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"Antisense oligonucleotides treatment rescues elevated Prominin-1 and reduced Annexin A1 in Usher mice retina"

Purpose: Harmonin, a scaffolding protein encoded by the *USH1C* gene, is involved in the development and function of ciliated cells including photoreceptors; however, its role in the retina remains unclear. Mutations in harmonin lead to Usher syndrome (Usher), the most common genetic cause of deaf-blindness. Acadian Usher Type 1C is attributed to the *USH1C* c.216G>A splicing mutation (216A), and like patients, knock-in mice display severe hearing impairment, balance defects, and visual dysfunction. Previously, we developed antisense oligonucleotides (ASO) targeting the 216A mutation and demonstrated that treatment in USH1C mice rescues hearing, balance, and visual function. To understand the effects of the 216A mutation on protein expression in the retina, quantitative discovery-based proteomics was performed on retinal extracts at 1, 3, 6 and 12 months of age from wild type (WT), Usher, and ASO-treated Usher mice. We hypothesize that Usher retinas have abnormal protein expression compared with WT. Furthermore, we predict that ASO treatment, which improves retinal *Ush1c* expression and restores visual function in Usher mice, also restores abnormal expression in the retina.

Methods: Juvenile Usher mice (P16) were treated with 216A-ASO by intravitreal injection (IVI) and allowed to recover for 2.5 months post-IVI. Visual function was measured in ASO-Usher, Usher, and WT mice at different ages using electroretinogram (ERG) analysis. Retinas were then harvested and processed for proteomic analysis using liquid chromatography and mass spectrometry (LCMS). Results were confirmed with immunohistochemistry (IHC) and western blot analysis.

Results: ERGs were significantly increased in ASO-Usher mice compared with untreated Usher controls, and LCMS identified significantly differentially expressed proteins in Usher versus WT and ASO-Usher versus untreated Usher retinas. Among these, prominin-1 levels were elevated and annexin A1 levels were reduced in Usher retinas compared to WT. Expression levels were significantly improved following ASO treatment as measured by LCMS in previous studies and confirmed by IHC and western blot analyses in this study.

Conclusions: This data demonstrates that ASO treatment improves visual function in Usher mice, as well as restores WT prominin-1 and annexin A1 expression levels in the retina.