TMPRSS3 Gene Variants With Implications for Auditory Treatment and Counseling

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Objective: To identify and report novel variants in the TMPRSS3 gene and their clinical manifestations related to hearing loss as well as intervention outcomes. This information will be helpful for genetic counseling and treatment planning for these patients.

Methods: Literature review of previously reported TMPRSS3 variants was conducted. Reported variants and associated clinical information was compiled. Additionally, cohort data from 18 patients, and their families, with a positive result for TMPRSS3-associated hearing loss were analyzed. Genetic testing included sequencing and copy number variation (CNV) analysis of TMPRSS3 and the Laboratory for Molecular Medicine’s OtoGenome-v1, -v2, or -v3 panels. Clinical data regarding patient hearing rehabilitation was interpreted along with their genetic testing results and in the context of previously reported cochlear implant outcomes in individuals with TMPRSS3 variants.

Results: There have been 87 previously reported TMPRSS3 variants associated with non-syndromic hearing loss in more than 20 ancestral groups worldwide. Here we report occurrences of known variants as well as one novel variant: deletion of Exons 1–5 and 13 identified from our cohort of 18 patients. The hearing impairment in many of these families was consistent with that of previously reported patients with TMPRSS3 variants (i.e., typical down-sloping audiogram). Four patients from our cohort underwent cochlear implantation.

Conclusion: Bi-allelic variants of TMPRSS3 are associated with down-sloping hearing loss regardless of ancestry. The outcome following cochlear implantation in patients with variants of TMPRSS3 is excellent. Therefore, cochlear implantation is strongly recommended for hearing rehabilitation in these patients.

Keywords: TMPRSS3, cochlear implantation, sensorineural hearing loss, genetic counseling, hereditary hearing loss
1 INTRODUCTION

Autosomal recessive non-syndromic hearing loss (ARNSHL) is the most common form of hereditary hearing loss. It accounts for about 70–80% of congenital hereditary hearing loss. ARNSHL is an extremely heterogeneous condition as more than 98 loci have been mapped and 77 causative genes have been identified to date (http://hereditaryhearingloss.org/).

The TMPRSS3 gene encodes a type III transmembrane serine protease that is structurally defined by four functional domains: a transmembrane domain, low density lipoprotein receptor A domain, scavenger receptor cysteine rich domain, and a carboxyl terminal serine protease domain (Südhof et al., 1985; van Driel et al., 1987; Sarras et al., 2004; Rawlings et al., 2010). The TMPRSS3 gene is expressed in inner hair cells, spiral ganglion neurons (SGNs), the stria vascularis, and cochlear aqueducts of fetal cochlea (Guipponi et al., 2002). Four alternatively spliced transcripts have been described (DiStefano et al., 2018). The transmembrane serine protease 3 protein is thought to be involved in the development and maintenance of the inner ear, perilymph, endolymph and SGNs (Guipponi et al., 2002). While the function of the TMPRSS3 gene in the auditory system is not fully understood, its alteration has been linked with non-syndromic genetic hearing loss (DiStefano et al., 2018).

The incidence of TMPRSS3-associated ARNSHL is variable among different ancestral backgrounds but TMPRSS3 is a significant contributor in some populations. Pathogenic TMPRSS3 variants account for 0.7% of Japanese (Miyagawa et al., 2015), 3% of Pakistani (Ben-Yosef et al., 2001), 4.6% of Chinese (Gao et al., 2017), 5–6% of Tunisian (Masmoudi et al., 2001), 5.9% of Korean (Chung et al., 2014), and 11% of Turkish (Wattenhofer et al., 2005) ARNSHL cases. However, this gene has been reported in less than 1% of non-syndromic genetic deafness in White individuals (Wattenhofer et al., 2002). In contrast, pathogenic variants in the GJB2 gene are found in up to 50% of patients with ARNSHL. Despite the relatively low proportion of ARNSHL cases attributed to TMPRSS3, the gene remains a prime candidate for postlingual progressive ARNSHL in North European populations once GJB2 variants are ruled out (Seligman et al., 2021).

Patients with pathogenic variants in the TMPRSS3 gene have been described as having one of two discrete hearing phenotypes: severe, prelingual or progressive, post-lingual hearing loss. Weegerink et al. (2011) proposed that the phenotypic outcome of hearing loss is dependent on the combination and severity of TMPRSS3 variants (i.e., mild or severe). They assert that having two "severe" pathogenic variants leads to profound deafness with prelingual onset (DFNB10), whereas a single 'severe' pathogenic variant in trans with a milder TMPRSS3 pathogenic variant yields an initially less severe, but progressive and post-lingual onset hearing loss (DFNB8) (Weegerink et al., 2011). The TMPRSS3 gene encodes for a transmembrane serine protease which is expressed in SGNs (Guipponi et al., 2002). Therefore, the differential hearing phenotype may reflect the extent of loss of protease activity from a given variant.

In this study, we compile previously reported TMPRSS3 variants and present a novel variant along with their associated hearing phenotypes. We also aggregate reported outcomes and present new findings regarding the therapeutic effects of cochlear implantation (CI) in patients with pathogenic TMPRSS3 variants. Together, this information may assist with genetic counseling and treatment planning for patients with TMPRSS3 variants.

2 METHODS

2.1 Review of the Literature

Literature databases were searched using different combinations of keywords such as “transmembrane serine protease 3,” “TMPRSS3,” “ear,” “hearing loss,” “non-syndromic hearing loss,” and “cochlear implantation.” The databases searched were PubMed, Google Scholar, and two selected gene database websites (https://hereditaryhearingloss.org; https://www.ncbi.nlm.nih.gov/clinvar/). The titles and abstracts were screened using following inclusion criteria: 1) written in English, 2) dealing with non-syndromic hearing loss, and 3) reporting human data.

