Mutation Agnostic RNA Interference with Concomitant Engineered Gene Replacement Rescues Hearing in a Mature Murine Model of DFNA36

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Introduction

TMC1 encodes the pore-forming subunit of the hair cell mechanotransduction channel. Mutations in *TMC1* cause dominant (DFNA36) and recessive hearing loss (DFNB7/11) which comprise ~2% of genetic hearing loss. Murine models of DFNA36 have been successfully treated with mutation-targeted RNA interference (RNAi), but these strategies require individually validated constructs for each causative variant. We present a mutation-agnostic approach for RNAi with concomitant gene replacement for preservation of hearing in a murine model of DFNA36.

Construct design

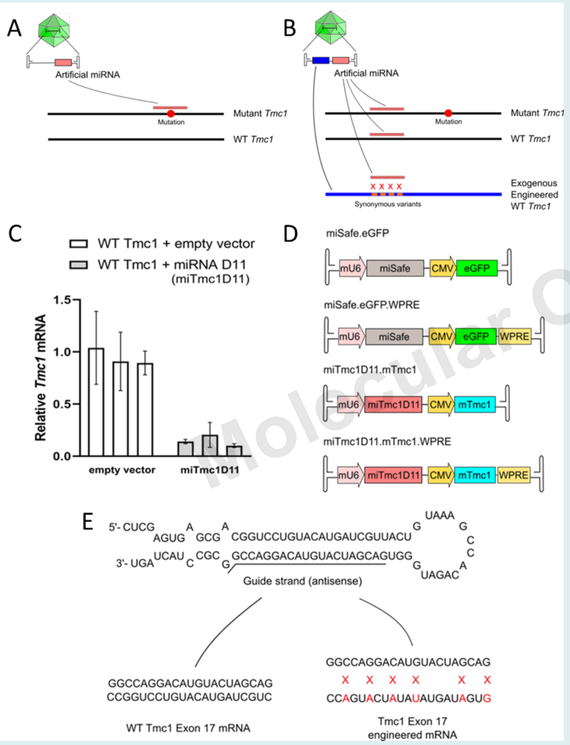

Figure 1. Overview of vector construct and development. (A-B) Comparison of mutation-specific RNAi and mutation-agnostic RNAi. (C) Real-time qPCR analysis from COS-7 cells cotransfected with wild-type *Tmc1* and either miTmc1D11 or empty vector. Knockdown efficiency of miTmc1D11 was >80%. (D) Outline of transgene control and gene therapy constructs. (E) miTmc1D11 sequence and diagram of knockdown-resistant exogenous *Tmc1*.

Acknowledgments

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Virus serotype selection

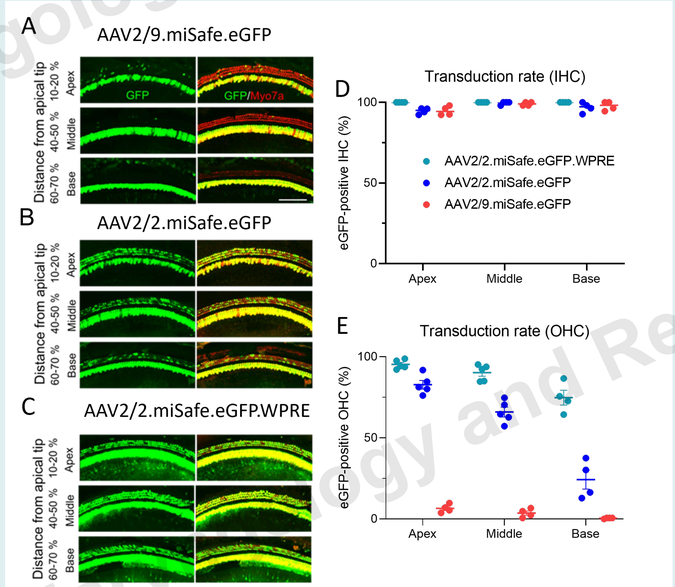

Figure 2. Transduction efficiency with marker vectors. (A-C) Cochlear whole mount and following delivery of AAV2/9.miSafe.eGFP, AAV2/2.miSafe.EGFP, and AAV2/2.miSafe.eGFP.WPRE. (D-E) Inner hair cell (IHC) and outer hair cell (OHC) transduction rates with each marker vector.

Experimental design

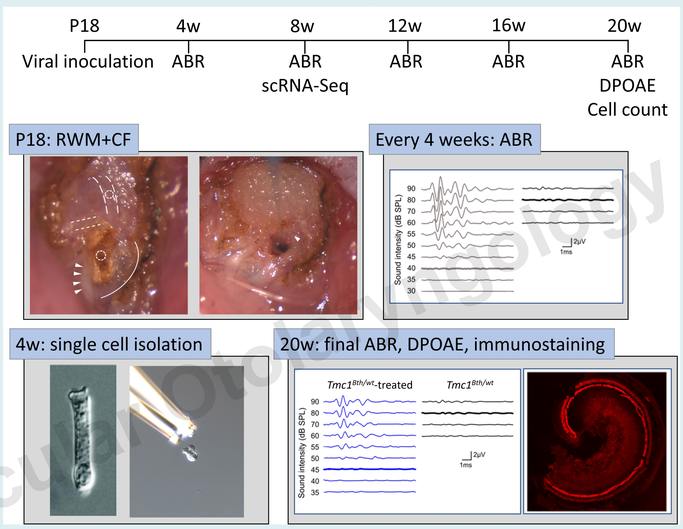

Figure 3. Summary of experimental timeline. Round window membrane injection with canal fenestration was performed at P16-18. Auditory brainstem response (ABR) was performed every 4 weeks, up to 20 weeks. Manual micropipetting was used to isolate outer hair cells (OHCs) for single-cell RNA sequencing for differential expression analysis. Cell counting was used to characterize hair cell preservation.

