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The Patients Associated With *TMPRSS3* Mutations Are Good Candidates for Electric Acoustic Stimulation

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Abstract

Objectives: To clarify the frequency of *TMPRSS3* mutations in the hearing loss population, genetic analysis was performed, and detailed clinical characteristics were collected. Optical intervention for patients with *TMPRSS3* mutations was also discussed.

Methods: Massively parallel DNA sequencing (MPS) was applied for the target exon-sequencing of 63 deafness genes in a population of 1120 Japanese hearing loss patients.

Results: Hearing loss in 5 patients was found to be caused by compound heterozygous *TMPRSS3* mutations, and their detailed clinical features were collected and analyzed. Typically, all of the patients showed ski slope type audiograms and progressive hearing loss. Three of the 5 patients received electric acoustic stimulation (EAS), which showed good results. Further, the onset age was found to vary, and there were some correlations between genotype and phenotype (onset age).

Conclusions: MPS is a powerful tool for the identification of rare causative deafness genes, such as *TMPRSS3*. The present clinical characteristics not only confirmed the findings from previous studies but also provided clinical evidence that EAS is beneficial for patients possessing *TMPRSS3* mutations.

Keywords

TMPRSS3, DFNB8/10, high-frequency hearing loss, massively parallel DNA sequencing, next generation sequencing, EAS

Introduction

Hearing impairment is a general sensory defect in humans. Based on the results of several etiological studies, it has been estimated that at least 50% of congenital hearing loss is of genetic etiology.[1] More than 80 genes have already been reported to be associated with sensorineural hearing loss (SNHL).

Cochlear implantation (CI), which electrically stimulates the spiral ganglion neurons, has been established as the standard therapy for severe to profound SNHL.[2] Electric acoustic stimulation (EAS) is a hearing implant system combining a cochlear implant and acoustic amplification technology in one device and has recently become a standard intervention for the patients with partial deafness, defined as a mild to moderate low-frequency sensorineural hearing loss sloping to a profound hearing loss in the higher frequencies.[3]

TMPRSS3 is responsible for autosomal recessive hearing loss, particularly high-frequency involved hearing loss. Interestingly, *TMPRSS3* is the cause of DFNB10 (severe and congenital) and DFNB8 (mild and postlingual) phenotypes.[4]

TMPRSS3 is a type-II transmembrane serine protease, structurally defined by a transmembrane domain located near the N terminus. In a previous study, *TMPRSS3* mRNA was detected in the cell bodies of spiral ganglion neurons, the entire epithelium supporting the organ of Corti, as well as the inner hair cells of the organ of Corti and in the lower levels of the stria vascularis.[5,6] *TMPRSS3* may be involved in processing proneurotrophins and, therefore, in the development and survival of cochlear neurons.

Twenty-five mutations in *TMPRSS3* were previously reported in the Middle East, Europe, and East Asia (Table 1).[7-17] The function of the *TMPRSS3* gene in the auditory system remains unclear, but it has been reported to play a crucial role in the morphological and functional maturation of the

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inner ear as well as in the maintenance of the contents of the perilymph and endolymph.[5,18]

Recent advances in targeted exon sequencing of selected genes using massively parallel DNA sequencing (MPS) technology have enabled the successful identification of causative mutations in relatively rare genes such as *TMPRSS3*. In this study, we describe 5 patients from 4 families with *TMPRSS3* detected by MPS. We present the clinical features of the patients and discuss the appropriate forms of intervention for hearing loss caused by *TMPRSS3*.

Subjects and Methods

Subjects

A total of 1120 Japanese hearing loss (HL) patients (autosomal dominant sensorineural hearing loss, 266; autosomal recessive sensorineural hearing loss, 600; unknown, 254) from 53 otolaryngology departments nationwide participated in this study. Written informed consent was obtained from all subjects (or from their next of kin, caretaker, or guardian on 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.

Amplicon Library Preparation

Amplicon libraries were prepared using an Ion AmpliSeq Custom Panel (Applied Biosystems, Life Technologies, Carlsbad, California, USA) for 63 genes reported to cause nonsyndromic hearing loss according to the manufacturer's instructions. The detailed protocol was described elsewhere.[10] 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 and Sequencing

Emulsion polymerase chain reaction (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 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.[19,20] 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,[21] (2) the 6500 exome variants,[22] (3) the Human Genetic Variation Database (data set for 1208 Japanese exome variants),[23] 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,[24] Sorting Intolerant from Tolerant (SIFT),[25] Polymorphism Phenotyping (PolyPhen2),[26] LRT,[27] MutationTaster,[28] and GERP++.[29]

Candidate mutations were confirmed by Sanger sequencing, and the responsible mutations were identified by segregation analysis using samples from among the patients' family members. In cases identified as heterozygous, Sanger sequencing of the coding region of the *TMPRSS3* was performed.

