Optimized dual promoter AAV gene therapy for lysosomal β-glucocerebrosidase with high mannose-6-phosphate content for treatment of neuronopathic Gaucher disease

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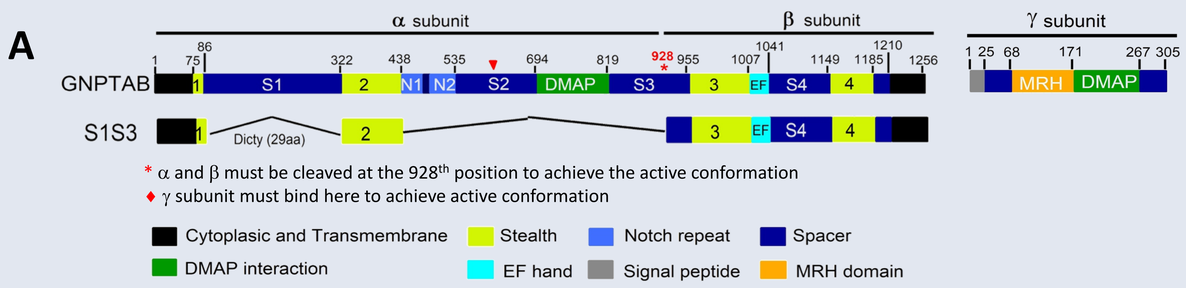
1. Introduction

Gaucher disease (GD) is a rare inherited metabolic disorder of defective lipid catabolism caused by deficient β-glucocerebrosidase (GCase) activity resulting in accumulation of glycosphingolipids in the peripheral and central nervous system (CNS). While there are multiple approved recombinant human β-glucocerebrosidase enzyme replacement therapies that address peripheral symptoms for Type 1 Gaucher disease, there is currently no effective treatment to address neuronopathic manifestations for Type 2 & 3 Gaucher disease. While enzyme replacement therapy improves hepatosplenomegaly and other peripheral symptoms in GD, it cannot address neurological manifestations of GD Types 2 and 3 patients because the enzyme is unable to cross the blood brain barrier. Here we demonstrate an AAV gene therapy approach to potentially treat neuronopathic GD, by co-expressing human GCase with a modified GlcNac-1-phosphotransferase (called S1S3 PTase) in one AAV vector. Co-expression of S1S3 PTase with GCase in our AAV vector allows for the generation of GCase enzyme with enhanced mannose-6-phosphate (Man-6-P) content. Soluble lysosomal enzymes, including GCase, that are modified with Man-6-P can be efficiently endocytosed by the cation-independent Man-6-P receptor (CI-MPR), which is abundantly expressed across tissues, including in the CNS. Highly phosphorylated GCase produced from the dual-promoter AAV binds the CI-MPR with high affinity (<10 nM), is efficiently internalized *in vitro* and reduces the substrate glucosylsphingosine (lyso-GL1). AAV vectors were optimized to maximize the Man-6-P content on GCase. Further, AAV9 was produced and injected into a Gaucher animal model intracerebroventricularly, highly phosphorylated GCase was produced in brain tissue, and the AAV vector shows broad GCase and S1S3 PTase expression, Man-6-P phosphorylation and substrate reduction in brain tissue. These results suggest that the dual-promoter AAV gene therapy approach could be a viable strategy to produce highly phosphorylated GCase *in vivo* to facilitate cellular uptake and cross-correction in the CNS as a novel treatment for Type 2/3 Gaucher disease.



