Insights into the Pathobiology of TMPRSS3-Related Hearing Loss and Implications for Cochlear Implant Patients with *TMPRSS3* Mutations.

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Abstract

OBJECTIVES

To review the audiological outcomes after cochlear implantation (CI) for *TMPRSS3*-associated autosomal recessive non-syndromic hearing loss (ARNSHL) and evaluate the spatial expression pattern of TMPRSS3 within the human cochlea.

METHODS

Review all published cases of CI in patients with *TMPRSS3*-associated ARNSHL to compare postoperative consonant-nucleus-consonant (CNC) word performance to published adult CI cohorts. Protein structural modeling of TMPRSS3 variants associated with post-lingual hearing loss. Determine TMPRSS3 expression pattern in human inner ear organoids and human cochlea.

RESULTS

Nine articles detailed 27 patients (30 total CI ears) with *TMPRSS3*-associated hearing loss treated with CI. Of these, 6 cases reported prelingual onset (< 2yo) and 24 cases reported post-lingual onset (≥2yo) of hearing loss. Subjectively, 85% of cases had a favorable outcome. Objectively, the postoperative mean (SD) post-operative CNC word score was not significantly different than other adults [66.2% (25.8%) correct vs. 50.1% (12.5%); F(1,6) = 1.97, P = 0.21]. In the *TMPRSS3* cohort, poor performers (CNC < 30% correct) were significantly older than good performers [49 (± 13.3) years vs. 17.4 (± 18.4) years; P < 0.01] and all harbored the A138E variant. TMPRSS3 immunostaining is restricted to the otic epithelial cells and is not expressed within auditory neurons of human cochlea and human inner ear organoids.

CONCLUSIONS

Patients with *TMPRSS3*-related hearing loss exhibit similar postoperative performance to other adult CI patients. TMPRSS3 is not expressed in human auditory neurons and the duration of hearing loss prior to CI likely contributes to poor performance.

Introduction

Transmembrane serine proteases are a large family of proteins that are indispensable regulators in many tissues, including the inner ear.[1] Broadly, they are classified based upon the way 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] One such type II transmembrane serine protease, TMPRSS3, is an integral protein 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 mouse and humans. Mice carrying, protein-truncating TMPRSS3 mutations display normal cochlear and vestibular hair cell (HC) development followed by rapid HC degeneration over 48 hours starting at postnatal day 12.[5] In humans, bi-allelic pathogenic variants in *TMPRSS3* are the 5th most common gene causally associated with hearing loss in a multiethnic cohort of human congenital deafness.[8,9] Most hearing loss associated variants have been linked to congenital profound deafness, while a few select missense mutations (e.g. A306T, A138E, A426T) are linked to post-lingual 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 (CI) remains the gold-standard treatment in inherited severe-profound sensorineural hearing loss (SNHL).[11,12] Implants function by directly stimulating spiral ganglion neurons (SGNs), permitting sound detection and speech recognition, circumventing the need for intact inner ear organs such as cochlear hair cells.[6,11-13] The postoperative CI outcomes in patients with *TMPRSS3*-associated hearing loss remain controversial in the published literature with some patients exhibiting good speech outcomes while other patients exhibit poor outcomes.[6,10,13] The poor performance has been hypothesized to be related to pathology within the spiral ganglion[13] based upon TMPRSS3 reported immunostaining within spiral ganglion neurons (SGNs) in mouse cochlear slices.[14] However, recent scRNA-seq studies demonstrate 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] These conflicting biological data and patient outcomes lead us to comprehensively review the postoperative CI outcomes of all patients with *TMPRSS3*-mediated hearing loss.

In this study, we show that TMPRSS3 is not expressed in SGNs of human cochlea or neuronal cell populations of human inner ear organoids. We show that patients with biallelic TMPRSS3 variants have similar postoperative performance outcomes to other reported cohorts and the allelic combination of patients with TMPRSS3-related hearing loss does not impact CI performance. Finally, we conclude patients with TMPRSS3 mutations have overall good CI outcomes, with age of implantation being the most likely factor for the few poor outcome reports.

Materials And Methods

TMPRSS3 hearing loss causing variants

We collected all reported TMPRSS3-deafness causing variants via a literature review from PubMed and by using the Deafness Variation Database (https://deafnessvariationdatabase.org/).[17] The protein structural domains were determined using NCBI for human TMPRSS3 (assession number AAQ88894). The protease catalytic triad was identified using homology to other serine proteases.

TMPRSS3 Model Building

Three-dimensional (3D) modeling of human wild type TMPRSS3 was performed using SWISS-MODEL protein structure homology-modelling software[18-22] and I-TASSER protein structure and function predictions software.[23-25] The human TMPRSS3 protein (AAQ88894) was uploaded in FASTA format to each online server and 3D renderings of TMPRSS3 were produced. Missense variants linked to HL were also modeled using both modeling software platforms.

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 included for final review. A total of nine unique articles were identified. Demographics, age of SNHL onset, phenotypic presentation, genetic 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 prelingual hearing loss whereas those with onset two years of age or older were designated as post-lingual hearing loss. Objective outcomes included pre-operative and postoperative pure tone averages (PTA), CNC word recognition scores (WRS), and sentence scores (SS). Subjective outcomes, when reported in published articles, were summarized as either favorable or poor. Postoperative implant-aided CNC WRS scores less than 30% were considered poor outcomes, and scores 50% or greater were considered favorable.