Outcome of EAS

Thirty-two consecutive hearing preservation surgeries in 30 of the 1120 patients with ski slope hearing loss were performed (for details, see Usami et al[30]). Twenty-nine ears in 27 patients received MED-EL PULSAR with a FLEX^{24} electrode (24 mm), 2 ears in 2 patients received a FLEX^{soft} electrode (31.5 mm), and 1 ear received a standard electrode (31.5 mm).

To evaluate speech perception outcomes, speech discrimination scores (using the 67S Japanese monosyllable test, 65dBSPL) preoperatively and at 12 months after the initial EAS stimulation were used. In this study, we compared the outcomes for 3 EAS patients with hearing loss resulting from *TMPRSS3* mutations with those for the remaining 27 patients with hearing loss from other etiologies.

Results

Detected Mutations

One nonsense and 5 missense mutations as well as 1 splice site mutation were identified (Table 1). The splice site mutation, c.617-4_-3dupAT (p.T205fs), was detected by additional Sanger sequencing. All of the detected mutations were confirmed by Sanger sequencing and were predicted to be pathologic by several software programs. Segregation analysis was consistent with them being plausible disease-causing mutations. All of the subjects with biallelic mutations were compatible with recessive inheritance patterns.

Table 1. *TMPRSS3* Mutations in Autosomal Recessive Sensorineural Hearing Loss (ARSNHL).

Exon	Domain	NM No.	Nucleotide Change	Amino Acid Change	Family Origin	Reference
4	Truncation agter TM	NM_032405	c.208delC	p.His70ThrfsX19	Spanish, Greek, Pakistani, Canada, Dutch	7, 8, 9
4	LDLRA domain	NM_032405	c.212T>C	p.F71S	Japanese	This study
4	LDLRA domain	NM_032405	c.268G>A	p.A90T	UK, Moroccan	11
4	LDLRA domain	NM_032405	c.280G>A	p.G94R	Japanese	This study
4	LDLRA domain	NM_032405	c.308A>G	p.D103G	Greek	7
4	LDLRA domain	NM_032405	c.310G>A	p.E104K	Pakistani	9
4	LDLRA domain	NM_032405	c.310G>T	p.E104X	Pakistani	9
Intron 4	SRCR	NM_032405	c.323-6G>A	p.Cys107fs	Pakistani	4
5	LDLRA domain	NM_032405	c.325C>T	p.R109W	Pakistani, Korea	12, 13
5	SRCR domain	NM_032405	c.413C>A	p.A138G	UK, Dutch	8
7	SRCR domain	NM_032405	c.581G>T	p.C194F	Pakistani	12
7	SRCR domain	NM_032405	c.595G>A	p.V199M	Dutch	8
Intron 8	Serine protease domain	NM_032405	c.617-4_-3dupAT	p.T205fs	Japanese	This study
8	Just before senine protease	NM_032405	c.646C>T	p.R216C	German	14
8	Serine protease domain	NM_032405	c.743C>T	p.T248M	Korea	13
8	Serine protease domain	NM_032405	c.753G>C	p.W251C	Tunisian	16
8	Serine protease domain	NM_032405	c.767C>T	p.A256V	Pakistani	9
9	Serine protease domain	NM_032405	c.916G>A	p.A306T	German, Korea, Dutch	8, 13, 14
12	Serine protease domain	AB038157	c.1221C>T	p.P404L	Turkish, Tunisian	16
12	Serine protease domain	NM_032405	c.1219T>C	p.C407R	Pakistani	9, 12
4	LDLRA domain	NM_032404	c.226C>T	p.Q76X	Japanese	10, this study
5	SRCR domain	NM_032404	c.390C>G	p.H130R	Japanese	This study
7	Just before serine protease	NM_032404	c.647G>T	p.R216L	Turkish, Japanese	15, this study
9	Serine protease domain	NM_032404	c.778G>A	p.A260T	Japanese	10, this study
9	Serine protease domain	NM_032404	c.830C>T	p.P277L	Turkish, Tunisian	16
Intron 8	Serine protease domain	NM_024022	c.782+8insT		Pakistani	17
11	Serine protease domain	NM_024022	c.1180_1187del8ins68		Palestinian	4
11	Truncation of serine protease	NM_024022	c.1192C>T	p.Q398X	Turkish	15
12	Serine protease domain	NM_024022	c.1273T>C	p.C425R	Pakistani	9

Abbreviations: LDLRA, low-density lipoprotain recrptor; SRCR, scavenger receptor cysteine-rich.

