A single injection of an optimized AAV vector into cerebrospinal fluid corrects neurological disease in a murine model of GM1 gangliosidosis

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Introduction

GM1 gangliosidosis is rare genetic disorder caused by mutations in the GLB1 gene, which encodes lysosomal beta galactosidase (β-gal). In the absence of β-gal, cells are unable to catabolize polysaccharides with terminal galactose residues. Keratan sulfate, a galactose-containing glycosaminoglycan, accumulates in a variety of tissues causing skeletal dysplasia, hepatosplenomegaly, and cardiomyopathy. In the central nervous system (CNS), the inability to catabolize GM1 ganglioside results in marked neuronal GM1 storage and subsequent neurodegeneration. The most common form of GM1 gangliosidosis is the infantile subtype, which occurs in patients with little or no residual β-gal expression. Infantile GM1 gangliosidosis is characterized by a uniformly rapid neurodegenerative course beginning in the first 6 months of life, with survival of less than 5 years.

Previous studies in murine and feline models of GM1 gangliosidosis demonstrated that adeno-associated viral (AAV) vectors can be used to deliver the normal GLB1 coding sequence to neurons, resulting in long-term expression of the β-gal enzyme. β-gal is secreted by transduced cells and can be taken up by surrounding cells via binding to the mannose-6-phosphate receptor, resulting in widespread distribution of the enzyme. This phenomenon allows for correction of storage lesions throughout the CNS following a small number of vector injections into the brain parenchyma. Studies in canine and feline models of other lysosomal storage diseases have shown that gene transfer to the brain and spinal cord can alternatively be achieved using AAV vector delivery into the cerebrospinal fluid (CSF). This approach allows for more widespread gene transfer with a single minimally invasive injection and appears to carry less risk of local toxicity at the injection site. AAV delivery into the CSF achieves efficient gene transfer to the brain and spinal cord even in the presence of systemic neutralizing antibodies to the vector capsid, making this approach applicable to patients regardless of the presence of pre-existing AAV antibodies.

Aim

The aim of this study was to develop an optimized AAV vector expressing human β-gal and evaluate the impact of vector administration into the CSF on brain enzyme activity, lysosomal storage lesions, and neurological signs using a murine disease model.

Vector selection

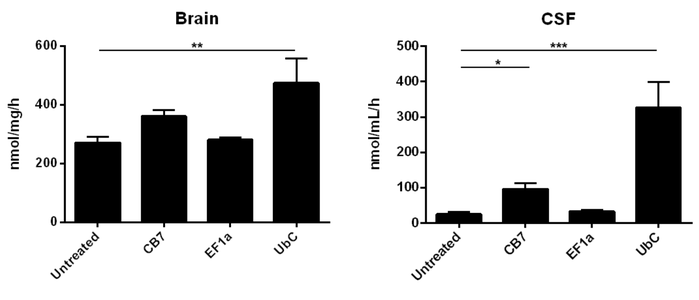

Figure 1: Selection of an optimized vector for β-gal expression in the CNS
Wild-type mice were treated with a single intracerebroventricular (ICV) injection of an AAV vector expressing a codon optimized human GLB1 cDNA from a CB7, EF1a, or UbC promoter (n = 10 per group). Untreated wild-type mice (n = 5) served as controls. Brain (frontal cortex) and CSF were collected 14 days after vector administration, and β-gal activity was measured using a fluorogenic substrate. *p < 0.05, **p<0.01, ***p<0.001, Kruskal-Wallis test followed by Dunn's test. Error bars = SEM.

- A vector expressing GLB1 from a human ubiquitin C promoter yielded the highest β-gal expression in brain and CSF, and was selected for further studies in a mouse model of GM1 gangliosidosis.

