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

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Introduction

- 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 sensorineural hearing loss and vestibular areflexia at birth and early-adolescent onset of retinitis pigmentosa.[1]
- 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.[2]
- 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.[2]
- Current research on genetic treatments for Usher syndrome has shown that both antisense oligonucleotide (ASO) and adeno-associated virus (AAV) genetic therapies transiently (ASO) or moderately (AAV) improve hearing, balance, and visual function in animal models of USH1C.[3,4,5] Optimization of these therapies is underway.
- Gene replacement therapy was previously tested in the Lentz laboratory using a synthetic AAV to express cDNA encoding a full-length retinal *Ush1c* RNA transcript in the mouse retina following subretinal injections.
- The Lentz laboratory has shown that intravitreal injection (IVI) of ASOs targeting the 216A mutation increases the expression of full-length (wild-type) transcripts in an USH1C mouse model that contains the 216A mutation.

Specific Aims:

1) Evaluate transduction efficacy of intravitreal AAV delivery to improve cellular targeting.
2) Investigate the efficacy of a novel chemical modification of ASO therapy to improve molecular targeting.

Schematic of Project

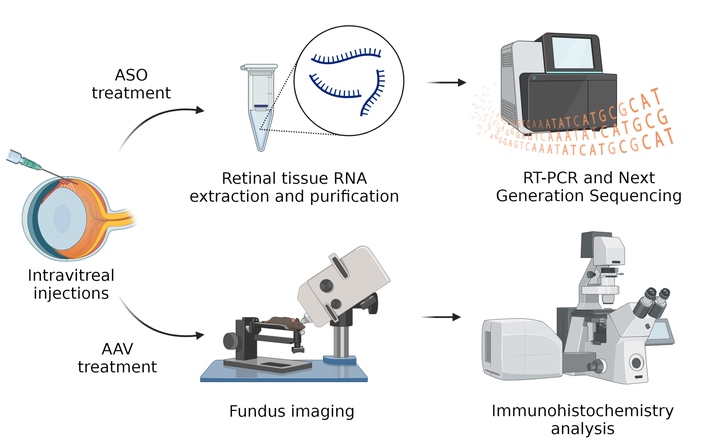

Fig. 1. Schematic of project design. Juvenile USH1C mice were treated by intravitreal injection (IVI) with either AAV44.9(E531D).CBA.EGFP, a 2'O-Methyl-ASO29 targeting the 216A mutation (2'OMe-ASO), or control-ASO29 (ASO-C). To assess the effect of AAV44.9 treatment, fundus imaging was performed using confocal scanning laser ophthalmoscopy at 2-, 3-, 4-, 6-, 8-, and 10-weeks post-IVI. Eyes were harvested at 10 weeks post-IVI for immunohistochemistry analysis. To assess the effect of the 2'OMe-ASO treatment, retinal tissues were harvested for molecular analysis 2 weeks post-IVI.

References

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AAV Delivery Optimization

Time course of retinal GFP expression *in vivo*

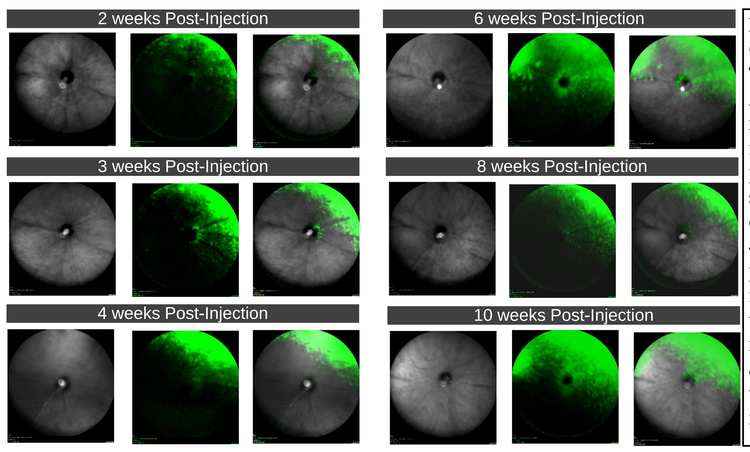

Fig. 2. Retinal AAV44.9-GFP expression *in vivo*. To assess the effect of AAV44.9 treatment on retinal cell transduction, fundus imaging was performed using confocal scanning laser ophthalmoscopy at 2-, 3-, 4-, 6-, 8-, and 10-weeks post-IVI. Analysis of the fundus images showed that IVI transduced retinal cells in 1 of 4 mice and that the area of transduced slowly increased over time.

Immunostaining analysis of GFP expression

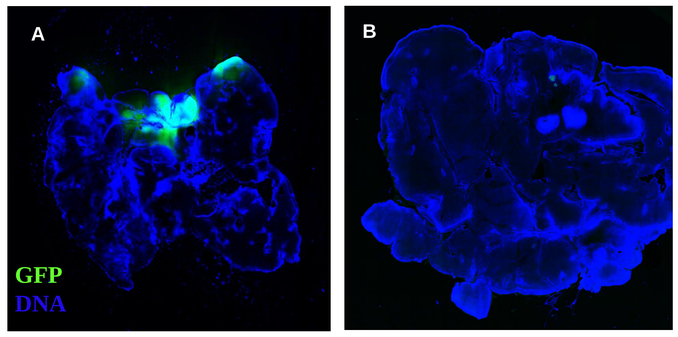

Fig. 3. Immunohistochemistry analysis of AAV44.9-treated retinas. Retinas were harvested at 10 weeks post-IVI, and retinal whole mounts were imaged using a confocal microscope 10x tile scan. An eye treated with the AAV44.9-GFP (**A**) showed expression of GFP in a small portion of the retina, while the retina of an un-injected control eye (**B**) showed no GFP fluorescence. Blue = DAPI; Green = GFP.

