Cochlear Explantation Prevents Hair Cell Degeneration in Transmembrane Serine Protease 3 (*Tmprss3)* Deficient Mice

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Background/Objective: Transmembrane serine protease 3 (*TMPRSS3*) is the most common causative hearing loss gene in adults undergoing cochlear implantation and a significant cause of non-syndromic hearing loss. However, the function of TMPRSS3 within the inner ear is unknown. In murine models, *Tmprss3*$^{Y260X/Y260X}$ mutants *(Tmprss3*$^{-/-}$*)* have normal hair cell development followed by rapid hair cell degeneration from postnatal day 12 (P12) to P14. The endocochlear potential *in vivo* rises from P7 to a peak at P12, when mice begin to hear, which temporally corresponds to the HC degeneration in *Tmprss3*$^{-/-}$ mice. We tested if hair cell death occurs after removal of the endocochlear potential through cochlear explantation.

Methods: P7 organ of Corti explants from control (*Tmprss3*$^{+/-}$) and *Tmprss3*$^{-/-}$ mice were cultured in 5 mM potassium solution for 7 days in vitro (equivalent to P14). Whole mounts of cultured (P7+7DIV) and *in vivo* P14 cochlea were immunostained for MYO7A (hair cell marker) and DAPI followed by quantification of inner and outer hair cell (IHC/OHC) counts per 20 μm. Statistical analysis included two tailed t-test with p-value of (<0.01, n=6).

Results: As expected, there is complete loss of hair cells at P14 *in vivo* in *Tmprss3*$^{-/-}$ mice. Compared to P14 *in vivo, Tmprss3*$^{-/-}$ OC explants displayed significantly improved IHC and OHC survival (P<0.001). IHC and OCH survival was similar between control and *Tmprss3*$^{-/-}$ OHC explants *in vitro* (P=0.99, n=6)

Conclusion: These results suggest that degeneration of *Tmprss3*$^{-/-}$ hair cells is due to factors related to the endocochlear potential and implicate TMPRSS3 function in regulation of epithelial tight junctions. Future directions include confirming that hair cell death is potassium-mediated by crossing *Tmprss3*$^{-/-}$ mice with *Pou3f4*$^{del-J}$ mice, which have a decreased endocochlear potential without hair cell degeneration.