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

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Introduction

- **Usher Syndrome** (USH) is the **leading genetic cause** of combined **deaf-blindness** in the world.
- Mutations in the *USH1C* gene are responsible for approximately 3.5% of USH cases. Notably, *USH1C c.*216G>A (216A) mutation accounts for nearly all Type 1 USH 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.
- Currently, the Lentz Lab is developing an **antisense** therapeutic approach to **correct the splicing defect** using antisense oligonucleotides (ASOs) targeting the 216A mutation.
- In this study, we aim to develop **FISH** as a novel tool to **localize expression** of truncated and full-length *Ush1c* RNA transcripts in the retina.

USH1C *c.216G>A* (216A) mutation

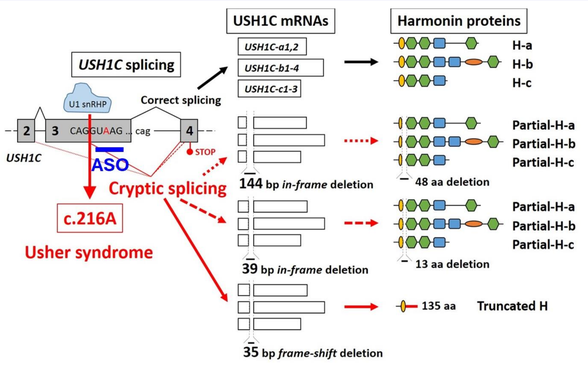

Fig 1. *USH1C c.*216G→A mutation is a splicing mutaion. Diagram of exons 2-4 of *USH1C* (encoding harmonin protein) and RNA transcripts. Correct splicing leads to three isoforms of harmonin (a, b, c) whereas the 216A mutation causes aberrant splicing that result in truncated transcripts.

ASO Treatment Design

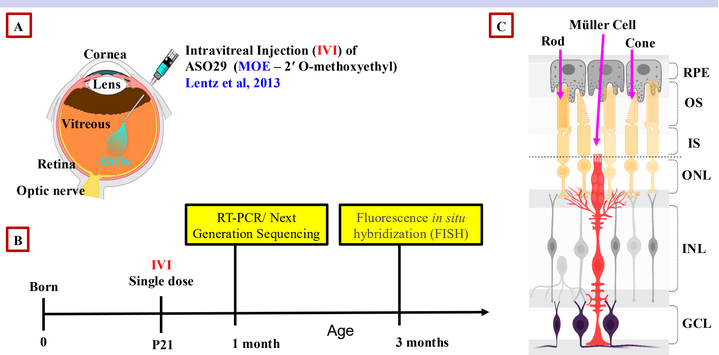

Fig 2. Schematic diagram of ASO treatment in USH1C mouse eye. (**A**) Schematic diagram of intravitreal injection (IVI) in eye; (**B**) experimental time course including a single dose of ASO, followed by outcome measures (retina tissue collection for RT-PCR/NGS and FISH). (**C**) Schematic diagram of mice retina, showing Rod, Cone photoreceptor, RPE and Müller Cells. **RPE**, Retinal Pigment Epithelium; **OS**, outer segment of photoreceptor cells, **IS**, Inner segment of Photoreceptor cells; **ONL**, Outer Nuclear layer of photoreceptor cells; **INL**, Inner Nuclear layer; **GCL**, Ganglion cell layer.

