A novel AAV9 gene therapy for producing β-glucocerebrosidase enzyme with high mannose 6-phosphate content to treat Gaucher disease

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1. Introduction

- Gaucher disease is a rare inherited Gaucher disease is a rare inherited metabolic disorder of defective lipid catabolism caused by deficient β-glucocerebrosidase (GCase) encoding by *GBA1* gene resulting in accumulation of glycosphingolipids in the periphery and central nervous system (CNS). Multiple recombinant human GCase enzyme replacement therapies have been approved to treat type 1 Gaucher disease, but there is no effective treatment to address the neuronopathic manifestations for Gaucher type 2&3 disease. Gene therapy could be a potential therapeutic approach to treat all three types of Gaucher disease.
- AAV gene therapy for soluble lysosomal enzymes to treat lysosomal storage disorders largely relies on enzyme cross-correction of non-transduced cells to achieve high efficacy during the treatment. Different approaches are employed to improve the targeting of the overexpressed enzymes (e.g., enzyme fusion with IGF2 peptide, or an antibody fragment) are under investigation in the field. Data from our previous publications show that co-expression of truncated GlcNAc-1-phosphotransferase (designated as S1S3 PTase, Figure 1; Liu et al 2017), greatly increases the levels of mannose 6-phosphate (M6P) on lysosomal enzymes to enable efficient cellular uptake via the cation-independent mannose 6-phosphate receptor (CI-MPR) that is the broadly expressed on most cells including neurons and other cell types in the CNS.
- Here, we report a novel AAV gene therapy approach using a dual promoter construct design for co-expression of *GBA* gene with the truncated S1S3 PTase to produce highly phosphorylated GCase as a potential treatment for Gaucher disease. The produced hGCase with S1S3 PTase co-expression is shown to have high M6P content and enhanced binding to CI-MPR as compared to the enzyme produced without S1S3 PTase. GCase uptake and distribution in the CNS was also evaluated by immunohistochemical staining.

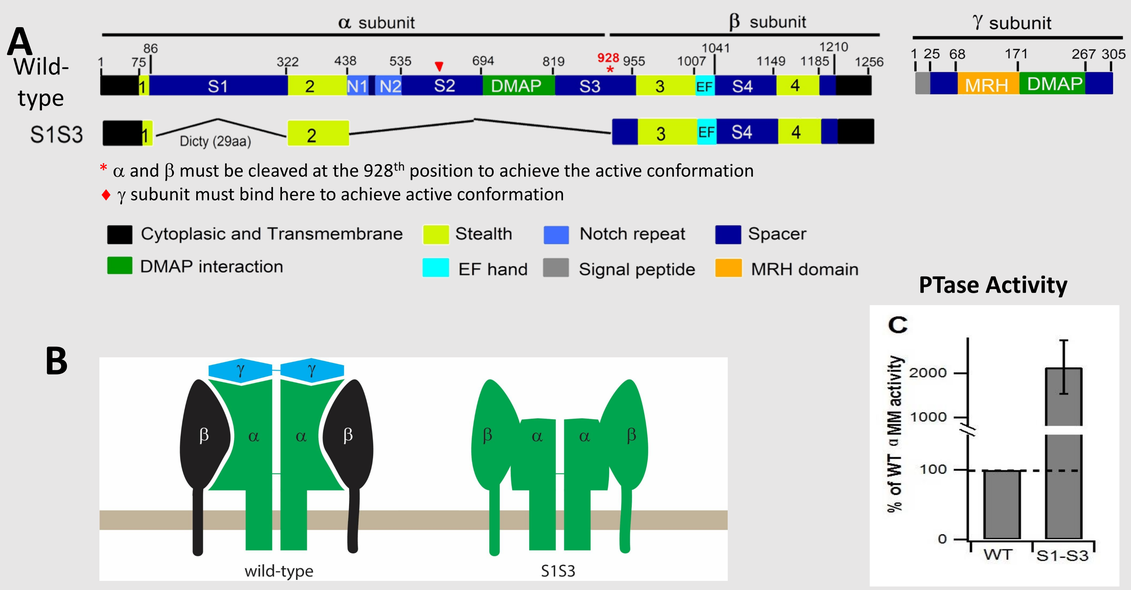
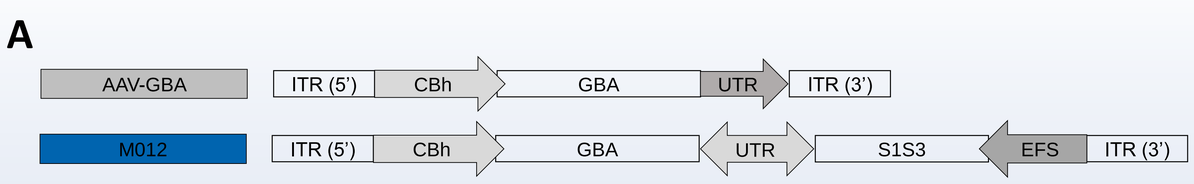
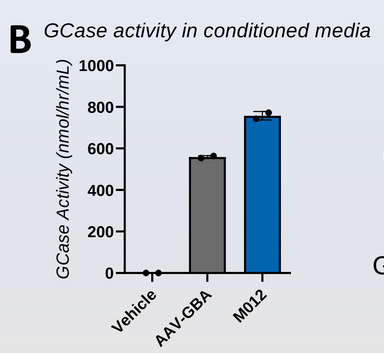