The compound heterozygote mutations, c.[226C>T]; [778G>A](p.[Q76X];[A260T]), found in 1 family (patient 4541, 4540), were previously reported.[10,31] However, the other 4 mutations (c.212T>C [p.F71S], c.280G>A [p.G94R]), c.390C>G [p.H130R], and c.617-4_-3dupAT [p.T205fs]) were novel causative mutations.

Clinical Findings

The clinical features and genotypes for the 5 patients are shown in Figures 1, 2, 3, and 4 and Table 2. All pedigrees showed typical autosomal recessive inheritance patterns, and all affected patients displayed progressive, symmetrical

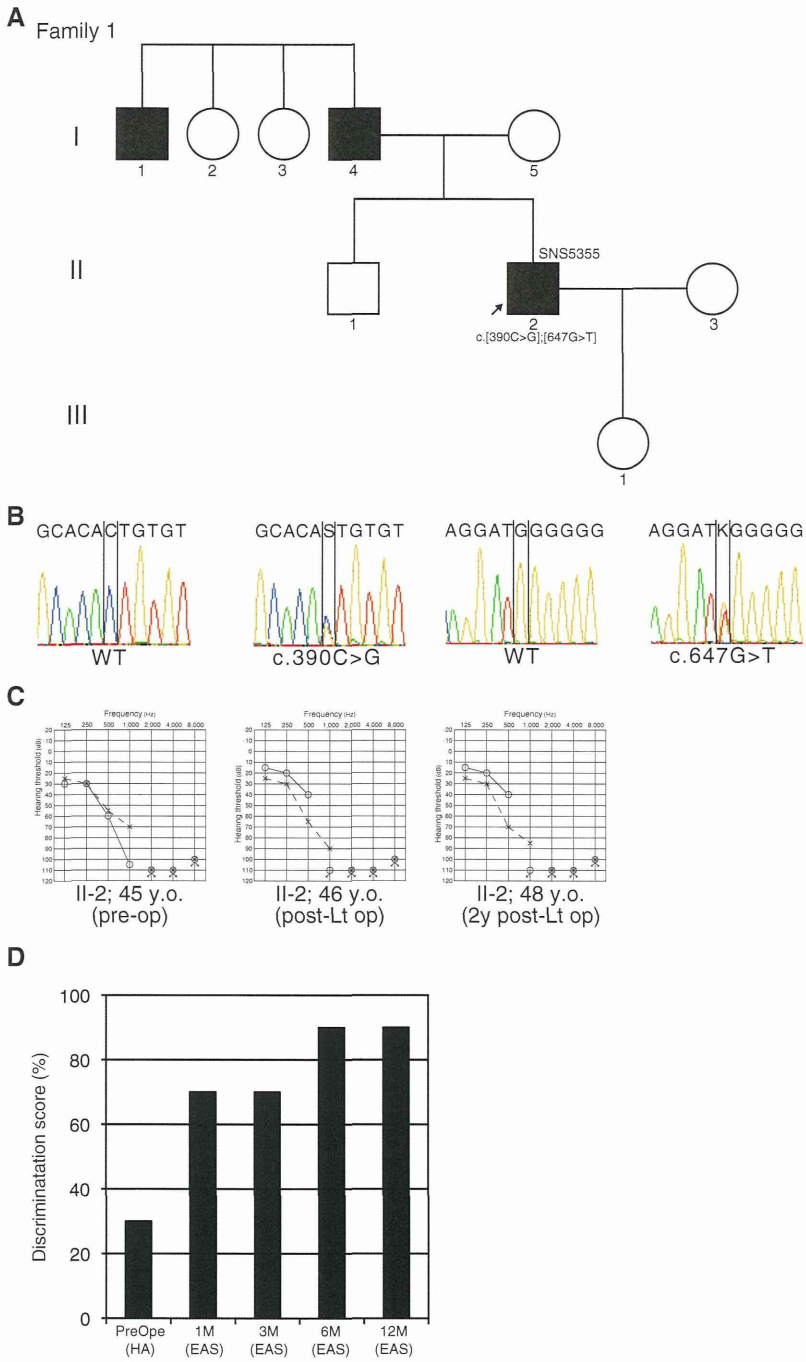

Figure 1. (A) The patient (SNS5355) shows compound heterozygous *TMPRSS3* mutations, c.[390C>G];[647G>T](p.[H130R];[R216L]). His father also developed age-related hearing loss with a different type of audiogram (not shown). (B) The results of Sanger sequencing. (C) Pre- and postoperative audiograms indicating the progressive nature of hearing loss and achievement of hearing preservation after EAS. (D) Japanese monosyllable test (65dB SPL in quiet) with bilateral EAS showing a good speech discrimination outcome after EAS. EAS, electric acoustic stimulation.

