M002, a Novel AAV9-Mediated Gene Therapy in a Mucolipidosis Type II Mouse Model Utilizing a Truncated Phosphotransferase

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Abstract

Mucolipidosis type II (MLII) is a rare genetic lysosomal storage disorder for which there is currently no treatment. The disease is caused by mutations in the GNPTAB gene encoding the α /β subunits of GlcNAc-1 phosphotransferase (PTase)[1], which generates the mannose 6-phosphate (M6P) recognition marker on lysosomal enzymes (LE). In the absence of PTase, LEs are secreted into the plasma of patients[2] and undegraded substrates accumulate in lysosomes, resulting in premature death by age 10. Using a mouse model of MLII, the efficacy of M002, an AAV9-mediated gene therapy for delivery of a truncated PTase (S1S3) was assessed in both short-term and long-term efficacy studies. S1S3 exhibits high PTase activity in cells[3], and the reduced length of its cDNA (1.8 kb) makes it highly amenable to insertion in the AAV9 vector. The results of our study demonstrate that relative to untreated controls, MLII mice administered M002 1) showed S1S3 PTase expression in tissues; 2) resulted in secretion of phosphorylated LEs with increased binding to the cation-independent mannose 6-phosphate receptor (CI-MPR) in a dose-dependent manner; 3) had partially decreased levels of LEs secreted into the serum whereas intracellular levels of enzymes within tissues increased; 4) showed significant improvement of lysosomal storage as assessed by histopathology of the secretory lesions of exocrine glands; 5) exhibited improved visual acuity and time on the inverted screen test. The ability of M002 to increase the levels of circulating LEs with high M6P content confers a distinct advantage to our gene therapy platform because of its potential to increase the cross-correction by allowing the secreted enzymes to be internalized by other tissues/cells. The efficacy of M002 in correcting cognitive defects associated with loss of PTase function will be determined by assessing additional motor functions of the MLII mice using a variety of behavioral tests. In conclusion, our pilot proof-of-concept data demonstrates that M002, an AAV9 mediated gene therapy for treatment of MLII mice increases LE binding to the CI-MPR, restores M6P content of LEs and decreases serum levels of LEs. M002 is a promising gene therapy for MLII that has the potential to reduce MLII symptoms, including some of the associated cognitive defects.

3. Methods

❖ Treatment of *GNPTAB*$^{-/-}$ mice with M002

I. *GNPTAB*$^{-/-}$ mice at 5 weeks of age were administered M002 via tail vein injection at increasing doses of: 0.1x10^{13} vg/kg (*M002 Low*), 0.5x10^{13} vg/kg (*M002 Mid*), and 2x10^{13} vg/kg (*M002 High*). Serum was collected the day before virus injection, and subsequently at weeks 2, 4, 6 and 12 post-injection.
- Animals were sacrificed at 12 weeks and tissues harvested for biochemical and histopathological analysis.

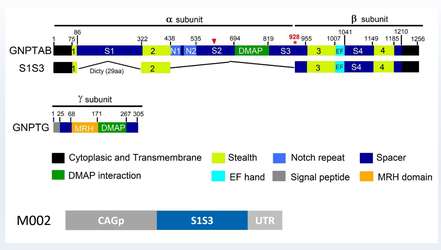
II. *GNPTAB*$^{-/-}$ mice at 4 weeks of age were administered M002 via tail vein injection at a dose of 2x10^{13} vg/kg. Serum was collected the day before virus injection, and subsequently at weeks 2, 6, 10 and 14 post-injection.
- Animals at 13 weeks after injection were subject to a limited behavioral analysis to assess sensorimotor activity.
- Animals were sacrificed at 14 weeks and tissues harvested for biochemical and histopathological analysis

- Serum lysosomal enzyme levels were measured with the collected serum and the degree of phosphorylation determined by binding immobilized cation-independent mannose 6-phosphate receptor(CI-MPR) in a 96-well format for both *in vivo* studies.

4. Results

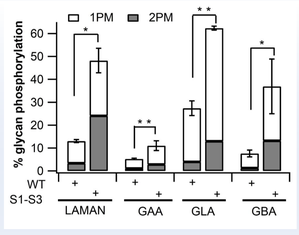
4.1. S1S3 PTase's small gene size and increased phosphorylation of several lysosomal enzymes makes it suitable for AAV gene therapy.

a.



