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

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Introduction

- Harmonin is encoded by the *USH1C* gene and functions in ciliated cells including photoreceptors, but its role in the retina remains unclear[3]
- Mutations in harmonin lead to Usher syndrome (Usher), the most common genetic cause of deaf-blindness[1]
- Acadian Usher Type 1C is attributed to the c.216G>A splicing mutation (216A) in the *USH1C* gene[4,5,6]
- Patients and knock-in mice display hearing, balance, and visual impairments[1,7]
- Antisense oligonucleotides (ASO) targeting the 216A mutation rescue hearing, balance, and visual function in Usher mice[2]

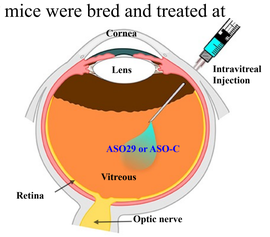
In this study-

- 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 and that ASO treatment restores abnormal expression in the retina

Methods

Mice: Ush1c c.216G>A knock-in (Usher) and littermate WT mice were bred and treated at LSUHSC.

Mouse treatment: Usher mice were treated with 54µg 216A-targeted ASOs by intravitreal injection (IVI) one time at postnatal day 16 (P16).



Assessment of visual function: Visual function was measured in WT, Usher, and ASO-Usher, mice at different ages using electroretinogram (ERG) analysis.

Retina tissue preparation for proteomics: Eyes were harvested from WT, Usher, ASO29-Usher, and Usher mice treated with a control-ASO (ASO-C) at 1, 3, 6 and 12 months of age. Retinas were then harvested and processed for proteomic analysis using liquid chromatography and mass spectrometry (LCMS).

Immunohistochemistry: Eyes were harvested from WT, Usher, and Usher ASO29 mice at 3 months of age. The eyes were then fixed, sliced, and prepared in primary (prominin-1 and annexin A1, 1:100) and secondary antibodies (1:200). Images were captured by Zeiss Confocal Microscope LSM 710 and analyzed with ImageJ.

Western blot analysis: Eyes were harvested from WT, Usher, and Usher ASO29 mice at 3 months of age. Retinas were then processed for protein extraction. Proteins (30µg) were separated on a 4-12% SDS-PAGE gel and transferred to nitrocellulose blots, followed by primary (prominin-1 and annexin A1, 1:1000) and HRP-secondary antibodies (1:15000). Blot is detected by ECL Prime Western blotting detection reagents. Images were captured by LAS 4000 gel doc and analyzed with Image Quant TL .

Statistical analysis: All data are shown as mean ± SEM fluorescence intensity. Statistical analysis was performed using one-way ANOVA (software).

References

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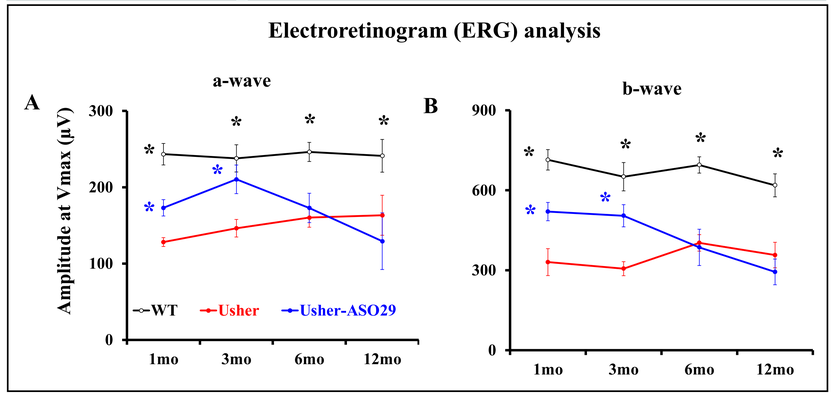
ASO29 Improves Usher Retina Function

