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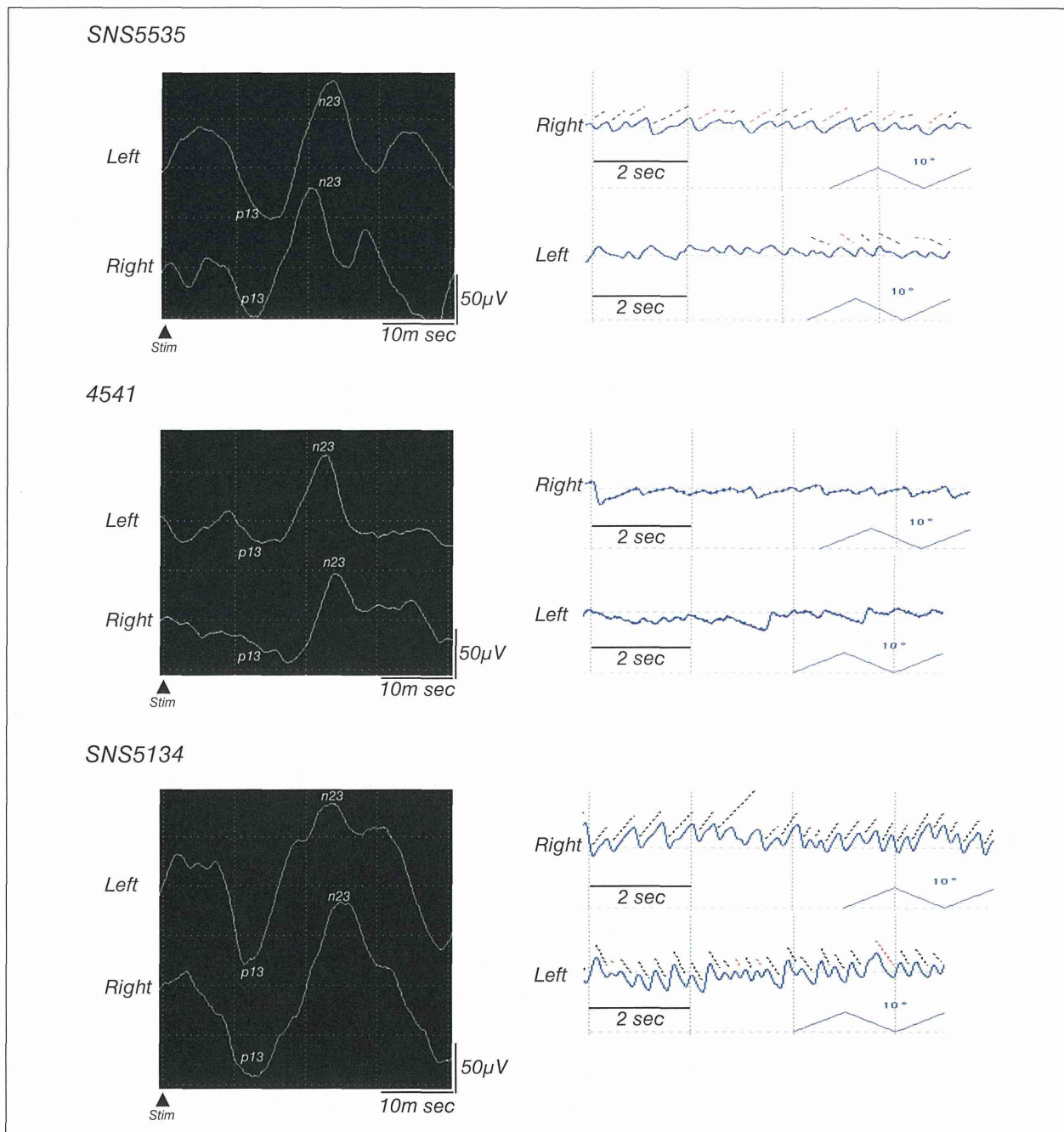
Figure 5. Three patients with *TMPRSS3* mutations (SNS5355, 4541, SNS5134) showed normal vestibular function on (left) caloric tests and (right) vestibular evoked myogenic potentials (VEMP).