Post-lingual cochlear implant outcomes

Two independent searches of PubMed for articles published on adult cochlear implant outcomes were conducted. Search terms included the combination of *"cochlear implant outcomes"* with the major subheading topic *"hearing loss".* Articles that contained more than 10 patients and had postoperative CNC word scores were searched. Demographics, age of SNHL onset, phenotypic presentation, patient age at time of CI, and audiologic outcomes were recorded.

Statistical Analysis

Objective data were evaluated by measures of central tendency using Microsoft Excel, 2016. Mean age difference between good and poor post-lingual CI performers was evaluated by T-test using SPSS Statistics, 2020. *TMPRSS3*-associated hearing loss CI outcomes were compared to postoperative outcomes of eight CI studies in the general hearing loss population. Mean postoperative CNC WRS analysis of variance (ANOVA) of the two populations was performed using SPSS Statistics, 2020.

Human Organoids and Human Cochlea

Human stem-cell derived inner ear organoids were a kind give of Eri Hashino, PhD. Human cochlea tissue was harvested according to the IRB approved protocol (2006345852) from an adult male during resection of a skull base cholesteatoma that eroded the cochlea.

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, Coralville, IA). The construct p3XFLAG-CMV 7.1_Tmprss3 were 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. The NEB® 5-alpha Competent *E. coli* (New England Biolabs, Ipswich, MA; C2987H) were used for transformation. *TMPRSS3* missense mutations were generated using the Q5 site-directed mutagenesis kit (New England Biolabs).

Immunoblotting

HEK293 cells (ATCC, CRL-1573), plated in 6 well plates, were transfected with p3XFLAG-CMV 7.1_Tmprss3 plasmid using Lipofectamine 3000 Reagent (Thermo Fisher, L3000015) for 24 hrs as described by the manufacturer. Cells were washed once with ice-cold PBS and lysed with RIPA Lysis and Extraction Buffer (Thermo Fisher, Waltham, MA; 89901) supplemented with protease (Thermo Fisher, 78430). The protein concentration of the samples was measured using BCA Protein Assay Kit (Thermo Fisher, 23225). 40 µg of total protein per sample were resolved by SDS-PAGE (4%–20% gels) and transferred to Immun-Blot® PVDF Membranes (Bio-Rad, Hercules, CA; 1620174). Membranes were blocked for 1 hour at room temperature in 5% milk with TBST (.05 % Tween-20 in 1x TBS). Primary antibodies were incubated overnight at 4°C. The antibodies used were mouse anti-FLAG (Sigma Aldrich, F1084, 1:16,000) and rabbit anti-Tmprss3 (Thermo Fisher, PA5-35325, 1:200). 1 wash with TBST and followed by HRP-linked goat anti-rabbit (Bio-Rad, 1705046, 1:25,000) and rat anti-mouse (Abcam, Ab131368, 1:1,000) secondary antibodies were incubated for 1 hour at room temperature. Three washes with TBST after incubation followed by development of blots using Clarity Western ECL Substrate (Bio-Rad, 1705061) and imaged with ChemiDoc Touch Imaging System (Bio-Rad, 1708370)

Immunofluorescence

Human cochlea samples were harvested and fixed in 10% formalin for 24 hours. Fixed cochleae were then decalcified for 2 hrs at room temperature with 120 mM EDTA in PBS and 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. Sections of 6 µm were cut on a cryostat and air-dried for 2 hr. The tissue was blocked with 10% goat serum with 0.1% Tween-20 in PBS for 30 mins at 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 antibodies we used were rabbit anti-TMPRSS3 (Thermo Fisher, PA5-35325, 1:50), mouse anti-TUJ1 (BioLegend, 801202, 1:100), rabbit anti-myosin VIIA (Proteus, 25-6790, 1:100), and mouse anti-SOX2 (BD Pharmingen, 561469, 1:50). 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. Sections were washed and mounted with ProLong™ Gold Antifade Mountant with DAPI (ThermoFisher, P36931). Staining was visualized on Leica DMi8 microscopy.

Results

The Mutational landscape of *TMPRSS3*-related hearing loss.

To date there are 85 variants in *TMRPSS3* that are associated with hearing loss (**Figure 1A, Supp Table 1**). Mutations within all domains of TMPRSS3 have been linked to hearing loss (**Figure 1A**). Of these, 59 (~69%) are missense variants, 9 (~10.6%) are nonsense, 9 (~10.6%) are frameshift indels and 8 (~9.4%) are splice-altering (**Figure 1A and 1B**). Non-protein truncating variants contribute to ~69% of hearing loss associated variants in TMPRSS3, whereas only ~31% are protein truncating (**Figure 1C**).

CI outcomes of patients with *TMPRSS3*-related hearing loss

A total of nine articles describing 27 patients who received CI for *TMPRSS3*-associated hearing loss (**Table 1**). Three patients were implanted bilaterally yielding a total of 30 CI outcomes. There were over twice as many females compared to males (70% vs. 30%) and the cohort included patients from various ethnic backgrounds (**Table 1**). Hearing loss onset was reported in all patients with 80% reporting post-lingual and 20% reporting prelingual onset.

