Results

We identified 4 patients, from 2 families, who had causative mutations in *LOXHD1* in 1314 hearing loss Japanese.

Case Details

Family No. 963; AH 4029, AH 4028. AH 4029 was a 9 year-old boy. He had not undergone newborn hearing screening. At the age of 6, hearing loss was detected at a school check-up. He had been referred to the Iwate Medical University Hospital, Department of Otolaryngology for further examinations. His tympanic membranes were normal. An auditory brain stem response (ABR) with click stimuli showed bilateral hearing loss that was approximately 40 dBnHL in both ears, and pure-tone audiometry (PTA) showed bilateral moderate sensorineural hearing loss (Figure 1). He was promptly fitted with a left hearing aid when he was 7 years, 3 months old. He showed 100% in word scores at the 50 dB sound field threshold. His hearing was unchanged from 6 to 9 years old. He didn't have any episodes of dizziness or vertigo attacks. Computed tomography (CT) findings of the middle and inner ear showed no abnormalities.

AH 4028 was a 3-year-old girl. She was a younger sister of AH 4029, and her parents noticed that she didn't have any intelligible words until she was 1 year, 6 months old. She visited the Iwate Medical University Hospital at the same time that AH 4029 received his check-up. Her bilateral tympanic membranes were normal. A previous ABR test at 2 years of age had shown bilateral severe hearing loss that was approximately 80 dBnHL in both ears, and PTA had also shown bilateral severe SNHL at the age of 3 (Figure 1). Her hearing had been unchanged from 3 to 5 years old. She started to wear a right hearing aid when she was 3 years old. CT findings of the middle and inner ear showed no abnormalities.

Family No. 143; 2061, 2059. Patient 2061 was a 70-year-old male. He was born in 1929. PTA showed bilateral profound SNHL (Figure 2). In his medical examination by interview, he demonstrated bilateral congenital nonprogressive hearing loss. He didn't have any episodes of vertigo. He had not undergone CT of the temporal bone.

Patient 2059 was a 60-year-old female. She was the younger sister of patient 2061 and was born in 1939. PTA showed bilateral profound SNHL (Figure 2). In her medical examination by interview, she also demonstrated congenital nonprogressive hearing loss. She didn't have any episodes of vertigo.

As shown in Figure 2, the pedigree tree showed consanguineous parental ancestry of patient 2061 and patient 2059 with profound hearing loss. They were both unmarried. We didn't obtain any information about the symptoms of hearing loss during childhood because of World War II.

Mutation Analysis

We identified compound heterozygous mutations, c.5869G>T, p.E1957X and c.4480C>T, p.R1494X in the *LOXHD1* gene in 2 patients from family No. 963.

We also identified homozygous splice site mutations: c.879+1G>A in the *LOXHD1* gene in 2 patients from family No. 143.

Discussion

We identified causative compound heterozygous p.R1494X and p.E1957X and homozygous c.879+1G>A mutations in the *LOXHD1* gene. These are the first cases found in the Japanese population.

As shown in Table 1, we summarized the mutations in *LOXHD1* that have been previously reported. The mutations of p.E1957X and c.879+1G>A were novel, and p.R1494X had been reported in an American family.[22]

LOXHD1 is expressed along the plasma membrane of the stereocillia. It is considered that *LOXHD1* may couple the plasma membrane to the underlying F-actin cytoskeleton. Although stereocilliary development was unaffected in a mouse model (*samba* mouse), hair cell function was disturbed and hair cells eventually degenerated.[2]

In this study, affected individuals in family No. 143 showed profound congenital SNHL, but affected individuals in family No. 963 showed different degrees of SNHL; hearing loss in AH 4028 was about 30 dB more severe than in her older sibling. Vozzi et al[3] reported 3 patients, in a consanguineous family, who had early-onset progressive SNHL, which differed in degree in spite of having the same genotype, homozygous nonsense alleles (c.1588G>T, p.E530X). On the other hand, nonprogressive congenital SNHL was also reported in other homozygous nonsense alleles (c.4714C>T, p.R1572X).[4] In *samba* mice, a homozygous missense mutation in *Loxhd1* caused profound deafness shortly after birth. Nevertheless, homozygous nonsense mutations in *Loxhd1* caused progressive hearing loss.[2] The cases in family No. 143 had splice site mutations and were totally deafened by their sixties. It is possible that their hearing deteriorated in childhood, resulting in profound hearing loss at a younger age. The genotype-phenotype correlation in *LOXHD1* is still unclear. The differences in phenotypes in each affected individual might be the result of the nature of the mutations and the location on the gene, or result from agenetic modifier.[23]

Two affected individuals in family No. 963 were fitted with hearing aids, and both of them were able to benefit from them. If their hearing loss progresses in future, a cochlear implant could be considered for them to acquire hearing ability. Eppsteiner et al[22] reported that a patient with compound heterozygous mutations in *LOXHD1* was a good CI performer (HINT[90], CNC[73], combined[81.3]).

