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Expression-Functional Correlation and Validation of a Surrogate Marker for DAPC Restoration in a Mouse Model of LGMD2E

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BACKGROUND

- Limb-girdle muscular dystrophy type 2E (LGMD2E) is an autosomal recessive disease caused by mutations in β-sarcoglycan (SGCB) leading to protein deficiency, loss of formation of the sarcoglycan complex, and loss of stabilization of the dystrophin-associated protein complex (DAPC).
- Individuals who have a single pathogenic variant are asymptomatic (carriers), and therefore able to compensate for the defective gene copy.
- Sarcoglycanopathies present as progressive muscular dystrophies starting in the girdle muscles before extending to lower and upper extremity muscles, and can also present in the diaphragm and heart, resulting in respiratory and cardiac failure in specific patient subtypes.
- The sarcoglycans and sarcospan are integral proteins critical for stabilizing the DAPC and providing mechanical support to the sarcolemma.
- Adeno-associated virus (AAV)-mediated gene replacement therapy has shown early signs of potential to treat sarcoglycanopathies. Key considerations include a systematic and stepwise evaluation of safety, transduction, expression, localization, cellular impact, and clinical function.
- With these considerations in mind, the self-complementary AAV.MHCK7.hSGCB construct was designed to restore functional β-sarcoglycan to muscles:
  - AAVrh74 vector: Displays robust muscle (skeletal and cardiac) tissue tropism and has relatively low level of pre-existing immunity
  - MHCK7 promoter: Regulates and drives transgene expression selectively in skeletal and cardiac muscle; includes an alphamyosin heavy chain enhancer to drive especially strong expression in cardiac muscle
  - hSGCB transgene: Carries full-length β-sarcoglycan cDNA.
- *SGCB-/-* mice have been shown to concurrently display loss of additional sarcoglycans (α, γ, and δ). Evidence suggests sarcospan may also be lost in the absence of SGCB.
- We hypothesize that other sarcoglycan and sarcospan expression may be able to serve as a surrogate marker for functional restoration of DAPC following SGCB gene transfer.

OBJECTIVES

- Characterize SGCB heterozygous mice and determine the downstream effects from one wild-type (WT) copy of the *SGCB* gene, ultimately assessing if these mice presented asymptomatic as human carriers do;
- Assess the ability for *SGCB* gene transfer to restore sarcoglycan and sarcospan expression in *SGCB-/-* mice; and
- Test their utility as a surrogate marker for DAPC restoration.

METHODS

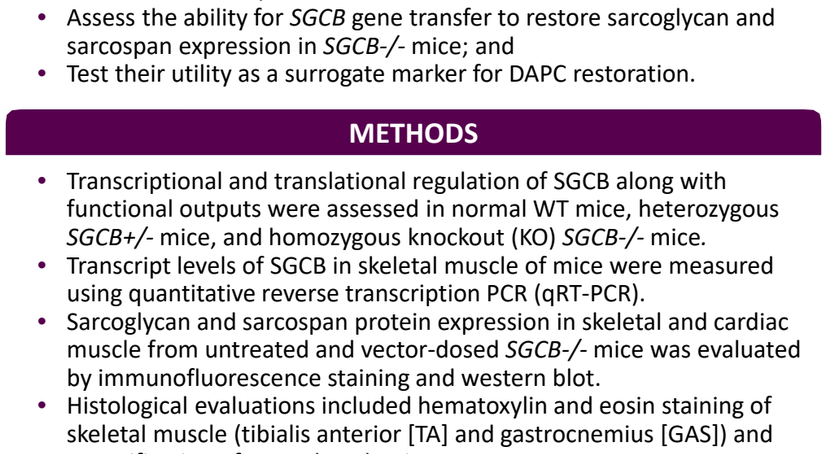
- Transcriptional and translational regulation of SGCB along with functional outputs were assessed in normal WT mice, heterozygous *SGCB+/-* mice, and homozygous knockout (KO) *SGCB-/-* mice.
- Transcript levels of SGCB in skeletal muscle of mice were measured using quantitative reverse transcription PCR (qRT-PCR).
- Sarcoglycan and sarcospan protein expression in skeletal and cardiac muscle from untreated and vector-dosed *SGCB-/-* mice was evaluated by immunofluorescence staining and western blot.
- Histological evaluations included hematoxylin and eosin staining of skeletal muscle (tibialis anterior [TA] and gastrocnemius [GAS]) and quantification of central nucleation.
- Functional assessments included measurement of force production and resistance to contraction-induced injury in the TA muscle along with laser monitoring of open-field cage activity to assess overall ambulation (movement around the cage) and vertical activity (rearing onto hind limbs).
- Animal models: All procedures were conducted in accordance with approval by the Research Institute at the Nationwide Children's Hospital Institutional Animal Care and Use Committee. C57BL6 WT, *SGCB+/-*, and *SGCB-/-* mice were maintained under standardized conditions on a 12:12-hour light:dark cycle, with food and water provided ad libitum.

