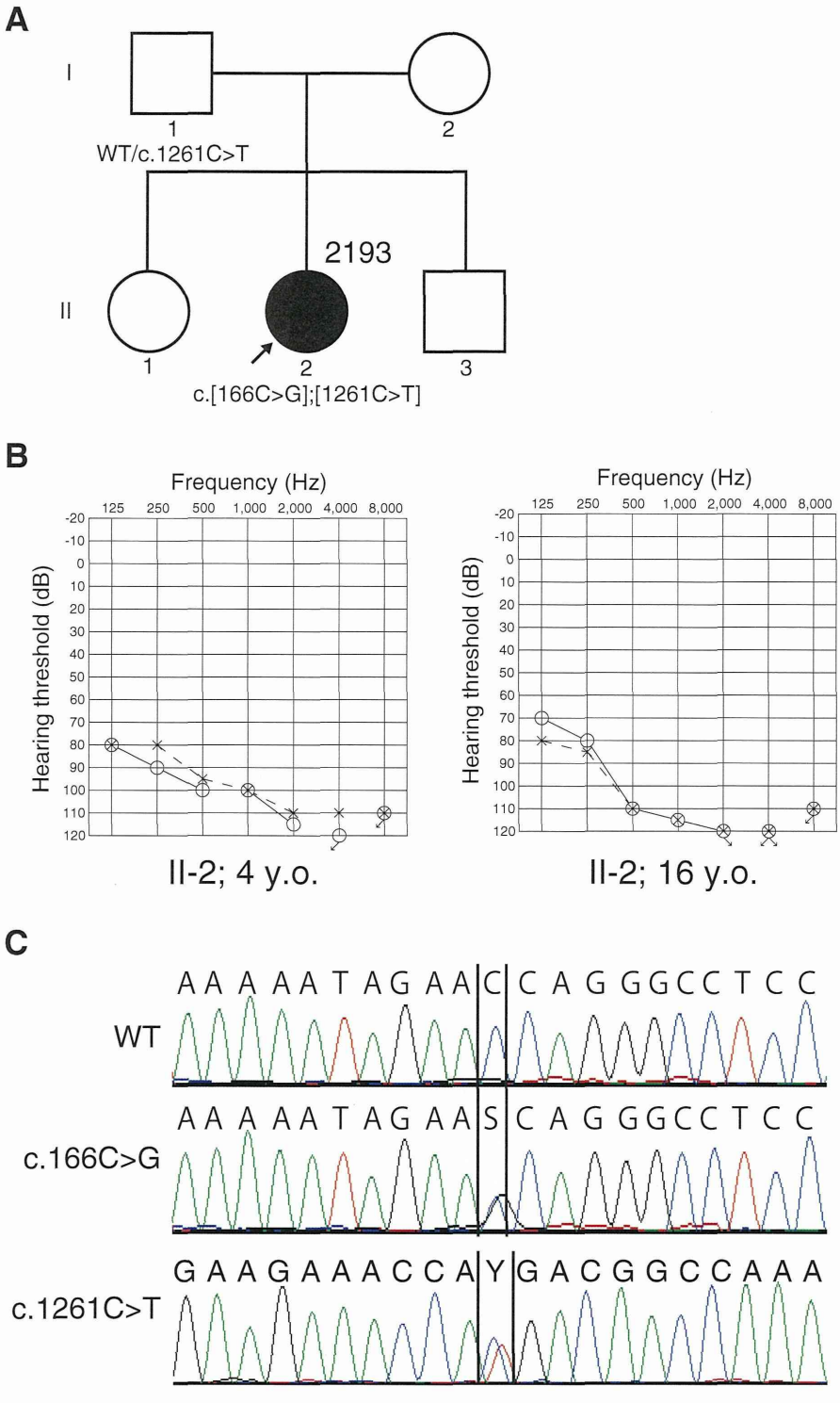

Figure 2. Pedigree and clinical findings for Case 2: SNS 2193. (A) Pedigree showed a single case in this family. (B) Pure-tone audiometry showed nonprogressive profound sensorineural hearing loss (SNHL). (C) Sanger sequencing and segregation analysis showed Case 2 had the compound heterozygous mutation, and her father had the heterozygous mutation.