Subjectively 85% of patients had a favorable outcome with CI. Objectively, the mean (SD) PTA improved from 81.9 (±17.6) dB to 26.7 (±6.4) dB while the mean CNC word score improved from 17.4% (±15.3%) correct to 66.2% (±25.8%) correct (**Table 1**). These outcomes were comparable to CI outcomes of the general hearing loss population (**Table 2**). Eight large studies of more than 10 patients reported post-lingual adult CI outcomes for 565 individuals and these patients exhibited a mean (SD) post-operative CNC word score of 50.1% (12.5%).[26-33] 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)[34]. A one-way ANOVA was conducted to compare mean postoperative CNC WRS between the *TMPRSS3* cohort and the eight adult hearing loss studies [66.2% (25.8) vs. 50.1% (12.5)]. There was not a significant difference between CI outcomes in the *TMPRSS3* cohort and outcomes of the eight adult hearing loss studies at the $P<0.05$ level for the seven conditions [$F(1,6) = 1.97$, $P=0.21$].

Genotype and allelic combination do not impact CI outcomes

In the cohort, 15 different alleles contributed to the genetic diagnosis of TMPRSS3 (**Figure 1D**). The most common allele was the frameshift variant c.208delC (p.His70fs) which account for ~1/3 of total alleles (17/54). This is followed by c.916G>A (p.Ala306Thr), c.1276G>A (p.Ala426Thr), c.413C>A (p.Ala138Glu) which comprised 10, 6 and 5 alleles, respectively. The rest of the 11 variants accounted for 2 or 1 alleles.

The most common variant type combination was PTxNPT accounting for 15 cases (14 favorable outcomes vs 1 poor outcome), followed by 8 NPTxNPT (5 favorable outcomes vs 3 poor outcomes), and 4 PTxPT (4 favorable outcomes vs 0 poor outcomes). Statistical analysis (Chi-Square test) could not be performed because several conditions had less than 5 events.

The four poor outcomes all carried the c.413C>A (p.Ala138Glu) variant (**Table 1**), but the second allele was different in each case (p.His70fs, p.Arg216Cys, p.Ala306Thr, and p.Ala426Thr). The c.413C>A (p.Ala138Glu) variant was also seen in one favorable outcome, opposite the c.595G>A (p.Val199Met) variant. Alleles p.His70fs, p.Ala306Thr, and p.Ala426Thr alleles were also seen in patients with favorable outcomes (**Table 1**).

Age of implantation correlates with CI performance.

The mean age of implantation for individuals with prelingual HL was 1.4 (±0.8) years and 24.1 (±21.6) years for individuals with post-lingual HL. The overall mean age of implantation of the entire cohort, irrespective of age of onset was 20.1 (±21.5) years (**Table 1**). The mean (SD) age of poor post-lingual performers was 49 (±13.3) whereas the mean (SD) age of good performers was 17.4 (±18.4). Poor post-lingual performers were significantly older than good performers [$t(17.4) = 4.7$, $P=0.018$]. In addition, poor performers were significantly older than good performers (49 (13.3) years vs. 17.4 (18.4) years; $P<0.01$).

TMPRSS3 Protein Modeling

The I-TASSER human TMPRSS3 model demonstrated a plausible orientation in which the primary structure of TMPRSS3 folds upon itself and highlighted three important domains: the serine protease, scavenger receptor cysteine-rich, and low-density lipoprotein receptor like domains (**Figure 2A**). Modeling of the protease domain with substrate ligand demonstrates the mouth-like binding pocket (**Figure 2B**).

The SWISS MODEL software produced a 3D rendering of human TMPRSS3 that was similar to I-TASSER model. This model allows for highlighting the numerous cysteine residues within the protein, which can form disulfide bonds (**Figure 3**). Cysteine residues that are distant from each other within the primary TMPRSS3 sequence come in close proximity within the tertiary structure to form disulfide bonds (**Figure 3B**). Critical catalytic triad residues (His257, Asp304, and Ser401) are in close proximity within the tertiary structure (**Figure 3B**).

The point mutations most frequently identified in post-lingual patients, Ala138, Ala306, and Ala426 (**Figure 1A**) were mapped to the 3D TMPRSS3 model (**Figure 3B & C**). These point mutations are located within 2 amino acids of either a cysteine residue involved in disulfide bonding or a key amino acid of the catalytic triad (**Figure 3B**). Amino acid Ala306 resides near Asp304, one of the three key amino acids involved in the TMPRSS3 catalytic triad and Ala426 resides next to Cys425 which formed a disulfide bond with Cys397 (**Figure 3B & 3C**). The Ala138Glu mutation was identified within a conserved alpha-helix of

the SRCR domain and near Cys142 which forms disulfide bond with Cys204 (**Figure 3B**). Thus, post-lingual point mutations likely disrupt the tertiary structure and disulfide bonds within the TMPRSS3 protein resulting in lower enzymatic function or substrate binding.

TMPRSS3 antibody is specific in human TMPRSS3

The human TMPRSS3 gene was cloned into a FLAG-epitope expression vector and expressed in HEK293 cells. Four other TMPRSS3 point mutations known to cause human hearing loss or that deletes the catalytic serine residue were generated and expressed in HEK293 cells. Western blot analysis demonstrates expression of FLAG-TMPRSS3 and point mutants using the FLAG antibody (**Figure 4A**). The TMPRSS3 antibody displays high specificity for the TMPRSS3 protein, which directly overlaps the FLAG immunostaining (**Figure 4A**).