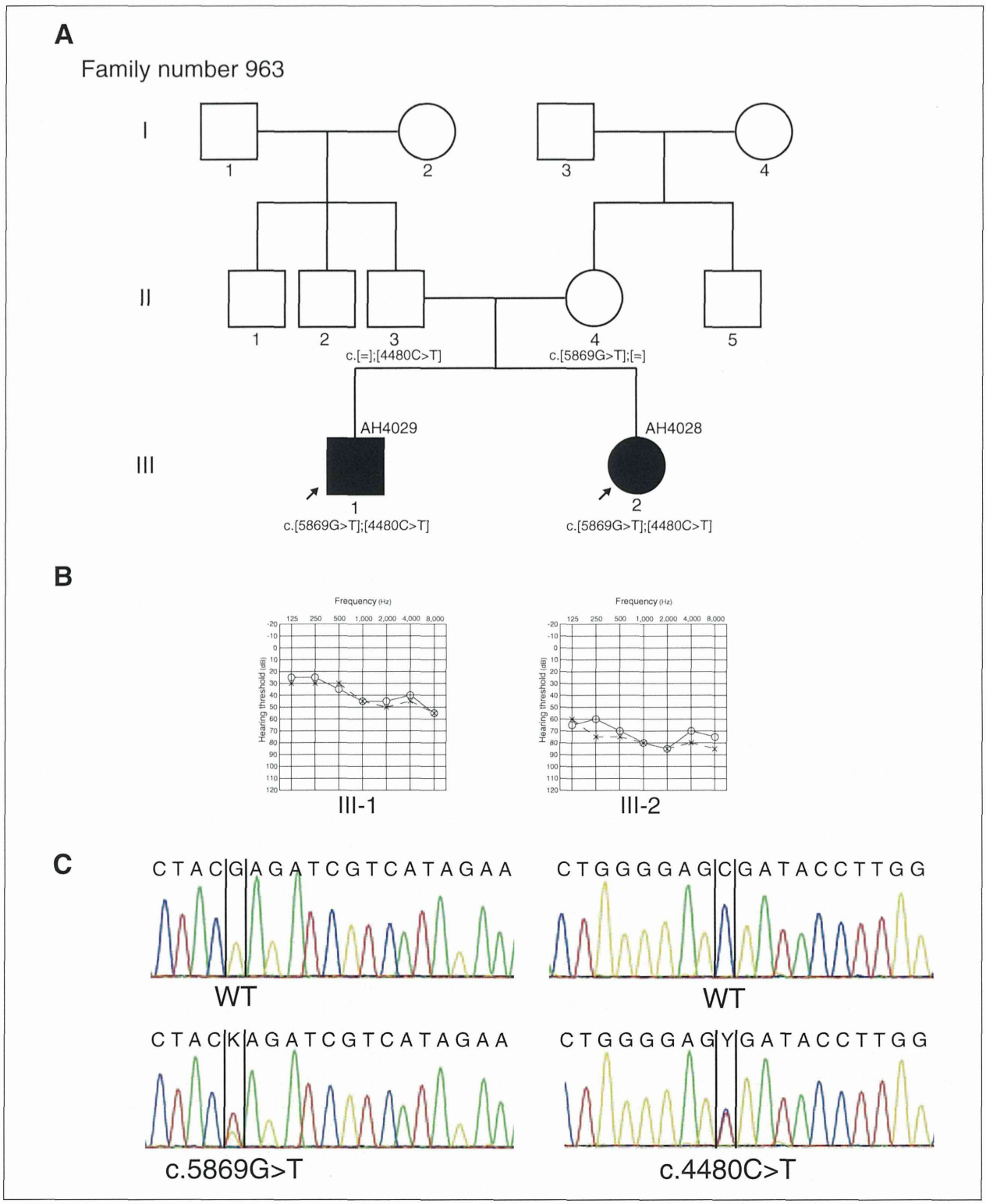

Figure 1. Pedigree and clinical findings of family No. 963. (A) Pedigree shows autosomal recessive inherited cases in this family. (B) Pure-tone audiometry (PTA) shows bilateral moderate sensorineural hearing loss in AH 4029 and bilateral severe sensorineural hearing loss in AH 4028. (C) The electropherogram shows mutations in 2 cases. AH 4029 and AH 4028 had identical compound heterozygous mutations, c.5869G>T, p.E1957X and c.4480C>T, p.R1494X. Each parent had 1 of the heterozygous mutations.