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.
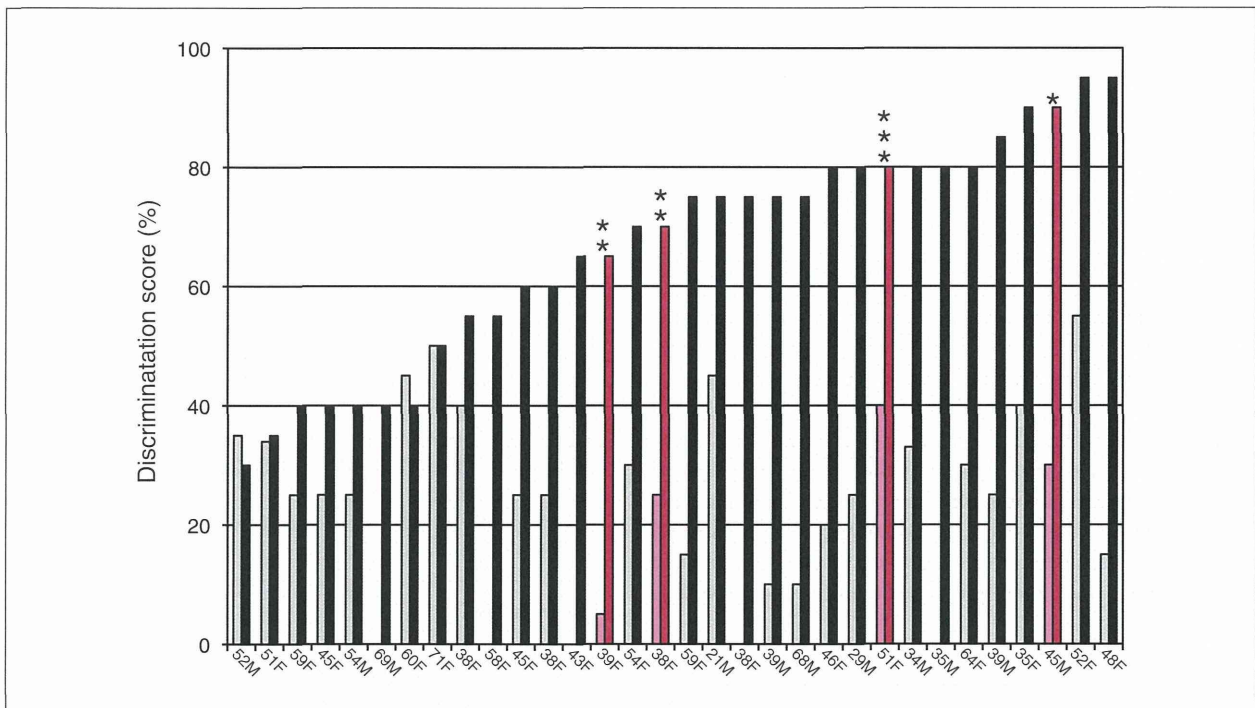
Figure 6. Speech discrimination scores (using the 67S Japanese monosyllable test, 70 dBSPL) preoperatively (grey) and at 12 months after the initial EAS (black). The 3 patients with *TMPRSS3* mutations (*SNS5355, **4541, ***SNS5134) showed significant improvement.

Discussion

In previous studies, the frequency of *TMPRSS3* mutations in hearing loss patients was found to be <0.5% in Europe, 3% in Pakistan, and 5.9% in Korea.[7,12,13] In this study, MPS technology successfully identified *TMPRSS3* mutations and the frequency of *TMPRSS3* mutations in a Japanese population. The rate of patients with *TMPRSS3* mutations was 0.36% (4/1120) among Japanese and 0.7% (4/600) in autosomal recessive sensorineural hearing loss (ARSNHL) patients, which are similar to the figures for Europe.

Five patients were detected with compound heterozygous mutations in this study, with no specific mutation found to occur at a significantly higher frequency. The existence of certain frequent mutations, such as c.916G>A (p.A306T), has been reported.[8,13,14] The mutation spectrum found in Japanese is quite different from those reported in other populations. Based on the present results, the carrier rate of *TMPRSS3* mutations is extremely low in Japan in comparison with *GJB2* or *SLC26A4*.