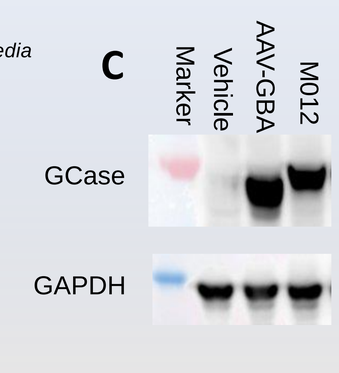
Figure 1. A) Wild-type and S1S3 GlcNAc-1-phosphotransferase. The wild-type phosphotransferase contains three subunits (α,β and γ). Several spacer domains (S1, S2, S3, and S4) in the α subunit including the γ bindings site are removed in S1S3[2]. B) Schematic of the wild-type and S1S3 phosphotransferase. S1S3 lacks cleavage between alpha and beta subunits and is shown without the gamma subunit. C) the S1S3 phosphotransferase activity is 20 times higher than then wild-type enzyme.

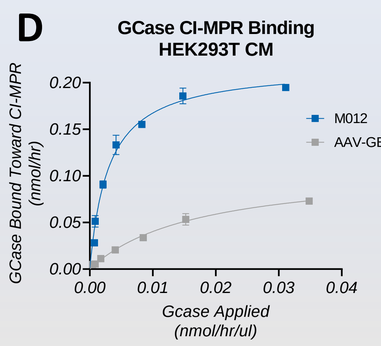
2. Results

2.1. Dual-promoter design co-expressing GBA gene with S1S3 PTase achieves high level expression of GCase enzyme with enhanced CI-MPR binding