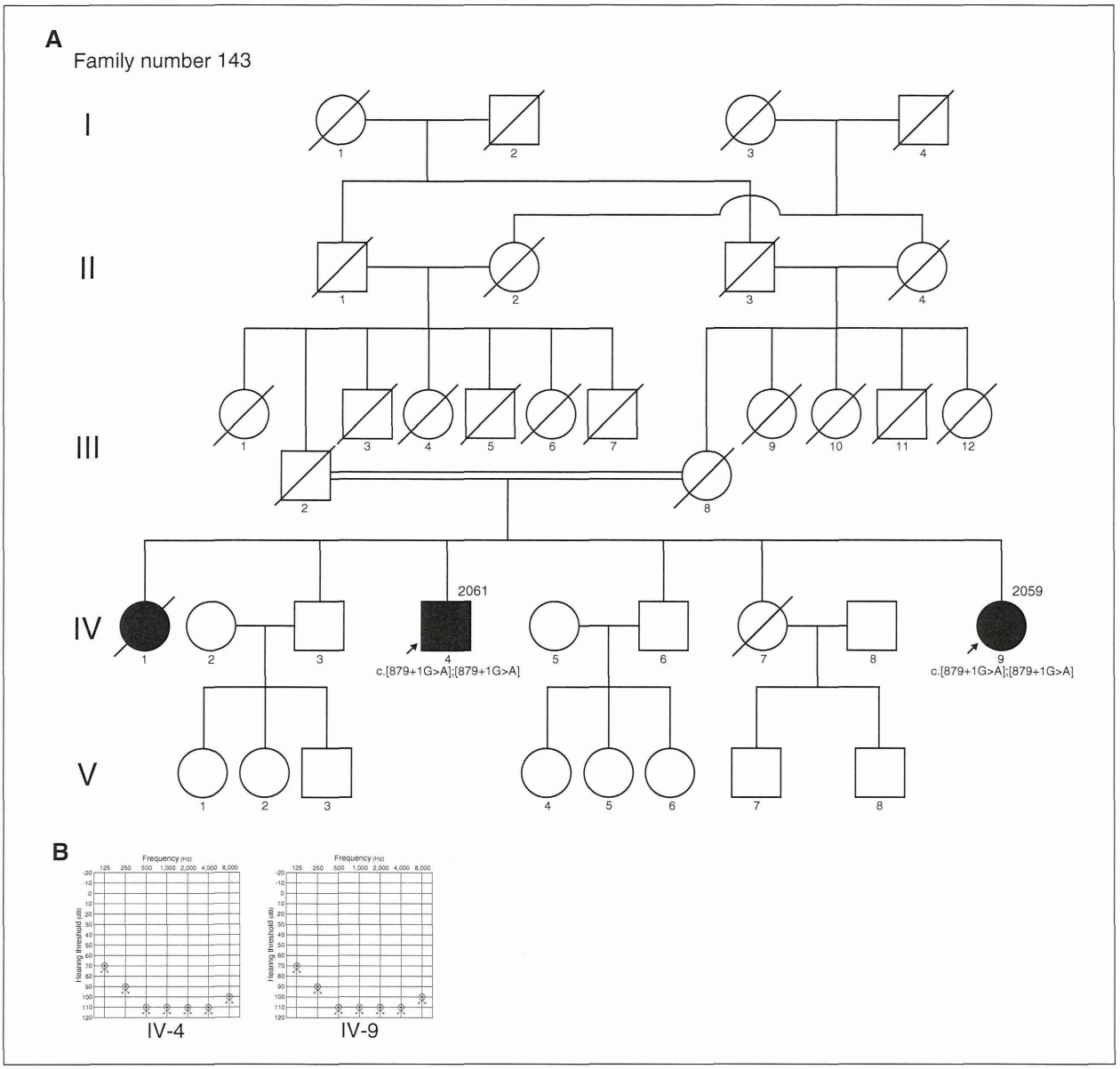

Figure 2. Pedigree and clinical findings of family No. 143. (A) Pedigree shows autosomal recessive inherited cases in this family. (B) Pure-tone audiometry (PTA) shows bilateral profound sensorineural hearing loss in both cases.

Affected individuals in family No. 963 and family No. 143 had no episodes of dizziness or vertigo attacks. No vestibular dysfunction is yet to be reported. Grillet et al[2] described *Loxhd1* expression was detected in the stereocillia of vestibular hair cells. However, under immunofluorescence microscopy, the expression level in vestibular hair cells was much weaker than that in cochlear hair cells.[2] Thus, *LOXHD1* might not associate with the vestibular system.

Mutations in *LOXHD1* have been reported to be responsible for late-onset Fuchs corneal dystrophy (FCD).[24] FCD is a genetic disorder of the corneal endothelium and is the most common cause of corneal transplantation. Recently, Stehouwer et al[25] reported that the rate of hearing disability in the FCD group was higher than that in the control group. This suggested an association between FCD and hearing loss. It is important that patients with hearing loss caused by *LOXHD1* have an ophthalmology check-up. Furthermore, FCD cases with hearing loss should be screened for *LOXHD1* mutation. These phenotypic features are important for genetic counseling. Further following examination should be necessary for the relevance to hearing loss and FCD caused by *LOXHD1* mutations.

Table 1. Known Mutations in the *LOXHD1* Gene in Hearing Loss.

