Retinal Degeneration and Macroglial Function: Contributions of Müller Cells in Usher Syndrome

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Introduction

Usher Syndrome (USH) is an inherited disorder that causes visual, vestibular, and hearing loss. Of the three main clinical forms (USH1-3), USH1 is the most severe characterized by congenital sensorineural hearing loss, vestibular areflexia, and early-onset retinitis pigmentosa, a progressive degenerative eye disorder that severely impairs vision. Patients experience gradual deterioration of photoreceptor cells that can eventually lead to blindness.[1]

Microglial and macroglial cells are involved in the maintenance of the retina's long-term health and function. Microglial cells extend throughout every retinal layer and are activated in response to injury or disease. Macroglia cells, which include astrocytes and Müller cells, are critical for retinal neuron and photoreceptor survival. Ush1c gene and harmonin protein expression have been detected in photoreceptors and glial cells within the mouse retina.[2]

Our long-term goals are to advance our understanding of the role of harmonin in vision and retinal disease. The *objective of this study* is to characterize macroglial cells, specifically Müller glia, in USH1C mice.

Materials & Methods

Mice: *USH1C* c.216>A knock-in (USH1C) and littermate mice were bred and treated at LSUHSC.

Immunohistochemistry: Eyes were harvested from 3-month-old USH1C and wild-type (WT) littermate mice and fixed in 4% paraformaldehyde at 4°C overnight. Cryoprotection in an ascending sucrose solution was performed, then tissues were embedded in OCT using isopentane over liquid nitrogen. Frozen sections (~ 14 µm) were permeabilized with 100% MeOH for 10 min., blocked with 10% donkey serum and 2% BSA in 1x PBS at room temperature for 2 hours, then incubated with primary antibodies (see diagram of cell surface markers) in 1:4 dilution of blocking buffer and incubated overnight at 4°C. The sections were washed with 0.1% PBST and incubated with secondary antibodies in 1:4 dilution of blocking buffer and incubated for 2 hours in the dark at room temperature. Washed sections were mounted in Prolong Diamond mounting media (Life Technologies Inc.). A Zeiss confocal microscope was used to capture images. Fluorescence intensity was analyzed using ImageJ software.

Statistical Analysis: Data is shown as mean ± SEM fluorescence intensity. Statistical analysis was performed using unpaired two-tailed Student's t-test.

Cell Surface Markers

- **Activated Microglial Cells**
  - Ionized Calcium Binding Adapter molecule (IBA-1)
- **Activated Astrocytes**
  - Glial Fibrillary Acidic Protein (GFAP)
  - Glutamine Synthetase (GS)
- **Wound Healing**
  - Vimentin

