A Novel Retinal Gene Therapy Strategy for Batten Disease and Beyond

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INTRODUCTION

Neuronal Ceroid Lipofuscinoses, or Batten Disease, is a group of fatal, neurodegenerative disorders primarily affecting children. Of the 13 subtypes, CLN3 Batten Disease is the most common with an incidence of one in 100,000. A common first presenting symptom is vision loss which progresses to blindness, rapid developmental and cognitive delay, seizures, loss of movement, and death. Our group has developed several intrathecal AAV9 based gene replacement therapeutic constructs for different forms of Batten Disease [1,2]. While preliminary results from preclinical studies and potentially clinical trials are promising, the visual component of the disease might not be fully addressed. Recent studies in mice suggest that certain retinal cell types must be rescued to prevent vision loss [3,4].

Goal: Develop an AAV9-based retinal gene therapy for Batten Disease that can be safely and effectively combined with CSF based therapy that is already in clinic.

RATIONALE

Despite photoreceptor cells being the primary affected cell type in Batten Disease related vision loss (Figure 1), recent studies suggest cells of the inner nuclear layer, such as bipolar cells, need to be rescued to prevent photoreceptor degeneration [3,4].

One step further: We performed 10X Single-Cell RNA Sequencing on Wild-Type Non-Human Primate (Macaque) retinas to identify CLN expressing cell types (Figure 2).

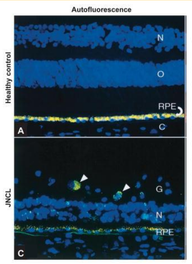


Figure 1: Human Control vs. CLN3 Retina. Diseased retina is characterized by significant retinal thinning of ganglion (G) and inner nuclear (N) cell layers with complete photoreceptor/ outer nuclear (O) layer degeneration. Significant autofluorescent lipofuscin accumulation is visible in remaining cells of the CLN3 retina. Figure provided by Ouseph et.al, 2017 [5].

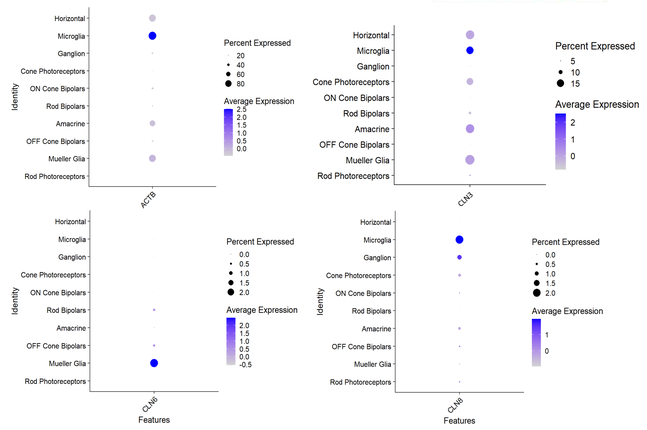


Figure 2: CLN3, CLN6, and CLN8 are all lowly expressing genes within the NHP retina. CLN3 and CLN8 are broadly expressed in almost every retinal cell type, indicating the need for successful AAV transduction in every major retinal layer. CLN6 has specific expression in bipolar cells, as previously reported, and in Mueller glia. Beta-Actin expression is shown for reference.

Problem: Intravitreal injection of AAV9 results in transduction of a limited number of cell types, mainly ganglion cells and Mueller glia with limited targeting of the inner nuclear layer.

Potential Solution: Co-deliver AAV9 with Neuraminidase enzyme, previously used by Wilson et.al [6] to improve targeting of other organs, to enhance retinal transduction of additional cell types.

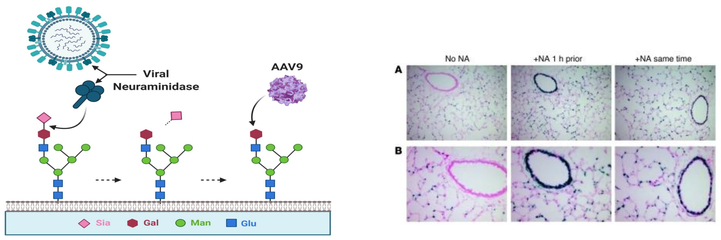
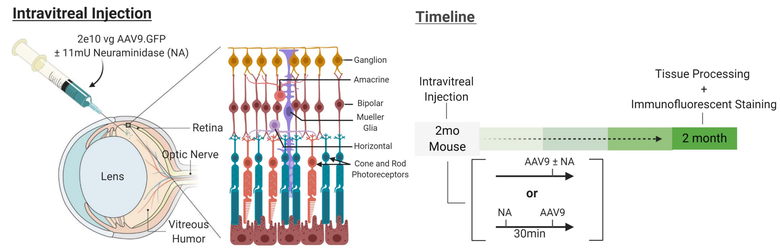


Figure 3: Neuraminidase enhances AAV9 transduction in vivo. Left; Diagram depicting the mechanism of Neuraminidase. The cleavage of sialic (Sia) acid residues from cell surface receptors allows for better access to galactose, the primary entry receptor for AAV9. Right; A figure from Wilson et. al. 2011 [6] ,that shows improved transduction of AAV9.LacZ to murine lung airways after pre-treatment or combined treatment with Neuraminidase .