Nucleotide Change	Amino Acid Change	Domain	Type of Mutation	Zygosity	NM No.	HL Onset	Type of HL	Progressiveness	Population	Reference
c.879+1G>A			Canonical-splice	Homozygous	NM_001145472	Congenital	Profound	Nonprogressive	Japanese	This study
c.1588C>T	p.E530X	PLAT4	Nonsense	Homozygous	NM_144612	Childhood	Severe-profound	Progressive	Qatar	Vozzi et al,[3] 2014
c.2008C>T	p.R670X	PLAT_repeat	Nonsense	Homozygous	NM_144612	Childhood	Moderate-profound	Progressive	Iranian	Grillet et al,[2] 2009
c.2863G>T	p.E955X	PLAT 7	Nonsense	Homozygous	NM_144612	na	na	na	Turkey	Diaz-Horta et al,[26] 2012
c.4480C>T	p.R1494X	PLAT 11	Nonsense	Homozygous	NM_144612	na	na	na	Turkey	Diaz-Horta et al,[26] 2012
c.4480C>T	p.R1494X	PLAT 11	Nonsense	Heterozygous	NM_144612	40 y.	Severe-profound	Progressive	American	Eppsteiner et al,[22] 2012
c.4480C>T	p.R1494X	PLAT 11	Nonsense	Heterozygous	NM_144612	na	Moderate-severe	Nonprogressive	Japanese	This study
c.4526G>A	p.G1509E	PLAT 11	Missense	Heterozygous	NM_144612	40 y.	Severe-profound	Progressive	American	Eppsteiner et al,[22] 2012
c.4714C>T	p.R1572X	PLAT 11	Nonsense	Homozygous	NM_144612	Prelingual	Severe-profound	Nonprogressive	Ashkenazi Jews	Edvardson et al,[4] 2011
c.5869G>T	p.E1957X	PLAT 14	Nonsense	Heterozygous	NM_144612	na	Moderate-severe	Nonprogressive	Japanese	This study

Abbreviations: HL, hearing loss; na, not available; PLAT, polycystin/lipoxygenase/alpha-toxin.

In summary, we analyzed 1314 Japanese and identified 4 patients, and 3 mutations in *LOXHD1*. It seems extremely rare, 0.30% in Japanese hearing loss patients. Candidate gene testing is not applicable for such rare genes. MPS makes it possible to detect rare genes like *LOXHD1*.

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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.

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References

1. Hilgert N, Smith R, Van Camp G. Forty-six genes causing nonsyndromic hearing impairment: which ones should be analyzed in DNA diagnostics? *Mutat Res*. 2009;681 (2-3):189-196.
2. Grillet N, Schwander M, Hildebrand MS, et al. Mutations in *LOXHD1*, an evolutionarily conserved stereociliary protein, disrupt hair cell function in mice and cause progressive hearing loss in humans. *Am J Hum Genet*. 2009;85(3):328-337.
3. Vozzi D, Morgan A, Vuckovic D, et al. Hereditary hearing loss: a 96 gene targeted sequencing protocol reveals novel alleles in a series of Italian and Qatari patients. *Gene*. 2014;542(2):209-216.
4. Edvardson S, Jalas C, Shaag A, et al. A deleterious mutation in the *LOXHD1* gene causes autosomal recessive hearing loss in Ashkenazi Jews. *Am J Med Genet A*. 2011;155A(5): 1170-1172.
5. Miyagawa M, Nishio SY, Ikeda T, Fukushima K, Usami S. Massively parallel DNA sequencing successfully identifies new causative mutations in deafness genes in patients with cochlear implantation and EAS. *PLoS One*. 2013;8(10):e75793.
6. Shearer AE, Hildebrand MS, Smith RJ. Solution-based targeted genomic enrichment for precious DNA samples. *BMC Biotechnol*. 2012;12(1):20.
7. Chang X, Wang K. wANNOVAR: annotating genetic variants for personal genomes via the web. *J Med Genet*. 2012;49(7):433-436.
8. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variation from high-throughput sequencing data. *Nucleic Acid Res*. 2010;38(16):e164.
9. Abecasis GR, Auton A, Brooks LD, et al. An integrated map of genetic variation from 1,092 human genomes. *Nature*. 2012;491(7422):56-65.
10. NHLBI Exome Sequencing Project (ESP) Exome Variant Server. http://evs.gs.washington.edu/EVS/. Accessed February 10, 2015.
11. Narahara M, Higasa K, Nakamura S, et al. Large-scale East-Asian eQTL mapping reveals novel candidate genes for LD mapping and the genomic landscape of transcriptional effects of sequence variants. *PLoS One*. 2014;9(6):e100924
12. Pollard KS, Hubisz MJ, Rosenbloom KR, et al. Detection of nonneutral substitution rates on mammalian phylogenies. *Genome Res*. 2010;20(1):110-121.
13. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding nonsynonymous variants on protein function using the SIFT algorithm. *Nat Protoc*. 2009;4(7):1073-1081.
14. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. *Nat Methods*. 2010;7(4):248-249.
15. Chun S, Fay JC. Identification of deleterious mutations within three human genomes. *Genome Res*. 2009;19(9):1553-1561.
16. Schwarz JM, Rodelsperger C, Schuelke M, et al. MutationTaster evaluates disease-causing potential of sequence alterations. *Nat Methods*. 2010;7(8):575-576.
17. Cooper GM, Stone EA, Asimenos G, et al. Distribution and intensity of constraint in mammalian genomic sequence. *Genome Res*. 2005;15(7):901-913.
18. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*. 2009;25(14):1754-1760.
19. McKenna A, Hanna M, Banks E, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res*. 2010;20(9):1297-1303.
20. Frommolt P, Abdallah AT, Altmuller J, et al. Assessing the enrichment performance in targeted resequencing experiments. *Hum Mutat*. 2012;33(4):635-641.
21. Shearer AE, Black-Ziegelbein EA, Hildebrand MS, et al. Advancing genetic testing for deafness with genomic technology. *J Med Genet*. 2013;50(9):627-634.
22. Eppsteiner RW, Shearer AE, Hildebrand MS, et al. Prediction of cochlear implant performance by genetic mutation: the spiral ganglion hypothesis. *Hear Res*. 2012;292(1-2):51-58.
23. McHugh RK, Friedman RA. Genetics of hearing loss: allelism and modifier genes produce a phenotypic continuum. *Anat Rec A Discov Mol Cell Evol Biol*. 2006;288(4):370-381.
24. Riazuddin SA, Parker DS, McGlumphy EJ, et al. Mutations in *LOXHD1*, a recessive-deafness locus, cause dominant late-onset Fuchs corneal dystrophy. *Am J Hum Genet*. 2012;90(3):533-539.
25. Stehouwer M, Bijlsma WR, Van der Lelij A. Hearing disability in patients with Fuchs' endothelial corneal dystrophy: unrecognized co-pathology? *Clin Ophthalmol*. 2011;5:1297-1301.
26. Diaz-Horta O, Duman D, Foster J II, et al. Whole-exome sequencing efficiently detects rare mutations in autosomal recessive nonsyndromic hearing loss. *PLoS One*. 2012;7(11):e50628.