RESULTS

HISTOPATHOLOGY ANALYSIS IN SGCB HET MICE

- *SGCB+/-* mice were not found to have any significant dystrophic phenotype as shown by histopathology and quantification of central nucleation in the TA and GAS. Levels of central nucleation are similar to WT and dramatically different to SGCB KO mice (**Figure 1**).

Figure 1. No difference in histopathology between WT and *SGCB+/-* muscle



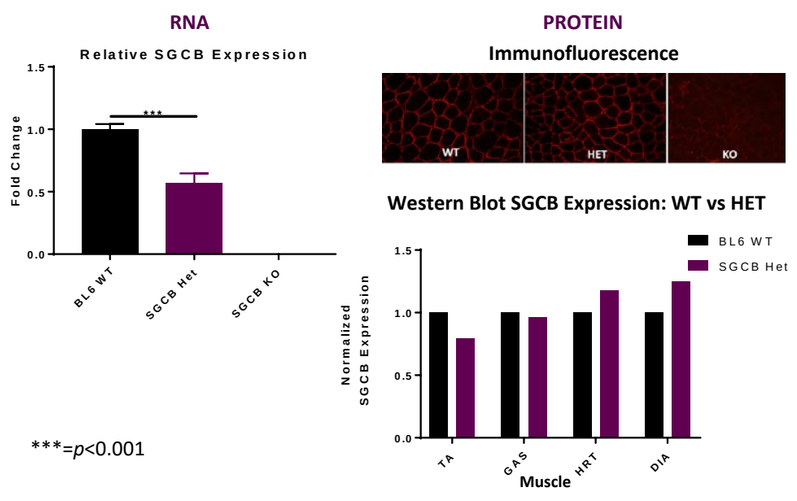
Absence of central nucleation in SGCB Het muscle
****=p<0.0001

RESULTS

RNA TRANSCRIPT AND PROTEIN EXPRESSION LEVELS

- Sarcoglycan expression in *SGCB+/-*, *SGCB-/-*, and WT mice was determined at the transcript level and protein level. Transcript mRNA levels were measured by qRT-PCR, and protein production was measured by western blot (immunofluorescence images also shown). SGCB expression is significantly reduced for the *SGCB-/-* mice as expected. SGCB mRNA levels are reduced for the *SGCB+/-* mice compared to WT mice, but no detectable differences are observed in protein production (**Figure 2**).

Figure 2. SGCB transcript and protein levels in *SGCB+/-* mice: qRT-PCR, immunofluorescence, and western blot

