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TMPRSS3 expression is limited in spiral ganglion neurons: implication for successful cochlear implantation

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Abstract

Background: It is well established that biallelic mutations in *TMPRSS3* cause hearing loss. Currently, there is controversy regarding the audiological outcomes after cochlear implantation (CI) for *TMPRSS3*-associated hearing loss. This controversy creates confusion amongst healthcare providers regarding the best treatment options for individuals with *TMPRSS3*-related hearing loss.

Methods: A literature review was performed to identify all published cases of patients with *TMPRSS3*-associated hearing loss who received a CI. CI outcomes of this cohort were compared to published adult CI cohorts using post-operative consonant-nucleus-consonant (CNC) word performance. *TMPRSS3* expression in mouse cochlea and human auditory nerves (ANs) was determined by using hybridization chain reaction (HCR) and single-cell RNA-sequencing analysis.

Results: In aggregate, 27 patients (30 total CI ears) with *TMPRSS3*-associated hearing loss treated with CI, and 85% of patients reported favorable outcomes. Post-operative CNC word scores in patients with *TMPRSS3*-associated hearing loss were not significantly different than those seen in adult CI cohorts (8 studies). Robust *Tmprss3* expression occurs throughout the mouse organ of Corti, the spindle and root cells of the lateral wall, and faint staining within <5% of the ANs, representing type II spiral ganglion neurons. Adult human ANs express negligible levels of *TMPRSS3*.

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Contributorship Statement:

Conception and Design: KTB and RFN. Data collection and analysis: YC, EC, BJT, TJS, JVM, DJT, KTB, RFN. Performed experiments: YC, EC, BJT, TJS, JVM, DJT, RFN. Drafted original manuscript: YC, BJT, KTB and RFN. All authors have reviewed and approved the final manuscript.

Declarations

Competing interests: The authors have no competing interests and declare that the research was conducted in the absence of any potential conflict of interest.

Ethics approval statement: The care and use of animals were approved by the Indiana University School of Medicine's Institutional Animal Care and Use Committee and by the the Indiana University School of Medicine IRB (IRB approval 200634852).

Conclusion: The clinical features after CI and physiologic expression of *TMPRSS3* suggest against a major role of *TMPRSS3* in auditory neurons.

Keywords

Cochlear implant; *TMPRSS3*; Neurons; Genetic; Hearing loss; Speech recognition

Introduction

Transmembrane serine proteases are a large family of proteins and indispensable regulators within many tissues, including the inner ear[1]. Broadly, they are classified based upon how they are anchored to the cellular membrane. Type II transmembrane serine proteases are anchored by their N-terminus to the cellular membrane and contain a group A scavenger receptor domain and an active serine protease domain[1 2]. *TMPRSS3* (Transmembrane Protease, Serine 3) is a type II transmembrane serine protease required in the mammalian auditory system for proper hearing[3 4].

TMPRSS3 is essential for cochlear hair cell survival[5–7]. Defects or ablation of *TMPRSS3* results in hearing loss in both mice and humans. Mice carrying a protein-truncating *TMPRSS3* mutation exhibit normal cochlear and vestibular hair cell (HC) development followed by rapid HC degeneration over 48 hours starting at postnatal day 12 (P12)[5]. In humans, bi-allelic pathogenic variants of *TMPRSS3* are the most common causative hearing loss gene in adults undergoing traditional and hybrid cochlear implantation (CI)[8]. Additionally, *TMPRSS3* is the 5th most common gene causally associated with deafness in a multiethnic cohort of human congenital deafness[9]. Furthermore, select missense mutations (e.g. A306T, A138E, A426T) are linked to post-lingual onset high-frequency hearing loss[10]. Despite this robust and dramatic phenotype, the function of *TMPRSS3* and the pathomechanism(s) that underlie *TMPRSS3*-related deafness remains elusive.

Cochlear implantation is the gold-standard treatment for inherited severe-to-profound sensorineural hearing loss (SNHL)[11 12]. Implants function by directly stimulating spiral ganglion neurons (SGNs), permitting sound detection and speech recognition while circumventing inner ear organs such as cochlear hair cells[6 11–13]. Currently, the post-operative CI outcomes in patients with *TMPRSS3*-associated hearing loss remain controversial in the published literature[6 10 13]. *TMPRSS3*-related damage to the SGN has been proposed as one possible explanation for poor CI outcomes in these patients[13], evidenced by *TMPRSS3* immunostaining within SGNs in the murine cochlea[14]. However, recent single-cell RNA sequencing (scRNA-seq) studies have demonstrated that *Tmprss3* is not expressed in type I SGNs, but only in type II SGNs—which do not transmit auditory signals and make up only 4% of the SGN population[15 16]. This disconnect between biological data and patient outcomes prompted us to critically review published reports regarding *TMPRSS3* patient CI outcomes and perform in-depth expression studies of *TMPRSS3* in the human and mouse auditory system.

Here we show that patients with *TMPRSS3*-related hearing loss have overall good post-operative performance and a *TMPRSS3* genetic diagnosis should not be a reason to restrict cochlear implantation. In addition, using two different methods, we show that *TMPRSS3* is

not expressed in the sound transducing neurons of the cochlea and is primarily localized to the sensory epithelium portion of the cochlea. Finally, we call into question the correlation between negative CI performance and *TMPRSS3*-related hearing loss.