Schematic of full-length PTase , S1S3, and M002 construct.

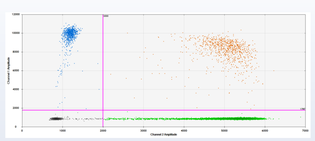
b.



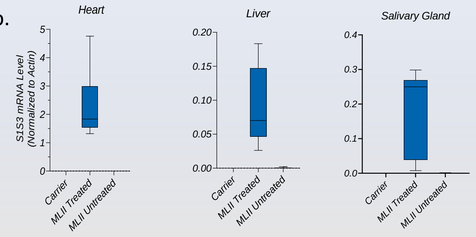
HeLa cells were co-transfected with either WT or S1S3 α/β precursor cDNAs, along with expression plasmids for four lysosomal enzymes. Cells co-transfected with S1S3 PTase showed a significantly higher percentage of glycans phosphorylated.
*p < 0.05, **p < 0.01.

4.2. M002 administration results in S1S3 expression in *GNPTAB*$^{-/-}$ treated mice.

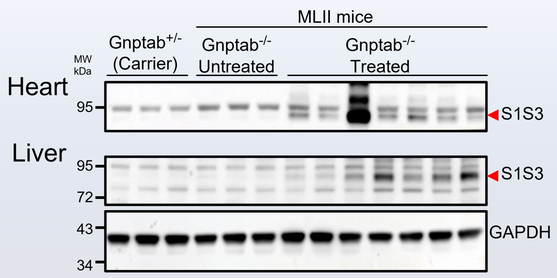
a. An example of ddPCR showing the profiles of S1S3 and β–actin signals



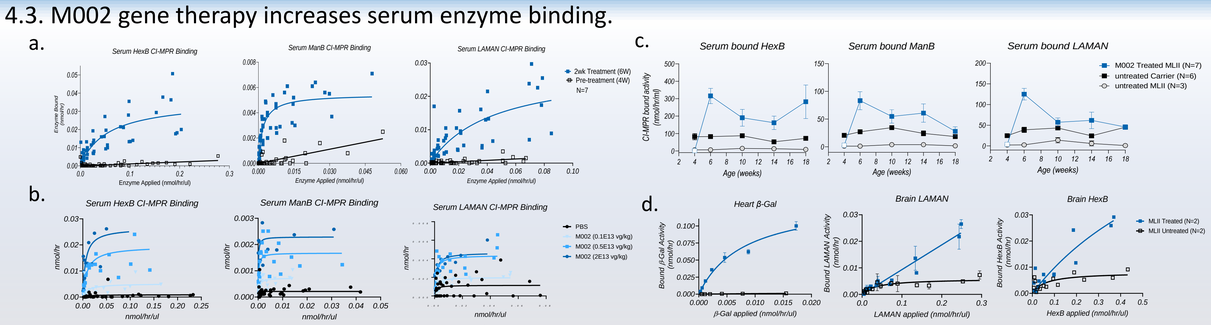
b.



c. Immunoblot analysis of S1S3 expression in heart and liver.