TMPRSS3 is Not Expressed in Human Auditory Neurons

Human inner ear organoids were culture to day 60, which is when POU4F3^{+}-hair cells within otic vesicles are detected. TMPRSS3 displays highly specific staining for the otic vesicles containing otic hair cells (**Figure 4B**). Human cochlea sensory epithelium was obtained intraoperatively from a patient with an invasive cholesteatoma. The human organ of Corti contains MYO7A^{+}- and Phalloidin^{+}-hair cells, SOX2^{+}-supporting cells and TUJ1^{+}-auditory neurons (**Figure 4C&D**). TMPRSS3 displays highly specific expression within the hair cells, but there is no observed staining of TMPRSS3 within the TUJ1^{+}-auditory neurons (**Figure 4D**).

Discussion

Elucidating the determinants of cochlear implantation performance is critical for identifying good candidates for CI and managing patient expectations. There is an increasing amount of data to support that CI outcome is linked to both the age of implantation[35-37] and the origin of the primary lesion (cochlear vs neuronal).[38] For the latter, knowing the genetic cause of deafness can help predict CI outcomes. Here we add *TMPRSS3* to the growing list of deafness-associated genes in which patients have good CI performance and call into question the association between TMPRSS3-related hearing loss and poor CI performance.

Poor CI outcomes in *TMPRSS3*-associated hearing loss were originally attributed to TMPRSS3 expression in spiral ganglion or auditory neurons.[13] However, these data were obtained with mouse tissue using an antibody that was not validated for specificity.[14] Although our staining of human tissue and published scRNA-seq data[15,16] contradict these previous data, the functional outcomes of TMPRSS3 patients with CI also indicate that TMPRSS3 has no clinically relevant biological role in the auditory nerve.

As a whole, CI outcomes were favorable and improvement in postoperative CNC word scores were comparable to prior studies of CI outcomes in the general hearing loss population.[26-29,39] The selected TMPRSS3 patients who exhibited poor performance all exhibited post-lingual hearing loss and all were implanted at a significantly older age. Two patients had onset of HL at age 5 or 6 with implantation at age 47 and 32, respectively. Two other poor performers did not have an age of onset reported but were implanted in the 5th and 6th decade of life. This suggests that duration of deafness in TMPRSS3 patients likely plays a significant role in the poor performance. It is known that duration of deafness prior to CI is associated with poor postoperative performance.[40] Regardless of the four poor performers in this study, our post-operative CNC word scores remained comparable to CI outcomes of the general hearing loss population.[26-33]

Interestingly, all poor performers carry the p.Ala138Glu mutation, but with different alleles in trans. The p.Ala138Glu variant occurs in the SRCR domain. While the exact pathomechanism of this variant remains unknown, it is unlikely that this specific variant, and no other variants examined are associated with poor CI outcomes. Three of the four alleles seen in trans with the p.Ala138Glu variant were also seen in individuals with good CI outcomes and the p.Ala138Glu allele was identified in one individual with good outcomes. For many deafness-associated genes, variant location, variant type and allelic combination can result in different phenotypes.[10,41,42] To see if allelic combination plays a role in CI outcomes for TMPRSS3 patients we compared patients with PTxPT, NPTxPT or NPTxNPT and found there is no difference in performance between these groups.

TMPRSS3 3D-modeling revealed that amino acids affected in post-lingual variants reside near either the catalytic triad or cysteine residues. Prior studies agree that cysteine-rich domains likely play a critical role in TMPRSS3 tertiary protein structure.[5,43-45] Across all mammalian proteins, 2.2% of the amino acids are cysteine residues, however the TMPRSS3 protein contains 4.4% cysteine residues (20 cysteines out of 454 amino acids).[46] Of all TMPRSS3 point mutations linked to hearing loss, 11.3% (6 of 53) of the altered amino acids involve a cysteine residue (Cys73, Cys194, Cys242, Cys386, Cys407, Cys425; (**Figure 1**). This may further highlight the importance of cysteine residues and structural disulfide bonds in TMPRSS3. However, further studies are needed to determine the precise role of these amino acids in structure and function.

In summary, these data suggest that patients with *TMPRSS3*-associated ARNSHL have generally favorable outcomes following CI. Furthermore, the CI outcomes among the *TMPRSS3*-hearing loss population are similar to CI outcomes among the general hearing loss population. Patients with pre-lingual onset hearing loss, such as those harboring c.208delC (p.H70fs) mutations, appeared to yield better CI outcomes when implanted early. Post-lingual hearing loss patients also tended to produce favorable CI outcomes so long as they were implanted early. Poor performers were all implanted late with prolonged duration of deafness. In general, the cause of deafness in patients harboring *TMPRSS3* mutations remains poorly understood, however, the etiology is likely multifactorial. Regardless of this mechanism, patients with *TMPRSS3*-associated ARNSHL benefit from CI.

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Tables

Table 1. Outcomes of cochlear implantation with or without acoustic stimulation in patients with DFNB8 or DFNB10 phenotypes of TMPRSS3 mutations based on literature review. 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.