Materials and Methods

TMPRSS3-associated cochlear implant outcomes

Two independent searches of PubMed for articles published on *TMPRSS3*-associated hearing loss treated with CI were conducted. Search terms included the combination of “*TMPRSS3*” with the major subheading topics “hearing loss” and “cochlear implant”. All articles reporting CI outcomes for *TMPRSS3*-associated hearing loss were identified. Articles that contained subjective (favorable or unfavorable) or objective (speech scores) outcome measures were reviewed. For the adult CI cohort analysis, articles that contained more than 10 patients and had post-operative CNC word scores were reviewed. Nine articles fit these criteria and were included in this study. Demographics, age of SNHL onset, phenotypic presentation, documented *TMPRSS3* mutations, patient age at time of CI, and audiologic outcomes were recorded. Individuals with hearing loss prior to two years of age were classified as having prelingual hearing loss whereas those with onset two years of age or older were designated as having post-lingual hearing loss. Objective outcomes included pre-operative and post-operative pure tone averages (PTA), consonant-nucleus-consonant (CNC) word recognition scores (WRS), and sentence scores (SS). Subjective outcomes, when reported in published articles, were classified as either favorable or poor: post-operative implant-aided CNC WRS scores less than 30% were considered poor outcomes, and scores 50% or greater were considered favorable. Change in PTA and CNC WRS scores were also calculated.

Statistical analysis

Objective data were evaluated by measures of central tendency using Microsoft Excel, 2016. The mean age difference between favorable and poor post-lingual CI performers was evaluated using a t-test using SPSS Statistics, 2020. CI outcomes in patients with *TMPRSS3*-associated hearing loss were compared to post-operative outcomes of eight representative CI studies in the general hearing loss population. Logistic regression was performed to determine the effects of possible predictor variables on favorable CI outcomes in the *TMPRSS3* population. Linear regression was similarly performed to evaluate possible predictor effects on average change (delta) dCNC and dPTA. Mean post-operative CNC WRS analysis of variance (ANOVA) of the two populations was performed using SPSS.

Animal handling

Tmprss3-mutant mice (*Tmprss3*^{Y260X}) have been described previously[5]. All mice were housed in the animal facility of Indiana University School of Medicine. All procedures were carried out in accordance with the approval of the Indiana University Institutional Animal Care and Use Committee protocol. Genotyping was performed as previously described[3].

Human tissue harvest

Human auditory nerves were harvested during vestibular schwannoma operation after obtaining patient consent (IRB approval 200634852). During the tumor removal, the auditory nerve was dissected away from the schwannoma and facial nerve within the internal auditory canal (IAC). The auditory nerve between the modiolus of the cochlea and porus of the IAC was harvested. Immediately after harvest, the tissues were flash-frozen in liquid nitrogen and stored at -80°C before RNA extraction for qRT-PCR. For HCR experiments, the auditory nerves were immediately fixed in 10% formalin for 24 hours prior to embedding.

Plasmid Construct

The coding sequence of human *TMPRSS3* isoform 1 from transcript variant A (NM_024022.4) with added 5' EcoRI and 3' KpnI restriction sites was generated as gBlocks™ Gene Fragments (Integrated DNA Technologies, Corralville, IA). The construct p3XFLAG-CMV 7.1_Tmprss3 was generated from the backbone of p3XFLAG-CMV 7.1_syn6 (Addgene, 50012). The *Syntaxin-6* gene was replaced by *TMPRSS3* gBlocks with EcoRI and KpnI restriction enzymes.

Cell culture

HEK293 cells (ATCC, Manassas, VA; CRL-1573), plated on 1% gelatin-coated cover slide in 6 well plates and were transfected with p3XFLAG-CMV 7.1_Tmprss3 plasmid using Lipofectamine 3000 Reagent (Thermo Fisher, Waltham, MA; L3000015) for 24 hours as described by the manufacturer. Cells were washed once with ice-cold PBS and fixed with 4% paraformaldehyde for 20 mins at room temperature.

HCR Immunofluorescence

Human auditory nerves were fixed in 10% formalin for 24 hours. Then, fixed samples were cryoprotected in 30% sucrose in PBS. After embedding in TFM™ Tissue Freezing Medium (General Data Company, TFM-5), the auditory nerve was frozen and stored at -30°C . Sections of 6 μm were cut on a cryostat and air-dried for 2 hours.

Mouse cochleae were dissected from P11 mice anesthetized with isoflurane. The mice were perfused with 1X PBS through left ventricle. The cochleae were dissected and fixed in 4% paraformaldehyde overnight at room temperature and decalcified overnight at room temperature in solution containing 120 mM EDTA buffered in 1X PBS. Fixed cochleae were cryoprotected in 30% sucrose in PBS. After embedding in TFM™ Tissue Freezing Medium (General Data Company, TFM-5), cochleae were frozen and stored at -30°C . Midmodiolar cross sections of 6 μm were cut on a cryostat and air-dried for 2 hr.

The human auditory nerve sections and *TMPRSS3*-transfected HEK cells were stained with human *TMPRSS3* HCR RNA-FISH probes while the mouse cochleae sections were stained with mouse *Tmprss3* and mouse *MBP* HCR RNA-FISH probes (Molecular Instruments, Los Angeles, CA) following the manufacture's protocol with incubations at 37°C (Corning LSE mini 6800). Twenty probes were designed for human *TMPRSS3* transcript variant A (NM_024022.4), mouse *Tmprss3* transcript variant 1 (NM_001163776), and mouse *MBP*

transcript variant 1 (NM_001025251), which recognizes 5'-UTR, 3'-UTR, and coding sequence (CDS). Sections were mounted with ProLong™ Gold Antifade Mountant with DAPI (ThermoFisher, P36931) and visualized on Leica DMI8 microscopy.