Localization of GFP in retinal layers

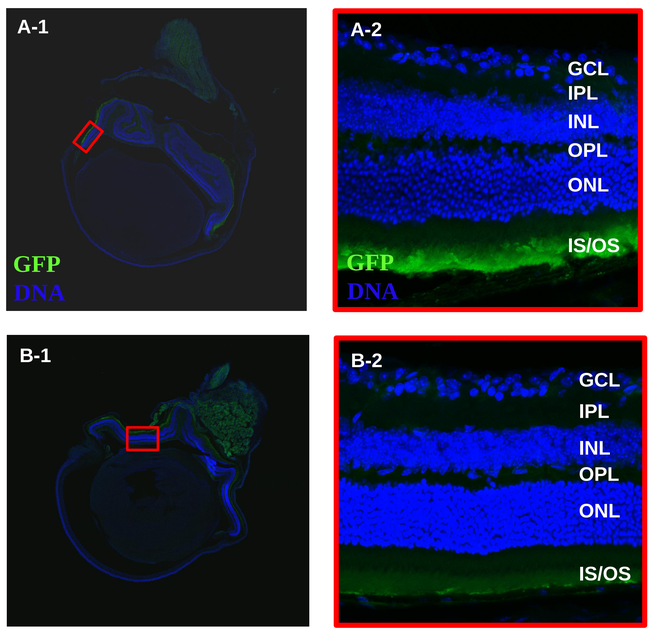

Fig. 4. Localization of GFP expression in retinal cells. Eyes were harvested at 10 weeks post-IVI, and transverse cryosections were imaged using a confocal microscope (**A-1** and **B-1**: 10x tile scan; **A-2** and **B-2**: 40x z-stack). AAV44.9-mediated GFP expression was detected in an injected eye (**A**) in the photoreceptor outer segment layer, but it was not detected in other layers of the retina. **A** corresponds to the fundus images in **Fig. 2**. An un-injected control eye (**B**) showed no significant fluorescence in any layers of the retina.

Blue = DAPI; Green = GFP.
GCL: ganglion cell layer; IPL: inner plexiform layer; INL: inner nuclear layer; OPL: outer plexiform layer; ONL: outer nuclear layer; IS/OS: photoreceptor inner segments/outer segments.

ASO Chemistry Optimization

USH1C splice site mutation

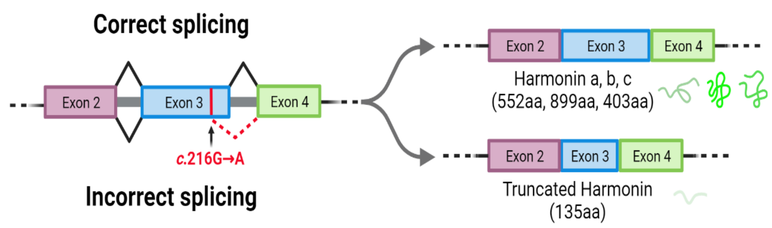

Fig. 5. Cryptic splice site mutation in *USH1C*. The wild-type *USH1C* gene undergoes alternative splicing, resulting in three different isoforms of the harmonin protein (harmonin a, b, and c). The cryptic splice site mutation, *USH1C* c.216G>A (216A), causes a 35-bp frame-shift deletion in exon 3 in the pre-mRNA transcript, resulting in a truncated harmonin protein. This mutation is the cause of almost all USH1 cases in Acadian populations in the United States and Canada.[2,3]

Ush1c splicing in ASO-treated retinas

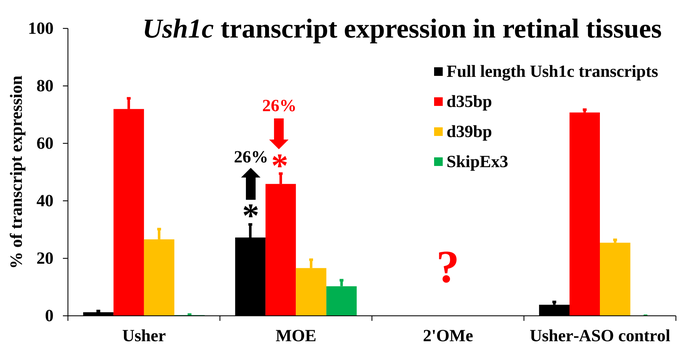

Fig. 6. Next Generation Sequencing analysis of *Ush1c* transcripts. Previously, the Lentz lab has shown that ASO treatments administered via IVI have successfully improved splicing in retinas of 1-month-old Usher mice. ASO29 (MOE) increased the percent of full-length *Ush1c* transcripts by 26% and reduced the percent of mutant transcripts by 26% (*P<0.05, student's t-test). In the present study, a group of juvenile mice were treated with a 2'OMe ASO. Retinal tissues were harvested 2 weeks post-IVI for Next Generation Sequencing (NGS). Results are pending.

Conclusions

- Intravitreal injection of AAV44.9-GFP vectors transduced photoreceptor cells in 1 of 4 mice. This experiment will be repeated to increase the number of mice tested to further assess the potential efficacy of AAV treatment via IVI.
- ASO-MOE treatments administered via IVI significantly improved the gene expression of wild-type full-length *Ush1c* transcripts and decreased the expression of mutant truncated transcripts in retinas of USH1C mice. NGS analysis of gene expression in ASO-OMe-treated retinas is ongoing.
- Future studies will aim to further optimize AAV delivery route and ASO chemistry for the treatment of vision loss in USH1C.

Acknowledgments

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