Tmc1 p.D569N Mutation Induces Tip-Link Aberrant Morphology & Outer Hair Cell Loss

Corentin Affortit, Miles J. Klimara, Hela Azaiez, Richard J.H. Smith

corentinaugustepierre-affortit@uiowa.edu

Molecular Otolaryngology & Renal Research Laboratories, University of Iowa,Iowa City, IA, USA

Introduction:

TMC1 encodes transmembrane channel-like protein isoform 1 (TMC1), a major component of the hair cell mechano-transduction channel. Both dominant and recessive *TMC1* mutations (associated with DFNA36 and DFNB7/11, respectively), are reported and in aggregate represent ~2% of genetic hearing loss. In this project, we focused on p.D572N mutation which is associated postlingual progressive sensorineural hearing loss. To decipher the mechanisms involved in this type of *TMC1*-associated hearing loss, we studied a *Tmc1* mouse model with an orthologous mutation (p.D569N).

Methods:

Auditory function was assessed at postnatal day p15, p21, p30 and p60 in *Tmc1*$^{D569N/+}$ and WT mice by measuring auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE). Cochlear cell morphology was analyzed using scanning and transmission electron microscopy (SEM and TEM) and hair cell loss was quantified by immunolabeling methods.

TMC1 p.D572N induces early and progressive hearing loss

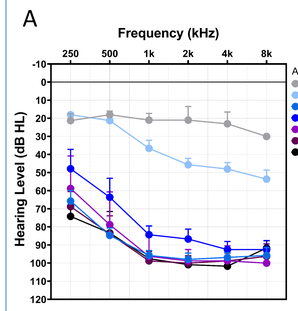

Figure 1: *TMC1* p.D572N induces early-onset hearing loss with severe and fast progression.
A: Age-related typical audiograms demonstrate the progression of hearing loss associated with the p.D572N mutation in different life decades. Audiograms have been collected from affected individuals in 7 families. Data are expressed as mean ± SEM. **Note:** TMC1 p.D572N induce early onset of hearing impairment with a loss ~25dB in high frequency in the first 5 years and progresses quickly during the second decade to 60-90dB. **B:** Sequence alignment across species, shows that p.D572N is located in a highly conserved region of the *TMC1* gene.

Early onset of hearing impairment

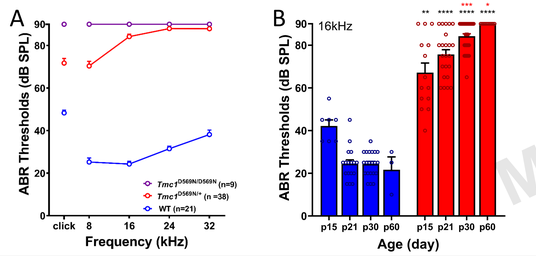

Figure 2: *Tmc1*^{D569N} induces early onset of hearing impairment that quickly progresses.
A: 1-month ABR thresholds recorded in WT (blue) and Tmc1$^{D569N/+}$ (red) and Tmc1$^{D569N/D569N}$ (purple) mice.
B: Average of threshold at 16kHz from p15 to p60. Data are expressed as mean ± SEM. One-way ANOVA test was followed by Dunn's test. *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.005, **** P ≤ 0.0001. Black asterisks, Tmc1$^{D569N/+}$ *vs* WT mice of the same age; red asterisks, older Tmc1$^{D569N/+}$ *vs* p15 -old Tmc1$^{D569N/+}$.