Real-time quantitative RT-PCR

Total RNA was extracted from human auditory nerve, HEK cells and *TMPRSS3*-transfected HEK cells with PureLink® RNA Mini Kit (Invitrogen) and cDNA was synthesized from 50 ng total RNA with SuperScript IV VILO Master Mix (Invitrogen). qRT-PCR was performed using SYBR-Green and primers: *TMPRSS3* sense: CAT CCA GGT GGG TCT AGT TTC; *TMPRSS3* antisense: AGC CTC TTT GGC TTG TAC TT; *MBP* sense: GGC AAG GTA CCC TGG CTA AA; *MBP* antisense: GGG TGG TGT GAG TCC TTG TA; *GAPDH* sense: CAT CAC TGC CAC CCA GAA GAC TG; *GAPDH* antisense: ATG CCA GTG AGC TTC CCG TTC AG. The amplification and detection were performed on QuantStudio 5 Real-Time PCR System (Invitrogen). Relative expression for *TMPRSS3* and *MBP* were adjusted to *GAPDH* levels.

Immunofluorescence

The cells on the slide or tissue sections were blocked with 10% goat serum with 0.1% Tween-20 in PBS for 30 mins at room temperature (RT). Following this, sections were treated with primary antibodies in 3% goat serum with 0.1% Tween-20 in PBS at room temperature for 1 hr. The antibody we used were mouse anti-TUJ1 (BioLegend, 801202, 1:100). After washing with PBS, sections were incubated with secondary antibodies or Alexa Fluor™ 568 Phalloidin (Thermo Fisher, A12380, 1:200) in 3% goat serum with 0.1% Tween-20 in PBS at RT for 1 hr. Finally, sections were washed and mounted with ProLong™ Gold Antifade Mountant with DAPI (ThermoFisher, P36931). Staining was visualized on Leica DMI8 microscopy.

TMPRSS3 hearing loss causing variants

Eighty-six *TMPRSS3* deafness-causing variants were curated from the published literature and the Deafness Variation Database (<https://deafnessvariationdatabase.org/>)[17] or via a Pubmed Search (July 2021). Variants were classified as Pathogenic, Likely Pathogenic or VUS according to the hearing loss specific ACMG[18].

Results

Variants throughout the *TMPRSS3* gene cause hearing loss.

Of 86 variants curated in this study, 62 were classified as pathogenic/likely pathogenic according to American College of Medical Genetics (ACMG) criteria while the remaining 24 are considered variants of uncertain significance (VUS). The 62 pathogenic/likely pathogenic *TMPRSS3* mutations map across all protein domains which are associated with hearing loss (Figure 1A, Supp Table 1). Of these, 35 (~56.5%) are missense or non-protein truncating (NPT) variants (Figure 1A–C). The remaining protein truncating (PT) mutations include 10 (~16%) nonsense variants, 8 (~13%) frameshift indels and 9 (14.5%) splice-altering variants (Figure 1A–C).

Patients with *TMPRSS3*-variants have excellent speech recognition outcomes with cochlear implants.

A total of nine articles describing 27 patients (30 ears) who received CI for *TMPRSS3*-associated hearing loss were identified (Table 1)[19–21]. After a review of the variants identified in individuals with reported CI outcomes, one individual reported in Miyagawa et al. 2015 was removed due to the reported variant c.212T>C (p.Phe71Ser), being present in >1% of control alleles in gnomAD[22]. Of the 19 patients with gender recorded, 13 (68%) were female, and 6 (32%) were male. Of 21 patients with data available, 14 (67%) were white, and 7 (33%) were Asian (Table 1). Post-lingual hearing loss occurred in 80% of patients.

The age of implantation was widely variable with a mean (standard deviation) age of 19.8 (± 20.2) years (Table 1). Favorable hearing outcome after implantation was reported by 85% of patients. The mean PTA improved from 82.4 (± 18.2) dB to 26.7 (± 6.4) dB with an average change in PTA (delta-PTA or dPTA), defined as post-op PTA minus pre-op PTA, of -55.7 (± 17.9). Multivariable linear regression found no significant difference between dPTA and age (0.954), although higher pre-operative PTA (worse hearing) was associated with a greater dPTA ($b = -0.284$, $p < 0.001$). Average dCNC, defined as post-op CNC score minus pre-op score, was 48.3% ($\pm 14.7\%$) (Table 1) with an average of 17.2% ($\pm 15.2\%$) pre-operatively and 65.5% ($\pm 26.3\%$) after implantation. Multivariable linear regression found no significant association between dCNC and age ($p = 0.973$) or pre-operative CNC ($p = 0.124$). Poor post-lingual performers were significantly older than good performers [$t(17.4) = 4.7$, $P = 0.018$]. Multivariable logistic regression found no significant association between age and subjective performance ($p = 0.413$).

TMPRSS3 mutations contributing to hearing loss occurred on 13 different alleles in our cohort (Figure 1D). Mutations were recorded for each of the two alleles present in each individual (Table 1). The most common allele was the frameshift variant c.208delC (p.His70fs) which accounts for $\sim 1/3$ of total alleles (17/52) present in this cohort. Other common variants included c.916G>A (p.Ala306Thr), c.1276G>A (p.Ala426Thr), and c.413C>A (p.Ala138Glu), which comprised 10, 6, and 4 alleles, respectively. The remaining 10 variants accounted for 2 or 1 alleles each (Figure 1D). Favorable and poor CI performance related to each variant was determined (Figure 1D).