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Novel Mutations in *GRXCR1* at DFNB25 Lead to Progressive Hearing Loss and Dizziness

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Abstract

Objective: We identified 2 patients in 1 family who had novel mutations in *GRXCR1*, which caused progressive hearing loss.

Methods: One thousand one hundred twenty Japanese hearing loss patients with sensorineural hearing loss from unrelated families were enrolled in this study. Targeted genomic enrichment with massively parallel sequencing of all known nonsyndromic hearing loss genes was used to identify the genetic causes of hearing loss.

Results: In this study, 2 affected individuals with compound heterozygous mutations—c.439C>T (p.R147C) and c.784C>T (p.R262X)—in *GRXCR1* were identified. The proband had moderate to severe hearing loss and suffered from dizziness with bilateral canal paralysis.

Conclusion: Our cases are the first identified in the Japanese population and are consistent with previously reported cases. The frequency of mutations in *GRXCR1* seems to be extremely rare. This study underscores the importance of using comprehensive genetic testing for hearing loss. Furthermore, longitudinal audiologic assessment and precise vestibular testing are necessary for a better understanding of the mechanisms of hearing loss and vestibular dysfunction caused by *GRXCR1* mutations.

Keywords

hearing loss, genetics, dizziness, *GRXCR1*, massively parallel sequencing

Introduction

Hearing loss is diagnosed in approximately 2 in every 1000 children in developed countries, and genetic causes account for at least 50% of all childhood nonsyndromic sensorineural hearing loss (SNHL).[1] Autosomal recessive (AR) SNHL occurs in 70% of cases and is characterized as typically congenital or prelingual, and severe to profound.[2] The most common causative gene is *GJB2*, which is affected with prelingual severe to profound SNHL, and most of these cases occur with nonprogressive hearing loss. However, the prevalence of SNHL increases to 2.7 per 1000 before the age of 5 years and reaches 3.5 per 1000 at adolescence.[3] This may suggest a potential for developing progressive SNHL with genetic causes. Several studies reported that only a few genetic causes of AR SNHL might be naturally occurring progressive hearing loss such as *SLC26A4* and *CDH23*.[4]

GRXCR1 is mapped to chromosome 4p13, which is known to be a cause of DFNB25.[5] Mutations in *GRXCR1* result in a progressive nonsyndromic SNHL. There have been only 2 reports in 6 families, from Pakistan, the Netherlands, and Iran.[5,6] According to these reports, SNHL caused by mutations in *GRXCR1* had a phenotypic feature showing early-onset progressive hearing loss and could be associated with vestibular dysfunction. The prevalence of *GRXCR1* hearing loss is deemed to be extremely rare, but the exact frequency is still unknown. Even though the phenotypic features are obtained, it could be hard to prioritize the candidate gene and move on to the sequencing of the whole *GRXCR1* gene. Recent advances in targeted genomic enrichment with massively parallel sequencing (TGE+MPS) have made possible the sequencing of all

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known causative genes simultaneously.[7-9] We performed genetic testing to analyze the genetic etiology of Japanese hearing loss patients using MPS. Here, we describe a family who was identified with novel mutations in *GRXCR1*, resulting in progressive hearing loss and vestibular dysfunction.

Patients and Methods

Patients

One thousand one hundred twenty Japanese hearing loss patients (autosomal dominant SNHL: 266, ARSNHL: 600, unknown: 254) from 53 ENT departments nationwide participated in this study. Written informed consent was obtained from all patients (or from their next of kin, caretaker, or guardian on the behalf of minors/children) prior to enrollment in the project. This study was approved by the Shinshu University Ethical Committee as well as the respective ethical committees of the other participating institutions.

