Development of a Gene Therapy Program for Barth Syndrome

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Background

- Barth syndrome (BTHS) is caused by a single gene mutation in tafazzin (TAZ) that results in abnormal mitochondria, skeletal myopathy and heart failure.
- Currently there are no effective therapies for BTHS other than supportive cardiac care.
- Our program aims to: 1) identify relevant clinical and physiologic outcome variables, 2) identify the optimal AAV-TAZ expressing vector in a) BTHS mice and b) human induced pluripotent stem cell (iPSC)-derived cardiomyocytes and myotubes, 3) test the safety of AAV9 TAZ expressing vectors through primate toxicology studies and 4) test the efficacy and safety of AAV9 TAZ gene therapy in patients with BTHS.

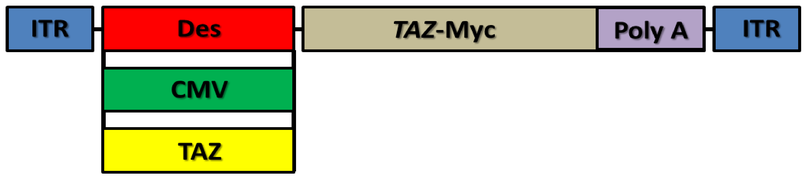
AIM 1: Clinical and Physiologic Outcome Variables

	Control (n=22)	BTHS (n=24)	P-value
Age (yrs)	15 ± 5	19 ± 8	0.04
Children (#)	15	14	
Height (cm)	162.9 ± 17.6	158.2 ± 20.8	0.21
Weight (kg)	61.0 ± 23.7	48.7 ± 20.7	0.07
FFM (kg)	48.7 ± 17.7	34.9 ± 11.4	0.003
Fat Mass (kg)	11.9 ± 8.4	18.0 ± 11.7	0.05
Peak VO_2 (ml/kg/min)	37.4 ± 7.5	13.1 ± 3.3	0.001
Cardiac PCr/ATP	2.0 ± 0.5	1.6 ± 0.4	0.001
Skeletal Muscle Oxidative Capacity (mmol/s)	1.0 ± 0.3	0.5 ± 0.2	0.01
FatOxEx (umol/kgFFM/min)	1.4 ± 1.0	0.7 ± 0.4	0.003
Strain (%)	-20.4 ± 2.9	-18.4 ± 3.7	0.05

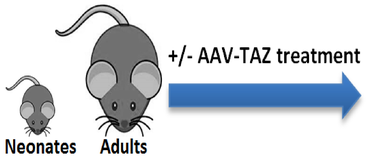
Participants traveled to St. Louis for 3-4 days of exercise testing, clinical metabolism studies, body composition assessment, echocardiography, and magnetic resonance spectroscopy.

AIM 2: Identify Optimal AAV-TAZ Expressing Vector

- We have generated the following AAV vectors:
- 1) TAZ driven by a desmin (Des) promoter to restrict expression to heart and skeletal muscle
- 2) TAZ driven by a cytomegalovirus (CMV) promoter to enable ubiquitous expression in a wide variety of tissues
- 3) TAZ driven by a natural tafazzin promoter (Taz) to establish whether the native promoter generates sufficient expression to enable restriction of expression to those tissues in which this gene would usually be transcribed.
- Each of these vectors were packaged into AAV serotype 9 (AAV9) due to its high natural affinity for heart and skeletal muscle and known long-term expression.



Using the BTHS Dox inducible shRNA knockdown mouse model and IV administration routes to promote global-treatment, each of the AAV viruses were delivered to BTHS mice (n=3-5 per group). IV administration through the superficial temporal vein in 1-3 day old neonatal mice and IV administration through the jugular vein in 9-12 week old adult mice.



- In vivo functional assessments
- Ex vivo functional assessments
- Tissue analyses
- Vector biodistribution (transgene expression – RNA transcripts)
- Mitochondrial assessments

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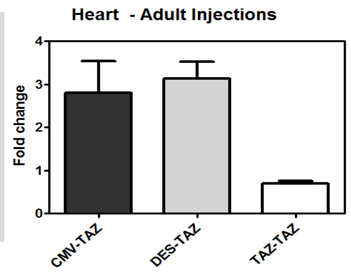
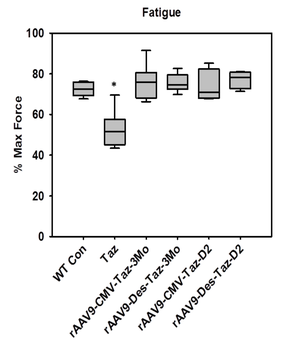
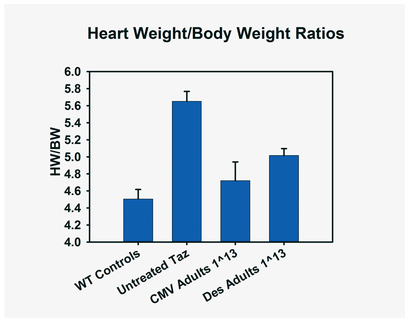
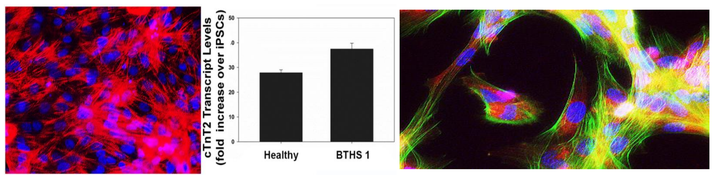
Right: Heart tafazzin expression in injected mice as compared to uninjected controls. Below left: skeletal muscle fatigue of solei muscle upon electric stimulation. Below right: heart weight/body weight ratio in treated and WT mice.









Differentiation of BTHS iPSCs into iPSC-CMs results in cTnT2 expression (red-left panel),~ 30 to 40 fold increases in cTnT2 mRNA transcript levels over that of undifferentiated iPSCs (middle), and distributions of mitochondria throughout iPSC-CMs indicative of typical cardiomyocytes (red [MTCO2 -Cytochrome C oxidase subunit II] green [f-actin] -right panel).

AIMS 3 & 4: Safety and Efficacy Trials

- IND-enabling and human studies have been planned

Conclusions

- Our current data have identified relevant cardio-skeletal muscle functional and physiologic outcomes in human subjects as well as suggested efficacy of the AAV9 expression vectors.