Novel AAV gene therapy produces β-glucocerebrosidase with high levels of M6P to enable cellular uptake and cross-correction in the CNS as a potential treatment for Type 2/3 Gaucher disease

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1. Introduction

- 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.
- 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 to improve targeting of 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.

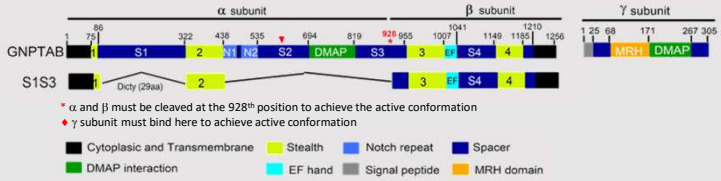
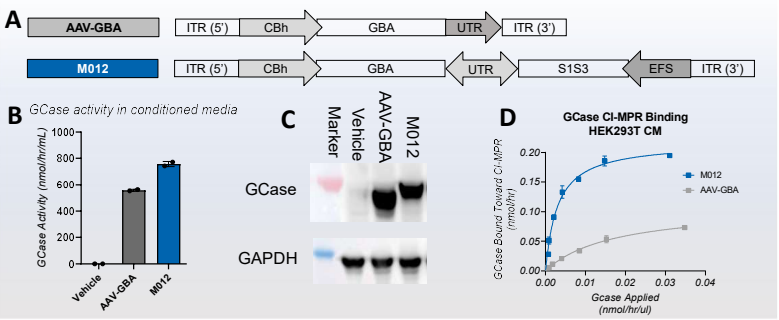


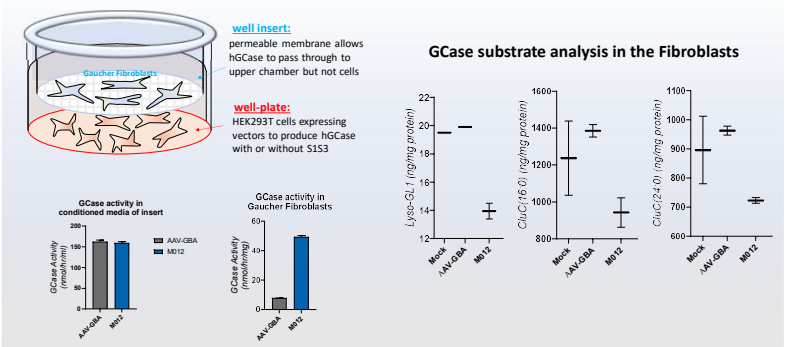
Figure 1. Wild-type and S1S3 GlcNAc-1-phosphotransferase. The wild-type phosphotransferase contains three subunits (α,β and γ). Several spacer domains (S1, S2, S3, and S4) in the α subunit including the γ bindings site are removed in S1S3

2. Results

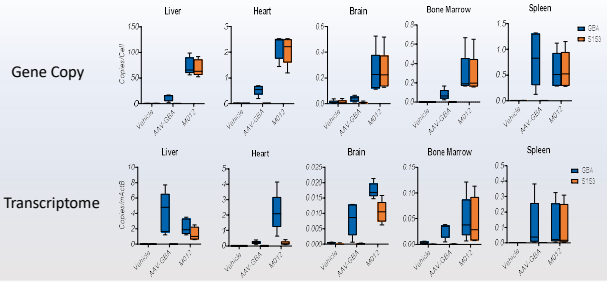
2.1. Dual-promoter design co-expressing GBA gene with S1S3 PTase achieves high level expression of GCase enzyme with enhanced CI-MPR binding



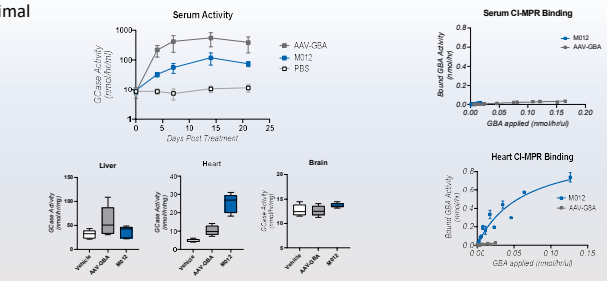
2.2. M012 GTx Produces Phosphorylated hGCase That Is Efficiently Internalized in Gaucher Fibroblasts; WT GBA Alone GTx Cannot



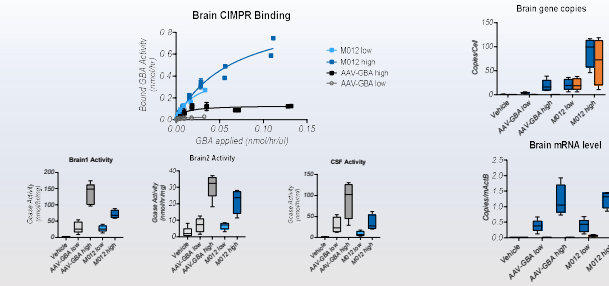
2.3. M012, the dual-promoter GBA AAV9 vector shows broad distribution across tissues detected by high gene copy and RNA level in treated animal



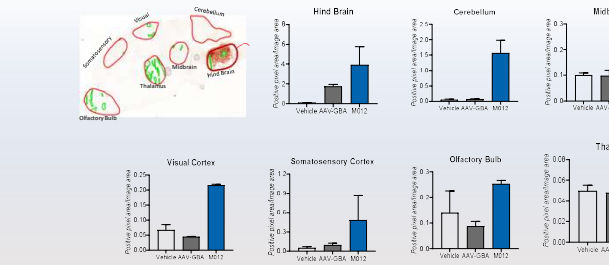
2.4. GCase enzyme is internalized into cells with high efficiency as evidence by lower serum enzyme activity, but higher enzyme level in tissues together with high CI-MPR binding in the M012 treated animal



2.5. ICV injection of M012 produced highly phosphorylated GCase enzyme in brain of neonate Gaucher Mice, proven with enhanced CI-MPR binding



2.6. Highly Phosphorylated GCase Produced by M012 AAV GTx is Broadly Distributed and Internalized in Brain Regions by ICM Administration in WT Animals



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 M6P *in vivo* which enables 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 was observed in peripheral tissues of treated animals. ICV injection of M012 AAV increase the CI-MPR binding in brains of neonate Gaucher animals. Brain samples were examined by BaseScope for mRNA and anti-GCase immunohistology for GCase protein. Strong GCase protein signal with broad distribution was detected in M012 AAV vector-treated brain samples. In contrast, only background signal was observed with the GBA only AAV vector. Taken together, these results strongly suggest M012 AAV enables effective GCase cross-correction in the CNS.