Methods

Amplicon library preparation. Amplicon libraries were prepared using an Ion AmpliSeq Custom Panel (Applied Biosystems, Life Technologies, Grand Island, New York, USA), according to the manufacturer's instructions, for 63 genes reported to cause nonsyndromic hearing loss. The detailed protocol was described elsewhere.[8] After preparation, the amplicon libraries were diluted to 20 pM and equal amounts of 6 libraries for 6 patients were pooled for 1 sequence reaction.

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

Base call and data analysis. 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.[10,11] 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 1000 genome database,[12] (2) the 6500 exome variants,[13] (3) the Human Genetic Variation Database (dataset for 1208 Japanese exome variants),[14] 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,[15] Sorting Intolerant from Tolerant,[16] Polymorphism Phenotyping (PolyPhen2),[17] LRT,[18] MutationTaster,[19] and GERP++.[20] Candidate mutations were confirmed by Sanger sequencing and the responsible mutations were identified by segregation analysis using samples from among the patients' family members.

Protein modeling. A 3-dimensional model was built by comparative modeling using the SWISS-MODEL workspace[21] and with coordinates of human GLRX5 (PDB accession number 2wul) as a structural template. The results of the 3-dimensional analysis were visualized by MacPyMOL version 0.99rc.6 (http://www.pymol.org).

Variant Confirmation

All pathogenic variants were confirmed by Sanger sequencing and segregation analysis using exon-specific custom primers.

Results

For the hearing loss patient cohort, we identified a family (family number 972) who had causative mutations in the *GRXCR1* gene.

Mutation Analysis

We identified compound heterozygous mutations—c.439C>T (p.R147C) and c.784C>T (p.R262X)—in *GRXCR1* in 2 hearing loss patients in the family (Figure 1A). c.439C>T (NM_001080476) is located in exon 2, which was strongly suspected as pathogenic. In silico prediction software (SIFT, PolyPhen2, LRT, and MutationTaster) indicated the mutation as pathogenic (0.98, 1.00, 1.00, and 1.00, respectively). c.784C>T (NM_001080476), which is located in exon 4, leads to a premature stop codon and truncation (p.R262X).

Case Details

Family number 972; II-1: AH 2888, II-5. The proband (II-1: AH 2888) was a 41-year-old male. He noticed bilateral hearing loss in infancy. He had normal motor development as an infant. He acquired spoken language through special classes for hearing loss students in his elementary school, but sometimes he needs lip-reading to communicate in his daily life. His pronunciation was clear, and his speech was almost completely intelligible. He began to use bilateral hearing aids around the age of 20. As shown in Figure 1C, his pure-tone audiometry (PTA) showed bilateral moderate sensorineural hearing loss at the age of 25 years. His