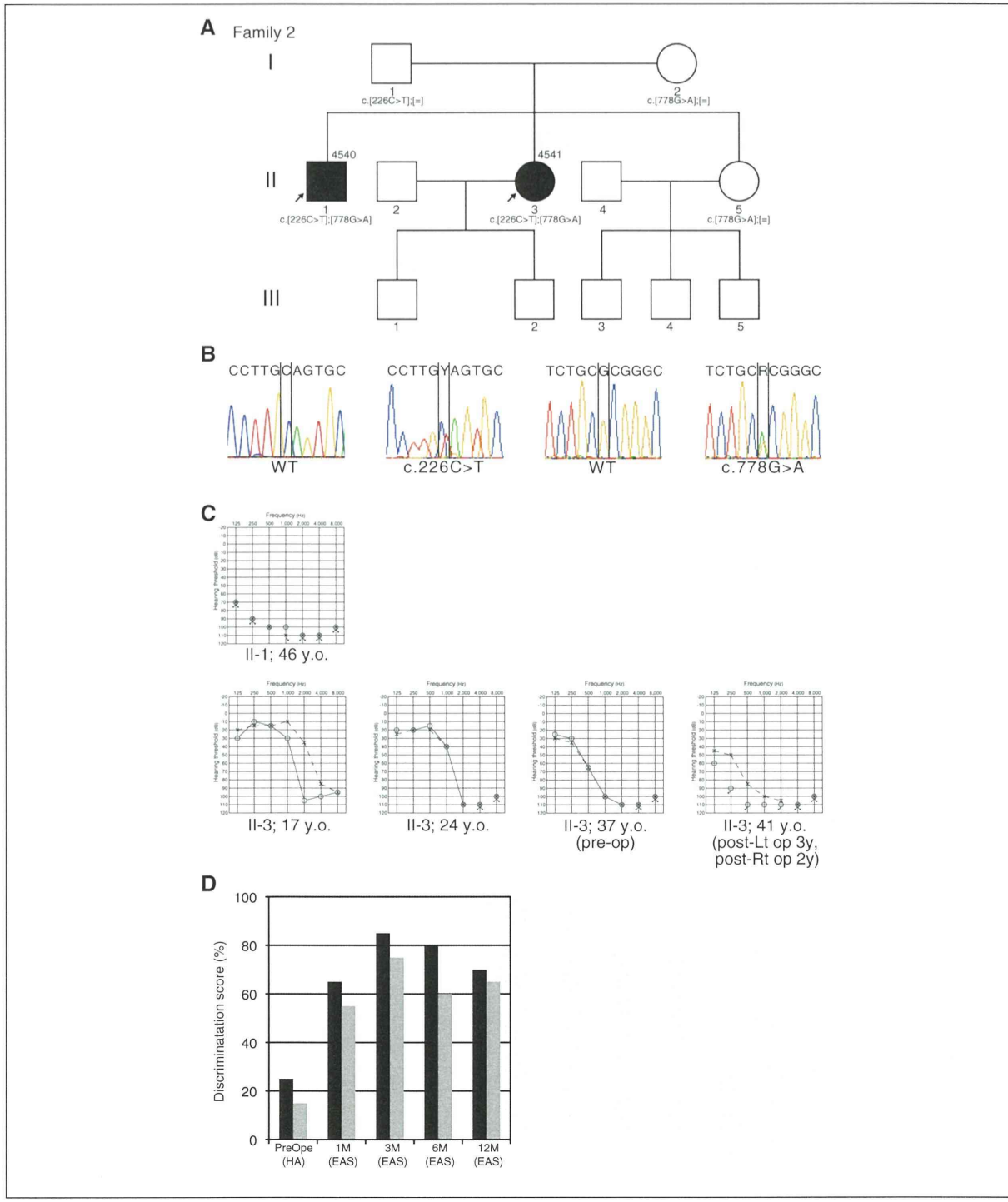

Figure 2. (A) The patient (4541) shows compound heterozygous *TMPRSS3* mutations, c.[226C>T];[778G>A](p.[Q76X];[A260T]), and the parents were found to be carriers for these mutations. The patient's brother (4540) has the same mutations. (B) The results of Sanger sequencing. (C) Audiograms of the 2 affected family members at different ages. Serial audiogram of the proband indicates the progressive nature of the hearing loss. (D) Japanese monosyllable test (65 dB SPL in quiet) for patient 4541 showing a dramatic improvement after bilateral EAS. Black, left side; gray, right side. EAS, electric acoustic stimulation.