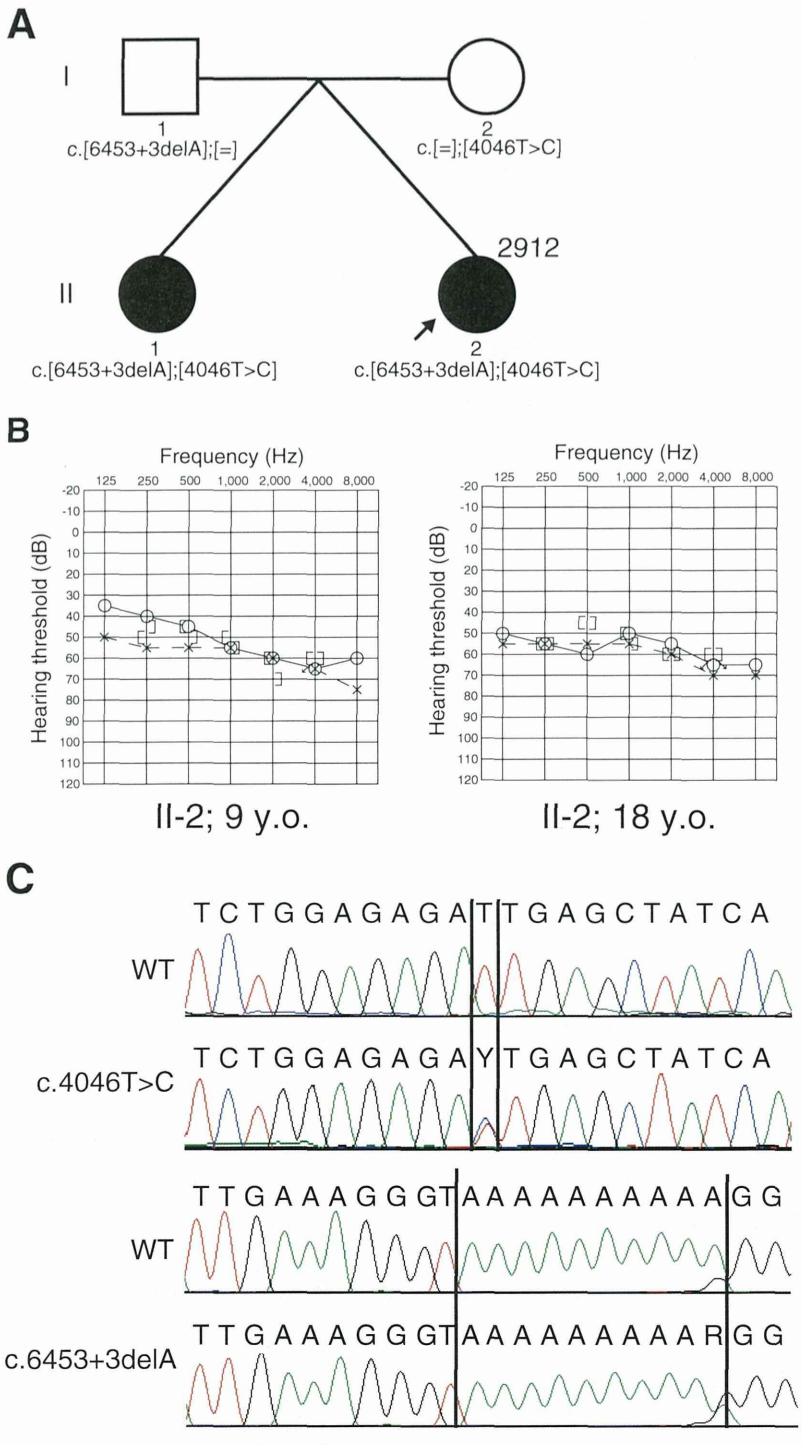

Figure 3. Pedigree and clinical findings for Case 3: SNS 2912. (A) Pedigree showed 2 cases in this family. (B) Pure-tone audiometry showed nonprogressive moderate sensorineural hearing loss (SNHL). (C) Sanger sequencing and segregation analysis showed Case 3 and her twin sister had the compound heterozygous mutation, and her parents had the heterozygous mutation.