The most common allelic combination was protein truncating (PT) by non-protein truncating (NPT), accounting for 14 cases (13 favorable outcomes vs 1 poor outcome). NPTxNPT accounted for the next highest allelic combination with 8 cases (5 favorable outcomes vs 3 poor outcomes), and 4 cases had PTxPT (4 favorable outcomes vs 0 poor outcomes). Statistical analysis (Chi-Square test) could not be performed because several conditions had fewer than 5 events.

Four patients reported subjectively poor outcomes. Three of the four patients carried the c.413C>A (p.Ala138Glu) allele (Table 1 and Figure 1D), although the second allele was different in each case (p.Arg216Cys, p.Ala306Thr, and p.Ala426Thr). The remaining patient also carried the c.1276G>A (p.Ala426Thr) allele along with the c.208delC (p.His70fs) allele. The c.1276G>A (p.Ala426Thr) allele was observed in 5 patients with favorable

outcomes, while the c.413C>A (p.Ala138Glu) allele was observed in one favorable outcome in combination with the c.595G>A (p.Val199Met) allele. Alleles p.His70fs and p.Ala306Thr were also seen in patients with favorable outcomes (Table 1). Of the four poor performers, two (both with p.Ala138Glu allele) had experienced hearing loss for greater than 25 years while the remaining two (one with p.Ala138Glu allele, both with p.Ala426Thr allele) did not have age at onset recorded, but were in their 50s and 60s, respectively, at time of implantation. The p.Ala138Glu and p.Ala426Thr mutations typically results in post-lingual progressive high-frequency sensorineural hearing loss prior to age 15[10]. Therefore, it is likely that both of these patients had a significant duration of hearing loss prior to implantation, which is associated with poor CI performance[23]. Both mutations are, however, typically associated with less severe hearing loss than other *TMPRSS3* mutations[10], such as p.Val199Met and p.Ala306Thr, which were not present in as high of a proportion of poor CI performers. Additionally, one patient (Case 8) who carries both the p.Ala306Thr and the p.Ala138Glu variants, required revision CI surgery due to hardware infection.

CI outcomes in patients with *TMPRSS3*-mediated hearing loss are similar to post-lingual adults

Nine large studies of more than 10 patients reported post-lingual adult CI outcomes for 565 individuals and these patients exhibited a mean post-operative CNC word score of 51.2% (22.2%) (Table 2)[24–31]. Of the seven studies with pre- and post-operative CNC scores recorded, the average improvement in CNC score (defined as final score minus initial score) was 40.4%. An ANOVA test found no significant difference in post-operative CNC WRS between the *TMPRSS3* and general hearing loss cohorts [66.2% (25.8) vs. 50.1% (12.5); $F(1,6) = 1.97, P=0.21$].

***TMPRSS3* expression is minimal in auditory neurons**

To determine the spatial and quantitative expression of *Tmprss3*, we performed hybridization chain reaction (HCR) on mouse cochlea at P11. We observe robust expression of *Tmprss3* in cells throughout the organ of Corti including Myo7A⁺ inner and outer hair cells (Figure 2A–D). *Tmprss3* is also detected in the spindle and root cells of the lateral wall, but no expression is detected in the stria vascularis (Figure 2A–C). Very faint expression is detected in <5% of the SGNs, but not in the peripheral neuronal processes near the habenula perforate (Figure 2E&F). Based upon the location and low quantity of these SGN cells, they most likely represent type II SGNs.

Next, we analyzed the expression of *Tmprss3* in the publicly available Gene Expression Analysis Resource (<http://umgear.org>)[32]. We compared the expression of *Tmprss3* across SGN subtypes (Type Ia, Ib, Ic and Type II) across three single-cell RNA-sequencing datasets from SGNs (Supplemental Figure)[15 16 33]. Consistent with our HCR data, we observed no expression of *Tmprss3* in all three subtypes (A, B, C) of Type I SGNs across all three datasets (Figure 2 and Supplemental Figure). The neuronal marker TuJ1 (*Tubb3* gene) was expressed across all subtypes of SGNs and all datasets (Figure 2 and Supplemental Figure). One dataset demonstrated expression of *Tmprss3* in Type II SGNs in P25 – P27 mice, but

expression in Type II SGNs was not seen in 9-week-old mice (Figure 2 and Supplemental Figure).

Next, we harvested human auditory nerves (HAN) from three patients undergoing translabyrinthine craniotomy for vestibular schwannoma surgery. As expected, these patients had preoperative hearing loss with speech reception thresholds (SRT) between 30–45 dB and word discrimination scores of 0% (patients 1 & 2) and 84% (patient 3) (Figure 3A). To confirm the specificity of our human HCR probe and for qRT-PCR, we cloned the human *TMPRSS3* gene into an expression vector and expressed *TMPRSS3* in HEK293 cells. Using quantitative RT-PCR on HAN, control HEK cells and transfected HEK293 cells we observed robust expression of *TMPRSS3* in the transfected cells and only minimal expression of *TMPRSS3* in the HAN (Figure 3B). As expected, we observed robust expression of myelin basic protein (*MBP*) in HAN and not in HEK cells (Figure 3C). Next, we performed *TMPRSS3* HCR and confirmed the specificity of the probe using *TMPRSS3* transfected HEK293 cells (Figure 3D&E). We did not detect *TMPRSS3* expression within the HAN of either patients 2 or 3 (Figure 3F&G). We confirmed the presence of auditory neurons using TuJ1 immunostaining (Figure 3H–I).