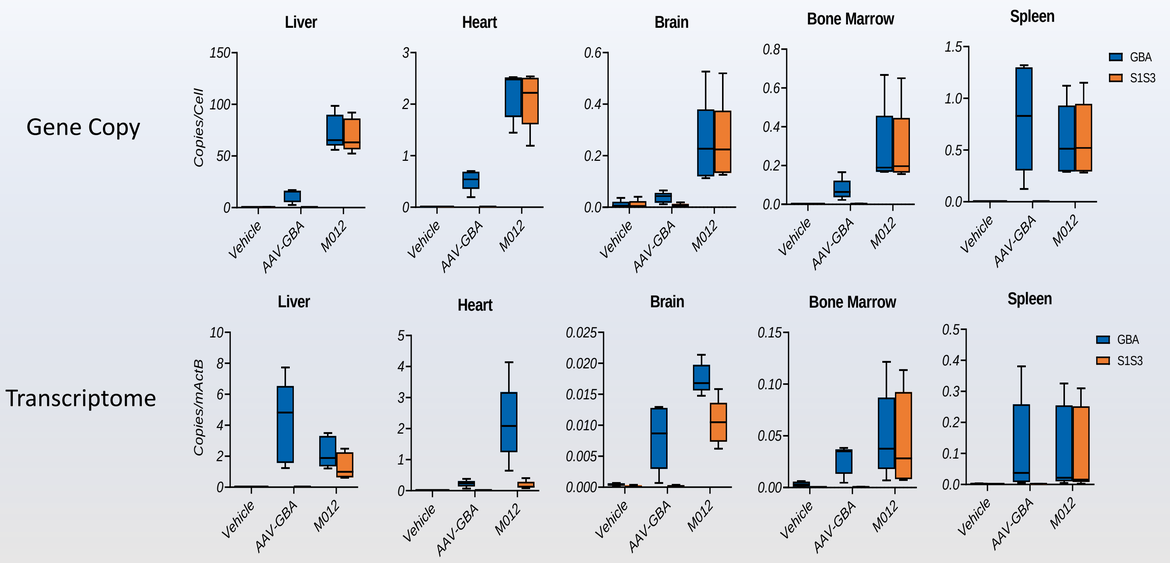




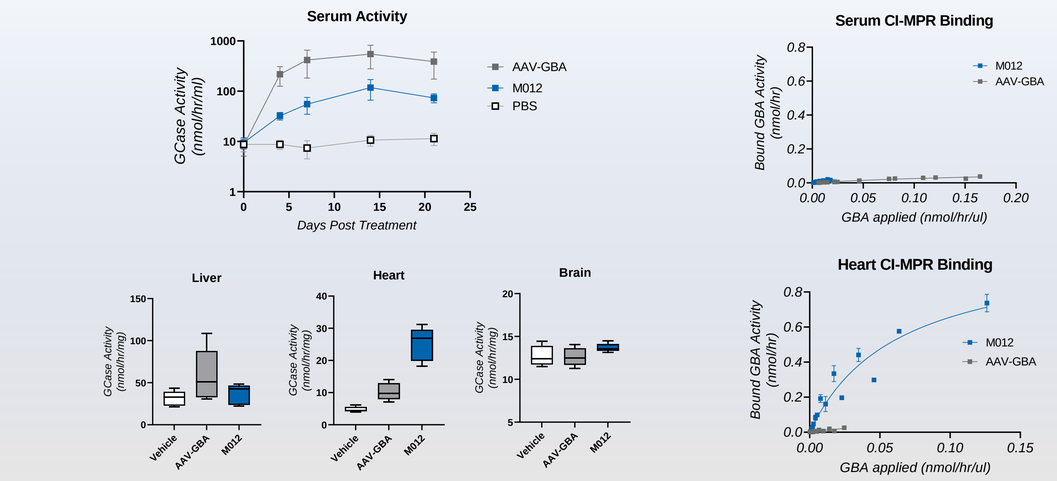




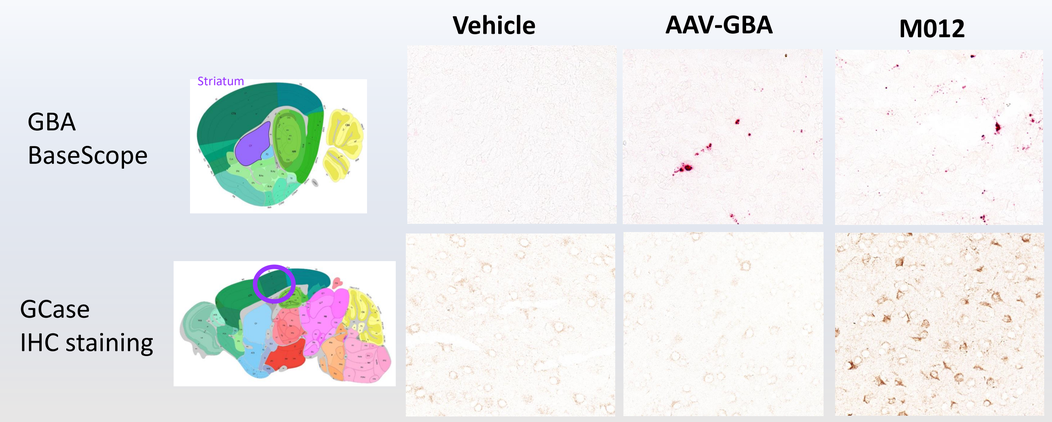
2.2. M012, the dual-promoter GBA AAV9 vector shows broad distribution across tissues detected by high gene copy and RNA level in treated animal



2.3. GCase enzyme is internalized into cells with high efficiency as evidence by lower serum enzyme activity, but high enzyme level in tissues together with high CI-MPR binding in the M012 treated animal



2.4. Highly phosphorylated GCase enzyme produced by M012 is shown to be broadly distributed and internalized in brain cells



3. Conclusion

Gene therapy for soluble enzyme/protein relies on cross-correction of non-transduced cells to achieve high *in vivo* efficacy. To effectively increase enzyme cross-correction, we utilized a novel dual-promoter AAV vector (M012) to co-express GBA and S1S3 PTase genes to produce therapeutic GCase with high levels of M6P *in vivo* to enable high binding affinity to CI-MPR. Gene copy and transcriptome analysis showed that the novel M012 AAV vector dosed at 2E13 vg/kg enabled transduction of peripheral tissues and various regions of brain in wild-type mice post-intravenous injection. Increased GCase enzyme activity with enhanced CI-MPR affinity were observed in peripheral tissues of treated animals. Brain samples were examined by BaseScope for mRNA and anti-GCase immunohistology for GCase protein. Strong GCase protein signal with broad distribution were detected in M012 AAV vector-treated brain samples. In contrast, only background staining was observed with the GBA only AAV vector. Taken together, these results strongly suggest M012 AAV enables effective GCase cross-correction in the CNS.

4. Acknowledgement

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