Table 1. All Known Causative Mutations in the *PTPRQ* Gene

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

Abbreviations: Age, age at the time of hearing examination; CP, cytoplasmic domain; EC, extracellular domain; HL, hearing loss; NR, not reported.

at an elementary school health check-up, and she was referred to a general hospital clinic of otolaryngology at the age of 7. PTA showed bilateral moderate hearing loss, approximately 60 dBHL in both ears. She started to wear hearing aids bilaterally. Over a period of 11 years, her hearing loss did not deteriorate. 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 or 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 3 cases identified in this study and 9 previously reported cases.[3,6] Each affected family had various degrees of hearing loss severity and progressiveness. With respect to Case 1: AG 8960, he had progressive and profound SNHL mainly affected at high frequencies, with a homozygous nonsense mutation. Four of the 7 cases with homozygous nonsense mutations were described as having severe to profound SNHL: 1 case with a flat audiogram and 3 cases with a down-sloping audiogram. Another 2 Palestinian cases with a homozygous nonsense mutation were described as having moderate SNHL, although clinical information on the deterioration of their hearing was lacking.[4] Schraders et al[3] also reported that hearing loss progressed over a period of 10 to 30 years, becoming profound SNHL in 2 Dutch cases with a homozygous nonsense mutation. 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 assessment (caloric test and 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[3] also reported that caloric test showed either no or hypo-responses on both sides in 4 cases with *PTPRQ* homozygous mutations. A *Ptprq* knockout mouse study revealed deformation of the stereocilia and hair bundles in the utricle and defects in the hair bundles in the saccule and ampullae.[26] Vestibular evoked potentials (VsEPs) were absent in the majority of *Ptprq* knockout mice.[26] These findings suggested that the *PTPRQ* mutations might cause dysfunctions in the vestibular organs. However, Case 1 had not experienced 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.[26]

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 mutation and missense mutation. Taken together with

the results of previous reports (Table 1), there does not appear to be any obvious genotype-phenotype correlation.

In summary, we performed TEG+MPS in this study, and we believe this method could be useful for identifying rare causative gene mutations, such as the *PTPRQ* gene. The *PTPRQ* mutations 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* mutations appeared to be congenital. With regard to the hearing levels and progression, we observed variations among 3 cases. More precise studies are necessary for better understanding the molecular basis 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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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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De Novo Mutation in X-Linked Hearing Loss–Associated *POU3F4* in a Sporadic Case of Congenital Hearing Loss

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Abstract

Objectives: In this report, we present a male patient with no family history of hearing loss, in whom we identified a novel de novo mutation in the *POU3F4* gene.

Methods: One hundred ninety-four (194) Japanese subjects from unrelated and nonconsanguineous families were enrolled in this study. We used targeted genomic enrichment and massively parallel sequencing of all known nonsyndromic hearing loss genes for identifying the genetic causes of hearing loss.

Results: A novel de novo frameshift mutation of *POU3F4* to c.727_728insA (p.N244KfsX26) was identified. The patient was a 7-year-old male with congenital progressive hearing loss and inner ear deformity. Although the patient had received a cochlear implant, auditory skills were still limited. The patient also exhibited developmental delays similar to those previously associated with *POU3F4* mutation.

Conclusion: This is the first report of a mutation in *POU3F4* causing hearing loss in a Japanese patient without a family history of hearing loss. This study underscores the importance of comprehensive genetic testing of patients with hearing loss for providing accurate prognostic information and guiding the optimal management of patient rehabilitation.

Keywords

hearing loss, genetics, *POU3F4*, cochlear implant, massively parallel sequencing

Introduction

The majority of genetic hearing loss is autosomal inherited (autosomal recessive: approximately 75%, autosomal dominant: approximately 20%), and X-linked hearing loss is estimated to account for 1% to 5% of genetic causes.[1] To date, 5 loci and 4 genes have been implicated in X-linked nonsyndromic hearing loss (DFNX).[2] The most common cause of X-linked hearing loss is mutation in the POU domain class 3 transcription factor 4 (POU3F4), which was mapped at DFNX2 loci on chromosome Xq21, and previous reports have described clinically heterogeneous disease phenotypes.[3-5] Hearing loss is severe—profound sensorineural hearing loss (SNHL), which varies from congenital to late onset, is often progressive, and may include a conductive hearing loss component in some cases. Anatomically, computed tomography (CT) of the temporal bone in such cases reveals an enlarged internal auditory canal that coalesces with the basal turn of the cochlea along with partial hypoplasia.[6] With these anatomical features, perilymphatic gusher is known to occur upon stapes surgery for correcting stapedial fixation as well as during cochlear implant surgery.[7,8]