Based on the search strategy, 39 TMPRSS3-associated papers published from May 2000 to Aug 2021 were reviewed and summarized (Figure 1; Table 1). Among those 39 studies, eleven studies described patients who underwent cochlear implantation (Table 2).

Previously reported variants and their associated hearing phenotypes and clinical outcomes following CI, when available, were compiled. Additionally, our own cohort of patients was genetically screened as described below.

2.2 Cohort Description

Our study included genetic and phenotypic data from 18 patients and their family members (when available), who were largely White, though Family A was a consanguineous White Egyptian family, Family B was “mixed,” and Families M and I were of Hispanic or Latino ethnicity. Of the patients with characterized hearing loss, the severity ranged from moderate to profound with some individuals experiencing congenital onset and others experiencing a childhood onset or an onset in the second decade of life. Patients were referred to the Laboratory for Molecular Medicine (LMM) at Mass General Brigham Personalized Medicine (Cambridge, MA, United States) from 2009 to 2017. Patients were referred from various clinics and hospitals across the United States. The LMM collected information pertinent to the nature of the hearing loss in the patients (if available) including family history of hearing loss and/or disease, audiological testing, temporal bone CT/MRI results, and CI status. Further information was requested through physicians via the Mass General Brigham Human Research Committee's IRB protocol for the study of the genetics of hearing loss. Patients were selected based on whether they received a positive result for TMPRSS3-associated hearing loss with the intent of follow up of the outcome of CI, if received.

2.3 TMPRSS3 Screening and Otogenome Next-Generation Sequencing Testing

Patient DNA was extracted from whole blood from patients who were referred to the LMM for hearing-loss genetic testing. Our
cohort contains patients from 2009 to 2017. The genetic testing varied for each patient based on the judgment of the ordering physician and the nature of the patient’s hearing loss. Testing was performed by single gene sequencing that included TMPRSS3, or LMM’s OtoGenome-v1, -v2, or -v3 panels.

The LMM’s bioinformatics pipeline for targeted next generation sequencing (NGS) panels has been described previously (Pugh et al., 2016). Patients with hearing loss who underwent genetic testing between 2010 and 2014 were tested with the OtoGenome-v1 which included the following 71 genes: ACTG1, ATP6V1B1, BSND, CCDC50, CDH23, CLDN14, CLRN1, COCH, COL11A2, CRYM, DFNA5, DFNB31, DFNB59, DIAPH1, ESPN, ESRRB, EYA1, EYA4, GIPC3, GJB2, GJB3, GJB6, GBP98, GPSM2, GRHL2, GRXCR1, HGF, ILDR1, KCNE1, KCNQ1, KCNQ4, LHFPF5, LOXHD1, LRTOMT, MARVELD2, MIR183, MIR96, MSRB3, MTRNR1 (12S rRNA), MTTSI (tRNAser(UCN)), MYH14, MYH9, MYO1A, MYO1A, MYO3A, MYO6, MYO7A, OTOA, OTOF, PCDH15, PDZD7, POUS3F4, POUS4F3, PRPS1, RDX, SERPINB6, SLC17A8, SLC26A4 (PDS), SLC26A5, TECTA, TIMM8A, TJP2, TMCI, TMIE, TMPRSS3, TPRN, TRIOBP, USH1C, USH1G, USH2A, and WFS1.

OtoGenome-v2 was used in patients who underwent testing at the LMM from 2014 to 2015. For this iteration, PDZD7 and SLC26A5 genes were removed and the STRC gene was added. In addition, copy number variant (CNV) detection was added using VisCap as previously described (Pugh et al., 2016; Tayoun et al., 2016).

OtoGenome-v3, used from 2015 to 2017, included 87 genes but did not include the following genes included in v2: CRYM, GJB3, MIR182, MYO1A, SLC17A8, and TJP2. The following 23 genes were added CACNA1D, CATSPER2, CEACAM16, CIB2, CLPP, DIABLO, EDN3, EDNRB, HARS2, HSD17B4, KARS, LARS2, MITF, OTOG, OTOLG, P2RX2, PAX3, SIX1, SMPX, SOX10, SYNE4, TBC1D24, and TSPEAR. Parents and other unaffected/affected family members, when available, were tested for detected variants. Variants were confirmed via Sanger sequencing for single-nucleotide variants (SNVs), or droplet digital PCR for CNVs called by VisCap (Pugh et al., 2016; Tayoun et al., 2016).

2.4 LMM Variant Classification
The LMM’s early variant classification methods are as previously described (Duzkale et al., 2013) and were subsequently updated to conform to more recent professional guidelines (Richards et al., 2015). Data used to classify variants included that from population databases (e.g., Exome Aggregation Consortium (ExAC); gnomAD), internal or external disease databases (e.g., ClinVar, LOVD, HGMD), the literature, functional studies, segregation, allelic observations and in silico missense and splicing prediction tools. Variants were classified as pathogenic (P), likely pathogenic (LP), of uncertain significance (VUS), likely benign, or benign. The VUS category was further subdivided into VUS-5, -4, and -3 where VUS-5 indicated leaning towards pathogenic, and VUS-3 indicated leaning towards benign. Likely benign and benign variants are not reported in this article but were submitted to ClinVar (www.ncbi.nlm.nih.gov/clinvar/) along with all other variants observed at the LMM.