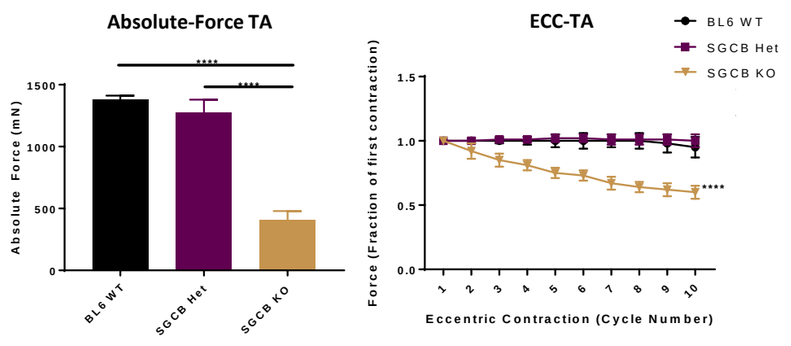


***=p<0.001

ANALYSIS OF FUNCTIONAL OUTPUTS IN SGCB HET MICE

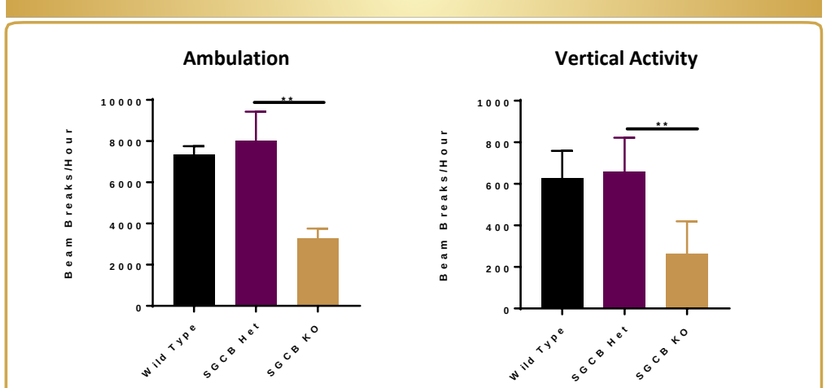
- Absolute force and resistance to eccentric contraction were similar to WT mice in *SGCB+/-* TA muscle and significantly different compared to *SGCB-/-* mice (**Figure 3**). Analysis of open-field cage activity shows that both ambulation and vertical activity is not affected in the *SGCB+/-* mice compared to WT mice (**Figure 4**).

Figure 3. Physiology assessments in skeletal muscle (TA)



****=p<0.0001 ****=p<0.0001

Figure 4. Open-field cage activity

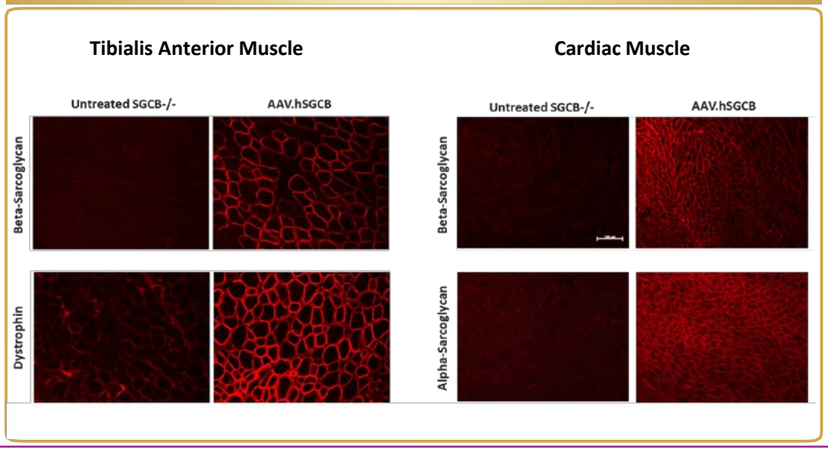


**=p<0.01 **=p<0.01

DAPC RESTORATION

- Dystrophin and alpha-sarcoglycan expression was restored following AAV.hSGCB gene transfer in *SGCB-/-* mice in both TA and cardiac muscle (**Figure 5**).

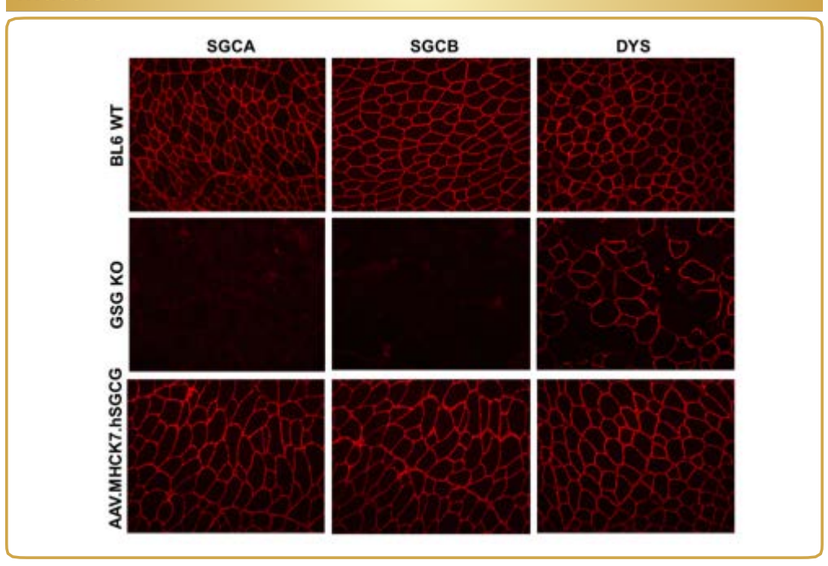
Figure 5. Immunofluorescence staining of *SGCB-/-* mouse muscle



