AAV9.LAMP-2B Reverses Metabolic and Physiologic Multigorgan Dysfunction in a Murine Model of Danon Disease
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INTRODUCTION
Danon disease (DD) is a rare, X-linked autophagic vascular myopathy caused by loss of function mutations in the gene encoding lysosomal Associated Membrane Protein type 2 (LAMP-2), a lysosomal transmembrane protein critical for autophagy. (1)

Persistence of disease-causing mutations in DD is nearly 100%. Most male patients develop several cardiac hypertrophy and arrhythmias in early adolescence. In addition to cardiomyopathy, patients also suffer from liver dysfunction, skeletal myopathies, retinal disease, and cognitive impairment. (1)

Recent registry-based studies of hyperophic cardiomyopathy (HCM) patients suggest that LAMP-2 mutations underlie between 7-14% of HCM, suggesting a worldwide prevalence of 15,000-30,000 in North America and Europe. (1, 2)

LAMP-2 protein consists of three splice isoforms (LAMP-2A, B, and C) that are distinguished by a 13 amino acid cytoplasmic tail. LAMP-2B has been associated with macro autophagy and is also the predominant LAMP-2 isoform expressed in cardiomyocytes. (3,4)

PRIMARY OBJECTIVE
To evaluate the efficacy of gene transfer of AAV9-carrying the wild-type human LAMP-2B (AAV9.LAMP-2B) in a previously established mouse model of DD, the LAMP-2 KO mouse. We specifically evaluated the effect of AAV9.LAMP-2B for treatment of cardiac, skeletal, and hepatic derangements and we determined whether treatment could improve the metabolic and physiologic abnormalities noted in this model.

METHODS
To determine whether AAV9.LAMP-2B is efficacious for the treatment of DD, we used an established LAMP-2 KO murine model of DD. These mice accumulate autophagic vacuoles in multiple organs including the heart, skeletal muscle, and liver; findings also observed in Danon patients.

RESULTS
AAV9 LAMP-2B Improves Cardiac Structure and Function in LAMP-2 KO Mice

Administration of AAV9.LAMP-2B in 6-month old mice shows dose-dependent expression of human LAMP-2B in heart tissue from LAMP-2 KO mice together (A-C), with an improvement in autophagic flux (D) and hepatic structure (E) and function (F). *P<0.05 vs WT and #P<0.05 vs PBS. Similar results noted in 3-month cohort (data not shown).

AAV9 LAMP-2B Improves Hepatic Structure and Function in LAMP-2 KO Mice

Administration of AAV9.LAMP-2B in 6-month old mice shows dose-dependent expression of human LAMP-2B in hepatic tissue from LAMP-2 KO mice together (A-C), with an improvement in autophagic flux (D) and muscle structure (E), *P<0.05 vs WT and #P<0.05 vs PBS. Similar results noted in 3-month cohort (data not shown).

AAV9 LAMP-2B Improves Skeletal Muscle Structure and Function in LAMP-2 KO Mice

Administration of AAV9.LAMP-2B in 6-month old mice shows dose-dependent expression of human LAMP-2B in skeletal muscle from LAMP-2 KO mice together (A-C), with an improvement in autophagic flux (D) and muscle structure (E), *P<0.05 vs WT and #P<0.05 vs PBS. Similar results noted in 3-month cohort (data not shown).

AAV9 LAMP-2B Improves Survival in LAMP-2 KO Mice

Kaplan Meier curve shows increased survival rate in 6-month old mice injected with higher doses (14.1 x10^9 and 28.2 x10^9 vg/kg) of AAV9.LAMP-2B. *P<0.05 vs LAMP-2 KO + PBS. Dose of 14.1 x10^9 vg/kg was selected for subsequent studies.

SUMMARY and CONCLUSIONS
• LAMP-2 KO mice receiving AAV9.LAMP-2B demonstrated dose-dependent normalization of human LAMP-2B protein levels in heart, liver, and skeletal muscle tissues in both cohorts.
• Improved autophagic flux was abrogated by LAMP-2 KO gene transfer all tissues in both cohorts.
• Cardiac function was also significantly improved, and transaminase levels were significantly reduced in AAV9.LAMP-2B-treated KO mice, indicating favorable effects on the heart and liver.
• Results from the 2-month cohort are not shown here but are similar across organs.
• Survival was significantly higher in 6-month cohort mice receiving > 5x10^12 vg/kg dose of the vector.
• Data from both cohorts together demonstrate, restoration (6-month cohort) and durable prevention (2-month cohort) of cardiac, muscle and liver structure and function.

In summary, LAMP-2B gene transfer improves metabolic and physiologic function in a DD murine model. Our findings have direct implications for clinical gene therapy development of AAV9.LAMP-2B for Danon disease.

REFERENCES

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