Based solely on frequency, if a male patient has hearing loss with an apparent X-linked form of inheritance segregation in the family, and/or if a temporal bone CT abnormality were found, the *POU34* gene could be the most likely cause. However, sporadic cases of SNHL with no family history can be difficult to recognize as a candidate and move on to the sequencing of the entire *POU3F4* gene. Recent advances in targeted genomic enrichment with massively parallel

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sequencing (TGE+MPS) have facilitated the simultaneous sequencing of all known causative genes.[9,10]

Here, we describe a male with no family history of hearing loss in whom we identified a novel de novo mutation in the *POU3F4* gene. This is the first report of a diagnosis of hearing loss caused by *POU3F4* in a patient with no family history of hearing loss and highlights the importance of comprehensive genetic testing for optimal diagnostic rates for nonsyndromic hearing loss.

Subjects and Methods

Subjects

One hundred ninety-four (194) Japanese subjects (114 females) from unrelated and nonconsanguineous families were ascertained through 33 otolaryngology clinics in 28 prefectures across Japan. All subjects had presumed nonsyndromic hearing loss. 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 from each proband and from all consenting affected and unaffected relatives.

Targeted Genomic Enrichment and Massively Parallel Sequencing

Genomic DNA was assessed for quality by gel electrophoresis and spectrophotometry (Nanodrop 1000; Thermo Fisher Scientific, Waltham, Massachusetts, USA; 260/280 ratio of 1.8-2.2) and for quantity by fluorometry (Qubit 2.0 Fluorometer; Life Technologies, Carlsbad, California, USA). TGE of all exons of all genes implicated in SNHL 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, California, USA).[11]

In brief, 3 μg gDNA was randomly fragmented to an average size of 250 bp (Covaris Acoustic Solubilizer; Covaris Inc, Woburn, Massachusetts, USA), 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 were followed by a second amplification before pooling for sequencing. Minimal amplification was used—typically 8 cycles for the prehybridization polymerase chain reaction (PCR; range, 8-10 cycles) using NEB Phusion HF Master Mix (New England BioLabs Inc, Ipswich, Massachusetts, USA), and 14 cycles for the posthybridization 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, California, USA) in pools of 4 to 6 or 48, respectively, using 100-bp paired-end reads.

Bioinformatics Analysis

Data were analyzed as described using a local installation of the open-source Galaxy software and the following open-source tools: BWA[12] for read mapping, Picard for duplicate removal, GATK[13] for local realignment and variant calling, and NGSRich[14] for enrichment statistics.[10] We reported and annotated variants with custom software.

Variant Confirmation

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

Results

We identified 1 case with a causative mutation in the *POU3F4* gene in the cohort of this study (194 hearing loss patients).

Case Details

The affected patient was a 7-year-old male with no particular perinatal events but failed newborn hearing screening. He was referred to Niigata University Hospital, Department of Otolaryngology for further examinations at the age of 2 months. An auditory brainstem response (ABR) revealed a bilateral hearing loss of approximately 70 dBnHL in both ears and clear responses were observed at 100 dBnHL. Behavioral observation audiometry demonstrated thresholds of 30 to 50 dB between 500 and 2000 Hz. Bilateral otitis media with effusion was observed on otoscopic examination. Bilateral high frequency sloping and mild-moderate SNHL was suspected at the age of 1 year. CT findings of the middle and inner ear showed partial cochlear hypoplasia and dilatation of the fundus of the internal auditory canal, which was also incompletely separated from the basal turn of the cochlea. At 2 years of age, his parents noticed that he did not respond to their voices and had only spoken a few words. Therefore, congenital and progressive SNHL was suspected, and the patient was promptly fitted for bilateral hearing aids. However, his hearing subsequently deteriorated, and ABR was absent at the age of 3 years, 6 months; the hearing aids were insufficient for adequate hearing.

Therefore, cochlear implant (CI) surgery was performed in the left ear at the age of 4 years, 3 months. Perilymphatic gusher occurred during cochleostomy, which was performed