Auditory outcomes

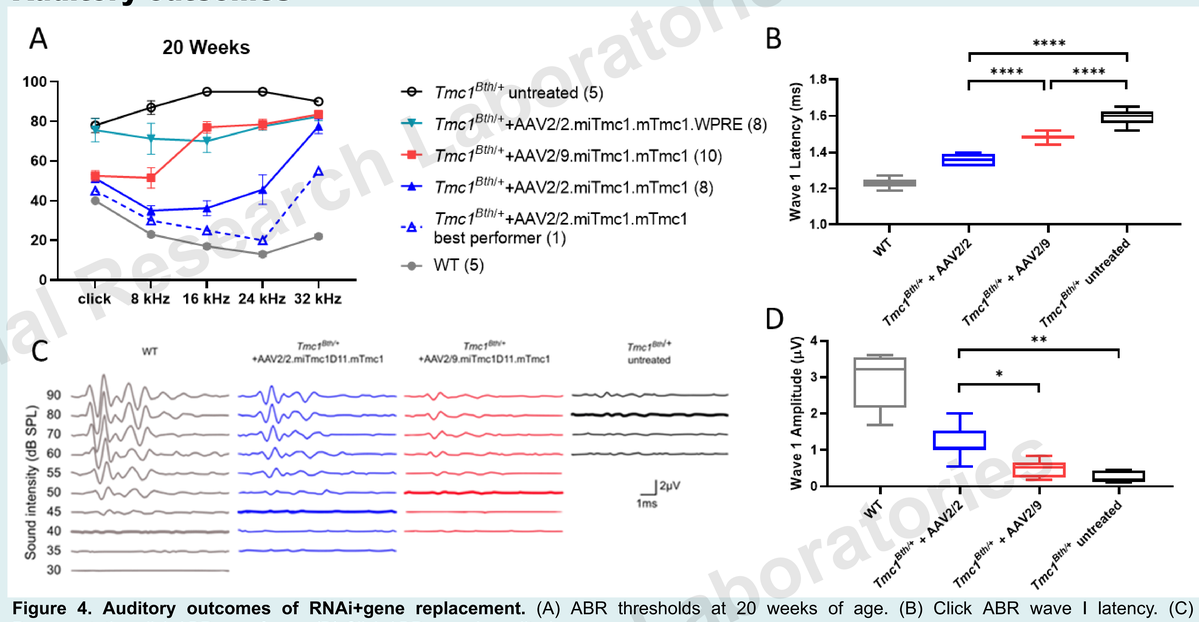

Figure 4. Auditory outcomes of RNAi+gene replacement. (A) ABR thresholds at 20 weeks of age. (B) Click ABR wave I latency. (C) Representative click ABR waveforms. (D) Click ABR wave I amplitude.

Hair cell preservation

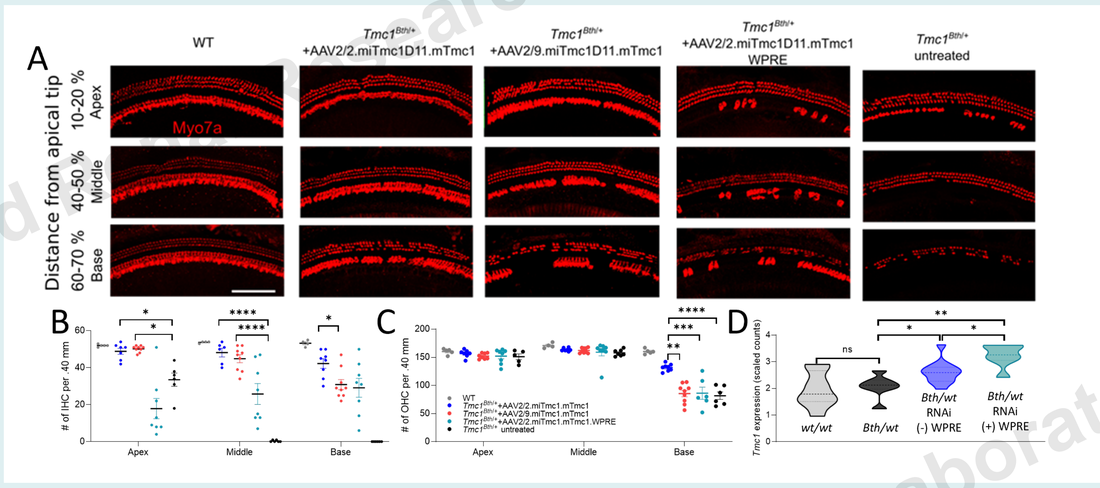

Figure 5. Hair cell preservation in animals which received gene therapy. (A) Cochlear whole mount of treated animals and wild-type and heterozygous controls. (B) Inner hair cell (IHC) counts in 0.4 mm segments of the apical, middle basal turns of the cochlea. (C) Outer hair cell (OHC) counts in apical, middle basal turns of the cochlea. (D) RNAi+gene replacement vectors resulted in increased *Tmc1* expression relative to untreated animals; expression was significantly higher in mice which received WPRE-carrying vector.

Conclusions

Mutation-agnostic RNAi with engineered, exogenous replacement achieved preservation of cochlear hair cells and auditory function in the treatment of *Tmc1*-related hearing loss. This strategy is likely to be broadly applicable in autosomal dominant nonsyndromic hearing loss. The inferior performance of WPRE-carrying vectors suggests that optimization of transgene dosage is critical for optimal auditory outcomes in gene therapy for *TMC1*-related hearing loss.

References →