β-gal expression in AAV-treated GLB1$^{-/-}$ mice

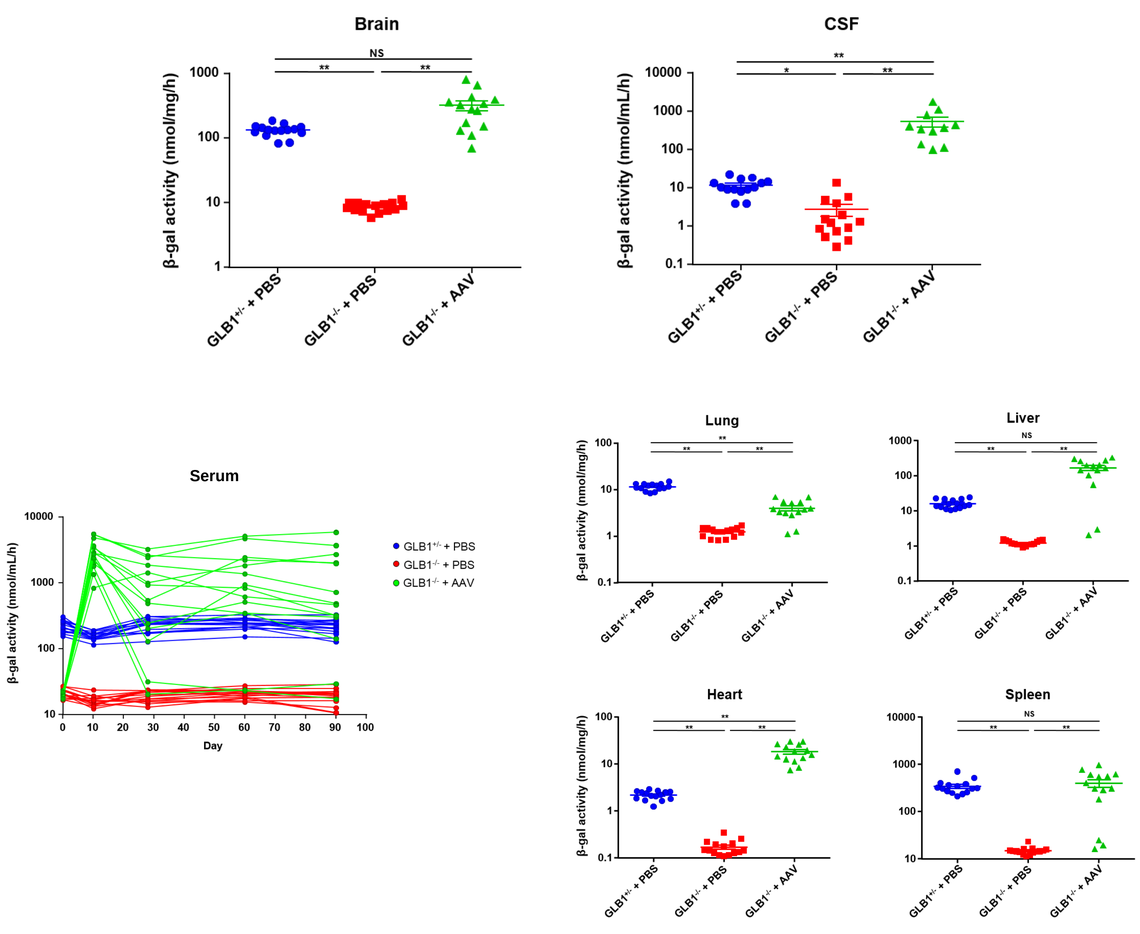

Figure 2: β-gal activity in AAV-treated GLB1$^{-/-}$ mice
GLB1$^{-/-}$ mice were treated with a single ICV injection of 10^{11} GC of AAV.UbC.hGLB1 (n = 15) or vehicle (n = 15). A group of heterozygous (GLB1$^{+/-}$) mice treated with vehicle (n = 15) served as normal controls. β-gal activity was measured in brain and CSF as well as serum, lung, liver, heart, and spleen samples using a fluorogenic substrate. PBS: phosphate buffered saline (vehicle), AAV: Adeno-associated virus (AAV.UbC.hGLB1). *p < 0.05, **p<0.01 Kruskal-Wallis test followed by Dunn's test. NS: not significant.