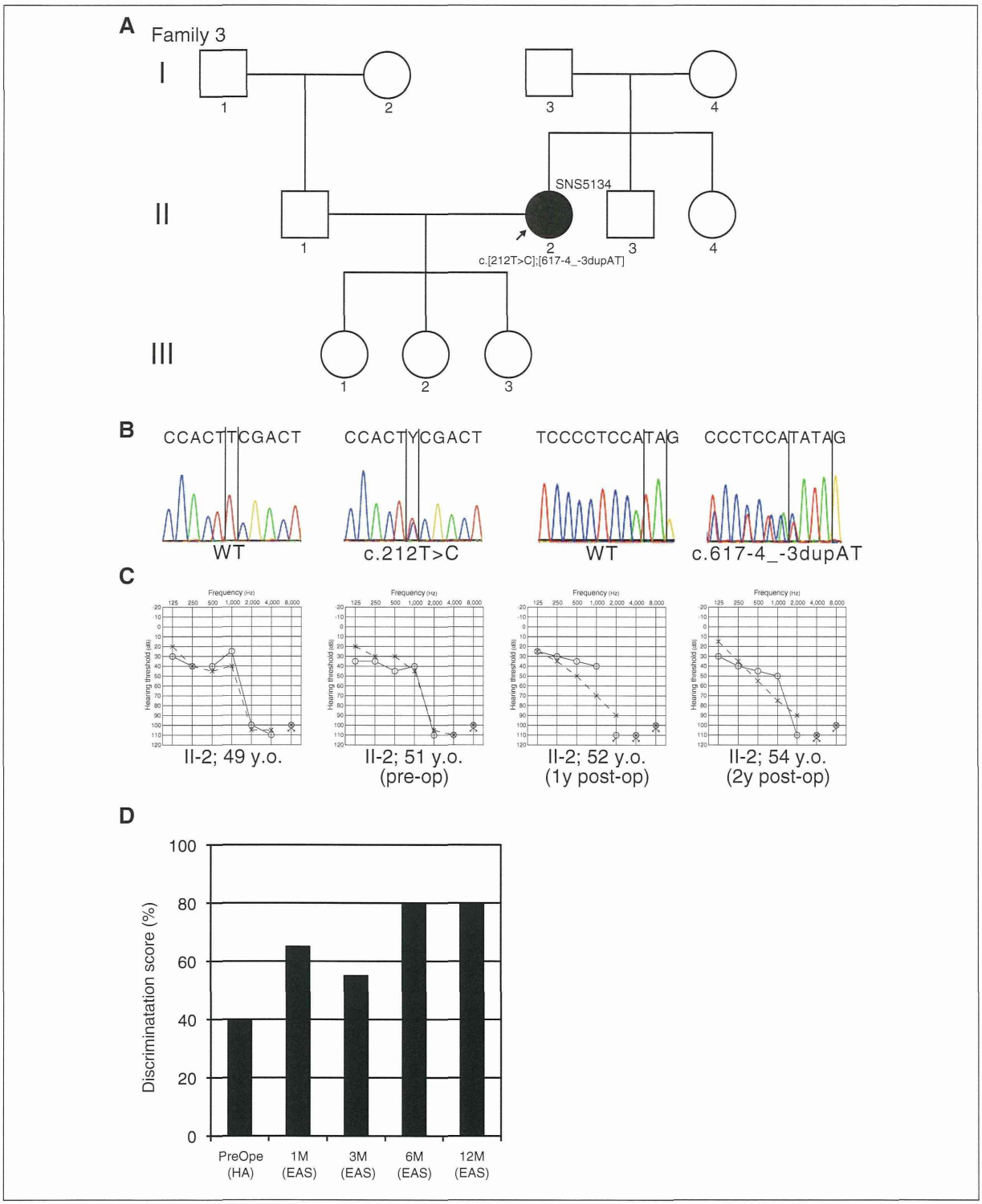

Figure 3. (A) The patient (SNS5134) shows compound heterozygous *TMPRSS3* mutations, c.[212T>C];[617-4_-3dupAT] (p.[F71S];[T205fs]). (B) The results of Sanger sequencing. (C) Pre- and postoperative audiograms indicating good hearing preservation after EAS. (D) Japanese monosyllable test (65 dB SPL in quiet) after bilateral EAS showing a dramatic improvement. EAS, electric acoustic stimulation.

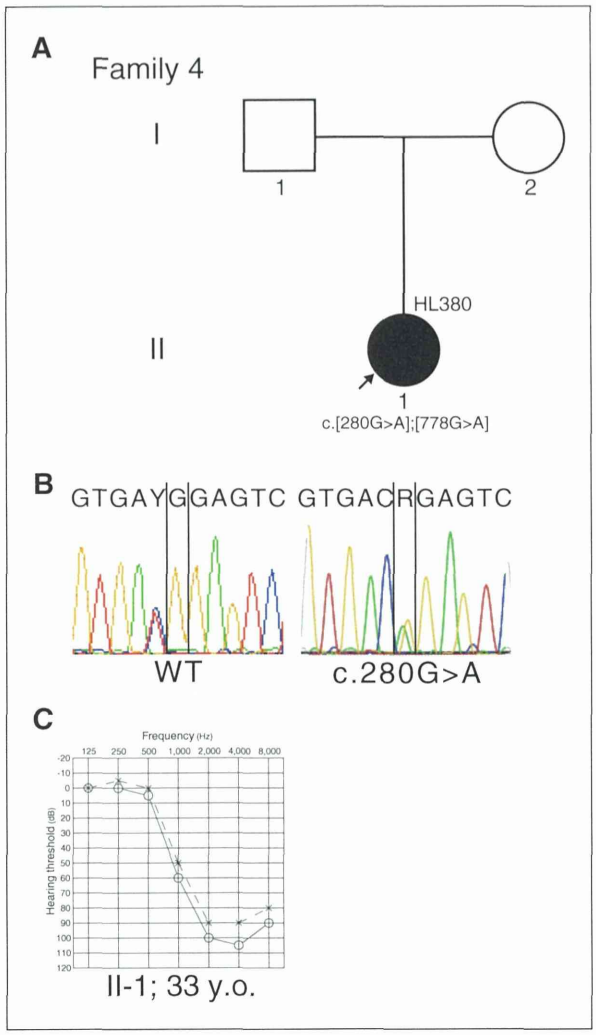

Figure 4. (A) The patient (HL0380) shows compound heterozygous *TMPRSS3* mutations, c.[280G>A];[778G>A] (p.[G94R];[A260T]). (B) The results of Sanger sequencing. (C) Audiogram at 33 years old.

sensorineural hearing loss with tinnitus. There were large variations in age of onset, although the hearing loss in patient 4541 and her brother (4540) developed in early childhood.

Family 1 (Figure 1: SNS5355). Patient SNS5355 (48-year-old male) had compound heterozygous mutation, c.[390C>G];[647G>T](p.[H130R];[R216L]). The patient noticed he could not hear his electric alarm at 33 years old due to high-frequency progressive hearing loss, and he started to use a hearing aid at 39 years old. Due to the inconvenience associated with using a hearing aid, he received EAS (MEDEL PULSAR FLEX^{24}) at 45 years old. His residual hearing in low frequencies was completely preserved, and his discrimination score was improved after EAS.