Methods and Materials

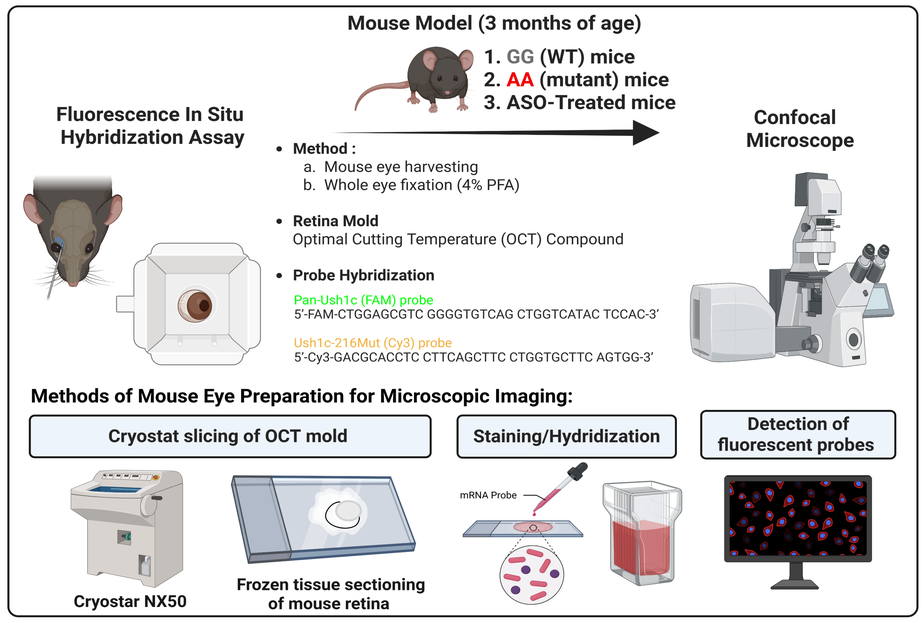

Fig 3. Schematic diagram of the Fluorescence In Situ Hybridization (FISH) assay. The eyes of 3-month-old wild-type mice (GG), untreated (AA), and ASO-treated (USH1C-ASO29) USH1C mice were obtained. Eyes were fixed in 4% paraformaldehyde overnight and cryopreserved following a sucrose gradient. Next, OCT molds were sectioned using a cryostat machine at 20-µm thickness. Retinal tissue sections were rehydrated, digested using proteinase K, and denatured followed by hybridization overnight with the following probes (each at 250 nM concentration): Pan-*Ush1c*-FAM and 216Mut-*Ush1c*-Cy3. Slides were thoroughly washed to reduce non-specific binding of probes, counterstained with DAPI, and mounted on cover slips for imaging. Confocal imaging was used to assess the localization and abundance of *Ush1c* mRNA transcripts. ImageJ software was used to analyze the relative fluorescence intensity.

ASO treatment improves *Ush1c* splicing

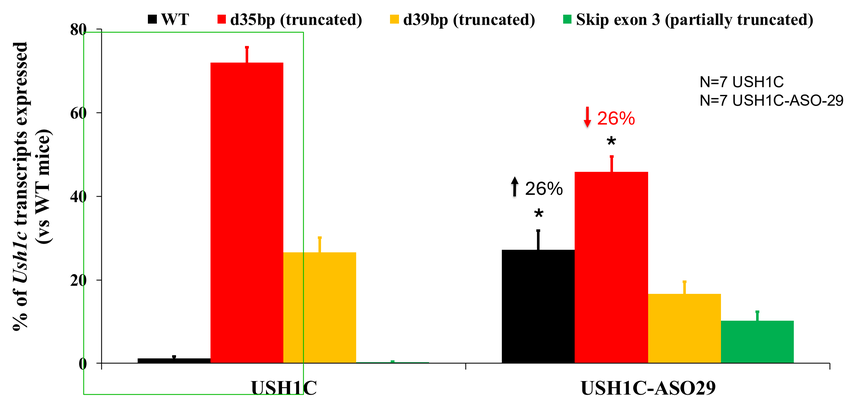

Fig 4. ASO therapy improved *Ush1c* splicing in USH1C mice retina. RT-PCR /NGS showed a significant increase in full-length *Ush1c* mRNA transcripts (**black bar, 26%**) and significantly reduced mutant *Ush1c* transcripts (d35bp, red bar, 26%) in ASO29-treated USH1C retinas compared with untreated/ASO-C treated USH1C mice. Also, reduced d39bp (yellow bar) *Ush1c* transcripts and increased SkipEx3 (green bar) *Ush1c* transcripts were observed in ASO29-treated USH1C mice compared with untreated/ASO-C treated USH1C mice. *P<0.05 (Students t-test, Excel). **d**, deletion; **bp**, base pair; **SkipEx3**, skipped *Ush1c* exon 3; **ASO**, Antisense Oligonucleotides; **ASO-C**, control ASO;