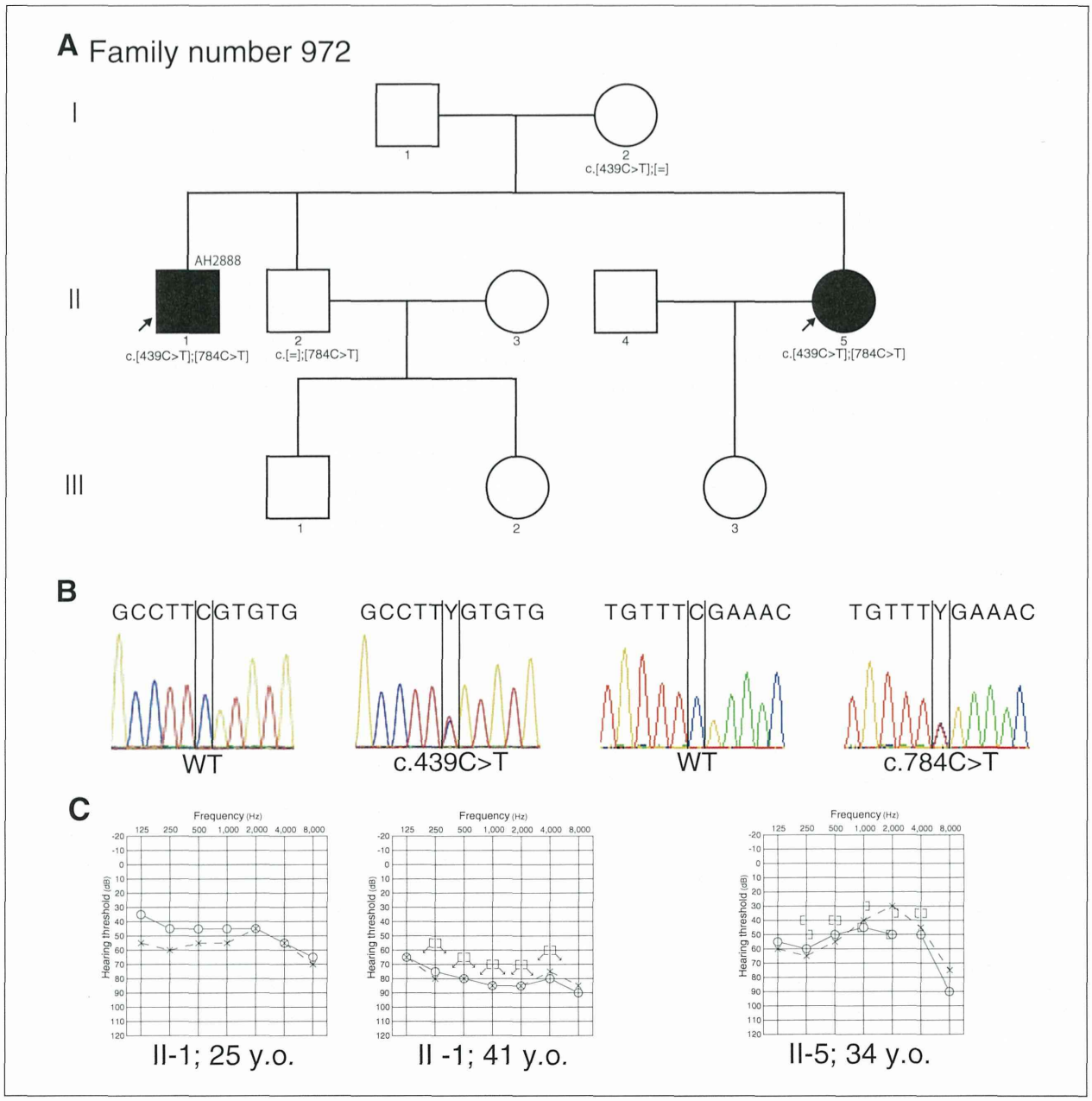

Figure 1. Pedigree and clinical findings of family number 972. (A) Pedigree shows autosomal recessive inherited cases. (B) The electropherograms show mutations in 2 cases. Target genome enrichment and massively parallel sequencing were carried out for II-1: AH 2888. Sanger sequencing for family segregation identified mutations in II-5. c.439C>T, (p.R147C) and c.784C>T, (p.R262X) were segregated in the family. (C) Pure-tone audiometry (PTA) shows bilateral sensorineural hearing loss in II-1: AH 2888 and II-5. PTA revealed that II-1: AH 2888 had progressive hearing loss.

hearing loss gradually deteriorated to become severe around the age of 40 years. For further examination of his progressive SNHL, he was referred to Kagoshima University Hospital, Department of Otolaryngology. He had some occasional episodes of dizziness, but no attacks of vertigo. Caloric testing with cold water irrigation (20°C, 5 mL) revealed his vestibular dysfunction; maximum slow phase velocity of the right side was 5°/sec, and that of the left side was 7°/sec. The computed tomography (CT) findings of the middle and inner ear were normal.

Table 1. Known Mutations in the *GRXCR1* (DFNB25) Gene in Hearing Loss.

Nucleotide Change	Amino Acid Change	Domain	Zygosity	NM Number	Hearing Loss Onset	Type of Hearing Loss	Progression	Vestibular Dysfunction	Population	Reference
c.113C>T	p.P38L	—	Homozygosity	NM_001080476	Congenital	Severe to profound	No evidence	No evidence	Iranian	Odeh et al, 2010
c.190G>A	p.G64S	—	Homozygosity	NM_001080476	Congenital	Severe to profound	No evidence	No evidence	Iranian	Odeh et al, 2010
c.229C>T	p.Q77X	—	Homozygosity	NM_001080476	Very early-onset or congenital	Severe	No evidence	No evidence	Pakistani	Schraders et al, 2010
c.412C>T	p.R138C	Glutaredoxin domain	Homozygosity	NM_001080476	Very early-onset or congenital	Severe	No evidence	No evidence	Pakistani	Schraders et al, 2010
c.439C>T	p.R147C	Glutaredoxin domain	Heterozygosity	NM_001080476	Childhood	Moderate to severe	Yes	Yes	Japanese	This study
c.457T>G	p.F153V	Glutaredoxin domain	Homozygosity	NM_001080476	Congenital	Severe to profound	No evidence	No evidence	Iranian	Odeh et al, 2010
c.627+19A>T	p.G210VfsX14	Glutaredoxin domain	Homozygosity	NM_001080476	Very early-onset or congenital	Mild to moderate	Yes	No	Dutch	Schraders et al, 2010
c.628-9C>A	p.G210LfsX5	Glutaredoxin domain	Homozygosity	NM_001080476	Very early-onset or congenital	Severe to profound	Yes	Yes	Dutch	Schraders et al, 2010
c.784C>A	p.R262X	—	Heterozygosity	NM_001080476	Childhood	Moderate to severe	Yes	Yes	Japanese	This study

