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"Development of Fluorescence In Situ Hybridization (FISH) in Usher Syndrome Type 1C Murine Retina"

Usher Syndrome (USH) is the leading genetic cause of combined deaf-blindness in the world. Three clinical types (USH1-3) and 10 genes are associated with the disease. USH1 is the most severe type of Usher with congenital sensorineural hearing loss and early onset of retinitis pigmentosa, a form of progressive vision loss. Mutations in the *USH1C* gene are responsible for approximately 6–15% of USH1 cases. Notably, *USH1C c.*216G>A (216A) mutation accounts for nearly all USH1 cases in the Acadian population in the U.S. and Canada. The 216A mutation causes abnormal splicing of the RNA transcript resulting in a frameshift deletion of 35 base pairs at the end of exon 3, producing a severely truncated harmonin protein. Harmonin is a key scaffolding protein essential for proper functioning of the inner ear and retina. The improper formation of harmonin due to the 216A mutation leads to dysfunctional cochlear hair cells and photoreceptors. We have previously shown that antisense oligonucleotides (ASOs) targeting the 216A mutation correct the aberrant *Ush1c* splicing and partially restore vision, hearing, and balance in USH1C^{216AA} mice. However, the effects of ASO treatment in modifying the localization and distribution of *Ush1c* mRNA transcripts in the retina are currently not well understood. Additionally, there is a critical shortage of commercially available antibodies targeting Harmonin that are well-validated, proving gene expression analysis to be difficult. In this study, we aim to develop FISH as a novel tool to visualize *Ush1c* mRNA gene expression *in situ*.

USH1C mice were treated via intravitreal injection at 3 weeks of age with ASO-29 targeting the 216A mutation. As controls, untreated wild-type mice and USH1C mice were kept in parallel. At 3-months of age, all animals were sacrificed, and the eyes were harvested and cryopreserved. Frozen sections of the eyes were obtained using a cryotome and subjected to FISH assay using fluorescently-labeled probes designed to target the full-length or 216A mutant *Ush1c* mRNA transcripts in the mouse retina.

Preliminary data show abundance of full-length *Ush1c* transcripts in the wild-type mouse retina as compared to that of USH1C mouse retinas. Interestingly, ASO treatment in mutant mice led to an increase in full-length *Ush1c* transcripts as compared to the untreated USH1C mice, primarily near the inner and outer nuclear layers. These findings show that FISH is a powerful tool that provides quantitative, spatial, and temporal understanding of *Ush1c* mRNA gene expression *in situ.* Future studies will aim to optimize the FISH protocol to better *understand Ush1c* mRNA gene expression at different time points (3-, 6-, and 12-months of age).