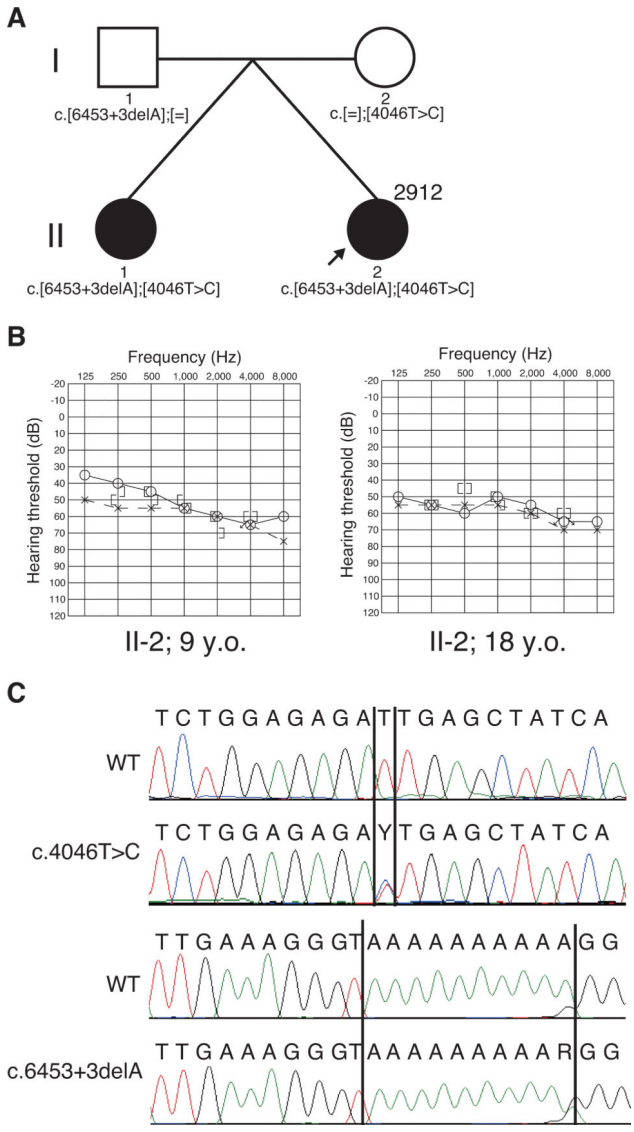

Figure 3.

Pedigree and clinical finding of case 3. (A) Pedigree showed two cases in this family. (B) Pure-tone audiometry showed non-progressive moderate SNHL. (C) Sanger sequencing and segregation analysis showed case 3: ID 2912 and her twin sister had the compound heterozygous mutation, and her father had the heterozygous mutation.

Table 1

Known mutations including our cases in the *PTPRQ* gene and associated phenotypes.

Nucleotide change	Protein change	Domain	Type of mutation	Zygosity	Time of onset	Type of HL (Age)	Progression	Family origin	Refference
c.166C>G	p.Pro56Ala	EC	Missense	Compound heterozygous	Congenital	Profound (16 y.o.)	Stable	Japanese	**This study**
c.1261C>T	p.Arg421Stop	EC	Nonsense						
c.1261C>T	p.Arg421Stop	EC	Nonsense	Homozygous	Congenital	Profound (19 y.o.)	Progressive	Japanese	**This study**
c.1285C>T	p.Gln429Stop	EC	Nonsense	Homozygous	NR	Moderate (11 y.o.)	NR	Palestinian	Shahin et al. 2010
c.1285C>T	p.Gln429Stop	EC	Nonsense	Homozygous	NR	Moderate (15 y.o.)	NR	Palestinian	Shahin et al. 2010
c.1285C>T	p.Gln429Stop	EC	Nonsense	Homozygous	NR	NR	NR	Palestinian	Shahin et al. 2010
c.1285C>T	p.Gln429Stop	EC	Nonsense	Homozygous	NR	Severe (5 y.o.)	NR	Palestinian	Shahin et al. 2010
c.1285C>T	p.Gln429Stop	EC	Nonsense	Homozygous	NR	Severe (14 y.o.)	NR	Palestinian	Shahin et al. 2010
c.1369A>G	p.Arg457Gly	EC	Missense	Homozygous	Congenital	Moderate (9 y.o.)	Stable	Moroccan	Schraders et al. 2010
c.1369A>G	p.Arg457Gly	EC	Missense	Homozygous	Congenital	Moderate (6 y.o.)	Stable	Moroccan	Schraders et al. 2010
c.1491T>A	p.Tyr497Stop	EC	Nonsense	Homozygous	Congenital	Profound (61 y.o.)	Progressive	Dutch	Schraders et al. 2010
c.1491T>A	p.Tyr497Stop	EC	Nonsense	Homozygous	Congenital	Profound (56 y.o.)	Progressive	Dutch	Schraders et al. 2010
c.4046T>C	p.Met1349Thr	EC	Missense	Compound heterozygous	Congenital	Moderate (18 y.o.)	Stable	Japanese	**This study**
c.6453+3delA		CP	Splice site						

HL: Hearing loss, NR: Not reported, EC: Extracellular domain, CP: Cytoplasmic domain Type of HL (Age); Age at the time of hearing examination