3 RESULTS
We reviewed the type, position, origin, and variant classification of 87 previously reported TMPRSS3 variants and present one novel variant identified from our cohort (Figure 1; Table 1). Compiled variants are associated with non-syndromic hearing loss in more than 20 ancestral groups worldwide. Fourteen of the identified variants were predicted loss-of-function (pLOF) (frameshift, stop-codon, or splice-site variants) with either prematurely terminated protein products or nonsense-mediated
| DNA change | Protein change | Exon | Domain | Variant classification | Origin | Phenotype severity at testing | References |
|------------|----------------|------|--------|------------------------|--------|-------------------------------|------------|
| Deletion of E1-5 and 13 | — | E1-5 and E13 | — | Pathogenic | United States | Severe | This study |
| c.36delC | p.Pro12fs | E2 | | | Chinese | Severe | Gao et al. (2017) |
| c.39delCpG | p.Phe13fs | E2 | | | Turkish | — | Diaz-Horta et al. (2012) |
| c.157G>A | p.Val53ile | E3 | TM | | Palestinian | — | Scott et al. (2001) |
| c.205+38C>T | — | Intron3 | — | | United States | — | Lee et al. (2013) |
| c.207delC | p.Thr70fs | E4 | | | Turkish | — | Weegerink et al. (2011) |
| c.208delC | p.Thr70fs*19 | E4 | Pathogenic | | Slovenian | Severe | Battelino et al. (2016) |
| c.212T>C | p.Phe71Ser | E4 | LDLRA | | Japanese | — | Miyagawa et al. (2015) |
| c.218G>A | p.Cys73Tyr | E4 | LDLRA | | Polish | — | Lechowicz et al. (2017) |
| c.225C>T | p.Gln76X | E4 | | | Japanese | — | Miyagawa et al. (2013) |
| c.238C>T | p.Arg80Cys | E4 | LDLRA | Likely pathogenic | European | — | Capalbo et al. (2019) |
| c.239G>A | p.Arg80His | E4 | LDLRA | | Taiwanese | — | Wong et al. (2020) |
| c.236G>A | p.Ala90Thr | E4 | LDLRA | | UK Caucasian | — | Charif et al. (2012) |
| c.280G>A | p.Gly94Arg | E4 | LDLRA | | Japanese | — | Miyagawa et al. (2015) |
| c.296G>A | p.Ser99X | E4 | LDLRA | | Chinese | Severe | Gu et al. (2015) |
| c.310G>A | p.Glu104Lys | E4 | LDLRA | | Pakistani | — | Lee et al. (2012) |
| c.316C>T | p.Arg106Cys | E4 | LDLRA | | Japanese | Mild | Miyagawa et al. (2013) |
| c.323-6G>A | — | Intron4 | — | Pathogenic | Polish | — | Gao et al. (2017) |
| c.325C>T | p.Arg109Trp | E5 | SRCR | Pathogenic | Pakistani | — | Scott et al. (2001) |
| c.326G>A | p.Arg109Gln | E5 | SRCR | | Korean | — | Ahmed et al. (2004) |
| c.331G>A | p.Gly111Ser | E5 | SRCR | | Czech | — | Weegerink et al. (2011) |
| c.346G>A | p.Val116Met | E5 | SRCR | | Pakistani | Mild | Gao et al. (2017) |
| c.371C>T | p.Ser124Leu | E5 | SRCR | | United States | Severe | This study |
| c.390G>G | p.His130Arg | E5 | SRCR | | Polish | — | Ben-Yosef et al. (2001) |