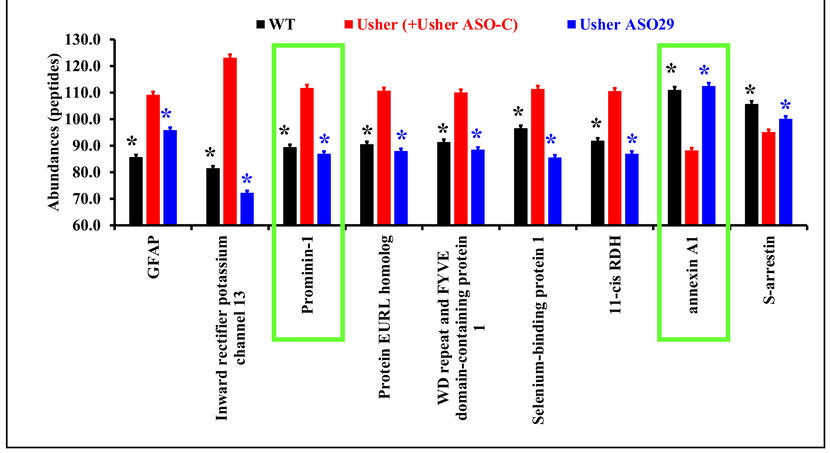


Significantly Different Proteins in Mouse Retina

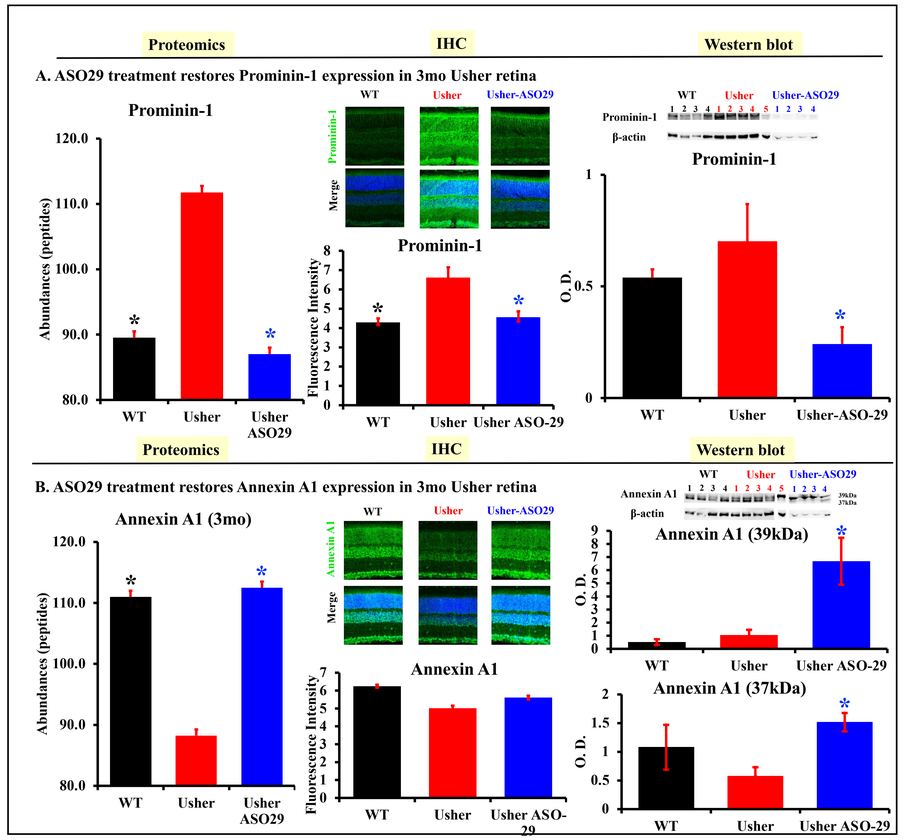
Retina	Usher vs. WT	ASO-Usher vs Usher (ASO-C)*
1mo	245	1055
3mo	155	124
6mo	165	430
12mo	153	159

* ASO-C is a control-ASO that does not target anywhere in the mouse genome

ASO29 Restores Some Proteins to WT Levels in 3 Mo. Usher Retina



Proteomic Proteins Validation with IHC and Western Blot Analysis



Conclusions

- Usher mice have reduced ERGs (a- and b-waves) at all observed ages. A single ASO29 treatment at P16 significantly improves Usher retinal function (both a-wave and b-wave ERG) up to 3 months of age.
- Significantly different proteins were identified in Usher vs WT and Usher-ASO29 vs Usher-control retinas up to 12 months of age.
- Proteomics analysis at 3 months of age shows 9 proteins restored to WT levels following a single ASO treatment at P16 in Usher mice. Of these, 7 protein were up-regulated and 2 were down-regulated.
- Retinal homeostasis proteins, Prominin-1 (up) and Annexin A1 (down) identified by proteomics as up- or down-regulated in 3-month-old Usher retinas, were restored to WT levels and validated by IHC and western blot analyses.
- ASO treatment improves visual function in Usher mice, as well as restores retinal homeostasis proteins Prominin-1 and Annexin A1 expression to WT levels in the retina, suggesting a new role for harmonin in the healthy retina.
- Future studies will investigate additional retinal proteins and pathways identified by proteomic analyses to improve our understanding of the role of harmonin in vision and the mechanism of disease when it is mutated.

This research project was supported through the LSU Health Sciences Center, School of Medicine.