- ICV AAV delivery resulted in robust β-gal expression in the CNS and peripheral organs of GLB1$^{-/-}$ mice.

Reduction of brain storage lesions

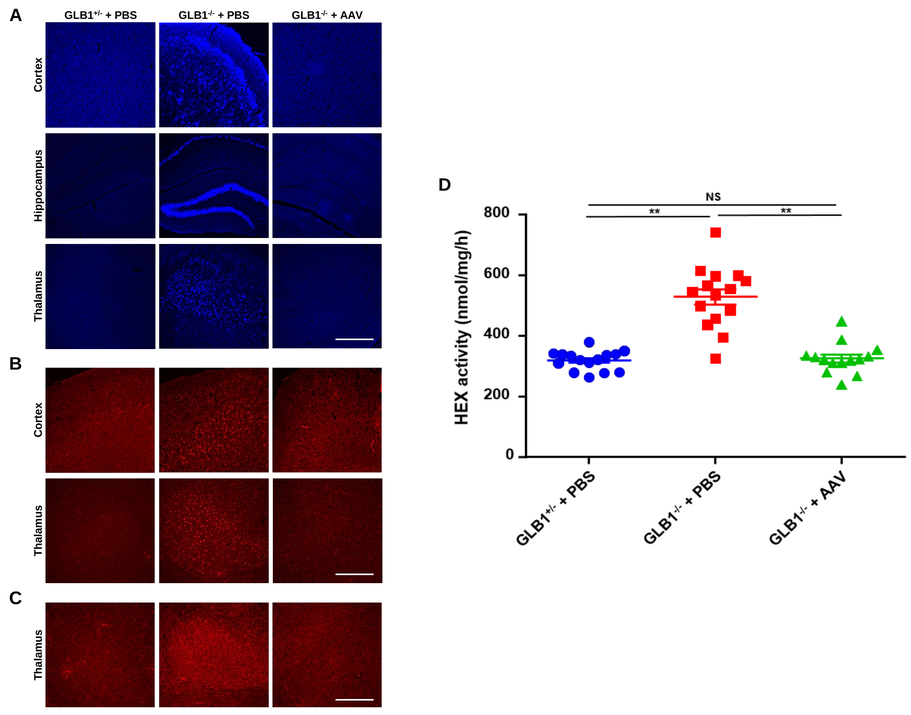

Figure 3. Brain histology and biochemistry of AAV-treated GLB1$^{-/-}$ mice
90 days after ICV administration of 10^{11} GC of AAV.UbC.hGLB1 or vehicle, GLB1$^{-/-}$ mice were sacrificed and brain sections were stained with filipin (A) which binds to GM1 ganglioside and unesterified cholesterol, antibodies against LAMP1 (B), a lysosomal marker or GFAP (C) an astrocyte marker. Hexosaminidase activity was measured in samples of frontal cortex (D). **p<0.01 Kruskal-Wallis test followed by Dunn's test. NS: not significant. Scale bars = 250 µm (A, cortex) and 500 µm (A, hippocampus and thalamus, B, C).

- ICV administration of an AAV vector expressing human GLB1 reduced storage lesions and gliosis in brains of GLB1$^{-/-}$ mice and normalized aberrant overexpression of lysosomal enzymes.

