POST-SYMPTOMATIC REVERSAL OF MUSCLE PATHOLOGY IN A MODEL OF POMPE DISEASE USING GENE THERAPY

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Introduction

Pompe disease is a lysosomal storage disorder where mutations in the acid alpha-glucosidase (*Gaa*) gene causes GAA enzyme deficiency and accumulation of glycogen in multiple tissues, leading to a neuromuscular disorder with severe weakness and respiratory failure. Enzyme replacement therapy (ERT) is currently the only approved treatment for patients with Pompe disease. Although ERT significantly improves survival in patients with classic infantile Pompe disease, this treatment cannot fully reverse the skeletal muscle pathology, in part due to autophagic buildup, which inhibits the enzyme from reaching the lysosome. We believe that adeno-associated viral (AAV) vector-mediated gene therapy using a pantropic capsid, a ubiquitous promoter, and a transgene encoding an optimized protein engineered to increase cross correction can improve treatment outcomes and muscle pathology correction.

Aim

We have previously reported the therapeutic effect of an engineered human acid α-glucosidase gene therapy AAV vector using different routes of administration among aged mice that model the advanced stages of Pompe disease. Here we aim to further characterize the efficacy of this approach to reverse several features of muscle pathology and compare the treatment effect in young pre-symptomatic vs. old post-symptomatic mice.

Study Overview & Methods

Young Mice

- Pompe mice **1-1.5 months old**
- **AAV.hGAA eng**

Age at necropsy 3-4.5 months

D0 – IV
PBS
1e11 GC (5e12 GC/Kg); n = 4M & 4F
2e11 GC (1e13 GC/Kg); n = 4M & 4F
5e11 GC (2.5e13 GC/Kg); n = 4M & 4F
1e12 GC (5e13 GC/Kg); n = 4M & 4F
2e12 GC (1e14 GC/Kg); n = 4M & 4F

D60
Euthanasia
Histology

Aged Mice

- Pompe mice **6-7 months old**
- **AAV.hGAA eng**

Age at necropsy 13-14 months

IV
HD 1e12 GC (5e13 GC/Kg)
LD 2e11 GC (1e13 GC/Kg)

ICV
HD 1e11 GC (5e12 GC/Kg)
LD 5e10 GC (2.5e12 GC/Kg)

IV + ICV
HD + HD 1e12 GC + 1e11 GC
LD + LD 2e11 GC + 5e10 GC

D0-D180
Neurobehavior testing

D210
Euthanasia
Histology

- To study muscle pathology, quadriceps muscle sections were analyzed by immunohistochemistry using staining with WGA (cell membrane; to allow measuring muscle fiber diameter), DAPI (nucleus; to quantify presence of central nuclei), and LC3b antibody (autophagosome; to quantify autophagic buildup). The sections were scanned and automatically digitized, and then analyzed using the Visiopharm software.
- To quantify differences in fiber sizes when compared to wild-type control animals, the fiber diameters were assigned to classes of small (<30 um), medium (30-50 um) and large (>50um).

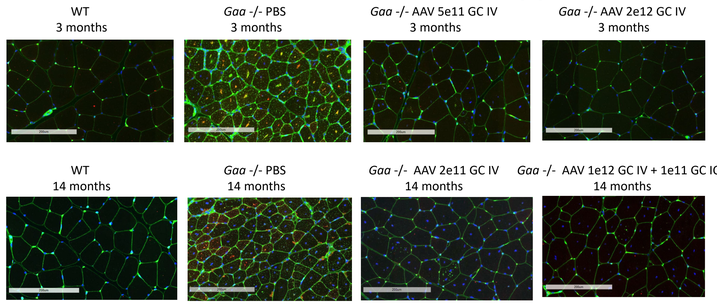
Conflict of Interest Statement

JMW is a paid advisor to and holds equity in Scout Bio and Passage Bio; he also has sponsored research agreements with Amicus Therapeutics, Biogen, Elaaj Bio, FA212, Janssen, Passage Bio, Regeneron, and Scout Bio, which are licensees of Penn technology. JMW and JH are an inventor on patents that have been licensed to various biopharmaceutical companies and for which they may receive payments. ST, NM, PT, JW, and HD are employees of Amicus Therapeutics. This research was supported by Amicus Therapeutics.

Acknowledgements

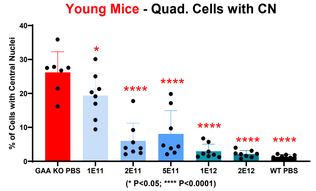
We would like to thank the GTP Penn vector core for vector production, GTP pathology core for histopathology analyses, and Dr. Kristofer Michalson for his invaluable assistance with VisioPharm analyses.