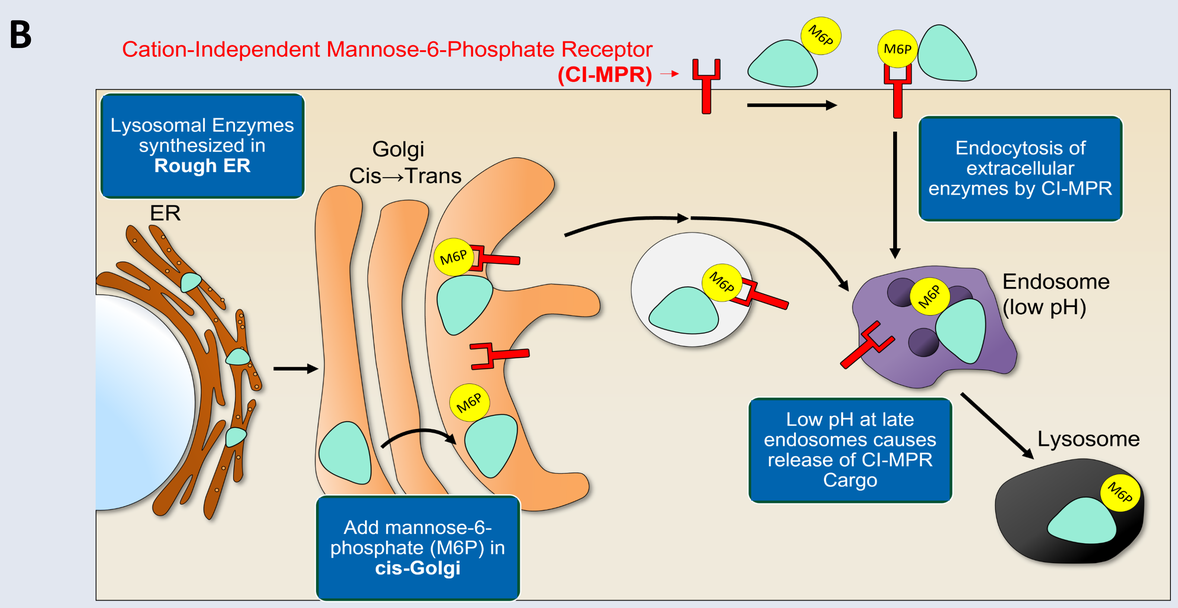
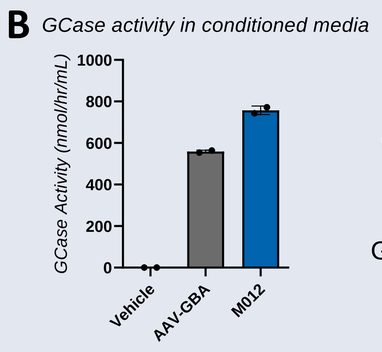


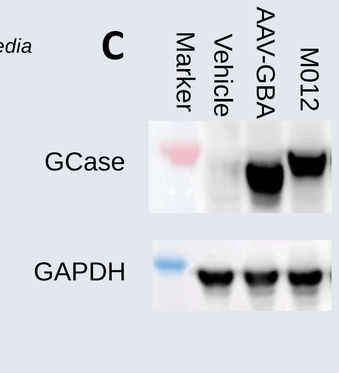
Figure 1. A) Wild-type (GNPTAB) and S1S3 GlcNAc-1-phosphotransferase (PTase). The wild-type phosphotransferase contains three subunits (α, β and γ). Several spacer domains (S1, S2, S3 and S4) in the α subunit including the γ bindings site are removed in S1S3 PTase.

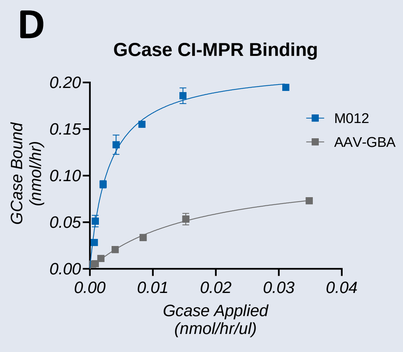
B) Summary of Man-6-P mediated lysosomal trafficking. In the Golgi, PTase enzyme adds Man-6-P (M6P) glycan to proteins which is recognized by the CIMPR to facilitate lysosomal trafficking.

2. Results

2.1. Dual-promoter design co-expressing human GBA1 gene with S1S3 PTase achieves high level expression of GCase enzyme with enhanced CI-MPR binding.