For such rare causative mutations/genes, targeted exon sequencing of selected genes using MPS technology was extremely useful. In fact, in this study we successfully identified 7 *TMPRSS3* mutations among 4 families. All of the patients with *TMPRSS3* mutations showed typical ski slope

hearing loss with postlingual onset. However, the clinical characteristics varied among the patients.

Weegerink et al[8] hypothesized that *TMPRSS3* mutations are associated with 2 types of hearing impairment phenotypes, (1) DFNB10: a severe congenital or early childhood onset type with prelingual hearing impairment caused by the presence of 2 severe mutations and (2) DFNB8: a later onset progressive but initially milder type with postlingual hearing impairment caused by the presence of 1 mild and 1 severe mutation. In this study, patient ID 4541 had early onset and relatively rapid progressive hearing loss, resembling the DFNB10 phenotype. Her brother (patient ID 4540) also had a more severe phenotype that demonstrated earlier onset and deteriorated to profound hearing loss from young adulthood. Therefore, the c.226C>T(p.Q76X) and c.778G>A(p.A260T) mutations identified in this family are thought to be associated with early onset hearing loss. Conversely, patients SNS5355 and SNS5134 showed late-onset hearing loss. Those mutations might be associated with milder mutations, leading to a DFNB8 phenotype.

With regard to the function of *TMPRSS3* in the inner ear, it was hypothesized that *Tmpress3* participates in the regulation of sodium homeostasis through its ability to activate the inner ear-expressed sodium channel (ENaC) in vitro.[5] In the *Tmpress3*-related deafness mouse model, degeneration

of hair cells in the organ of Corti starts at the basal turn at the onset of hearing loss (postnatal day 12) and progresses toward the apex within 2 days.[32] This phenomenon observed in the mouse model is in line with the human phenotype that presents as high-frequency hearing loss.

Concerning vestibular function, the patients showed normal vestibular function, as evaluated by caloric test and cVEMP (Figure 5), and none of the patients showed vestibular symptoms. Similarly, none of the patients reported previously were found to have vestibular symptoms, but the vestibular function in some patients was reported to be affected. Weegerink et al[8] reported that 5 out of 9 patients showed mild hyperreflexia/hyporeflexia on rotatory and caloric tests. It is not surprising to find this type of associated vestibular hypofunction as *Tmprss3* is expressed both in the cochlear and vestibular hair cells.[32] A similar discrepancy between gene expression, vestibular testing, and vestibular symptoms has also been reported for *GJB2*-associated deafness.[33] Vestibular compensation may be related to these complications, and this possibility should be further examined in future studies.

Three of the 5 patients with mutations detected in this study received EAS. A good outcome for EAS in a *TMPRSS3* patient (patient ID: 4541) was previously reported.[10] In this study, 2 additional patients with *TMPRSS3* mutations also showed good outcomes, further confirming that patients with *TMPRSS3* mutations are good candidates for EAS.

The outcomes for CI for *TMPRSS3* patients remain controversial.[8,10,14,34] A majority of cases (13/15) were reported to show good outcomes for CI, while 2 cases reported by Eppsteiner et al[34] showed a poorer performance. *Tmprss3* is reported to be expressed not only in the organ of Corti but also in the spiral ganglion, and the loss of ganglion cells has been reported.[32,34] Therefore, it is possible that neuronal cell loss may negatively affect CI performance. However, the majority of cases, including our 3 EAS cases, showed good performance, indicating that CI and/or EAS is a potential therapeutic option. If the progression of hearing loss results in the patients losing the benefits of EAS acoustic stimulation, it is possible to cover all frequencies by electric stimulation as is common in CI. A recent study on the human temporal bones indicated that the behavior of human ganglion cells is different from those in animals, being more resistant to degeneration.[35] Although no study of the temporal bone in relation to patients with *TMPRSS3* mutations is available, such behavioral differences may provide an explanation of why CI/EAS is effective in patients with *TMPRSS3* mutations.

Clinicians should keep in mind that hearing loss caused by *TMPRSS3* mutations may be progressive and should consider proper intervention for these patients.

In conclusion, the present study provided additional evidence that the patients associated with *TMPRSS3* mutations

are good candidates for EAS. Genetic testing based on next-generation sequencing will facilitate candidate selection and personalized intervention.

Declaration of Conflicting Interests

Funding

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