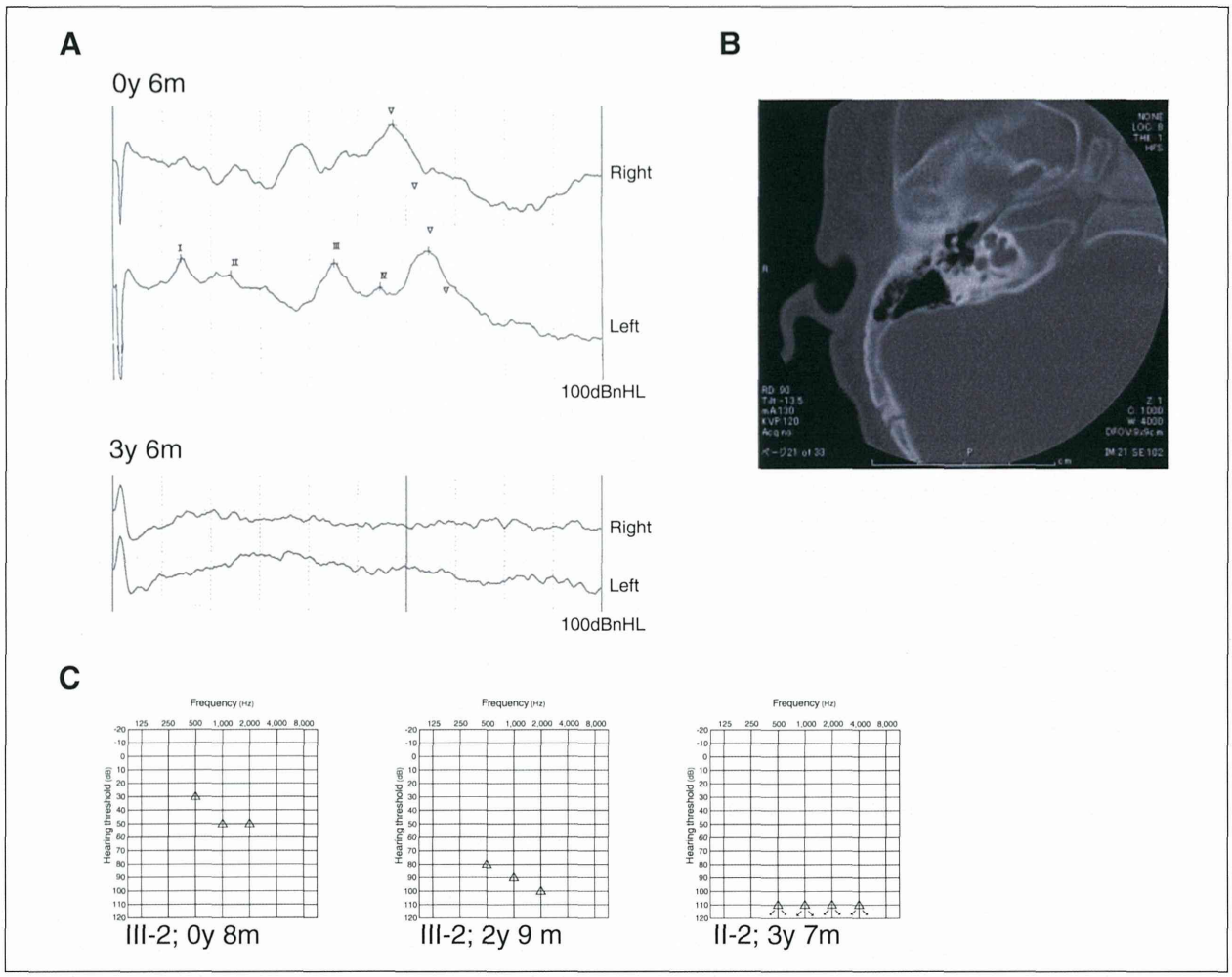

Figure 1. Clinical findings of the patient (ID 4750) (A) Auditory brain stem response (ABR) at the age of 6 months and 3 years with click stimuli at 100 dBnHL. A clear ABR was observed at the age of 6 months; however, ABR was absent in the following year. (B) Temporal bone computed tomography revealed partial cochlear hypoplasia, dilatation of the internal auditory canal fundus, and incomplete separation from the basal turn of the cochlea. (C) Behavioral observation audiometry (BOA) revealed deterioration of the threshold from 50 to 100 dB during the subsequent 3 years, suggesting progressive hearing loss.

using a conventional method. The patient underwent implantation with a Nucleous 24 device and strait array (Cochlear Ltd, Lane Cove, Australia); finally, electrode insertion was accomplished.

One year after CI, the patient's sound field threshold was 30 to 40 dB at low and mid frequencies, but he still could not perceive high frequency sounds with the electrode at the basal turn of the cochlea. He continued to exhibit delayed speech and low ability to interpret Japanese at 2 years after CI (age: 6 years, 6 months). His limited perceptual and communicative abilities led to a diagnosis of pervasive developmental disorder (PDD).