(Continued on following page)
| DNA change | Protein change | Exon | Domain | Variant classification | Origin | Phenotype severity at testing | References |
|------------|----------------|------|--------|------------------------|--------|-------------------------------|------------|
| c.413C>G   | p.Ala138Glu    | E5   | SRCR   | Pathogenic             | British | Mild                          | Weegerink et al. (2011) |
|            |                |      |        |                        | Korean  |                               |                         |
|            |                |      |        |                        | United States |                     |                         |
| c.432delA  | p.Gln144fs     | E5   | SRCR   | Pathogenic             | Korean  | Mild                          | Eppsteiner et al. (2012) |
| c.447-13A>G|                |      | Intron 5 | —                      | United States |                      | Lechowicz et al. (2017) |
| c.453G>A   | p.Val151Val    | E6   | SRCR   |                        | United States | Mild                | Shearer et al. (2018)  |
| c.511T>C   | p.Leu184Ser    | E6   | SRCR   |                        | United States | Mild                | Singh et al. (2020)    |
| c.581G>T   | p.Cys194Phe    | E7   | SRCR   | Pathogenic             | Polish  | Severe                        | Ahmed et al. (2004)    |
| c.579dupA  | p.Cys194Mfs*17 | E7   |        |                        | United States | Mild                | Lechowicz et al. (2017) |
| c.595G>A   | p.Val199Met    | E7   | SRCR   |                        | Dutch   | Severe                        | Weegerink et al. (2011) |
| c.607C>T   | p.Gln203X      | E7   |        |                        | Japanese | Severe                        | Miyagawa et al. (2013) |
| c.617-4_-3dupAT |           | Intron7  | —          |                        | Japanese | Mild                          | Miyagawa et al. (2015) |
| c.621T>C   | p.Cys207Cys    | E8   | Serine protease       |                        | Korean   | —                             | Lee et al. (2013)      |
| c.636C>T   | p.Gly212Gly    | E8   | Serine protease       |                        | German   | Mild                          | Elbracht et al. (2007) |
| c.646C>T   | p.Arg216Cys    | E8   | Serine protease       |                        | United States (Caucasian) | —                  | Eppsteiner et al. (2012) |
| c.647G>T   | p.Arg216Leu    | E8   | Serine protease       |                        | Turkish  | Severe                        | Wattenhofer et al. (2005) |
| c.726C>G   | p.Cys242Trp    | E8   | Serine protease       |                        | Japanese | Mild                          | Miyagawa et al. (2015) |
| c.727G>A   | p.Gly243Arg    | E8   | Serine protease       |                        | Indian   | —                             | Ganapathy et al. (2014) |
| c.734C>T   | p.Ser245Phe    | E8   | Serine protease       |                        | Pakistani | —                             | Khan et al. (2019)     |
| c.743C>T   | p.Met248Glu    | E8   | Serine protease       |                        | Czech    | —                             | Safka Brozko et al. (2020) |
| c.753G>C   | p.Trp251Cys    | E8   | Serine protease       |                        | Tunisian | —                             | Masmoudi et al. (2001) |
| c.757A>G   | p.Ile253Val    | E8   | Serine protease       |                        | Pakistani | —                             | Ben-Yosef et al. (2001) |
| c.767C>T   | p.Arg256Val    | E8   | Serine protease       |                        | Korean   | —                             | Lee et al. (2003)      |
| c.778G>A   | p.Val260Thr    | E8   | Serine protease       |                        | Taiwanese | —                             | Wong et al. (2003)     |
| c.782+8insT| —               | Intron8 | —          |                        | Pakistani | —                             | Lee et al. (2012)      |
| c.782+2T>A | —               | Intron8 | —          |                        | Polish   | —                             | Lechowicz et al. (2017) |
| c.783-1G>A | —               | Intron8 | —          |                        | Korean   | —                             | Kim et al. (2017)      |
| DNA change | Protein change | Exon | Domain | Variant classification | Origin | Phenotype severity at testing | References |
|------------|----------------|------|--------|------------------------|--------|-------------------------------|------------|
| c.809T>A   | p.Ile270Asn    | E9   | Serine protease | Chinese       | Severe | Gao et al. (2017)            |
| c.830C>T   | p.Pro277Leu    | E9   | Serine protease | Turkish       | —      | Masmoudi et al. (2001)      |
| c.871G>C   | p.Val291Leu    | E9   | Serine protease | Korean        | —      | Lee et al. (2013)            |
| c.916G>A   | p.Ala306Thr    | E9   | Serine protease | Likely pathogenic | German | Elbracht et al. (2007)      |
|            |                |      |         |                         | Dutch  | Wegerink et al. (2011)      |
|            |                |      |         |                         | United States (Caucasian) | —      | Eppsteiner et al. (2012)    |
|            |                |      |         |                         | Korean | —                            |
|            |                |      |         |                         | Turkish | Chung et al. (2014)        |
|            |                |      |         |                         | Chinese | —                            |
|            |                |      |         |                         | Korean | —                            |
|            |                |      |         |                         | —      | Gao et al. (2017)          |
| c.933C>T   | p.Ala311Ala    | E9   | Serine protease | United States | Mild   | This study                  |
| c.941T>C   | p.Leu314Pro    | E9   | Serine protease | Pakistani     | —      | Zhou et al. (2020)          |
| c.953-5A>G | p.Leu325Gln    | E10  | Serine protease | Polish        | —      | Lechowicz et al. (2017)    |
| c.974T>A   | p.Leu325Gln    | E9   | Serine protease | Polish        | —      | Lechowicz et al. (2017)    |
| c.988delA  | p.Glu330fs     | E10  | Serine protease | Pakistani     | Severe | Walsh et al. (2006)        |
| c.999delC  | p.Asp334Met+24 | E10  | Serine protease | Polish        | —      | Lechowicz et al. (2017)    |
| c.1019C>G  | p.Thr340Arg    | E10  | Serine protease | Italian       | Severe | Vozz et al. (2014)         |
| c.1025G>A  | p.Gly342Glu    | E10  | Serine protease | Turkish       | —      | Duman et al. (2011)        |
| c.1028G>C  | p.Trp343Ser    | E10  | Serine protease | Czech         | —      | Safka Brozko et al. (2020) |
| c.1039G>T  | p.Glu347X      | E10  | Serine protease | Korean        | —      | Song et al. (2020)         |
| c.1128C>T  | p.Tyr344Tyr    | E11  | Serine protease | United States | —      | Ben-Yosef et al. (2001)    |
| c.1151T>G  | p.Met341Val    | E11  | Serine protease | Chinese       | Severe | Gao et al. (2017)          |
| c.1156T>C  | p.Cys346Arg    | E11  | Serine protease | Indian        | —      | Ganapathy et al. (2014)    |
| c.1159G>A  | p.Ala347Thr    | E11  | Serine protease | Japanese      | Mild   | Miyagawa et al. (2013)     |
| c.1180_1187del8ins68 | — | E11 | Serine protease | Palestinian | Severe | Scott et al. (2001)        |
| c.1183G>C  | p.Asp349His    | E11  | Serine protease | United States | Severe | This study                  |
| c.1192C>T  | p.Glu348X      | E11  | Serine protease | Turkish       | Severe | Wattenhofer et al. (2005)  |
| c.1194+15C>A | p.Glu402Arg   | E12  | Serine protease | United States | Severe | This study                  |
| c.1204G>A  | p.Glu405Arg    | E12  | Serine protease | Taiwanese     | —      | Wong et al. (2020)         |
| c.1211C>T  | p.Pro404Leu    | E12  | Serine protease | Pakistani     | Severe | Norm et al. (2019)         |
| c.1219T>C  | p.Cys407Arg    | E12  | Serine protease | United States | Severe | Bowles et al. (2021)       |
| c.1244T>C  | p.Leu415Ser    | E12  | Serine protease | Chinese       | Severe | Gao et al. (2017)          |

