An innovative gene therapy approach to produce novel human GALC variant with enhanced protein stability and enzyme activity with high levels of mannose 6-phophate for Krabbe disease

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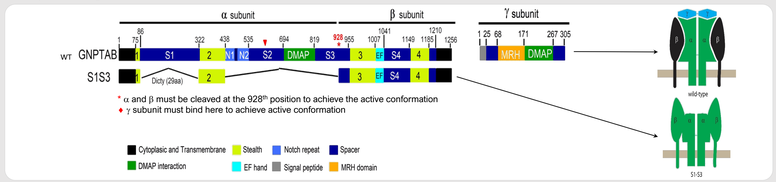
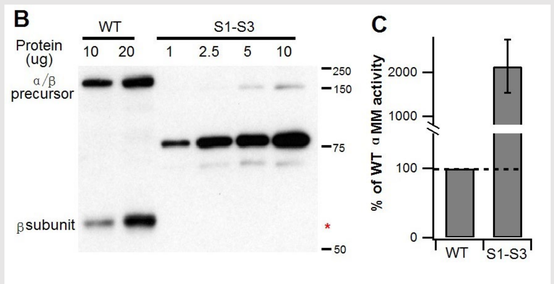
1. Introduction

- Krabbe disease is a severe neuromuscular lysosomal storage disorder caused by mutations in the galactosylceramidase (GALC) gene. This leads to galactosylceramidase (GALC) enzyme deficiency and results in continuous build-up of various lipids and glycolipids including cytotoxic psychosine. GALC deficiency causes loss of myelin sheath (protective layer around nerves) resulting in severe nerve damage. Krabbe has many debilitating symptoms including seizures, feeding difficulties, vomiting, deafness, blindness, slurred speech and loss of motor function. There is currently no cure or effective treatments for Krabbe. Human GALC enzyme is difficult to express because it is highly unstable and contains only modest amounts of mannose 6-phosphate (M6P). Development of an effective human GALC treatment must overcome these challenges- more stable and active enzyme with higher amounts of M6P for better lysosomal targeting to increase GALC levels and address clinical symptoms
- 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.

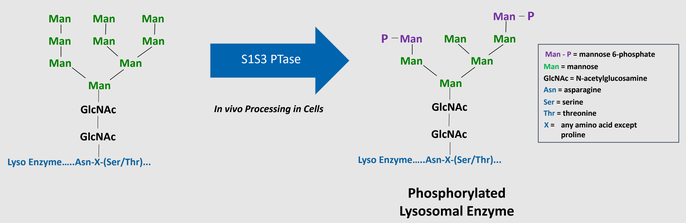
Wild-type and S1S3 GlcNAc-1-phosphotransferase. The wild-type phosphotransferase contains three subunits (α,β and γ). Several spacer domains (S1, S2, S3, and S4) in the α subunit including the γ bindings site are removed in S1S3



S1S3 PTase is a truncated, ***pre-activated*** variant with significant advantages:
- Has proper conformation without need for γ-subunit
- Has 20-fold higher specific activity
- Enables efficient phosphorylation of soluble lysosomal enzymes
- Small gene size enables its incorporation into expression constructs for S1S3 PTase co-expression with therapeutic enzymes within cells for ERT and gene therapy applications

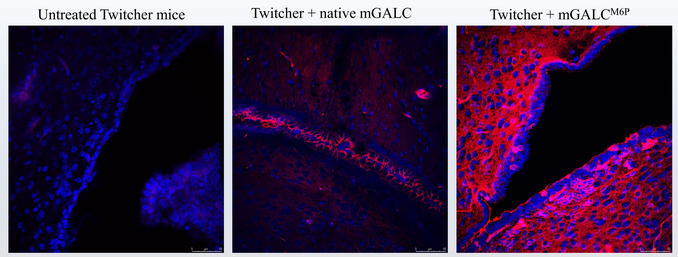


S1S3 PTase Co-Expression Platform Ensures Production of Therapeutic Lysosomal Enzymes with High Levels of M6P



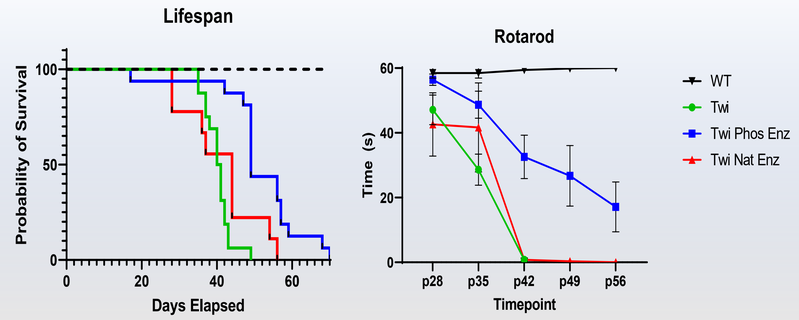
2. Results

2.1. Highly Phosphorylated Mouse GALC (mGALC) Enzyme Exhibits Much Broader Distribution in Brain of Krabbe Mice Post-ICV Injection.