His parents and 2 siblings had normal hearing, and there was no family history of hearing loss or other cognitive disorders. The patient's audiological assessment results, CT findings, and pedigree are shown in Figure 1.

Mutation Analysis

We performed comprehensive genetic testing using TGE+MPS of all known nonsyndromic hearing loss genes as well as nonsyndromic hearing loss mimic genes, as previously described.[10] We identified a novel frameshift mutation in the *POU3F4* gene, in which 1 adenine nucleotide was inserted. This mutation corresponded to c.727_728insA (NM_000307) and led to frameshift mutation and truncation (p.N244KfsX26). We also performed Sanger sequencing for the family segregation study and confirmed the gene

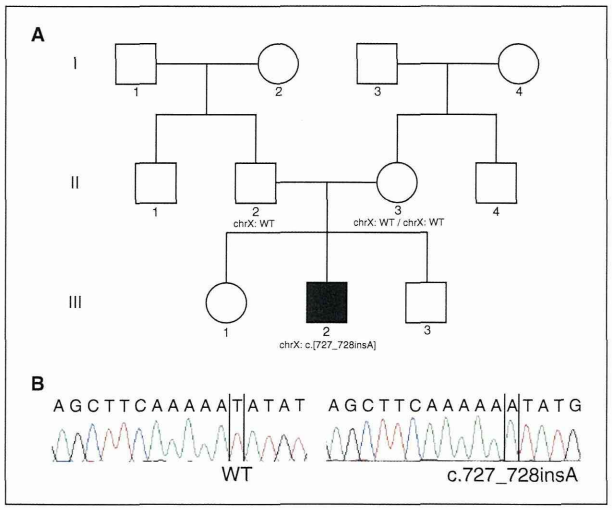

Figure 2. Pedigree of the patient (ID 4750) (A) Pedigree indicated a sporadic case in this family. (B) Electropherogram revealed a mutation only in this patient (on the right). Parents (4751 and 4752) did not carry the mutation.

variant in the proband. As shown in Figure 2, the Sanger sequencing revealed that the parents had no mutations, although his mother had been expected to be a carrier of the variant. Therefore, we diagnosed the patient with a de novo mutation that had resulted in hearing loss with inner ear deformity.

Discussion

In this study, we identified a novel de novo *POU3F4* mutation in a case of sporadic hearing loss. TGE+MPS allowed us to identify the causative mutation, based on all the known hearing loss genes screened. Although there has been only 1 previous report of a *POU3F4* mutation in the Japanese population,[15] suggesting a very rare event, many such cases have been reported in the Korean population, which harbors a very similar genetic background.[16-18] Thus, there may be more Japanese patients harboring *POU3F4* mutations even in sporadic cases that are not identified as X-linked inheritance.

As shown in Table 1, more than 30 mutations, point mutations, and genomic rearrangements involving large deletions and inversions on the upstream gene regulatory element have been reported.[4,19-21] However, it is difficult to detect such large genomic alterations using conventional PCR-based DNA sequencing. Anger et al[22] have reported normal genetic sequencing results in a DFNX2 case with chromosomal rearrangements in the vicinity of *POU3F4*, suggesting a failure of Sanger sequencing to identify mutations due to genetic causes. In contrast, it is possible to identify such genetic alterations using MPS instead of tests such as array comparative genomic hybridization and fluorescence *in situ* hybridization.

POU3F4 encodes a transcription factor that binds DNA using 2 specific domains: The POU-specific domain and POU homeodomain. These domains play essential roles in inner ear development and are expressed in both the brain and neural tube. Several studies of *POU3F4* knockout and mutant mouse models have been published; Parzefall et al[23] have clearly described an observed shortened cochlear duct as a possible Mondini malformation in a mouse harboring a *POU3F4* mutation. A case involving DFNX2 with *POU3F4* mutations was characterized as exhibiting an inner ear deformity of incomplete partition type III.[18] Our presented case harbored the same cochlear deformity as described in previous reports and was consistent with a de novo mutation in *POU3F4* that led to congenital progressive hearing loss.

