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P559 Single AAV gene therapy with mini-Glycogen Debranching Enzyme for glycogen storage disease type III

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Background

Glycogen storage disease type III (GSDIII) is a rare metabolic disorder that affects the liver, skeletal muscles, and heart. It is caused by mutations in the AGL gene, which encodes the glycogen debranching enzyme (GDE). However, the use of the full-length GDE cDNA in a single adeno-associated virus (AAV) vector strategy is a major challenge due to its large size (4.6 kb). Here, we developed a gene therapy strategy for GSDIII using a truncated version of the GDE enzyme delivered by a single rAAV vector to correct skeletal muscles and heart impairment in Agl^{-/-} mice and rats and to decrease glycogen content in skeletal myotubes differentiated from $Agl^{-/-}$ human pluripotent stem cells (hiPSC).





Correction of multiple models of GSDIII using our optimized vector







Rescue of muscle and heart impairment in the Agl^{-/-} mouse model with rAAV-ΔNter2-GDE vector. (A) 4-month-old male Agl^{-/-} mice were injected in the tail vein with an rAAV-MT vector encoding Δ Nter2-GDE, at the dose of 1 x 10¹⁴ vg/kg. PBS-injected Agl^{+/+} and Agl^{-/-} mice were used as controls. (B) Western Blot analysis of GDE and vinculin expression in heart and triceps, three months after | $Aal^{+/+}$ rats were used as controls. (B) Western Blot analysis of GDE and vinculin expression of specific myogenic markers (desmin.expression)



Glycogen reduction in human induced pluripotent stem cells (hiPSC)-derived muscle cells treated with rAAV-ΔNter2-GDE. (A) Transduction protocol of GSDIII^{CRISPR} hiPSC-derived skMb and isogenic controls with an rAAV-LK03 vector expressing either GFP or

CTRL1 GSDIII^{CRISPR}

CTRL1 GSDIII^{CRISPR}

The were used as controls. (b) western blot analysis of ODE and vincum expression in heart and theeps, three months after vector	Agr Tats were used as controls. (b) western blot analysis of ODE and vincum expression in heart and theeps, three months after	Anter 2-ODE at a multiplicity of infection (MOI) of either 15 000 of 75 000. (B) Expression of specific more destinin,
injection. (C) Histological analysis of triceps using HPS and PAS staining. Representative images are shown. (D) Glycogen content	vector injection. (C) Histological analysis of triceps using HPS and PAS staining. Representative images are shown. (D) Glycogen	myosin heavy chain (MHC) and titin) and GFP by immunofluorescence analysis, after rAAV transduction of GSDIII ^{CRISPR} hiPSC-derived
measured in triceps, heart and quadriceps three months after vector injection. (E) Western blot analysis of Myom3 fragments in	content measured in triceps, heart and quadriceps three months after vector injection. (E) Western blot analysis of Myom3	skMt at a MOI of 75 000. Representative images are shown (C) Glycogen content normalized to the number of viable cells measured
plasma of mice three months after vector injection. Plasma from mdx mouse was used as positive control. (F) Wire-hang test	fragments in plasma of rats three months after vector injection. Plasma from mdx mouse was used as positive control. (F)	in GSDIII ^{CRISPR} hiPSC-derived skMt and isogenic controls (CTRL1) transduced as described above. (D) PAS staining on GSDIII ^{CRISPR} hiPSC-
expressed as number of falls per minute, performed before and 3 months after vector injection. Statistical analyses were performed	Quantification of cardiac troponin I (cTnI) and NT-pro-BNP in plasma of Agl ^{-/-} or Agl ^{+/+} rats injected with PBS or rAAV vector encoding	derived skMt and isogenic controls (CTRL1) transduced as described above. Representative images are shown. (E) Quantification of
by one-way ANOVA in D (*p<0.05, **p<0.01 or ***p<0.001 vs. PBS-injected Agl ^{-/-} mice; #p<0.05, ##p<0.01 or ###p<0.001 vs. PBS-	for ΔNter2-GDE at 3 months after injection. The dash line represents the lower limit of detection (LLD). Statistical analyses were	PAS staining. Statistical analyses were performed by one-way ANOVA (*p<0.05, **p<0.01 or ***p<0.001 vs. GSDIII ^{CRISPR} cells
injected Agl ^{+/+} mice) and two-way ANOVA in F . n=7-9 mice per group coming from two independent experiments. All data are shown	performed by one-way ANOVA in panels D and F (*p<0.05, **p<0.01 or ***p<0.001 vs. PBS-injected Agl ^{-/-} rats; #p<0.05, ##p<0.01 or	transduced with rAAV-GFP; #p<0.05, ##p<0.01 or ###p<0.001 vs. CTRL1 cells transduced with rAAV-GFP, three replicates for
as mean ±SEM. HPS = hematoxylin phloxine saffron; PAS = periodic acid Schiff; Myom3 = myomesin 3.	###p<0.001 vs. PBS-injected Agl ^{+/+} rats; n=4-5 rats per group). All data are shown as mean \pm SEM. HPS = hematoxylin phloxine	experiments in panel B-C and 5 replicates for experiments in panel D-E). All data are shown as mean \pm SEM. PAS = periodic acid
	saffron; PAS = periodic acid Schiff; Myom3 = myomesin 3.	Schiff; skMb = skeletal myoblasts; skMt = skeletal myotubes.

Conclusions

The use of ΔNter2-GDE allowed us to generate a potent and high-quality product for GSDIII. rAAV-ΔNter2-GDE corrected muscle and heart phenotype in both mouse and rat models of GSDIII with extensive glycogen reduction, reflecting broader cell transduction. Treatment of Agl^{-/-} mice with rAAV-ΔNter2-GDE vector promoted the recovery of muscle strength 3 months post-injection. This suggests that in GSDIII, the myopathy can be reversed, at least in rodent models. We also demonstrated, in a recently developed human skeletal muscle model of GSDIII derived from hiPSC, that Δ Nter2-GDE clears the accumulated glycogen, suggesting that the truncation on the N-terminal region does not affect the enzyme function and regulation in a human cellular context. Our work provides proof-of-concept of the use of a single rAAV vector expressing a truncated form of GDE for the rescue of the muscle and heart impairment in two GSDIII rodent models as well as in a novel human muscle cellular model of GSDIII. This supports the clinical translation of this approach in GSDIII patients.



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