METHODS



RESULTS

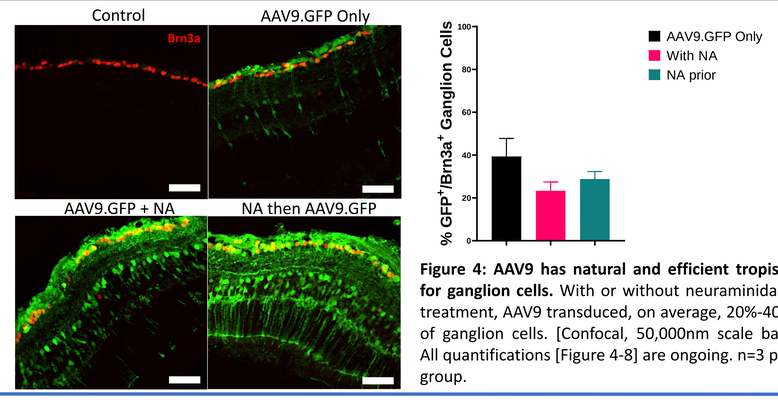


Figure 4: AAV9 has natural and efficient tropism for ganglion cells. With or without neuraminidase treatment, AAV9 transduced, on average, 20%-40% of ganglion cells. [Confocal, 50,000nm scale bar.] All quantifications [Figure 4-8] are ongoing. n=3 per group.

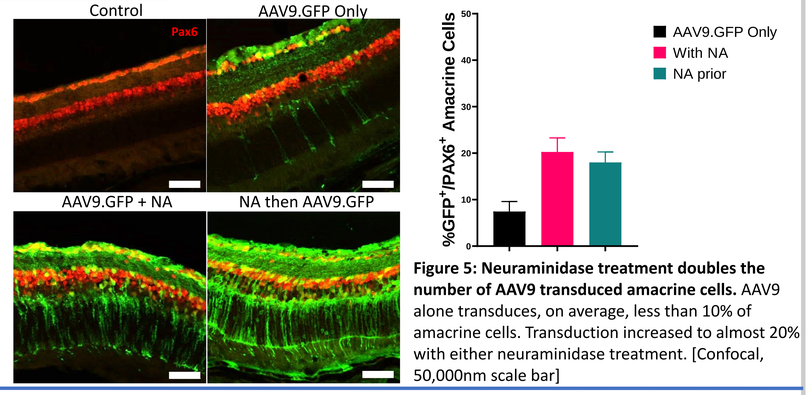


Figure 5: Neuraminidase treatment doubles the number of AAV9 transduced amacrine cells. AAV9 alone transduces, on average, less than 10% of amacrine cells. Transduction increased to almost 20% with either neuraminidase treatment. [Confocal, 50,000nm scale bar]

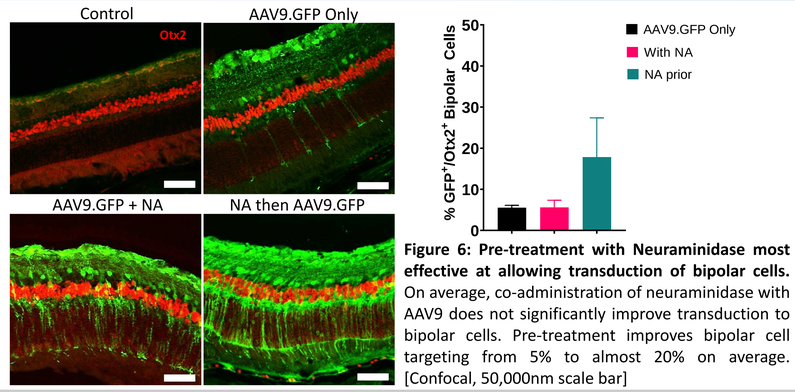


Figure 6: Pre-treatment with Neuraminidase most effective at allowing transduction of bipolar cells. On average, co-administration of neuraminidase with AAV9 does not significantly improve transduction to bipolar cells. Pre-treatment improves bipolar cell targeting from 5% to almost 20% on average. [Confocal, 50,000nm scale bar]