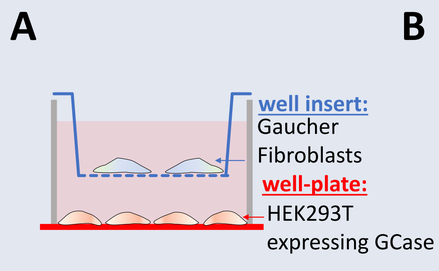


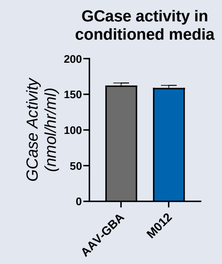


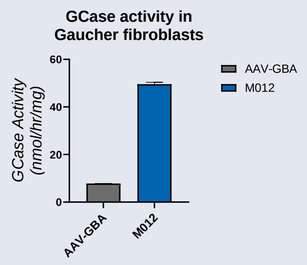


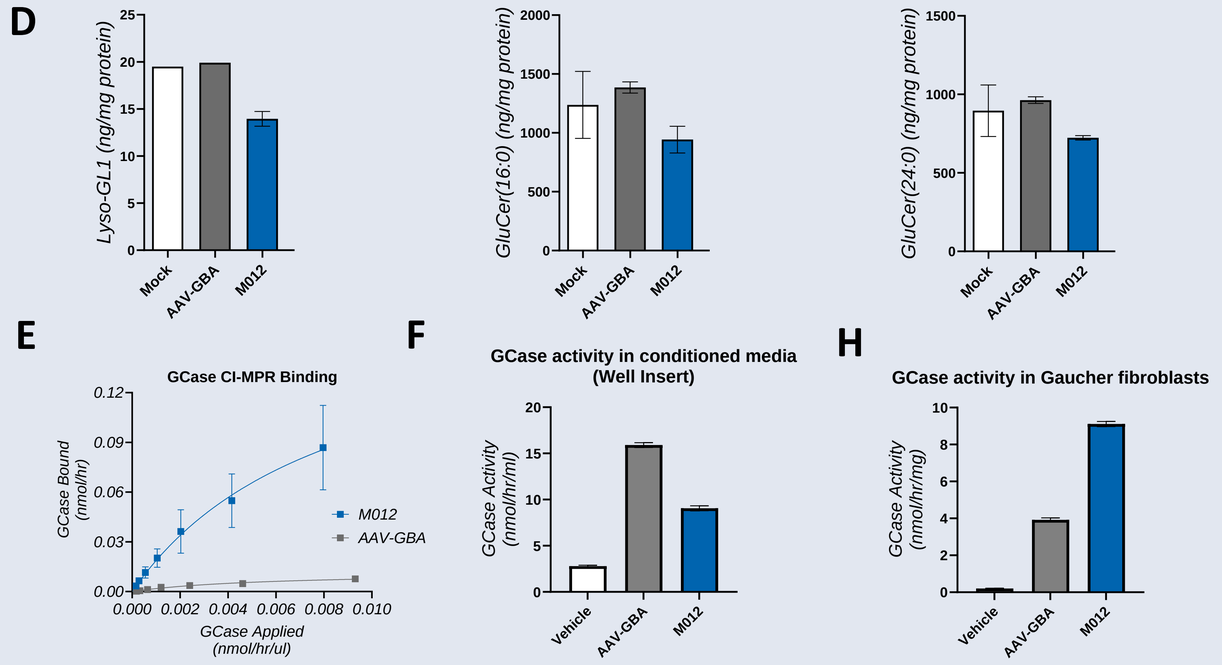
HEK293T cells were transfected with plasmids for (A) AAV-GBA, expressing GCase alone, or M012, for dual promoter co-expression of GCase and S1S3 modified PTase, and analyzed for (B) GCase activity in conditioned media, (C) Western blot of GCase expression and (D) binding of GCase from conditioned media to CI-MPR

2.2 M012 GTx produces phosphorylated GCase that is efficiently internalized in Gaucher fibroblasts and reduces Lyso-GL1 levels.