Case	Reference	Ethnicity	Age of Onset (years)	Sex	Genotype		Allele Combination	Age at CI	Preop PTA (dB)	Preop Word Score (%)	Preop Sentence Score (%)	Postop PTA (dB)
					Allele 1	Allele 2						
1	Battelino, 2016	Slovenian	<2		c.208delC (p.His70fs)	c.208delC (p.His70fs)	PTxPT	11 mo	80-110			25
2	Battelino, 2016	Slovenian	<2		c.208delC (p.His70fs)	c.208delC (p.His70fs)	PTxPT	30 mo	95-110			45
3	Battelino, 2016	Slovenian	<2		c.208delC (p.His70fs)	c.208delC (p.His70fs)	PTxPT	13 mo	80-100			25
4	Battelino, 2016	Slovenian	<2		c.208delC (p.His70fs)	c.208delC (p.His70fs)	PTxPT	11 mo	70-85			25
5	Chung, 2014	Korean	6	F	c.325C>T (p.Arg109Trp)	c.916G>A (p.Ala306Thr)	NPTxNPT	11 yo	70-80			
6	Chung, 2014	Korean	3	M	c.325C>T (p.Arg109Trp)	c.916G>A (p.Ala306Thr)	NPTxNPT	5 yo	90-100			
7	Eppsteiner, 2013	Caucasian	5	M	c.413C>A (p.Ala138Glu)	c.646C>T (p.Arg216Cys)	NPTxNPT	47 yo	93			
8	Eppsteiner, 2013	Caucasian	6	F	c.916G>A (p.Ala306Thr)	c.413C>A (p.Ala138Glu)	NPTxNPT	32 yo	98			
9	Miyagawa, 2013	Japanese	25	F	c.607C>T (p.Gln203Ter)	c.1159G>A (p.Ala387Thr)	PTxNPT	38 yo	90			30
10	Miyagawa, 2015	Japanese	33	M	c.647G>T (p.Arg216Lue)	c.771C>G (p.His257Gln)	NPTxNPT	45 yo*	93.8	30		
12	Miyagawa, 2015	Japanese	6	F	c.607C>T (p.Gln203Ter)	c.1159G>A (p.Ala387Thr)	PTxNPT	38 yo*	106.3	18		30
12	Miyagawa, 2015	Japanese	30	F	c.212T>C (p.Phe71Ser)	c.617-4dup	PTxNPT	51 yo*	78.8	40		
13	Shearer, 2018				c.208delC (p.His70fs)	c.413C>A (p.Ala138Glu)	PTxNPT	64 yo				
14	Shearer, 2018				c.413C>A (p.Ala138Glu)	c.1276G>A (p.Ala426Thr)	NPTxNPT	53 yo				
15	Shearer, 2018				c.1345-2A>G	c.1276G>A (p.Ala426Thr)	PTxNPT	38 yo				
16	Weegerink, 2011	Dutch	<2	M	c.208delC (p.His70fs)	c.916G>A (p.Ala306Thr)	PTxNPT					
17	Weegerink, 2011	Dutch	<2	F	c.595G>A (p.Val199Met)	c.916G>A (p.Ala306Thr)	NPTxNPT					
18	Weegerink, 2011	Dutch	17	F	c.208delC (p.His70fs)	c.1276G>A (p.Ala426Thr)	PTxNPT			20**		
19	Weegerink, 2011	Dutch	12	M	c.208delC (p.His70fs)	c.1276G>A (p.Ala426Thr)	PTxNPT			5**		
20	Weegerink, 2011	Dutch	7	F	c.208delC (p.His70fs)	c.1276G>A (p.Ala426Thr)	PTxNPT			0**		
21	Weegerink, 2011	Dutch	17	M	c.208delC (p.His70fs)	c.1276G>A (p.Ala426Thr)	PTxNPT			0**		
22	Weegerink, 2011	Dutch	15	F	c.413C>A (p.Ala138Glu)	c.595G>A (p.Val199Met)	NPTxNPT			2.5**		
23	Song, 2020	Korean	6	F	c.916G>A (p.Ala306Thr)	c.1039G>T (p.Glu347Ter)	PTxNPT	8 yo	70	20	50	
24	Song, 2020	Korean	5	F	c.916G>A (p.Ala306Thr)	c.1039G>T (p.Glu347Ter)	PTxNPT	6 yo				
25	Holder, 2021 (R)		3	F	c.208delC (p.His70fs)	c.916G>A (p.Ala306Thr)	PTxNPT	4.5 yo*	83	44		20
26	Holder, 2021 (L)							4.8 yo*	83	32		24
27	Holder, 2021 (R)		3	F	c.208delC (p.His70fs)	c.916G>A (p.Ala306Thr)	PTxNPT	4.8 yo*	67.5			23.3

28	Holder, 2021 (L)						3.9 yo*	73.3			25
29	Holder, 2021 (R)	2	F	c.208delC (p.His70fs)	c.916G>A (p.Ala306Thr)	PTxNPT	3.6 yo*	44			25
30	Holder, 2021 (L)						3.6 yo*	42			22.5
	AVERAGE						**20.1**	**81.9**	**19.2**	**50**	**26.7**
	(St.Dev)						**(21.5)**	**(17.5)**	**(16.0)**		**(6.4)**

Table 2. Cochlear implant outcomes in *TMPRSS3*-associated hearing loss (bold) and the general hearing loss population.