Regarding phenotypic features, previously reported manifestations and clinical histories are presented in Table 1. However, we did not observe considerable variations in the genotype-associated phenotypes. Therefore, it is difficult to determine whether these genotype-phenotype correlations exist as described in previous reports (Table 1). Cochlear deformities were observed in most cases, demonstrating mixed hearing losses that were independent of middle ear function and perilymphatic gusher. Several DFNX2 cases with large genomic deletions or chromosomal rearrangements (even single nucleotide variants) have exhibited developmental delays in addition to hearing loss (Table 1).[7,24,25] PDD was diagnosed in our patient, who was suspected of exhibiting some syndromic features. In general, hearing loss may impact early childhood development, and differences in hearing levels, social background, and parental factors may affect language acquisition and learning abilities. However, hearing loss–associated *POU3F4* mutation should be additionally considered as a cause of developmental delays involving communication skills, particularly in CI recipients.

Prior to cochlear implantation, we were unable to provide either genetic testing results or counseling for this patient as TGE+MPS technologies were unavailable for common clinical usage at that time. However, these technologies are currently applicable, and the further evolution of genetic testing will facilitate the accurate diagnosis of hearing loss. Furthermore, we must also rigorously establish phenotypes with respect to the hearing loss level, progression, and other manifestations. Regarding hearing loss caused by mutations in *POU3F4*, all clinicians and audiologists should provide optimal CI rehabilitation management and applicable educational support based on the phenotypic features described herein and elsewhere. This study supports the use of comprehensive genetic diagnosis for SNHL to provide the highest chance of diagnostic success, particularly in sporadic cases.

Table 1. Known Mutations in the *POU3F4* Gene and Associated Phenotypes.[a]

Nucleotide Change	Amino Acid Change	Inheritance	CT Findings	HL Onset	Type of HL	Progression	Other Features	Perilymphatic Gusher	Year	First Author
	Large delation	Inherited	IP3	Congenital	Mixed	Progressive		Gusher	2005	Vore[20]
	Large delation	Inherited	Mondini	Congenital	SNHL	Progressive			2000	Arellano[26]
	Large delation	Inherited			Mixed		Mental retardation	Gusher	1996	de Kok[19]
	Large delation	Inherited	IP3	Congenital		Progressive	Developmental delay, mental retardation		2010	Song[17]
	Large delation	De novo (sporadic)	IP3						2015	Choi[18]
	Deletion (upstream and gene)	Inherited	Bony defect		Mixed			Gusher	1996	de Kok[19]
	Upstream delation	Inherited	Bony defect		Mixed				1996	de Kok[19]
	Upstream delation		IP3	Congenital	Mixed				1996	de Kok[19]
	Upstream delation	Inherited		Congenital	SNHL	Progressive		Gusher	2010	Naranjo[21]
	Upstream delation	De novo (sporadic)	IP3	Early		No			2010	Song[17]
	Upstream duplication	Inherited	IP3		Mixed				1995	de Kok[4]
	Regulatory region deletion	Inherited	Bony defect	Early	Mixed	No			2014	Stanton[25]
	Regulatory region inversion	Inherited	IP3		Mixed	Progressive	Developmental delay		2013	Anger[22]
c.235C>T	p.G79X	De novo (sporadic)							2013	Parzefall[23]
c.346delG	p.A116fs	Inherited	IP3	Congenital	Mixed		Limited verbal communication		2009	Lee[16]
c.383delG	p.G128fs	Inherited	IP3	Early	SNHL	Progressive			2009	Lee[7]
c.499C>T	p.R167X	Inherited	IP3	Congenital	Mixed	No	Attention disorders, learning delay	Gusher	2010	Stankovic[8]
c.540C>A	p.C180X	De novo (sporadic)	IP3						2015	Choi[18]
c.601-606del	p.201-202del	Inherited	Bony defect	Early	Mixed	No		Gusher	1998	Hagiwara[15]
c.603delA	p.K202X	Inherited	Bony defect		SNHL				1995	de Kok[4]
c.623T>A	p.L208X	Inherited	IP3	Early	SNHL	Severe	Mental retardation		2009	Lee[7]
c.623T>A	p.L208X	Inherited	IP3		SNHL				2013	Choi[27]
c.632C>T	p.T211M	Inherited	IP3		Mixed				2013	Choi[27]
c.647G>A	p.G216E	Inherited	IP3	Congenital	SNHL	Progressive			2010	Li[5]
c.648delG	p.D215X		Bony defect		Mixed			Gusher	1995	de Kok[4]
c.683C>T	p.S228L	Inherited	IP3	Congenital	Mixed	Progressive	Developmental delay		2005	Vore et al[20]

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