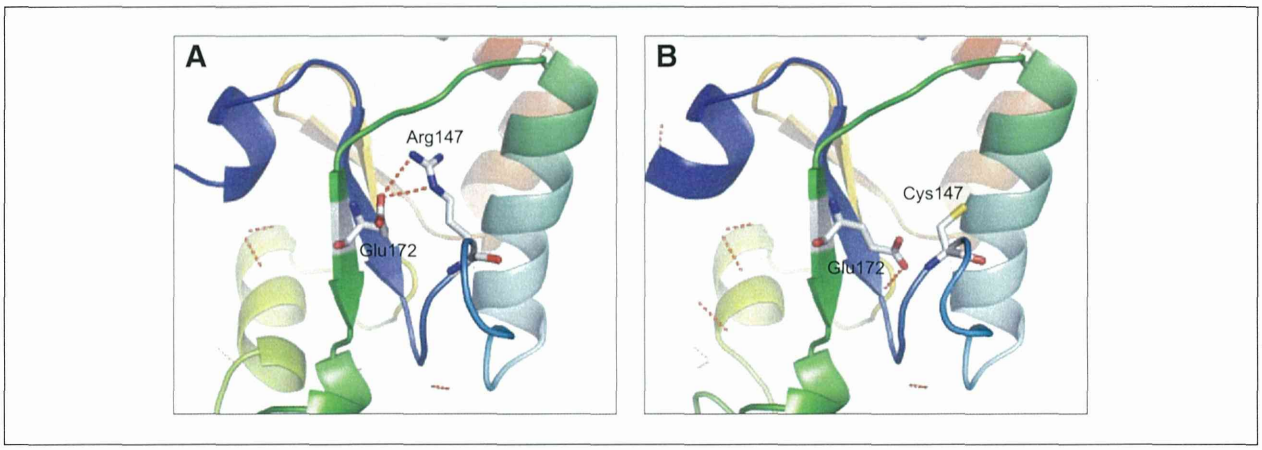

Figure 2. Three-dimensional model of the structure of glutaredoxin domain built by SWISS-MODEL workspace. (A) Wild type of the structure between arginine 147 (Arg147) and glutamic acid 172 (Glu172) showing a hydrogen bond. (B) The c.439C>T leads to a missense mutation from an arginine to a cysteine at the position 147 (Cys147), resulting in disruption of the hydrogen bond.

He had a younger sister (II-5): a 34-year-old female who began to use a hearing aid 2 years earlier. Her PTA showed bilateral moderate sensorineural hearing loss, but it was less severe than that of her brother (II-1) (Figure 1C).

Discussion

In this report, we identified novel causative mutations with compound heterozygous c.439C>T (p.R147C) and c.784C>T (p.R262X) mutations in the *GRXCR1* gene. *GRXCR1* encodes a 290-residue protein cysteine-rich C-terminal region and a predicted glutaredoxin (GRX) domain. GRX domains are the catalytic domains in glutaredoxins and are responsible for the modulation of the reversible S-glutathionylation of proteins.[22] We summarized the mutations in *GRXCR1* and the corresponding phenotypes that have been previously reported[5] in Table 1. Each affected family member had differing degrees of hearing loss severity and onset times. Mutations located near the N terminus may tend to be associated with a very early onset and severe SNHL. However, our study identified novel mutations that result in milder SNHL. We suggest that c.784C>T, located near the C terminus, might express an incomplete GRXCR1 protein with residual activity that appears to form in cases exhibiting moderate SNHL. We also suggest that c.439C>T, located in the GRX domain, might lead to the denaturation of glutaredoxins that reduce oxidized cysteines in cellular proteins and that this domain is crucial for the maintenance of enzymatic functions. In addition, 4 of the 7 previously reported mutations were located in the GRX domain. As shown in Figure 2A, our 3-dimensional model shows that arginine 147 and glutamic acid 172 are coupled by hydrogen bonds.[21] The c.439C>T mutation leads to a missense mutation from an arginine to a cysteine at position 147 of the protein, resulting in the disruption of the hydrogen bond and playing a role in the alteration of the protein structure (Figure 2B).

The GRXCR1 protein is localized in the hair bundles and stereocilia and plays a role in actin organization in hair cells toward the development of normal diameter and growth of stereocilia.[23] *Grxcr1* is expressed along the entire length of the stereocilia in the outer hair cells, inner hair cells, and all vestibular hair cells. In their study of the pirouette (*pi*) mouse model of *Grxcr1* mutants, Odeh et al[6] reported that loss of function of *Grxcr1* resulted in abnormally thin and slightly shortened stereocilia. It was also reported that a defective mouse with the *Grxcr1* gene had deafness and showed circling behavior. In this study, patient II-1 (AH 2888) had early-onset progressive SNHL and vestibular dysfunction. Schraders et al[5] reported that individuals with mutations in *GRXCR1* were also affected with vestibular dysfunction, and they concluded that mutations in *GRXCR1* caused congenital or early-onset moderate to severe hearing loss and could be associated with vestibular dysfunction. Our cases are consistent with previously reported cases.

We analyzed 1120 Japanese with hearing loss and identified only 1 patient (0.09%) with 2 novel mutations in *GRXCR1*. Candidate gene testing is not applicable for such a rare gene. Further evolution of genetic testing will make accurate diagnosis of hearing loss possible with less labor and expense. Furthermore, longitudinal audiologic assessment and precise vestibular testing are necessary for a better understanding of the mechanisms of hearing loss and vestibular dysfunction caused by *GRXCR1* mutation.

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