Study, year	Subjects (n)	Preop CNC WRS - % (SD)	Postop CNC WRS - % (SD)
Tucker et al, 2021	**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)

Figures

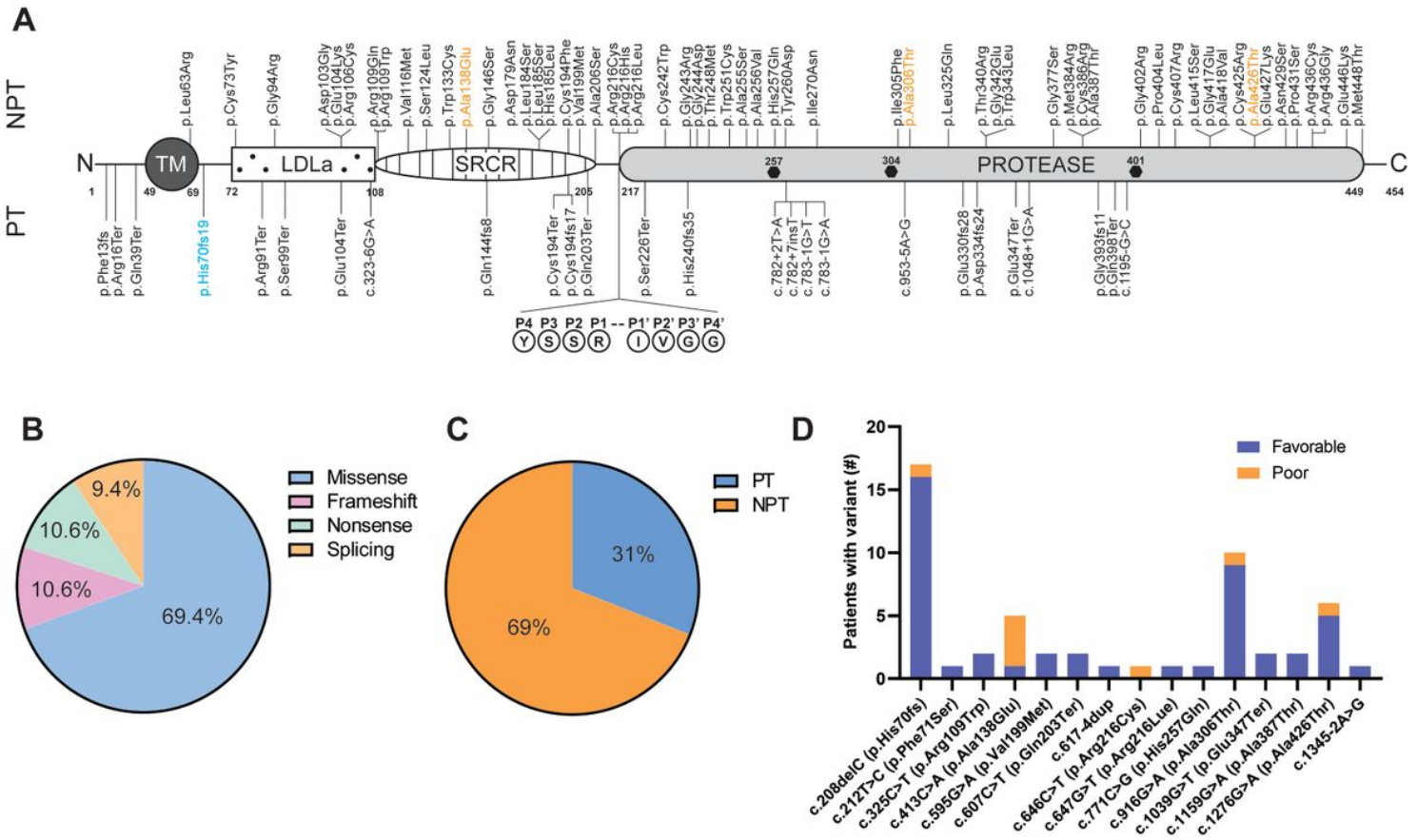

Figure 1

Human TMPRSS3 Gene Mutations Associated with Hearing Loss. (A) Schematic of TMPRSS3 protein and reported deafness causing variants. Frequent variants causing congenital or post-lingual 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. Amino acid number for start and end of protein domains are noted. Note: c.36del and c.36dup both result in a frameshift starting at residue Phe13 and result in terminations of 12 and 10 amino acids downstream, respectively. C771C>G and c.771C>G both create the same change p.His257Gln.; p.Gly393fs11 is an approximation based description of 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 that are PT or NPT. (D) In cochlear implant recipients, relationship between TMPRSS3 variants and number of patients with postoperative performance defined as poor or favorable.