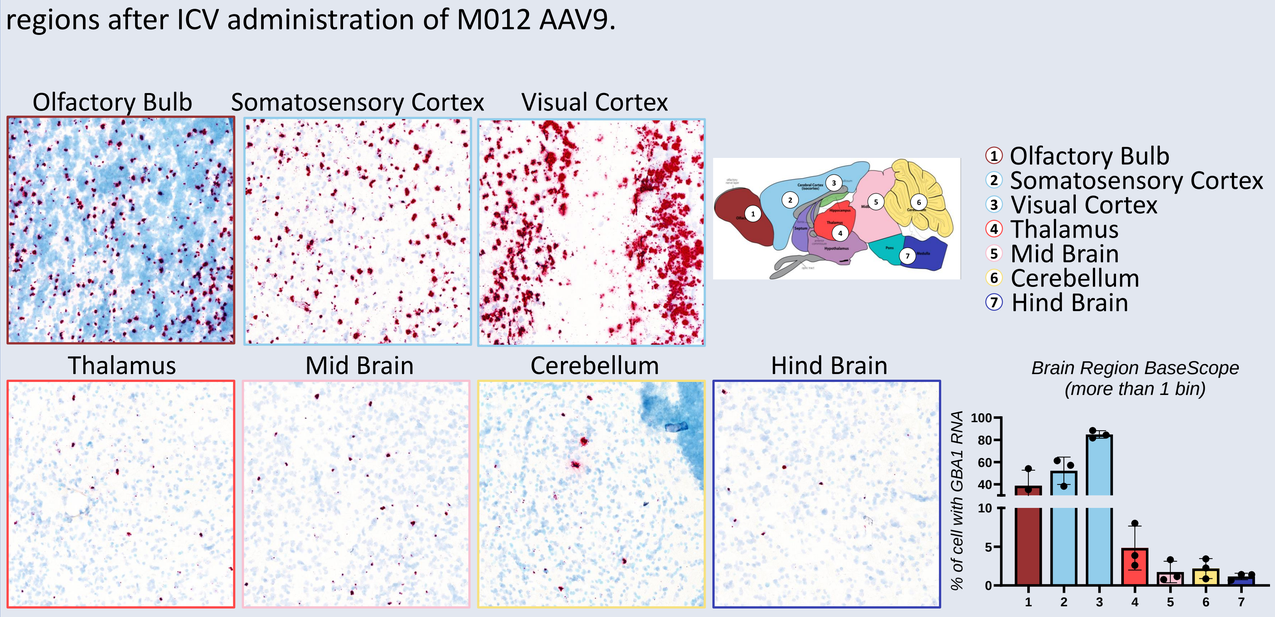






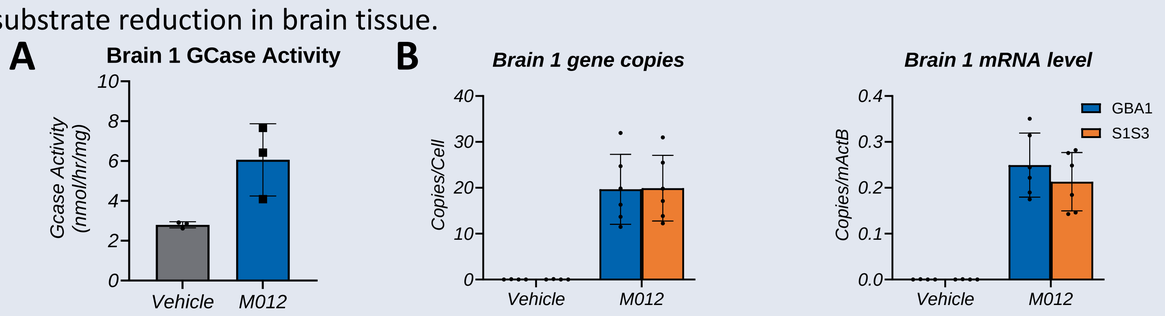
(A) Co-culture of HEK293T expressing vectors for GBA alone or M012 were co-cultured with Gaucher fibroblasts (GM20273). GCase activity, substrate levels or CI-MPR binding were measured after transfection (B-D) or AAV2 transduction (E-H).

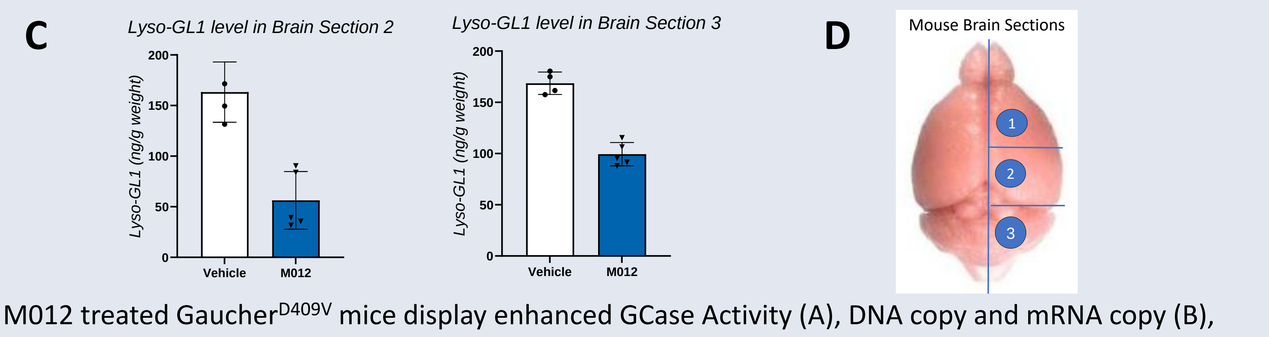
2.3. GBA^{D409V} mice show broad distribution of GBA1 mRNA detected by BaseScope in brain regions after ICV administration of M012 AAV9.



BaseScope analysis of GBA1 mRNA expression in the brain of M012 treated Gaucher^{D409V} mice.

2.4. GBA^{D409V} mice treated with M012 AAV9 enhances GCase activity, gene expression and substrate reduction in brain tissue.





M012 treated Gaucher^{D409V} mice display enhanced GCase Activity (A), DNA copy and mRNA copy (B), and reduced Lyso-GL1 levels (C). (D) shows brain sections that were analyzed.

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 GBA1 and S1S3 PTase genes to produce therapeutic GCase with high levels of Man-6-P *in vivo* which enables high binding affinity to CI-MPR. Gene copy and transcription analysis showed that the novel M012 AAV vector enabled broad transduction of the brain. M012 treated mice showed increased GCase enzyme activity with reduction of the substrate Lyso-GL1. Altogether M012 AAV produces GCase enzyme with enhanced Man-6-P content *in vivo* for potential cross correction. Future work will explore M012 vector in neuronopathic models of Gaucher disease.