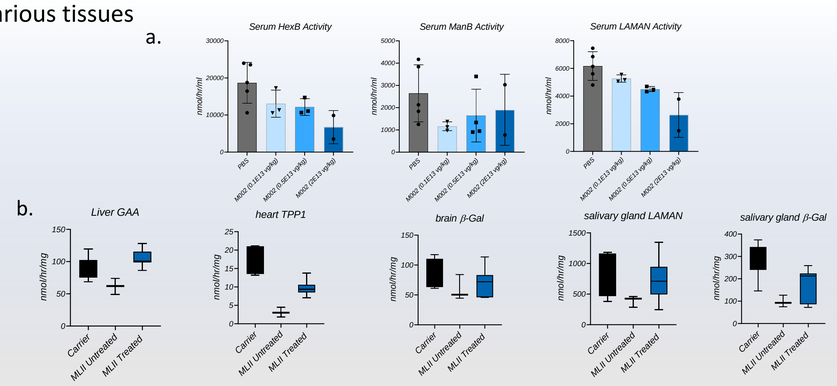


4.3. M002 gene therapy increases serum enzyme binding.



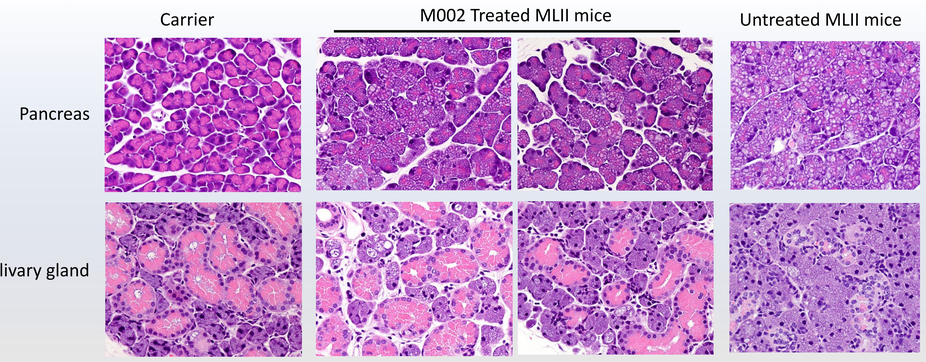
Relative to untreated controls (PBS), treated *GNPTAB*$^{-/-}$ mice showed a) increased CI-MPR binding of serum lysosomal enzymes b) in a dose-dependent manner, c) higher CI-MPR bound serum enzyme activity post injection, and d) higher CI-MPR bound lysosomal enzyme activity in tissues.

4.4. M002 gene therapy partially decreases serum lysosomal enzyme activity and restores lysosomal enzyme levels in various tissues



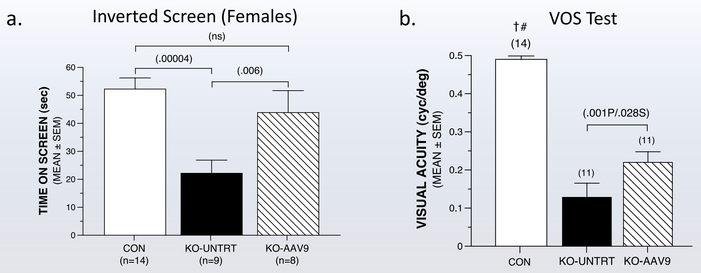
Relative to untreated controls, treated *GNPTAB*$^{-/-}$ mice showed a) a partial decrease of serum lysosomal enzyme activity in a dose-dependent manner, and b) increased lysosomal enzyme activity in several tissues analyzed.

4.5. M002 gene therapy decreases lysosomal vacuolation in pancreas and salivary gland tissues



Relative to untreated controls, treated *GNPTAB*$^{-/-}$ mice showed a partial decrease of lysosomal vacuolation in a) pancreas, and b) salivary gland.

4.6. M002 gene therapy improves visual acuity and strength in *GNPTAB*$^{-/-}$ mice



Relative to untreated controls, treated *GNPTAB*$^{-/-}$ mice performed better in a) the inverted screen test, and b) the VOS test.

5. Conclusion

GNPTAB$^{-/-}$ mice treated with gene therapy M002: 1) showed expression of S1S3 in a number of tissues; 2) had PTase activity which restored phosphorylation to a number of lysosomal enzymes; 3) showed partially decreased levels of lysosomal enzymes secreted into the serum with increased intracellular levels within tissues; 4) exhibited improvement in the phenotype of the secretory lesions of exocrine glands that is normally associated with loss of PTase; 5) displayed improved visual acuity and strength/coordination. The increased levels circulating lysosomal enzymes with high M6P content could potentially increase the cross-correction by allowing the enzymes to be internalized by other tissues/cells. More behavioral tests are planned with a larger cohort of treated and control mice in the near future to assess the efficacy of M002 in correcting more of the motor functions. In conclusion, our pilot proof-of-concept data demonstrates that M002, an AAV9 mediated gene therapy for treatment of MLII mice increases lysosomal enzymes binding to the CI-MPR, restores M6P content of lysosomal enzymes, and decreases serum lysosomal enzymes. M002 is a promising gene therapy for MLII that has the potential to reduce MLII symptoms, including some of the cognitive defects associated with MLII.

6. References

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2. Grayson, M. Lysosomal Storage disorders. *Nature* 2016 537: S145
2. Liu et al. Method for Producing Highly Phosphorylated Lysosomal Enzymes for Enzyme Replacement Therapy. *Mol. Ther. Methods Clin. Dev.* 2017 5: 59-65