(Continued on following page)
decay of mRNA. Fifty-eight of the identified variants were missense variants. Nearly all variants were predicted to disrupt the proteolytic activity of the protein. Both prelingual and postlingual hearing impairment was reported, with most patients showing a typical ski-slope audiogram configuration. CI outcomes were reported for 32 patients with bi-allelic variants in TMPRSS3 across 11 different studies (Table 2) (Weegerink et al., 2011; Eppsteiner et al., 2012; Miyagawa et al., 2013; Chung et al., 2020; Holder et al., 2021). While degree of hearing improvement varied between patients, the majority of those identified as Hispanic/Latino or mixed. We identified 12 different TMPRSS3 variants of which 1 has not been previously reported: deletion of Exons 1–5 and 13 (Table 3). This novel variant was classified as pathogenic as it met the criteria outlined by previous professional guidelines (Richards et al., 2015) with specifications provided by ClinGen (https://clinicalgenome.org/working-groups/sequence-variant-interpretation), specifically the combination of PVS1 (predicted loss of function), PM2 (absence in gnomAD), and PM3 (homozygous observation in an individual with phenotype matching the gene). The most commonly identified variants were p.Thr70fs*19 and p.Ala138Glu. Eight patients had congenital hearing loss, four of whom had biallelic pLOF variants. Four patients in our cohort underwent CI, and outcome information was available for two patients. The first patient, from family B, was found to have congenital profound hearing loss and was homozygous for p.Thr70fs*19. It is unclear when the patient underwent CI. However, at a follow up at 4 years of age, the patient had functional speech. Clinical records indicated that the patient had ongoing articulation errors and required speech therapy but was able to maintain adequate hearing. The second patient, from family K, was compound heterozygous for p.Glu104Lys and p.Ala306Thr. Clinical records have suggested positive CI outcome for her moderate-profound hearing loss. The remaining two patients who underwent CI were the siblings from family A who both had profound congenital hearing loss and were homozygous for a deletion of Exons 1–5 and 13. Their current hearing status is unknown.

### 4 DISCUSSION

The genotype-phenotype correlations of TMPRSS3 variants have not been well characterized. It has been previously shown that the frequency of TMPRSS3-induced ARSNHL was low in White

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**Table 1** (Continued) Overview of TMPRSS3 variants resulting in non-syndromic hearing loss, including those identified in the present study.

| DNA change   | Protein change | Exon | Domain          | Variant classification | Origin        | Phenotype severity at testing | References                  |
|--------------|----------------|------|-----------------|------------------------|---------------|------------------------------|-----------------------------|
| c.1250G>A    | p.Gly417Glu    | E12  | Serine protease | Chinese                | Severe        | Gao et al. (2017)            |
| c.1253C>T    | p.Ala418Val    | E12  | Serine protease | Taiwanese              | —             | Wong et al. (2020)           |
| c.1269C>T    | p.Ile423lle    | E12  | Serine protease | Taiwanese              | —             | Wong et al. (2020)           |
| c.1273T>C    | p.Cys425Arg    | E12  | Serine protease | Pakistani              | —             | Lee et al. (2012)            |
| c.1276G>A    | p.Ala426Thr    | E12  | Serine protease | Likely pathogenic      | Dutch         | Mid                          | Weegerink et al. (2011)    |
| c.1291C>T    | p.Pro431Ser    | E12  | Serine protease | Italian                | Severe        | This study                   | Vozzi et al. (2014)        |
| c.1306C>G    | p.Arg436Gly    | E12  | Serine protease | Polish                 | —             | Lechowicz et al. (2017)     | Czech                      |
| c.1343T>C    | p.Met448Thr    | E12  | Serine protease | Likely pathogenic      | United States | Severe                      | This study                   | Polish                     |
| c.1345-2A>G  | —              | E12  | —               | United States          | Mild          | This study                   | Lechowicz et al. (2017)     | Czech                      |

**Table 2**

| Origin          | Phenotype  |
|-----------------|------------|
| Taiwanese       | Severe     |
| Pakistani       | —          |
| United States   | —          |
| Taiwanese       | —          |
| United States   | —          |
| United States   | —          |

**Table 3**

| DNA change | Protein change | Exon | Domain          | Variant classification | Origin | Phenotype severity at testing | References                  |
|------------|----------------|------|-----------------|------------------------|--------|------------------------------|-----------------------------|
| c.1250G>A  | p.Gly417Glu    | E12  | Serine protease | Chinese                | Severe | Gao et al. (2017)            |
| c.1253C>T  | p.Ala418Val    | E12  | Serine protease | Taiwanese              | —      | Wong et al. (2020)           |
| c.1269C>T  | p.Ile423lle    | E12  | Serine protease | Taiwanese              | —      | Wong et al. (2020)           |
| c.1273T>C  | p.Cys425Arg    | E12  | Serine protease | Pakistani              | —      | Lee et al. (2012)            |
| c.1276G>A  | p.Ala426Thr    | E12  | Serine protease | Likely pathogenic      | Dutch  | Mid                          | Weegerink et al. (2011)    |
| c.1291C>T  | p.Pro431Ser    | E12  | Serine protease | Italian                | Severe | This study                   | Vozzi et al. (2014)        |
| c.1306C>G  | p.Arg436Gly    | E12  | Serine protease | Polish                 | —      | Lechowicz et al. (2017)     | Czech                      |
| c.1343T>C  | p.Met448Thr    | E12  | Serine protease | Likely pathogenic      | United States | Severe                      | This study                   | Polish                     |
| c.1345-2A>G | —              | E12  | —               | United States          | Mild   | This study                   | Lechowicz et al. (2017)     | Czech                      |