Reduction of the OHC function

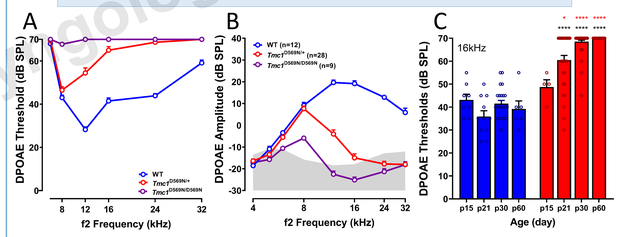

Figure 3: *Tmc1*^{D569N} induces loss of OHC function with reduced DPOAE amplitude.
A-B: DPOAE threshold (**A**) and DPOAE amplitude (**B**) recorded at 1 month in WT (blue), Tmc1$^{D569N/+}$ (red) and Tmc1$^{D569N/D569N}$ (purple) mice. **C:** Average DPOAE amplitude at 16kHz from p15 to p60. Data are expressed as mean ± SEM, one-way ANOVA test was followed by Dunn's test. *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.005, **** P ≤ 0.0001. Black asterisks, Tmc1$^{D569N/+}$ *vs* WT mice of the same age; red asterisks, older Tmc1$^{D569N/+}$ *vs* p15 -old Tmc1$^{D569N/+}$.

More severe OHC loss

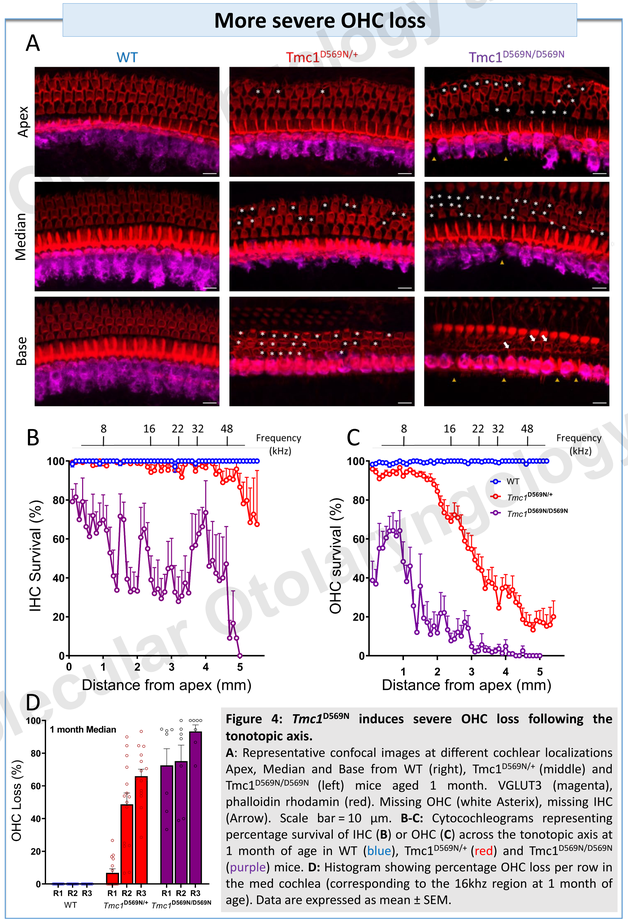

Figure 4: *Tmc1*^{D569N} induces severe OHC loss following the tonotopic axis.
A: Representative confocal images at different cochlear localizations Apex, Median and Base from WT (right), Tmc1$^{D569N/+}$ (middle) and Tmc1$^{D569N/D569N}$ (left) mice aged 1 month. VGLUT3 (magenta), phalloidin rhodamin (red). Missing OHC (white Asterix), missing IHC (Arrow). Scale bar = 10 µm. **B-C:** Cytocochleograms representing percentage survival of IHC (**B**) or OHC (**C**) across the tonotopic axis at 1 month of age in WT (blue), Tmc1$^{D569N/+}$ (red) and Tmc1$^{D569N/D569N}$ (purple) mice. **D:** Histogram showing percentage OHC loss per row in the med cochlea (corresponding to the 16khz region at 1 month of age). Data are expressed as mean ± SEM.