Discussion

Determining factors that impact CI performance is critical for identifying CI candidates and managing patient expectations. CI performance is linked to both age of implantation and hearing loss etiology[23 34 35]. As genomic sequencing has become more feasible, increasing efforts are being made to determine which genetic causes may predict good or poor outcomes after implantation—and, consequently, how these patients should be counseled pre-operatively. Much of this interest has centered on *TMPRSS3* and the still poorly-understood mechanism by which mutations to this gene adversely affect hearing and, possibly, CI outcomes. In this study, however, we find that patients with *TMPRSS3*-related hearing loss do not have worse CI outcomes compared to the general population. Furthermore, we demonstrate that *Tmprss3* is highly expressed in otic sensory epithelia with limited SGN expression.

Poor CI outcomes in *TMPRSS3*-associated hearing loss were originally attributed to *TMPRSS3* expression in SGNs[13]. However, these data were obtained with mouse tissue using an antibody that was not validated for specificity with methods such as *Tmprss3*-knockout tissue or western blot analysis[14]. Our compiled CI outcomes data here indicate that *TMPRSS3* does not play a significant physiologic role in the neuronal aspects of hearing. Our data using validated and highly specific probes for *Tmprss3* in mouse and *TMPRSS3* in human tissues corroborates published scRNA-seq datasets from SGNs (Supplementary Figure 1)[15 16 33], the sensory epithelia[36 37] and the lateral wall[38].

Post-implantation outcomes in *TMPRSS3* patients compared to the general population[24–27 39]. These outcomes compare similarly to a large systematic review of adult CI outcomes of 2798 patients across 46 studies and showed CNC scores improved from 8.3% (12.4) to 54.0% (22.5)[40]. The absence of significantly different outcomes between the *TMPRSS3*

cohort and the general population also suggest that *TMPRSS3* may have no clinically relevant biological role in the auditory nerve.

Duration of deafness is one of the most important factors to predict speech perception after cochlear implantation in postlingually deaf patients[23]. This may be due to progressive degeneration of SGNs. Mouse models mimicking human deafness with degeneration of sensory hair cells uniformly exhibit progressive neuronal death, a secondary effect to the primary lesion[41–43]. Loss of *TMPRSS3* in mouse does not alter the total number of sensory hair cells or total number of SGNs during development[3], yet we and others have shown that loss of *TMPRSS3* in mouse results in rapid HC degeneration at P12 leading to profound deafness[3 7]. Similar to other genetic causes of hearing loss in which the pathology affects the hair cell or the supporting cells of the sensory epithelium, *Tmprss3*-mutant mice also exhibit a delayed-onset progressive SGN degeneration started after P90[3]. The selected *TMPRSS3* patients who exhibited poor performance were all implanted at a significantly older age—and, in at least two cases, with prolonged duration of hearing loss, which most likely affected the overall number and health of residual SGNs. These factors likely played a substantial role in their poor outcome[44].

In order to assess possible genotype-phenotype correlations among various *TMPRSS3* allelic combinations, we compared hearing outcomes in patients with PTxPT, NPTxPT or NPTxNPT combinations. No difference in performance between these groups.

Taken together, patients with *TMPRSS3*-associated SNHL have comparable CI outcomes relative to the general population. Duration of deafness appears to be the key factor predictive of poor performance. While the cause of deafness in patients harboring *TMPRSS3* mutations remains poorly understood, our results suggest that *TMPRSS3* mutations largely affect cochlear hair cells and not the SGN, which would explain the positive CI outcomes in these patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Key messages**WHAT IS ALREADY KNOWN ON THIS TOPIC**

Bi-allelic variants in *TMPRSS3* result in congenital and post-lingual sensorineural hearing loss. Previous reports suggest *TMPRSS3* is expressed in spiral ganglion neurons (SGNs) and portend for poor cochlear implantation (CI) outcomes in patients with *TMPRSS3*-related hearing loss

WHAT THIS STUDY ADDS

We find that CI outcomes in patients with *TMPRSS3*-related hearing loss are similar to other cohorts of CI patients. *Tmprss3* is not expressed in acoustic stimuli-transmitting type I SNGs of mice or humans.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE AND/OR POLICY

This study shows that patients with bi-allelic *TMPRSS3*-related hearing loss have excellent CI performance and argues against the physiologic role of *TMPRSS3* in SGNs.