**References**

- Richards et al. (2015)
- Shearer et al. (2018)
- Vozzi et al. (2014)
- Wong et al. (2020)
- Lee et al. (2021)
- Holder et al. (2021)

**Abbreviations**

- TM, transmembrane domain; LDLRA, LDL receptor-like domain; SRCR, scavenger receptor cysteine-rich domain; serine protease, trypsin-like serine protease domain.
TABLE 2 | Overview of clinical characteristics and genotypes of patients with TMPRSS3 variants who have received cochlear implantation.

| Study (country) | DNA change | Protein change | Exon | Domain | Hearing loss severity | Age at CI (gender) | Age at severe-profound HL | Pre-operative hearing | CI type | CI outcomes |
|----------------|------------|----------------|------|--------|-----------------------|-------------------|--------------------------|------------------------|---------|-------------|
| Weegerink et al., 2011 (Netherlands) | c.207delC | p.Thr70fs | E4 | Serine protease | — | 4.5 years | — | Sloping HL 40–60–100–110–110 dB (0.25, 0.5, 1, 2, 4 kHz) | Nucleus Freedom (Cochlear) | 91% Phoneme (76% WRS) |
| | c.916G>A | p.Ala306Thr | E9 | Serine protease | — | 6 years | — | Sloping HL 40–50–110–110–110 dB (0.25, 0.5, 1, 2, 4 kHz) | Nucleus Freedom (Cochlear) | 80% Phoneme (65% WRS) |
| | c.595G>A | p.Val199Met | E7 | SRCR | — | 29 years | — | Decreasing HL 80–90–110–110–110 dB (0.25, 0.5, 1, 2, 4 kHz); 5% Phoneme | Nucleus Contour C224R (Cochlear) | — |
| | c.413C>G | p.Ala138Glu | E5 | SRCR | — | 49 years | — | Decreasing HL 70–95–110–110–110 dB (0.25, 0.5, 1, 2, 4 kHz); 20% Phoneme | Clarion AB-5100H (Advanced Bionics) | 89% Phoneme (75% WRS) |
| | c.207delC | p.Thr70fs | E4 | Serine protease | — | 45 years | — | Decreasing HL 80–90–110–110 dB (0.25, 0.5, 1, 2, 4 kHz); 5% Phoneme | Nucleus Contour C224R (Cochlear) | 76% Phoneme (60% WRS) |
| | c.1276G>A | p.Ala426Thr | E12 | Serine protease | — | 46 years | — | Flat 100–100–110–110–110 dB (0.25, 0.5, 1, 2, 4 kHz); 0% Phoneme | Clarion AB-5100H (Advanced Bionics) | 82% Phoneme (58% WRS) |
| | c.207delC | p.Thr70fs | E4 | Serine protease | — | 43 years | — | Flat 100–90–110–110–110 dB (0.25, 0.5, 1, 2, 4 kHz); 0% Phoneme | Clarion AB-5100H (Advanced Bionics) | 83% Phoneme (62% WRS) |
| Eppsteiner et al., 2012 (United States) | c.413C>G | p.Ala138Glu | E5 | SRCR | — | 51 years | — | Decreasing HL 80–90–110–110–110 dB (0.25, 0.5, 1, 2, 4 kHz); 2.5% Phoneme | Nucleus Contour C224R (Cochlear) | 88% Phoneme (68% WRS) |
| | c.646C>T | p.Arg216Cys | E8 | Serine protease | — | 30 years | — | Sloping HL 93 dB (PTA at 0.5, 1, 2, and 4 kHz) | Advanced Bionics CI | Poor performance (Combined CNC & HINT Score: 37) |
| Miyagawa et al., 2013 (Japan) | c.607C>T | p.Glu203X | E7 | Serine protease | Mild | 45 years (male) | 45 years | 98 dB (PTA at 0.5, 1, 2, and 4 kHz) | Advanced Bionics CI | Poor performance (Combined CNC & HINT Score: 23) |
| | c.1159G>A | p.Ala387Thr | E12 | Serine protease | — | 32 years (female) | 17 years | — | — |
| | c.232-6G>C | — | In4 | SRCR | — | 30 years | — | Sloping HL 50–90–110–110–110 dB (0.25, 0.5, 1, 2, 4 kHz); 10% Phoneme | Nucleus Freedom (Cochlear) | — |
| | c.916G>A | p.Ala306Thr | E9 | Serine protease | Mild | 45 years (male) | 45 years | — | — |
| | c.323-6G>C | — | In4 | SRCR | — | 30 years | — | — | — |
| | c.607C>T | p.Glu203X | E7 | Serine protease | Mild | 32 years (female) | 17 years | — | — |
| | c.1159G>A | p.Ala387Thr | E12 | Serine protease | — | 40 years (female) | — | — | — |