Early and progressive OHC loss

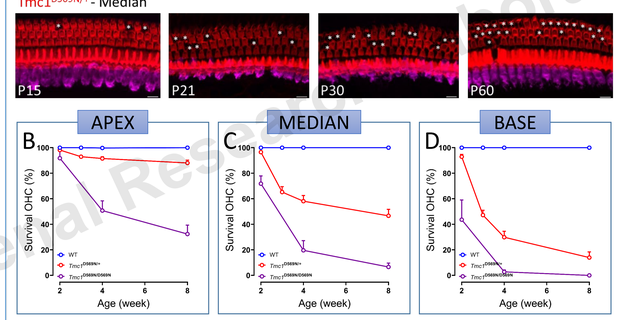

Figure 5: *Tmc1*^{D569N} induces early and progressive OHC loss.
A: Representative confocal images in mid cochlear region corresponding to 16kHz, from Tmc1$^{D569N/+}$ mice at p15, p21, 1 and 2 months of ages. VGLUT3 (magenta), phalloidin rhodamin (red), missing OHC (white Asterix). Scale bar = 10 µm.
B-D: Cytocochleograms representing percentage survival OHC from p15 to 2 months of age at different cochlear localizations, Apex (**B**), Median (**C**) and Base (**D**) in WT (blue), Tmc1$^{D569N/+}$ (red) and Tmc1$^{D569N/D569N}$ (purple) mice. Data are expressed as mean ± SEM.

Alteration of stereocilia morphology

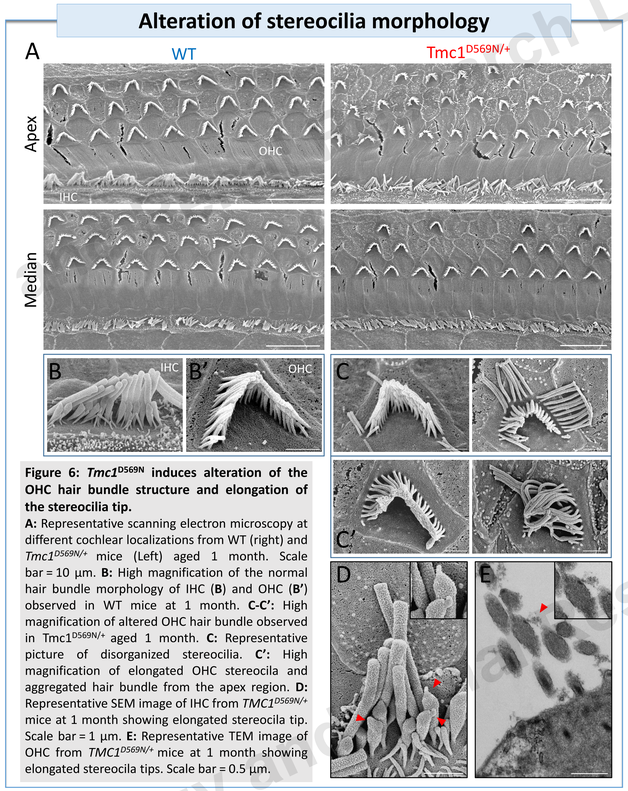

Figure 6: *Tmc1*^{D569N} induces alteration of the OHC hair bundle structure and elongation of the stereocilia tip.
A: Representative scanning electron microscopy at different cochlear localizations from WT (right) and *Tmc1*$^{D569N/+}$ mice (Left) aged 1 month. Scale bar = 10 µm. **B:** High magnification of the normal hair bundle morphology of IHC (**B**) and OHC (**B'**) observed in WT mice at 1 month. **C-C':** High magnification of altered OHC hair bundle observed in Tmc1$^{D569N/+}$ aged 1 month. **C:** Representative picture of disorganized stereocilia. **C':** High magnification of elongated OHC stereocilia and aggregated hair bundle from the apex region. **D:** Representative SEM image of IHC from *Tmc1*$^{D569N/+}$ mice at 1 month showing elongated stereocilia tip. Scale bar = 1 µm. **E:** Representative TEM image of OHC from *Tmc1*$^{D569N/+}$ mice at 1 month showing elongated stereocilia tips. Scale bar = 0.5 µm.