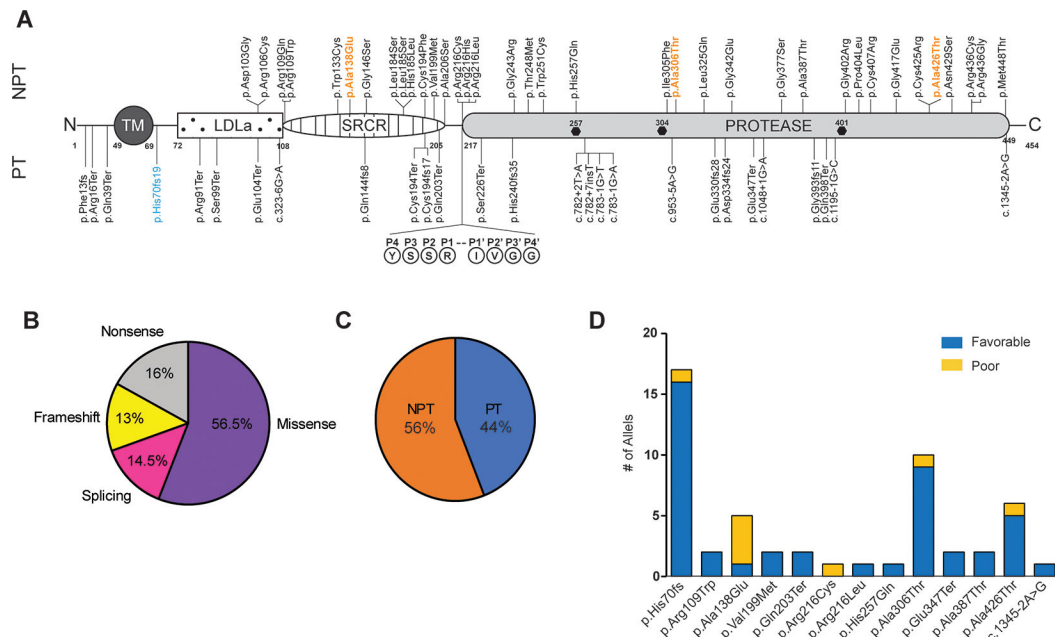


Figure 1: Human *TMPRSS3* Gene Mutations Associated with Hearing Loss.

(A) Schematic of *TMPRSS3* protein and pathogenic/likely pathogenic reported deafness causing variants. Frequent variants causing congenital or postlingual hearing loss denoted in blue and orange, respectively. The *TMPRSS3* autocleavage site (arrowhead) at R216 is shown and hexagons indicate the conserved serine protease catalytic triad (H-D-S). TM, transmembrane domain; LDLa, low-density lipoprotein receptor domain; SRCR, scavenger receptor cysteine-rich domain, PT, protein truncating; NPT, non-protein truncating. The amino acid number for the start and end of protein domains are noted.

Note: p.Gly393fs11 is an approximation-based description of the variant in Scott et al. (B) Pie chart of the percentage of human *TMPRSS3*-associated hearing loss variant types. (C) Pie chart of the percentage of human *TMPRSS3*-associated hearing loss variants PT vs. NPT. (D) In cochlear implant recipients, the relationship between *TMPRSS3* variants and the number of patients with post-operative performance defined as poor or favorable.

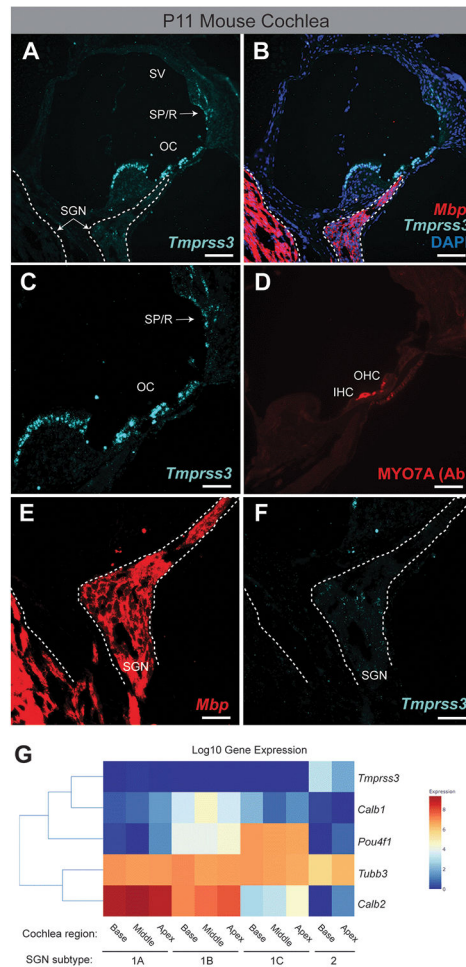


Figure 2: *Tmprss3* is robustly expressed in mouse otic sensory epithelium and not in type I SGNs.

(A) Hybridization chain reaction (HCR) labeling of *Tmprss3* in the P11 mouse cochlea. The SGN is outlined with a dotted line. (B) HCR labeling of *Tmprss3* and *Mbp* to label neurons of the SGN. (C) Higher magnification insets of the otic epithelium. (D) Antibody labeling of the *Myo7A*⁺ inner and outer hair cells. (E&F) Higher magnification of the SGN showing minimal expression of *Tmprss3* near the location of Type II SGNs. (G) Single-cell RNA-sequencing gene expression data for mouse SGN subtypes using gEAR (<http://umgear.org>). Expression data from the P25P27, mouse, scRNA-seq, cochlear sensory neurons dataset[16]. *Tmprss3* is only expressed in Type II SGNs. OC, organ of Corti; SGN, spiral ganglion neurons; SP/R, spindle and root cells; SV, stria vascularis; Ab, antibody. Scale bars: 25 μ m (A, B); 10 μ m (C-F).

