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"Drug development using genetic strategies for the treatment of Usher syndrome Type 1C"

Usher syndrome (Usher) is an autosomal recessive genetic disorder that is the most common cause of hereditary deaf-blindness. Of the four clinical types, Usher Type 1 (USH1) is the most severe, characterized by profound hearing loss and vestibular areflexia at birth and early-adolescent onset of retinitis pigmentosa. Mutations in the *USH1C* gene cause Usher Type 1C (USH1C), which accounts for 6-15% of all USH1 cases. The *USH1C* gene encodes harmonin, a protein vital for inner ear hair cell development and retinal photoreceptor function. The cryptic splice site mutation, *USH1C* c.216G>A (216A), is a founder mutation in Acadian populations in the U.S. and Canada and causes almost all USH1 cases in Acadian patients. The 216A mutation causes a splicing error in the pre-mRNA transcript, resulting in a truncated harmonin protein. Gene replacement therapy was previously tested using a synthetic adeno-associated virus (AAV) to express cDNA encoding a full-length retinal *Ush1c* RNA transcript in the mouse retina following subretinal injections. In addition, the Lentz laboratory has shown that intravitreal injection (IVI) of antisense oligonucleotides (ASOs) targeting the 216A mutation increases the expression of full-length (wild-type) transcripts in an USH1C mouse model that contains the 216A mutation. The goals of our project are two-fold: 1) to evaluate transduction efficacy of intravitreal AAV delivery, and 2) to investigate the efficacy of a novel chemical modification of ASO therapy. The evaluation of intravitreal AAV injection, if successful, will present a novel drug delivery route for gene replacement therapy in USH1C, and testing new modified ASO chemistries aims to optimize splicing correction for the treatment of USH1C.

Juvenile USH1C mice were treated by intravitreal injection (IVI) with either AAV44.9(E531D).CBA.EGFP, a 2'O-Methyl-ASO targeting the 216A mutation (2'OMe-ASO29), or control-ASO (ASO-C). To assess the effect of AAV44.9(E531D).CBA.EGFP treatment on retinal cell transduction, fundus imaging was performed using confocal scanning laser ophthalmoscopy at 2-, 3-, 4-, 6-, 8-, and 10-weeks post-IVI. Analysis of the fundus images showed that IVI transduced retinal cells in 1 of 4 mice and that the area of transduced retina slowly increased over time. Eyes were harvested at 10 weeks post-IVI to confirm the cell type within the retina that has been transduced, and immunohistochemistry analysis detected AAV44.9-mediated GFP expression in the photoreceptor outer segment layer. To assess the effect of the 2'OMe-ASO29 treatment on splicing, retinal tissues were harvested for molecular analysis (RT-PCR and Next Generation Sequencing) of *Ush1c* RNA transcripts 2 weeks post-IVI. Results are pending.

The results of these studies will determine the effect of AAV delivery route and ASO chemistry on the treatment of vision loss in USH1C.