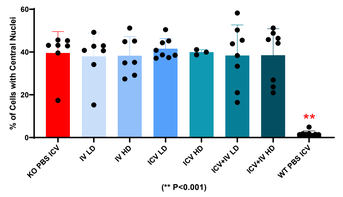
1/ Skeletal Muscle pathology in *Gaa*–/– Mice is progressive and reversible by AAV therapy



- Representative immunofluorescence images of quadriceps muscle sections from the WT, control PBS-treated *Gaa* -/-, and AAV.hGAA eng-treated *Gaa* -/- mice at different ages. **WGA (green; cell membrane), DAPI (blue; nucleus),** and **LC3b antibody (red-orange; autophagosome**).
- Increased number of central nuclei, central autophagosome buildup, and muscle atrophy with increased small fibers and decreased large fibers characterize muscle pathology in GAA -/- mice.
- AAV hGAA eng treatment of *Gaa* KO mice at various doses or routes of vector administration appears to prevent or reverse the muscle pathology in young and aged *Gaa* KO mice, respectively.

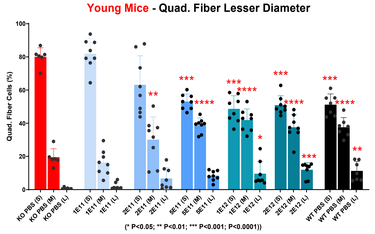
2/ Muscle degeneration and regeneration (central nuclei) is prevented by early gene therapy

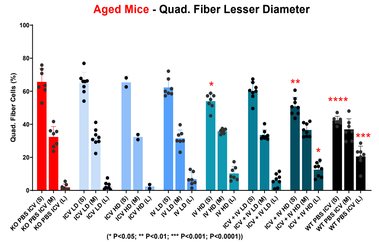




- Healthy muscle fibers rarely contain central nuclei (CN) (below 3% in WT muscle) and the presence of CN is indicative of muscle degeneration followed by regeneration.
- In "Young Mice", the % of fibers with CN was significantly decreased, indicating protection from degeneration in mice treated with 2e11 GC (1e13 GC/kg) dose and higher. In contrast, no treatment effect was seen in any of the "Aged Mice".
- In mice treated after 6 months, degeneration/regeneration cycles had already occurred before the treatment.

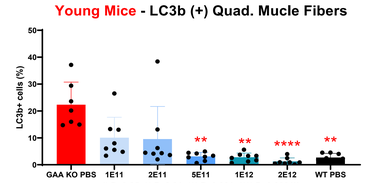
3/ Muscle atrophy is prevented by early gene therapy and reversed by late gene therapy

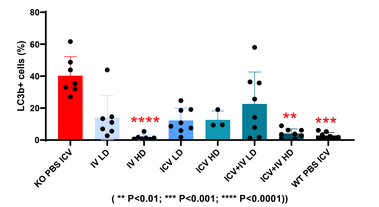




- In "Young Mice", the proportion of small fibers (S) was significantly decreased in *Gaa* -/- mice treated with 2e11 GC (1e13 GC/kg) dose and higher, indicating muscle atrophy prevention. Note PBS controls show significant atrophy at 3 months of age.
- In "Aged Mice" treated at 6 months of age, when muscle atrophy is already prominent, the proportion of small fibers (S) was decreased in ICV+IV HD treated group (ICV 1e11 GC and IV 1e12 GC = 5e13 GC/kg), and the proportion of large fibers (L) was improved in IV HD and ICV+IV HD treated groups.

4/ Autophagic buildup is prevented by early gene therapy and reversed by late gene therapy





- In "Young Mice", autophagic buildup (% of LC3b + cells) was prevented at all doses started from 5e11 GC (2.5e13 GC/kg). Note the significant autophagosome buildup in PBS controls at 3 months of age (20% of fibers).
- In "Aged Mice" with pre-existing pathology at treatment, the autophagosome accumulation was completely reversed in IV HD (1e12 GC = 5e13 GC/kg), and ICV+IV HD (ICV 1e11 GC and IV 1e12 GC) groups.

Conclusion

- Our optimized gene therapy candidate prevented the development of muscle fiber pathology in young Pompe mice and reversed pre-existing muscle fiber pathology in aged Pompe mice, including findings that are typically treatment-resistant such as the atrophy of fibers and autophagic build-up. Our results demonstrate nearly identical treatment effects in young pre-symptomatic vs. old post-symptomatic mice.
- The results support the pursuit of AAV gene therapy using a pantropic capsid, ubiquitous promoter, and engineered GAA protein as a promising clinical approach for treating patients with Pompe disease.