Correction of gait abnormalities

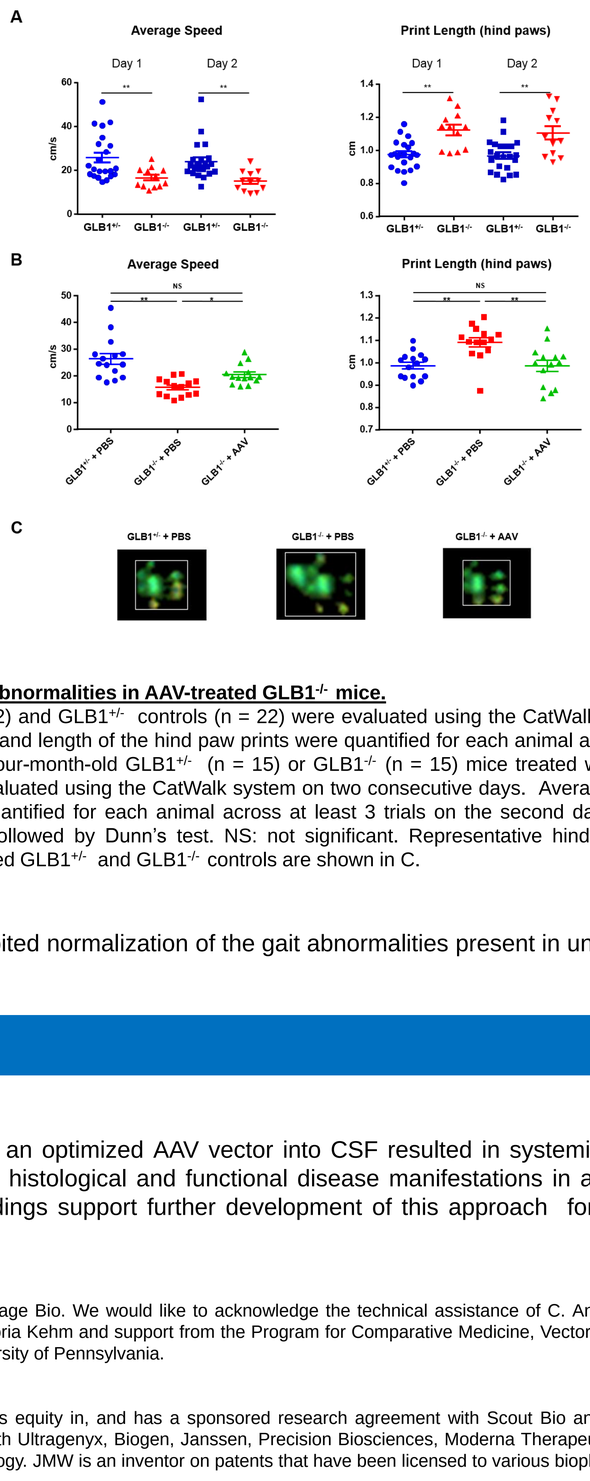

Figure 4: Correction of gait abnormalities in AAV-treated GLB1$^{-/-}$ mice.
Untreated GLB1$^{-/-}$ mice (n = 12) and GLB1$^{+/-}$ controls (n = 22) were evaluated using the CatWalk system on two consecutive days. Average walking speed and length of the hind paw prints were quantified for each animal across at least 3 trials (A). **p < 0.01 Mann Whitney test. Four-month-old GLB1$^{+/-}$ (n = 15) or GLB1$^{-/-}$ (n = 15) mice treated with vehicle and AAV-treated GLB1$^{-/-}$ mice (n = 14) were evaluated using the CatWalk system on two consecutive days. Average walking speed and length of the hind paw prints was quantified for each animal across at least 3 trials on the second day of testing (B). *p < 0.05, **p<0.01 Kruskal-Wallis test followed by Dunn's test. NS: not significant. Representative hind paw prints for AAV-treated GLB1$^{-/-}$ mice and vehicle-treated GLB1$^{+/-}$ and GLB1$^{-/-}$ controls are shown in C.

- AAV-treated mice exhibited normalization of the gait abnormalities present in untreated GLB1$^{-/-}$ mice.

Conclusion

A single administration of an optimized AAV vector into CSF resulted in systemic β-gal expression and correction of biochemical, histological and functional disease manifestations in a mouse model of GM1 gangliosidosis. These findings support further development of this approach for the treatment of GM1 gangliosidosis.

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Conflict of Interest Statement
J.M. Wilson is an advisor to, holds equity in, and has a sponsored research agreement with Scout Bio and Passage Bio; he also has a sponsored research agreement with Ultragenyx, Biogen, Janssen, Precision Biosciences, Moderna Therapeutics, and Amicus Therapeutics who are licensees of Penn technology. JMW is an inventor on patents that have been licensed to various biopharmaceutical companies.