Results

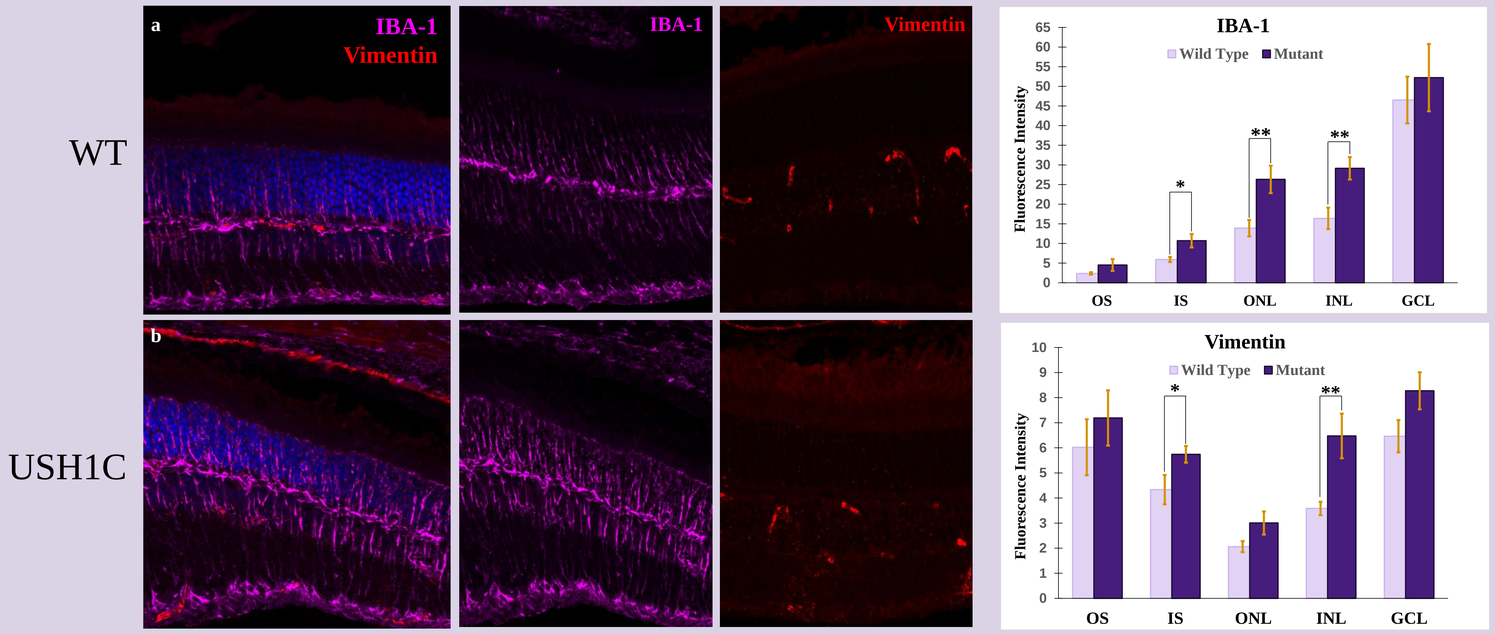

***Figure 1*:** An increase in activated microglial cells (IBA-1, pink) in the IS, ONL, and INL; and intermediate filaments (vimentin, red) in the IS and INL was detected in 3-month-old USH1C (b) retinas compared with WT (b) littermates. Nuclei were stained using DAPI (blue).

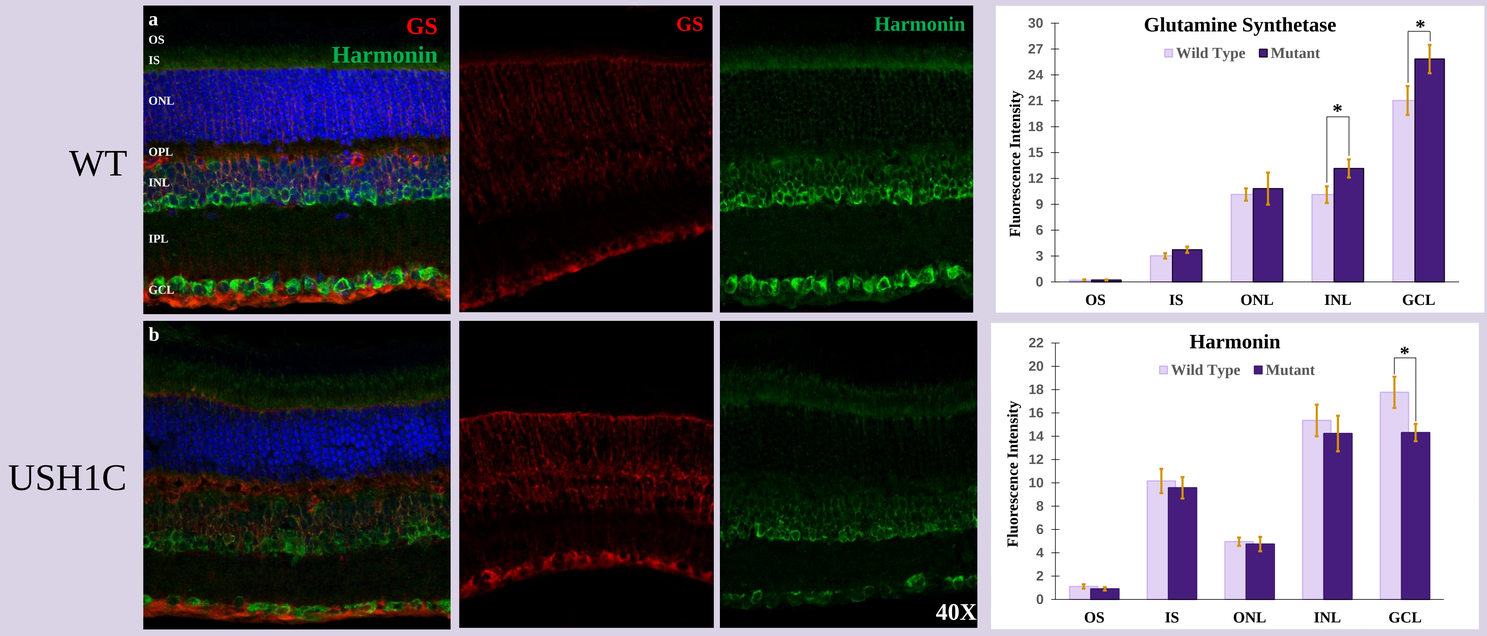

***Figure 2*:** Activated astrocytes were detected with an increase in glutamine synthetase (GS, red) in the INL and GLC of 3-month-old USH1C (b) retinas compared with WT (a) littermates. Reduced Harmonin (green), was observed in the GCL of 3-month-old USH1C (b) retinas compared with WT (a) littermates. Nuclei were stained using DAPI (blue).

Conclusion

- ✓ USH1C adult mice show increased inflammation and glial activation in the retina.
- ✓ USH1C mice have significantly reduced levels of harmonin protein localized in the retinal ganglion cell layer.
- ✓ These findings will guide future research in the development of novel therapies to prevent, slow, or cure the disease.
- ✓ Future research is needed to determine how the expression of these markers vary throughout the lifespan of an individual with Usher Syndrome.

References

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2. Nagel-Wolfrum, K., et al. (2023). Expression and subcellular localization of USH1C/harmonin in human retina provides insights into pathomechanisms and therapy. Human molecular genetics, 32(3), 431–449. https://doi.org/10.1093/hmg/ddac211

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