(Continued on following page)
| Study (country) | DNA change | Protein change | Exon | Domain | Hearing loss severity | Age at CI (gender) | Age at severe-profound HL | Pre-operative CI type | CI outcomes |
|----------------|------------|----------------|------|--------|-----------------------|-------------------|---------------------------|----------------------|--------------|
| Chung et al., 2014 (Korea) | c.325C>T | p.Arg109Trp | E5 | SRCR | 12 years | female | — | Flat (−sloping) | 100–110—110–110–110–110 dB (0.25, 0.5, 1, 2, 4, 8 kHz) |
| | c.916G>A | p.Ala306Thr | E9 | Serine protease | — | — | — | — | Mean open set sentence score at 6 months following CI was 88.5% |
| | c.325C>T | p.Arg109Trp | E5 | SRCR | 6 years | male | — | Decreasing HL | 70–80–90–100–110–110 dB (0.25, 0.5, 1, 2, 4, 8 kHz) |
| Miyagawa et al., 2015 (Japan) | c.390C>G | p.His130Arg | E5 | SRCR | — | 45 years | male | Sloping HL | MED-EL PULSAR FLEX24 |
| | c.226C>T | p.Gln76X | E4 | Serine protease | — | 39 years | female | Flat (−Sloping) | 90% discrimination score on Japanese monosyllable test at 24 months |
| | c.212T>C | p.Phe71Ser | E4 | LDLRA | — | 51 years | female | — | 80% discrimination score on Japanese monosyllable test at 12 months |
| Battelino et al., 2016 (Slovenia) | c.617-4_3dupAT | — | In7 | — | — | 11 months | male | Sloping HL | 80% discrimination score on Japanese monosyllable test at 12 months |
| | c.208delC* | p.Thr70fs | E4 | — | — | 11 months | male | — | 25 dB (unclear methodology) |
| Gao et al., 2017 (China) | c.916G>A | p.Ala306Thr | E9 | Serine protease | Severe | 3 years | female | Decreasing HL | — | Described as “improved” |
| | c.1250G>A | p.Gly417Glu | E12 | Serine protease | Severe | 14 years | female | Sloping HL | — | Described as “improved” |
| Kim et al., 2017 (Korea) | c.346G>A | p.Val116Met | E5 | SRCR | 4 years | female | — | Decreasing HL | — | Not described, unofficially good |
| | c.871G>A | p.Val291Leu | E9 | Serine protease | Profound | 10 years | female | Sloping HL | — | Not described, unofficially good |

(Continued on following page)
TABLE 2 | Overview of clinical characteristics and genotypes of patients with TMPRSS3 variants who have received cochlear implantation.

| Study (country) | DNA change | Protein change | Exon | Domain | Hearing loss severity | Age at CI (gender) | Pre-operative hearing | CI type | CI outcomes |
|----------------|------------|----------------|------|--------|-----------------------|-------------------|----------------------|---------|-------------|
| Shearer et al., 2018 (United States) | c.208delC | p.Thr70fs | E4 | — | 64 years | — | — | Nucleus Hybrid CI L24 Array | 80–90–110–110–110 dB (0.125, 0.25, 0.5, 1, 2 kHz) |
| | c.1276G>A | p.Ala426Thr | E12 | Serine protease SRCR | 53 years | — | — | Nucleus Hybrid CI S8 Array | 50–60–90–110–110 dB (0.125, 0.25, 0.5, 1, 2 kHz) |
| | c.1345–2A>G | — | In12 | — | 38 years | — | — | Nucleus Hybrid CI L24 Array | 35–30–55–110–110 dB (0.125, 0.25, 0.5, 1, 2 kHz) |
| Song et al., 2020 (Korea) | c.916G>A | p.Ala306Thr | E9 | Serine protease | 17 years (female) | 3–5 years | Stopping HL | — | 86% WRS at 12 months following implantation |
| | c.1039G>T | p.Glu347Ter | E10 | Serine protease | — | 40–90–100–110–110 dB (0.25, 0.5, 1, 2, 4, 8 kHz) | | | |
| Holder et al., 2021 (United States) | c.208delC | p.Thr70fs | E4 | — | 54 months (female) | — | Stopping HL | Cochlear Nucleus 522/522 (left/right) | CNC 84%; BabyBio Quiet 94%/92% (left/right) |
| | c.916G>A | p.Ala306Thr | E9 | Serine protease | 47 months (female) | — | Stopping HL | Cochlear Nucleus 522/522 (left/right) | CNC 72%; BabyBio Quiet 55% |
| | c.208delC | p.Thr70fs | E4 | — | 43 months (female) | — | Stopping HL | Cochlear Nucleus 522/522 (left/right) | LNT 92%/82% (left/right); HINT 62% |

HL, hearing loss; CI, cochlear implant; LDLRA, LDL receptor-like domain; dB, decibel; WRS, word-recognition score; SRCR, scavenger receptor cysteine-rich domain; serine protease, trypsin-like serine protease domain; PTA, pure tone average; CNC, consonant-nucleus-consonant; HINT, hearing in noise test; HA, hearing aid; LNT, lexical neighborhood test. Naming of variants and labeling of domains and exons are based on the NM_001256317.3 transcript. Of note, the phenotype severity is provided at the time of testing. While some patients may initially have milder phenotypes, the hearing loss can progress and become more severe.

*Patient is homozygous for the specified variant.
**FIGURE 2** Pedigree chart for enrolled patient families (A–N). Age at genetic testing, age at onset of hearing loss, and other relevant clinical information is provided, when available, for patients and family members. CI, cochlear implant; HL, hearing loss; HA, hearing aid.