Family 2 (Figure 2: 4540, 4541). Patient 4541 (41-year-old female) was identified with a compound heterozygous mutation, c.[226C>T];[778G>A](p.[Q76X];[A260T]). The mutation and brief clinical features have been reported previously.[10,31] Her hearing loss was first detected by mass screening in primary school. It appeared to slowly progress, and by age 25, she suffered some inconvenience in hearing and communication. Progressive, ski slope type hearing loss was noted (Figure 2C). The threshold level for 1000 Hz was preserved at 17 years old but thereafter decreased rapidly until 36 years old. The average rate of progression for 1000 Hz was 4.5 dB/year. EAS (MEDEL PULSAR FLEX^{24}) was applied at the ages of 38 and 39 bilaterally. Residual hearing for acoustic amplification was preserved, and the hearing level with bilateral EAS was around 30 dB. The patients showed a dramatic improvement in scores for the Japanese monosyllable test (65 dB SPL in quiet) after bilateral EAS, improving from 18% to 90% 1 year after receiving the second EAS.

The same compound heterozygous mutation, c.[226C>T];[778G>A](p.[Q76X];[A260T]), was identified in her brother (patient 4540), who had experienced postlingual hearing loss from 10 years old. His hearing loss was progressive, and he experienced profound hearing loss at 46 years old.

Family 3 (Figure 3: SNS5134). Patient SNS5134 (54-year-old female) had a compound heterozygous mutation, c.[212T>C];[617-3_-4dupAT](p.[F71S];[T205fs]). The patient's age at onset was 30; however, due to rapid progression of the hearing loss, she experienced some inconvenience in hearing and communication by 44 years old. She did not suffer any associated vertigo but did complain of tinnitus. She showed a ski slope type audiogram and received EAS (MEDEL PULSAR FLEX^{24}) when she was 51 years old. Her word discrimination score on the Japanese monosyllable test improved after receiving EAS.

Family 4 (Figure 4: HL0380). Patient HL0380 was found to have a compound heterozygous mutation, c.[280G>A];[778G>A](p.[G94R];[A260T]). She had noticed the onset of hearing loss, particularly involving high frequencies, when she was 15 years old. She showed ski slope type audiograms at age 33 when she visited an ENT clinic. As she cannot obtain sufficient amplification by use of a hearing aid, she is planning to have genetic counseling, including recommended intervention such as EAS.

Three patients with *TMPRSS3* mutations (SNS5355, 4541, SNS5134) showed normal vestibular function, as evaluated by caloric test and cervical vestibular evoked myogenic potentials (cVEMP) (Figure 5). In addition, no symptoms except hearing loss were confirmed.

Table 2. Clinical Features of 5 Patients With Hearing Loss Caused by *TMPRSS3* Mutations.

Family No.	Patient ID	Nucleotide Change	Amino Acid Change	Age	Onset Age	Intervention	Age at Time of Surgery	Hearing Level (dB)[a]	Hearing Level at Low Frequencies (dB)[b]	Progressiveness	Tinnitus	Vertigo	Caloric Test	cVEMP
1	SNS5355	c.[390C>G];[647G>T]	p.[H130R];[R216L]	48	33	EAS	45	93.8	33.3	+	+	−	Normal	Normal
2	4541	c.[226C>T];[778G>A]	p.[Q76X];[A260T]	41	6	EAS	38/39	106.3	81.7	+	+	−	Normal	Normal
2	4540	c.[226C>T];[778G>A]	p.[Q76X];[A260T]	46	10	None		105	86.7	+	+	−	N/A	N/A
3	SNS5134	c.[212T>C];[617-3_-4dupAT]	p.[F71S];[T205fs]	54	30	EAS	51	78.8	38.3	+	+	−	Normal	Normal
4	HL0380	c.[280G>A];[778G>A]	p.[G94R];[A260T]	33	15	None		57.5	−1.7	+	+	−	N/A	N/A

Abbreviations: EAS, electric acoustic stimulation; cVEMP, cervical vestibular evoked myogenic potential; +, existing symptoms; −,without symptoms.
[a]Average of 500, 1000, 2000, and 4000 Hz.
[b]Average of 125, 250, and 500 Hz.