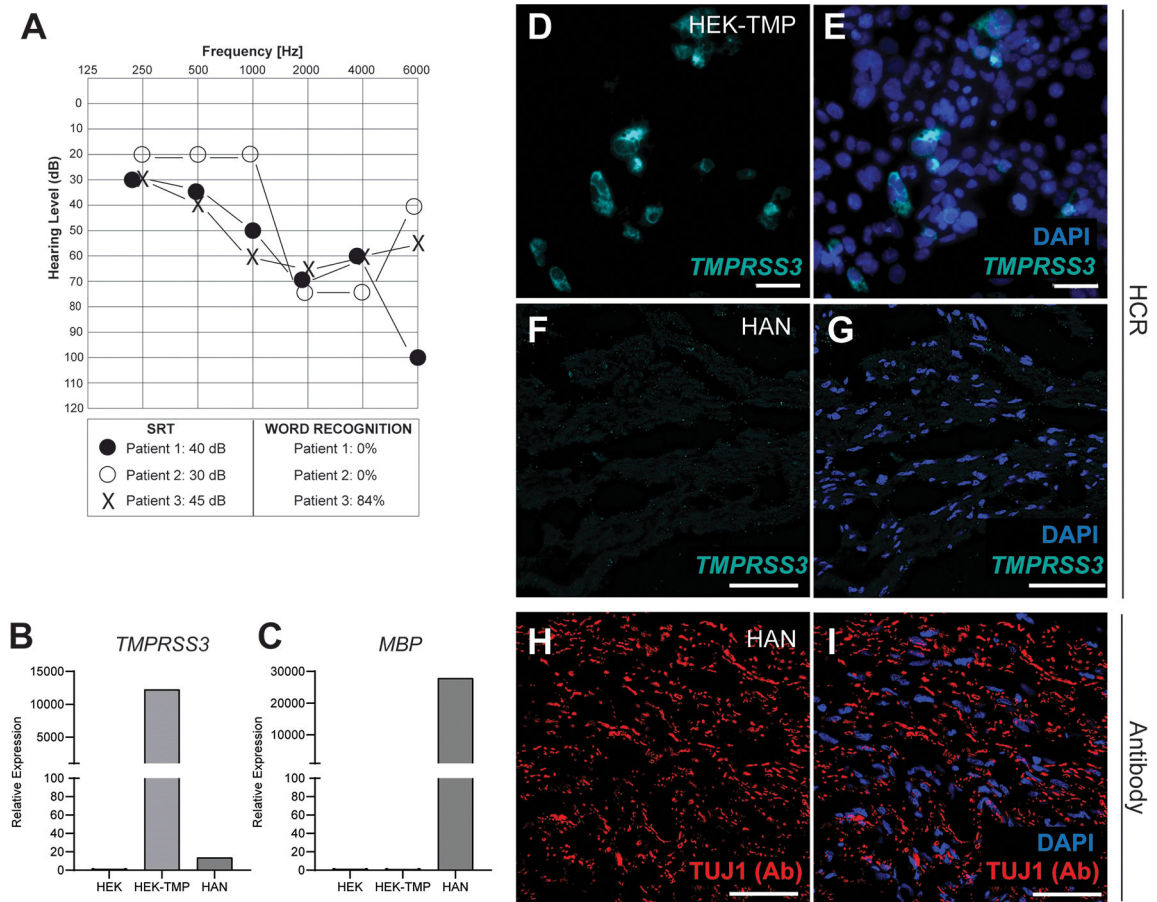


Figure 3: *TMPRSS3* expression is minimal in human auditory neurons (HAN).

(A) Audiogram from three patients whose auditory nerves were analyzed. Circles denote right ear and X denotes the left ear. (B&C) qRT-PCR analysis of *TMPRSS3* and *MBP* of patient #3 HAN shows minimal expression of *TMPRSS3*. (D&E) HCR labeling of human *TMPRSS3* in *TMPRSS3*-transfected HEK293 cells. (F&G) HCR of HAN with *TMPRSS3* shows minimal expression of *TMPRSS3*. (H&I) Antibody (Ab) labeling of HAN with neuron-specific TUJ1. SRT, Speech reception threshold. Scale bar, 10 μ m (D, E), 20 μ m (F - I).

Outcomes of cochlear implantation with or without acoustic stimulation in patients with DFNB8 or DFNB10 phenotypes of TMPRSS3 mutations based on literature review.

Table 1.

Case	Reference	Ethnicity	Age of Onset (years)	Sex	Genotype		Allele Combination	Age at CI	Preop PTA [34]	Preop Word Score (%)	Preop Sentence Score (%)	Postop PTA [34]	Postop Word Score (%)	Postop Sentence Score (%)	Outcome
					Allele 1	Allele 2									
1	Battelino, 2016	Slovenian	<2		c.208delC (p.His70fs)	c.208delC (p.His70fs)	PTxPT	11 mo	80–110			25			Favorable
2	Battelino, 2016	Slovenian	<2		c.208delC (p.His70fs)	c.208delC (p.His70fs)	PTxPT	30 mo	95–110			45			Favorable
3	Battelino, 2016	Slovenian	<2		c.208delC (p.His70fs)	c.208delC (p.His70fs)	PTxPT	13 mo	80–100			25			Favorable
4	Battelino, 2016	Slovenian	<2		c.208delC (p.His70fs)	c.208delC (p.His70fs)	PTxPT	11 mo	70–85			25			Favorable
5	Chung, 2014	Korean	6	F	c.325C>T (p.Arg109Trp)	c.916G>A (p.Ala306Thr)	NPTxNPT	11 yo	70–80					88.5	Favorable
6	Chung, 2014	Korean	3	M	c.325C>T (p.Arg109Trp)	c.916G>A (p.Ala306Thr)	NPTxNPT	5 yo	90–100					88.5	Favorable
7	Eppsteiner, 2012	Caucasian	5	M	c.413C>A (p.Ala138Glu)	c.646C>T (p.Arg216Cys)	NPTxNPT	47 yo	93				28	47	Poor
8	Eppsteiner, 2012	Caucasian	6	F	c.916G>A (p.Ala306Thr)	c.413C>A (p.Ala138Glu)	NPTxNPT	32 yo	98				21	25	Poor
9	Miyagawa, 2013 (L)	Japanese	25	F	c.607C>T (p.Gln203Ter)	c.1159G>A (p.Ala387Thr)	PTxNPT	38 yo	90			30	90		Favorable
10	Miyagawa, 2013 (R)	Japanese	25	F	c.607C>T (p.Gln203Ter)	c.1159G>A (p.Ala387Thr)	PTxNPT	38 yo	90			30	90		Favorable
11	Miyagawa, 2015	Japanese	33	M	c.647G>T (p.Arg216Leu)	c.771C>G (p.His257Gln)	NPTxNPT	45 yo*	93.8			30			Favorable
12	Miyagawa, 2015	Japanese	6	F	c.607C>T (p.Gln203Ter)	c.1159G>A (p.Ala387Thr)	PTxNPT	38 yo*	106.3			30	67.5		Favorable
13	Shearer, 2018***				c.208delC (p.His70fs)	c.413C>A (p.Ala138Glu)	PTxNPT	64 yo				2			Poor
14	Shearer, 2018***				c.413C>A (p.Ala138Glu)	c.1276G>A (p.Ala426Thr)	NPTxNPT	53 yo				29			Poor
15	Shearer, 2018***				c.1345–2A>G	c.1276G>A (p.Ala426Thr)	PTxNPT	38 yo				83			Favorable
16	Weegerink, 2011	Dutch	<2	M	c.208delC (p.His70fs)	c.916G>A (p.Ala306Thr)	PTxNPT					91**			Favorable