**TABLE 3** Genotype and phenotype overview of our patient cohort.

| Family | Age (in years) | Gender | DNA change | Protein change | Configuration | HL onset | HL severity |
|--------|----------------|--------|------------|----------------|---------------|----------|-------------|
| A      | 16 months      | F      | Deletion of Exons 1–5 and 13<sup>a</sup> | —              | Congenital    | —        | —           |
|        | 6 years        | M      | Deletion of Exons 1–5 and 13<sup>a</sup> | —              | Congenital    | —        | —           |
| B      | 3 months       | M      | c.208delC<sup>a</sup> | p.Thr70fs*19   | Congenital    | Profound | —           |
| C      | 9 months       | F      | c.208delC; c.1192C>T | p.Thr70fs*19; p.Gln398X | Congenital    | Profound | —           |
| D      | 8 years        | M      | c.208delC; c.1276G>A | p.Thr70fs*19; p.Ala426Thr | —              | —        | Profound    |
|        | 13 years       | F      | —              | —              | Sloping sensorineural hearing loss | —        | —           |
|        | 15 years       | M      | —              | —              | —              | 10 years old | Progressive sloping, moderate left, severe right |
| E      | 13 years       | F      | c.208delC; c.413G>G | p.Thr70fs*19; p.Ala138Glu | —              | —        | Progressive, sloping, severe |
| F      | 11 years       | F      | c.208delC; c.413G>G | p.Thr70fs*19; p.Ala138Glu | —              | 9 years old | Sloping, profound |
| G      | 22 years       | F      | c.208delC; c.413G>G | p.Thr70fs*19; p.Ala138Glu | —              | 19 years old | —           |
| H      | 6 years        | M      | c.323-6G>A; c.325C>T | p.Arg109Trp | —              | Trans Congenital | Moderately severe to profound |
| I      | 1 year         | M      | c.579dupA; c.1183G>C | p.Cys194MetfsX17; p.Asp396His | Trans Congenital | Severe to profound | —           |
| J      | 22 years       | M      | c.238C>T; c.1343T>C | p.Arg80Cys; p.Met448Thr | 12 years old | Progressive, moderate-severe left, severe right | —           |
|        | 24 years       | M      | c.238C>T; c.1343T>C | p.Arg80Cys; p.Met448Thr | 10–12 years old | — | — |
| K      | 3 years        | F      | c.310G>A; c.916G>A | p.Glu104Lys; p.Ala306Thr | —              | —        | Moderately severe at low frequencies, profound at high frequencies |
| L      | 17 years       | M      | c.325C>T; c.413G>G | p.Arg109Trp; p.Ala138Glu | 4 years old | Moderate-severe | —           |
| M      | 12 years       | M      | c.413G>G; c.916G>A | p.Ala138Glu; p.Ala306Thr | Congenital | Progressive, high frequency, moderate |
| N      | 36 years       | M      | c.208delC; c.1306C>T | p.Thr70fs*19; p.Arg436Gly | Congenital | Progressive, profound |

HL, hearing loss. Novel variant is bolded. Naming of variants is based on the NM_001256317.3 transcript.

<sup>a</sup> patient is homozygous for the specified variant.
individuals (Wattenhofer et al., 2002). However, a recent epidemiological study of patients undergoing CI revealed that 10% (13) of patients with positive genetic testing had TMPRSS3 gene variants (Seligmans et al., 2021). As adoption of genetic testing in clinical practice continues to grow, it is important to be aware of common TMPRSS3 variants and associated phenotypes to best counsel patients.

In our cohort of 18 patients, 15 of whom were White, the most frequently observed variants were p.Thr70fs*19 and p.Ala138Glu implying that those were either hot spots or founder variants. The combination of the p.Thr70fs*19 frameshift variant with a missense variant appeared to cause sloping hearing loss that varied in severity. Biallelic pLOF variants appeared to cause congenital profound hearing loss. This phenotype information is valuable when trying to understand potential patient prognosis based on genetic testing results. Previous studies on the role of CI in patients with TMPRSS3 variants have reported variable results. In one study, poor outcomes following CI in patients with TMPRSS3 variants were attributed to the expression of the TMPRSS3 gene in SGNs as opposed to other locations in the cochlea such as the membranous labyrinth (Eppsteiner et al., 2012). These authors also suggested that patients with pathogenic TMPRSS3 variants may have continued loss of SGNs over time which could contribute to ongoing hearing deterioration even after CI. However, recent studies have shown predominantly positive outcomes following CI in patients with TMPRSS3 variants (Weegerink et al., 2011; Miyagawa et al., 2013; Chung et al., 2014; Miyagawa et al., 2015; Battelino et al., 2016; Gao et al., 2017; Shearer et al., 2018; Song et al., 2020; Holder et al., 2021). This discrepancy might be related to the large duration of deafness and older age of the two patients in Eppsteiner et al. (2012) and Holder et al. (2021). In addition, a study of CI outcomes in pediatric patients with TMPRSS3 variants reported positive outcomes with no evidence of SGN degeneration leading to decreased performance over time (Holder et al., 2021). Furthermore, it was suggested that even if SGN degeneration does contribute to a longitudinal decline in performance, early CI may help slow or reverse this process (Holder et al., 2021). Even so, many clinics do not implant patients with precipitously sloping hearing loss as they do not meet labeled indications for CI. However, off-label implantation has been shown to be beneficial and is being employed more frequently at major academic medical centers (Carlson et al., 2015; Leigh et al., 2016; Carlson et al., 2018).

Taken together with the positive clinical outcomes following CI in two patients from our cohort, it is evident that CI is a promising treatment strategy for patients with TMPRSS3 variants. Active intervention with CI is likely to be beneficial, particularly in patients in whom residual hearing is preserved. It is imperative that the benefits of CI are made clear when counseling patients on their potential treatment options.

DATA AVAILABILITY STATEMENT

The evidence for all variants classified by the authors is included in submissions to ClinVar by the Laboratory for Molecular Medicine (Organization ID: 21766). All other data supporting the conclusions of this article, if not directly included in the paper, will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Mass General Brigham Human Research Committee’s IRB. Written informed consent to participate in this study was provided by the participants’ legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

IM and AG co-wrote the manuscript and prepared the tables and figures, VS edited the manuscript and prepared the tables and figures for submission, HR edited the manuscript and provided technical feedback, KS conceived, designed, and supervised the manuscript writing and editing.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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