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Ush1c mRNA detection using FISH

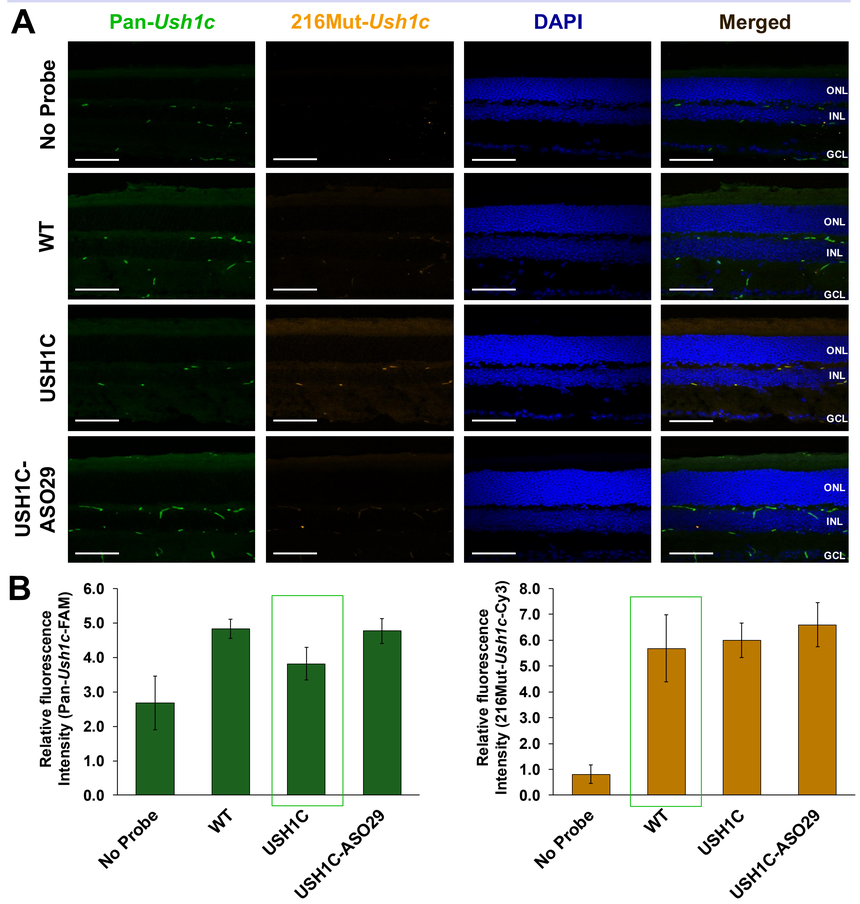

Fig 5. *Ush1c* mRNA detection using FISH in tissue sections from mouse retinas. (**A**) Maximum intensity Z-projections of Pan-*Ush1c* or 216Mut-*Ush1c* signals across Z-stack (same retina section) in 3-month-old wild-type (WT), USH1C, and ASO-treated USH1C mice. Retinal section of a WT mouse with no probe added was used as negative control. Green: FAM, Orange: Cy3, Blue: DAPI. Scale bars: 50 µm. **ONL**, Outer Nuclear layer; **INL**, Inner Nuclear layer; **GCL**, Ganglion cell layer. *Ush1c* mRNA transcripts are mostly localized near the INL and ONL. (**B**) Graphical illustrations of cumulative FISH data appear to show an increase in full-length *Ush1c* transcripts and a decrease in mutant *Ush1c* transcript in retinas of ASO-treated USH1C mice compared to those of untreated USH1C mice. However, statistical significance was not observed due to limited sample size (n=3).

Conclusion

- RT-PCR and NGS shows that ASO treatment in USH1C mice led to an increase in full-length *Ush1c* transcripts as compared to the untreated USH1C mice.
- Methods for in situ hybridization in retina tissues has been develop however In data with pilot pan- and mut-Ush1c probes suggest nonspecific binding.
- Future studies will focus to redesign probes that can distinguish full-length vs mutant transcripts with higher specificity.

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