Case	Reference	Ethnicity	Age of Onset (years)	Sex	Genotype		Allele Combination	Age at CI	Preop PTA [34]	Preop Word Score (%)	Preop Sentence Score (%)	Postop PTA [34]	Postop Word Score (%)	Postop Sentence Score (%)	Outcome
					Allele 1	Allele 2									
17	Weegerink, 2011	Dutch	<2	F	c.595G>A (p.Val199Met)	c.916G>A (p.Ala306Thr)	NPTxNPT						80**		Favorable
18	Weegerink, 2011	Dutch	17	F	c.208delC (p.His70fs)	c.1276G>A (p.Ala426Thr)	PTxNPT			20**			75		Favorable
19	Weegerink, 2011	Dutch	12	M	c.208delC (p.His70fs)	c.1276G>A (p.Ala426Thr)	PTxNPT			5**			60		Favorable
20	Weegerink, 2011	Dutch	7	F	c.208delC (p.His70fs)	c.1276G>A (p.Ala426Thr)	PTxNPT			0**			58		Favorable
21	Weegerink, 2011	Dutch	17	M	c.208delC (p.His70fs)	c.1276G>A (p.Ala426Thr)	PTxNPT			0**			62		Favorable
22	Weegerink, 2011	Dutch	15	F	c.413C>A (p.Ala138Glu)	c.595G>A (p.Val199Met)	NPTxNPT			2.5**			68		Favorable
23	Song, 2020	Korean	6	F	c.916G>A (p.Ala306Thr)	c.1039G>T (p.Glu347Ter)	PTxNPT	8 yo	70	20	50		100	86	Favorable
24	Song, 2020	Korean	5	F	c.916G>A (p.Ala306Thr)	c.1039G>T (p.Glu347Ter)	PTxNPT	6 yo							Favorable
25	Holder, 2021 (R)		3	F	c.208delC (p.His70fs)	c.916G>A (p.Ala306Thr)	PTxNPT	4.5 yo*	83	44		20	68		Favorable
26	Holder, 2021 (L)							4.8 yo*	83	32		24	75		Favorable
27	Holder, 2021 (R)		3	F	c.208delC (p.His70fs)	c.916G>A (p.Ala306Thr)	PTxNPT	4.8 yo*	67.5			23.3	80		Favorable
28	Holder, 2021 (L)							3.9 yo*	73.3			25	80		Favorable
29	Holder, 2021 (R)		2	F	c.208delC (p.His70fs)	c.916G>A (p.Ala306Thr)	PTxNPT	36 yo*	44			25	82		Favorable
30	Holder, 2021 (L)							36 yo*	42			22.5	92		Favorable
AVERAGE (St.Dev)								18.9 (20.7)	82.4 (18.2)	17.2 (15.2)	26.7 (6.4)	65.5 (26.3)	67 (29.4)		

CI = Cochlear Implantation, PTA = Pure Tone Average.

* Patients who underwent electric acoustic stimulation (both cochlear implantation and hearing aid use).

** Phoneme score rather than monosyllable word recognition score. PT, Protein truncating; NPT, nonprotein truncating.

*** Variants listed in Table 1 of Shearer 2018 are shifted up by 1 line. Grey shading denotes poor outcomes.

Cochlear implant outcomes in *TMPRSS3*-associated hearing loss (bold) and the general hearing loss population.

Table 2.

Study, year	Subjects (n)	Preop CNC WRS - % [14]	Postop CNC WRS - % [14]
This Study	30	17.4 (15.3)	66.2 (25.8)
Kelsall et al, 2021	100	14.6 (11.6)	65.2 (18.8)
Sullivan et al, 2019	60	8.4 (19)	33
Pillsbury III et al, 2018	73	30.4 (13.4)	66.9 (18.5)
Cusumano et al, 2017	26	6.6 (10.1)	42.1 (33.2)
O'Connell et al, 2016	220	n/a	46.6 (23.0)
Kamakura et al, 2016	17	n/a	40.3 (19.4)
Buchman et al, 2014	13	0	60
Zwolan et al, 2001	56	2.25 (3.95)	42.2 (23.5)