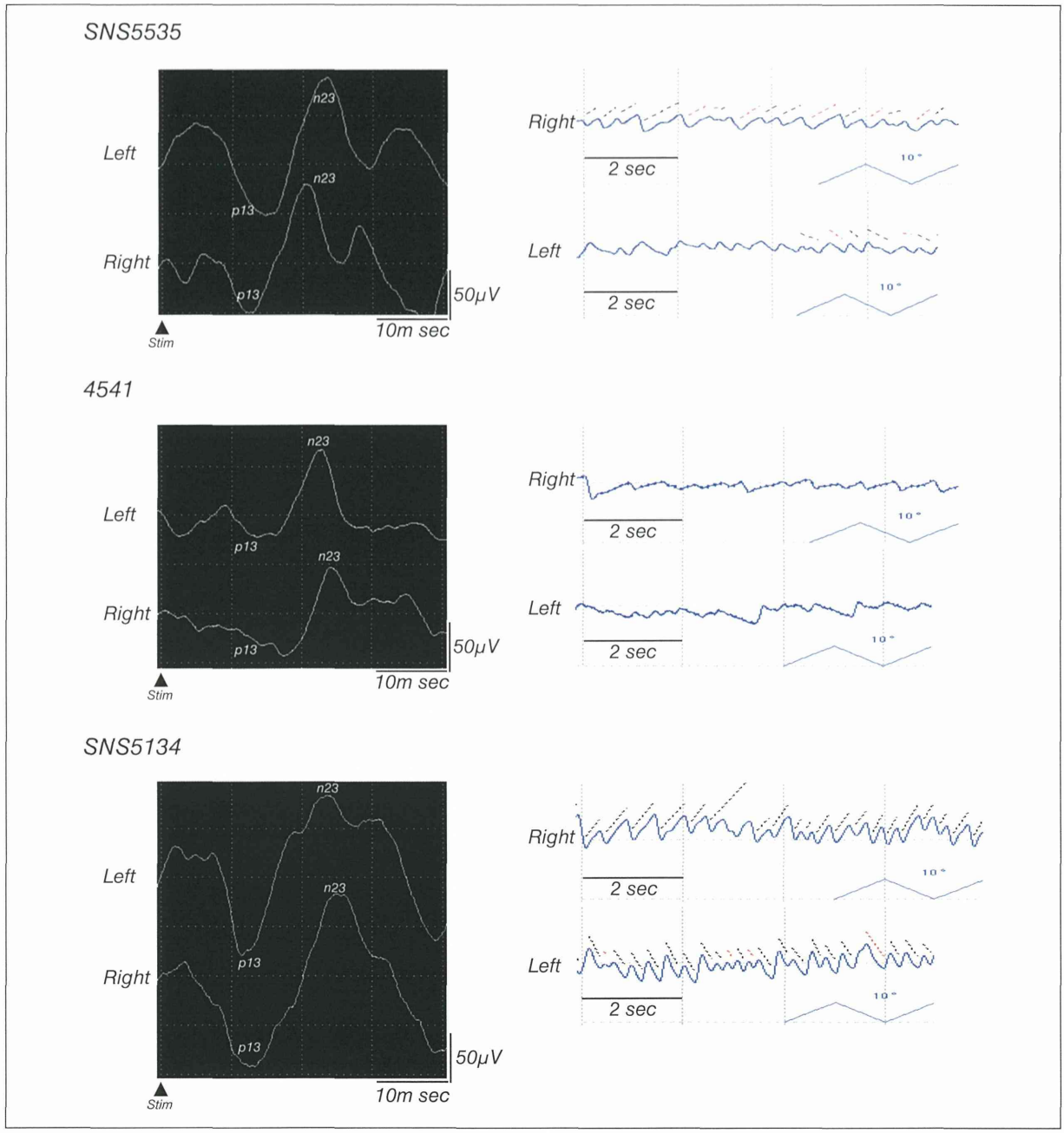

Figure 5. Three patients with *TMPRSS3* mutations (SNS5355, 4541, SNS5134) showed normal vestibular function on (left) caloric tests and (right) vestibular evoked myogenic potentials (VEMP).

Outcome of EAS

Three patients with *TMPRSS3* compound heterozygous mutations (SNS5355, 4541, SNS5134) received EAS. We evaluated the improvement in speech discrimination and perception scores (using the 67S Japanese monosyllable test) preoperatively and at 12 months after the initial EAS stimulation between 3 patients with *TMPRSS3* mutations who underwent EAS and the other 27 patients (Figure 6). Hearing preservation was achieved in all 30 patients (32 ears), with good speech perception observed for all patients. The *TMPRSS3* patients, in particular, showed good outcomes.