RESULTS (CONT'D)

- Alpha-sarcoglycan, β-sarcoglycan, and dystrophin expression were restored after AAV.hSGCG gene transfer in the TA muscle of *SGCG-/-* mice (**Figure 6**).

Figure 6. Immunofluorescence staining of *SGCG-/-*mouse muscle



SARCOSPAN AS SURROGATE BIOMARKER FOR RESTORATION OF DAPC

- Sarcospan was reduced or absent in the TA muscle of *SGCB-/-* mice and restored in *SGCB-/-* mice following AAV.hSGCB gene transfer measured by immunofluorescence (**Figure 7**) and western blot (**Figure 8**).

Figure 7. Immunofluorescence staining for sarcospan in LGMD2E mice

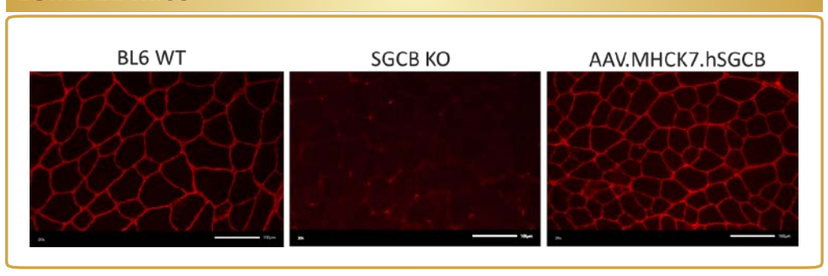
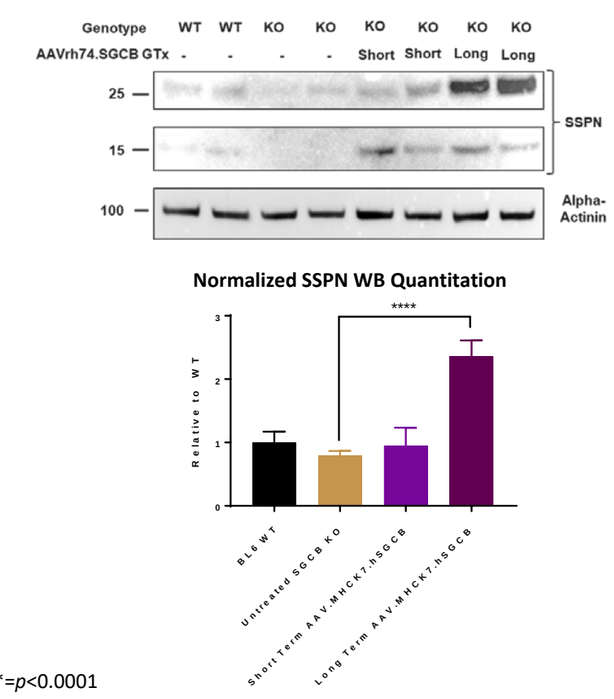


Figure 8. Western blotting for sarcospan in LGMD2E mice



****=p<0.0001

CONCLUSIONS

- Overall *SGCB+/-* mice present with normal muscle phenotype similar to WT mice and do not develop any dystrophic histopathology.
- RNA transcript levels of SGCB in Het mice were found to be about half the level of WT mice as expected; however, protein levels were normal and similar to WT mice.
- *SGCB+/-* mice do not present with any functional deficits.
- Further evaluation of lower doses in *SGCB-/-* will provide insight into a potential expression-function correlation.
- Preliminary co-localization studies confirmed restoration of the DAPC following SGCB or SGCG gene therapy. This data suggests the potential for additional sarcoglycans and sarcospan to serve as a surrogate marker for functional restoration of the DAPC.

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