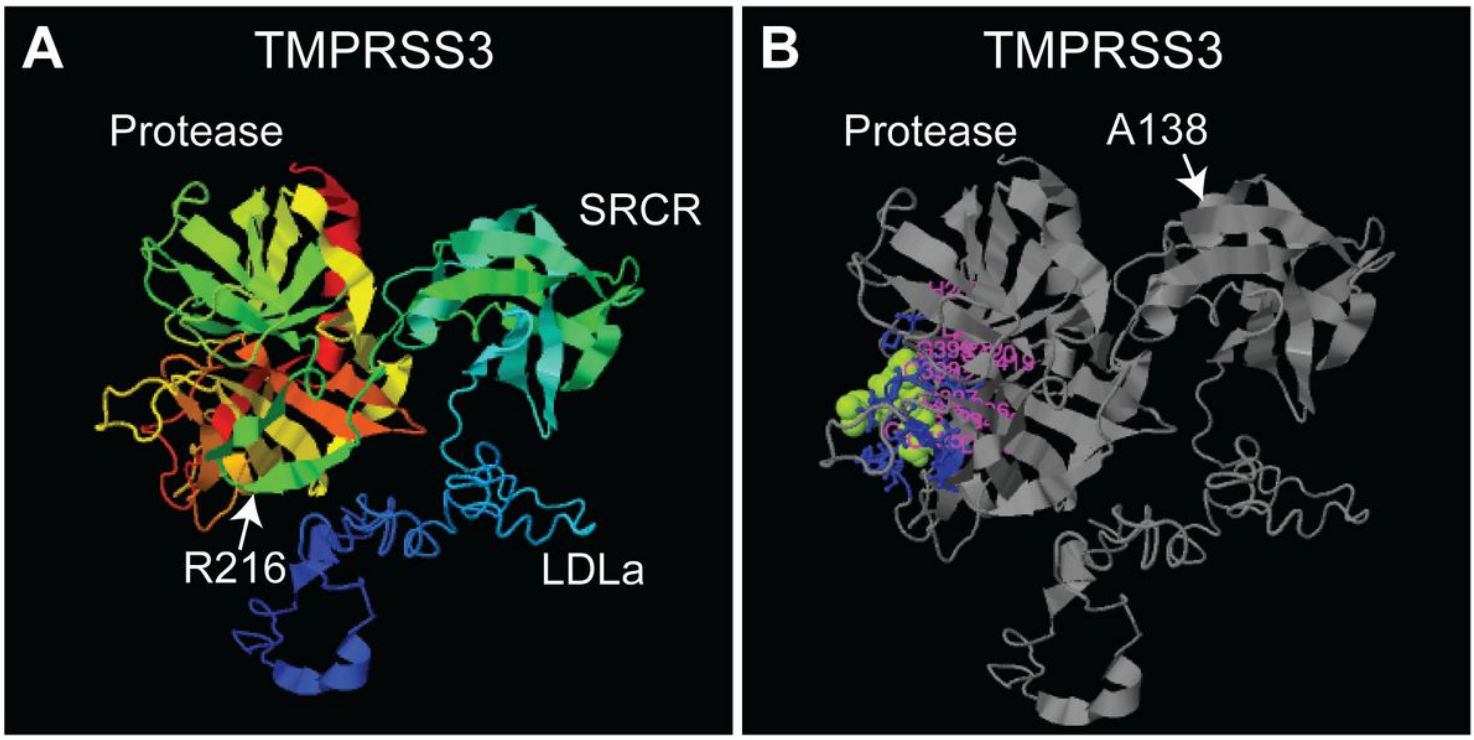

Figure 2

3D I-TASSER Model of TMPRSS3 Protein. (A) I-TASSER modeling of the 3D structure of TMPRSS3 with the mouth-like protease domain, low-density lipoprotein receptor domain (LDLa) and scavenger receptor cysteine-rich domain (SRCR). The autocleavage site R216 is noted. (B) I-TASSER 3D modeling of TMPRSS3 with substrate within protease pocket. The A138 residue is noted and is within the only alpha helix of the SRCR domain.