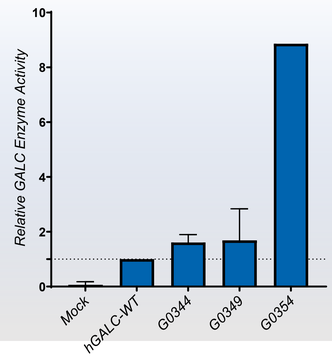


Native mGALC = recombinant mouse GALC expressed without S1S3 PTase
mGALC^{M6P} = recombinant mouse GALC expressed with S1S3 PTase

2.2. Single ICV Administration of Highly Phosphorylated mGALC Enzyme Improves Survival and Rotarod Performance in Twitcher Mice

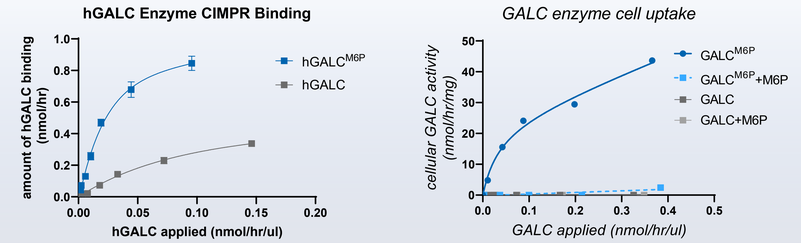


2.4. Novel Human GALC Variant with Two Strategic Amino Acid Substitutions Has Significantly Increased Protein Stability and Enzyme Activity

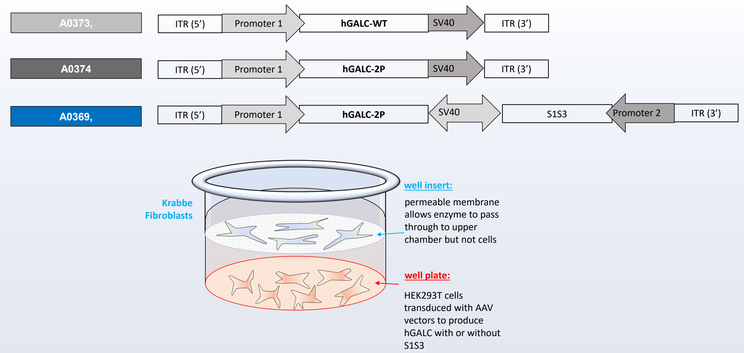


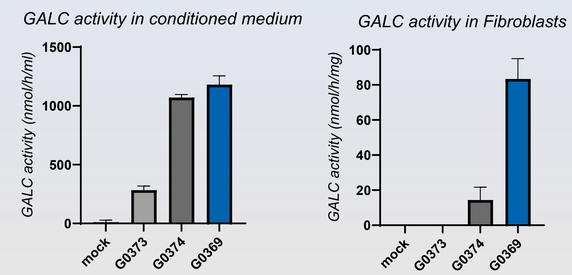
Novel hGALC is significantly more stable and has 8-10X higher enzyme activity than wildtype hGALC.

2.4. Combination of Novel hGALC Construct with S1S3 PTase Co-Expression Yields Much More Stable Enzyme with Improved M6P Receptor Binding and Cellular Uptake



2.5. AAV GTx Produces Novel Stable hGALC with High M6P Levels when Co-expressed with S1S3 for Efficient Cross-Correction of Krabbe Patient fibroblast cells





3. Conclusion

Human GALC (hGALC) is produced with only modest levels of M6P which would limit its cellular uptake and effectiveness as a therapeutic agent. There has been no effective way to increase endogenous phosphorylation process within cells for increasing M6P content on newly synthesized lysosomal enzymes. Our S1S3 PTase co-expression platform overcomes the inefficient endogenous phosphorylation process to ensure newly synthesized lysosomal enzymes are produced with high levels of M6P.
Wildtype hGALC is unstable and difficult to express which hinders development of an effective treatment for Krabbe. We have created a novel hGALC enzyme with 2 strategic amino acid substitutions that yields significantly higher protein stability and enzyme activity, and an innovative gene therapy approach for producing more stable hGALC with high M6P content via S1S3 PTase co-expression.
Novel highly phosphorylated, stable hGALC produced from AAV vector is shown to be efficiently internalized in patient-derived fibroblasts and suggests this could be a more effective gene therapy for Krabbe.

4. Future Studies

Evaluate GTx for cross-correction and substrate reduction, particularly psychosine, in cellular models of Krabbe
Determine whether GTx can improve various cellular dysfunction in Krabbe cellular models
Evaluate GTx in animal models of Krabbe disease for hGALC expression and biodistribution, including in CNS
Determine whether GTx increases survival in animal models of Krabbe disease
Determine whether GTx can reduce/prevent psychosine accumulation in Krabbe mouse models