Aberrant synapse morphology

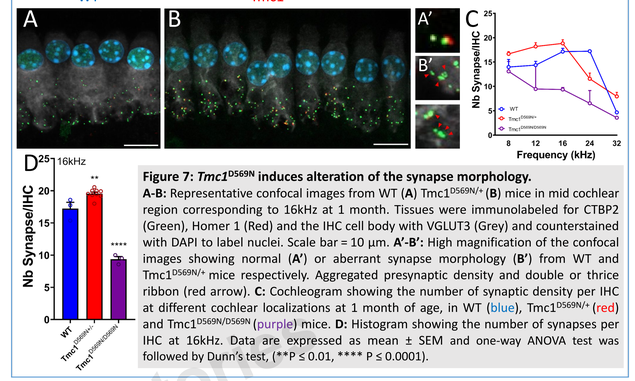

Figure 7: *Tmc1*^{D569N} induces alteration of the synapse morphology.
A-B: Representative confocal images from WT (**A**) Tmc1$^{D569N/+}$ (**B**) mice in mid cochlear region corresponding to 16kHz at 1 month. Tissues were immunolabeled for CTBP2 (Green), Homer 1 (Red) and the IHC cell body with VGLUT3 (Grey) and counterstained with DAPI to label nuclei. Scale bar = 10 µm. **A'-B':** High magnification of the confocal images showing normal (**A'**) or aberrant synapse morphology (**B'**) from WT and Tmc1$^{D569N/+}$ mice respectively. Aggregated presynaptic density and double or thrice ribbon (red arrow). **C:** Cochleogram showing the number of synaptic density per IHC at different cochlear localizations at 1 month of age, in WT (blue), Tmc1$^{D569N/+}$ (red) and Tmc1$^{D569N/D569N}$ (purple) mice. **D:** Histogram showing the number of synapses per IHC at 16kHz. Data are expressed as mean ± SEM and one-way ANOVA test was followed by Dunn's test, (**P ≤ 0.01, **** P ≤ 0.0001).

Tmc1^{D569N} and *Tmc1*^{M412K} induce distinct patterns of hair cell loss

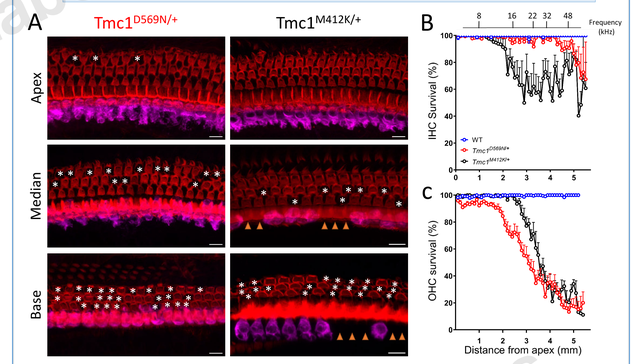

Figure 9: OHC loss is more severe in *Tmc1*^{D569N} than in *Tmc1*^{M412K} (Beethoven).
A: Representative confocal images at different cochlear localizations (Apex, Median and Base) from Tmc1$^{D569N/+}$ (right) and Tmc1$^{M412K/+}$ (left) mice aged 1 month. VGLUT3 (magenta), phalloidin rhodamin (red), missing OHC (white Asterix), missing IHC (arrow). Scale bar = 10 µm. **B-C:** Cytocochleograms representing percentage survival of IHC (**B**) or OHC (**C**) across the tonotopic axis at 1 month of age in WT (blue), Tmc1$^{D569N/+}$ (red) and Tmc1$^{M412K/+}$ (**Black**) mice.
Note: Tmc1$^{D569N/+}$ shows severe OHC loss mostly in the 3rd row of OHC with an early onset (p15). **Tmc1$^{M412K/+}$** show high IHC loss associated with OHC loss mostly in the 1st and 2nd row with later onset (p21 to p30).

Conclusion:

Tmc1$^{D569N/+}$ mice have early and rapid progression of the OHC loss associated with structural alterations.
These results suggest that restoring hearing by post-natal gene therapy in Tmc1$^{D569N/+}$ mice may be challenging.

Acknowledgements:

This study was supported in part by NIDCDs R01s DC003544, DC002842 and DC012049 to RJHS.