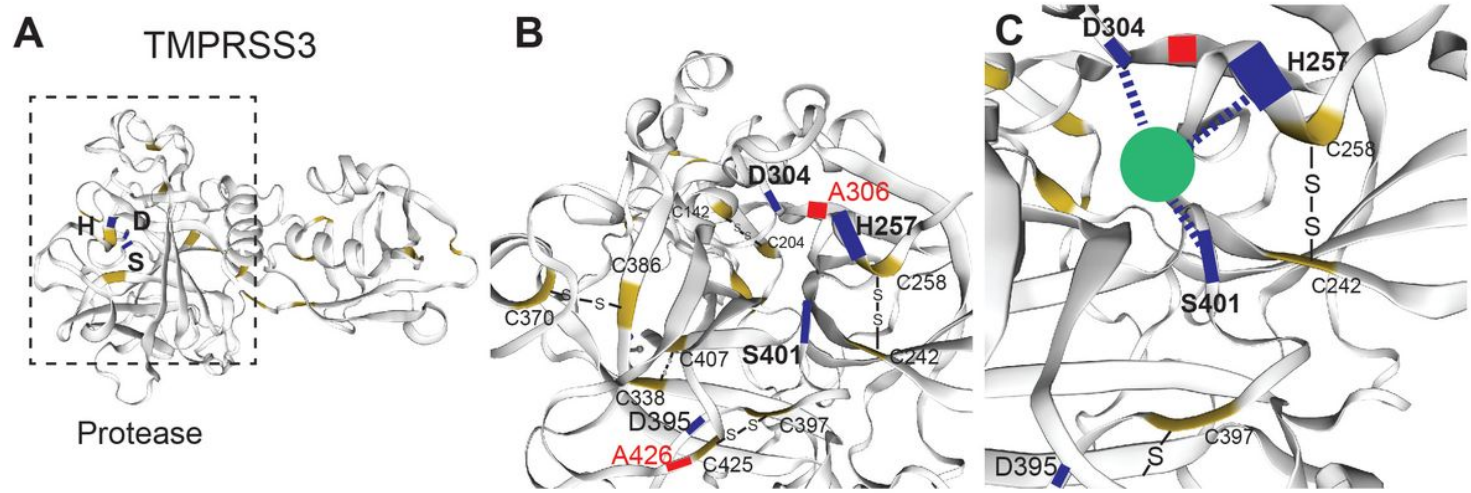

Figure 3

3D SWISS-MODEL of TMPRSS3 Protein. (A) TMPRSS3 3D structure model with cysteine residues highlighted in gold. The catalytic triad (H257, D304, and S401) are highlighted and are in proximity within the 3D structure. (B) Close up view of the protease domain in A, showing disulfide bonds between cysteine

residues (gold), the catalytic triad (blue) and known hearing loss mutations (red). (C) Catalytic triad (blue) with superimposed substrate binding (green ball) and disulfide bonds (gold).

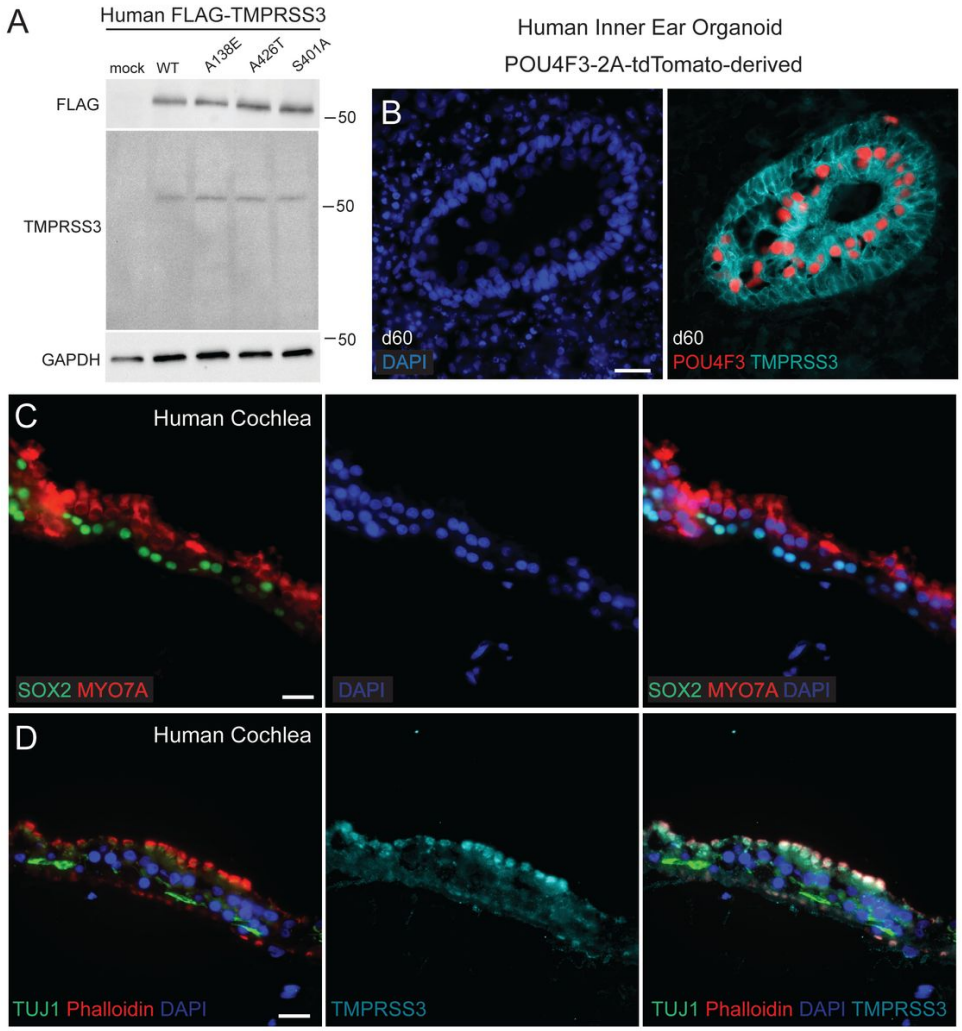

Figure 4

TMPRSS3 is Expressed in Human Otic Epithelium and Not Auditory Neurons. (A) Validation of TMPRSS3 antibody in transfected cells with various human TMPRSS3 mutant constructs. (B) Human ESC-derived inner ear organoids stained with TMPRSS3 antibody. Note the staining is limited to the otic vesicle containing POU4F3+ hair cells. (C) Human cochlea stained with SOX2 (support cells, green) and MYO7A (hair cells, red). (D) Human cochlea stained with TUJ1 (neurons, green), Phalloidin (hair cells, red) and TMPRSS3 (cyan). Note there is no overlap of TMPSS3 staining with TUJ1 positive auditory neurons. Scale bar, 25 μm (B), 20 μm (C, D).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementalTable1.xlsx