RESULTS

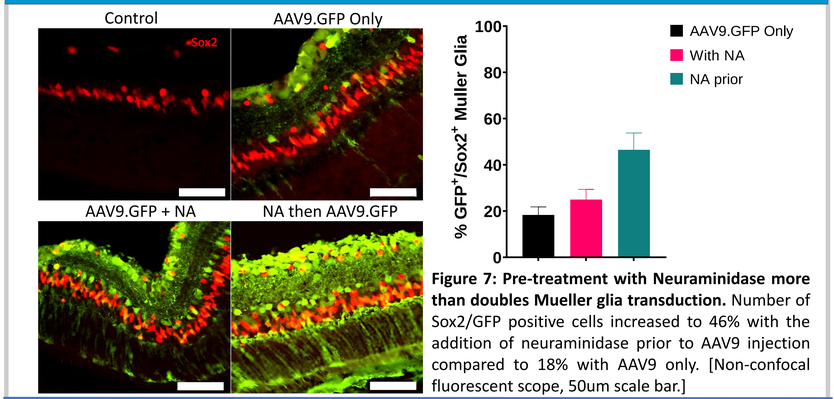


Figure 7: Pre-treatment with Neuraminidase more than doubles Mueller glia transduction. Number of Sox2/GFP positive cells increased to 46% with the addition of neuraminidase prior to AAV9 injection compared to 18% with AAV9 only. [Non-confocal fluorescent scope, 50um scale bar.]

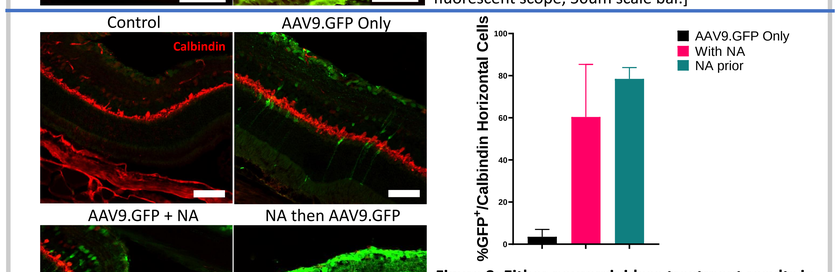


Figure 8: Either neuraminidase treatment results in significant improvement of horizontal cell targeting. Remarkably, horizontal cell transduction increased to up to 80% with both neuraminidase treatments compared to less than 5% transduction with AAV9 alone. [Confocal, 50,000nm scale bar]

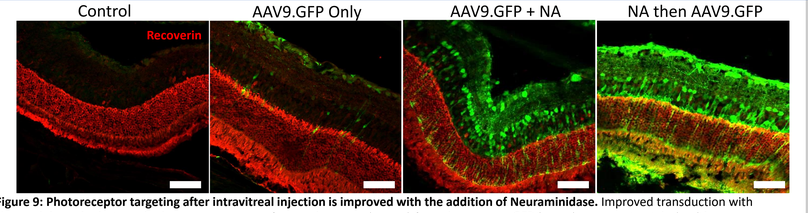


Figure 9: Photoreceptor targeting after intravitreal injection is improved with the addition of Neuraminidase. Improved transduction with Neuraminidase indicated with increased number of colocalized cells (yellow) following merged GFP (green) and counterstain (red) images. [Confocal, 50,000n, scale bar]

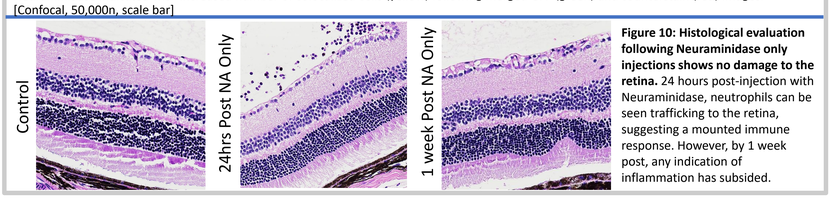


Figure 10: Histological evaluation following Neuraminidase only injections shows no damage to the retina. 24 hours post-injection with Neuraminidase, neutrophils can be seen trafficking to the retina, suggesting a mounted immune response. However, by 1 week post, any indication of inflammation has subsided.

CONCLUSIONS

- The addition of neuraminidase to intravitreal injections of AAV9.GFP drastically increased transduction to all layers of the retina.
- With the exception of ganglion cells, neuraminidase nearly doubled transduction of AAV9 in all retinal cell types. Ganglion cells are normally and efficiently transduced with AAV9 alone.
- Neuraminidase visibly increases transduction of AAV9 to photoreceptor cells, suggesting this treatment might be a safe and effective alternative to photoreceptor targeting with subretinal injection.
- Histology of Neuraminidase only injected retinas indicate a transient immune response with no detectable damage to the retina.
- **Future Directions**: Test this proof-of-concept therapy in large mammals and further determine efficacy and safety in Batten Disease mouse models prior to potential translation to clinic

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