Validation of a Novel Mouse Model of Cox6a2 Knockdown

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Abstract

Chronic limb threatening ischemia (CLTI) is characterized by ischemic pain at rest, tissue necrosis, and gangrene. There are currently no effective treatment options for CLTI patients, despite its association with high mortality rates and limb amputation. We identified a unique mitochondriopathy in CLTI patient limb skeletal muscles that was recapitulated in a preclinical mouse model of PAD, hindlimb ischemia (HLI). We further identified reductions in cytochrome oxidase 6a2 (Cox6a2), a regulatory protein subunit of cytochrome c oxidase (complex IV of the mitochondria) in the limb skeletal muscle of mitochondria of both CLTI patients and BALB/c mice. Cox6a2 is expressed only in mature, striated muscle (skeletal and cardiac) and is a potential genetic modifier of tissue loss in HLI. This project was designed to: 1) validate a novel and inducible model of skeletal muscle specific Cox6a2 knockdown in ischemia resistant C57BL/6J mice, and 2) determine whether Cox6a2 was required for muscle survival and regeneration after the onset of HLI. After following a prescribed breeding scheme, genotyping confirmed the creation of the desired HSA-MCM;Cox6a2fl/fl mice. Subsequent experiments established the validity of skeletal muscle specific complex IV deficiency as central to the ischemic mitochondripathy and myopathy that occurs in the peripheral limb of mice. Together, this data suggests that skeletal muscle Cox6a2 is a new target for therapeutic intervention.

Research Hypothesis

Cox6a2 loss results in greater susceptibility to ischemic muscle myopathy, creating a local muscle environment insufficient to support the vasculature

Fig 1. Breeding Scheme

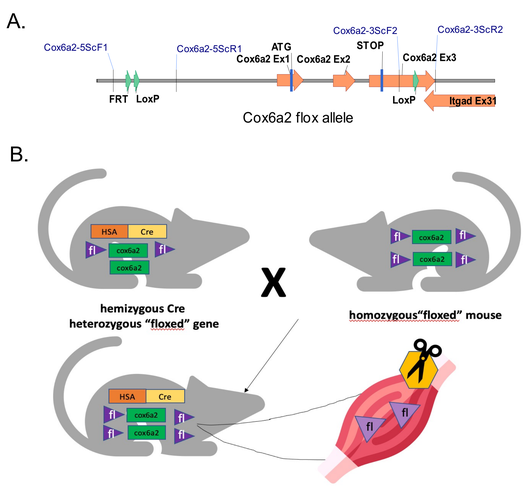
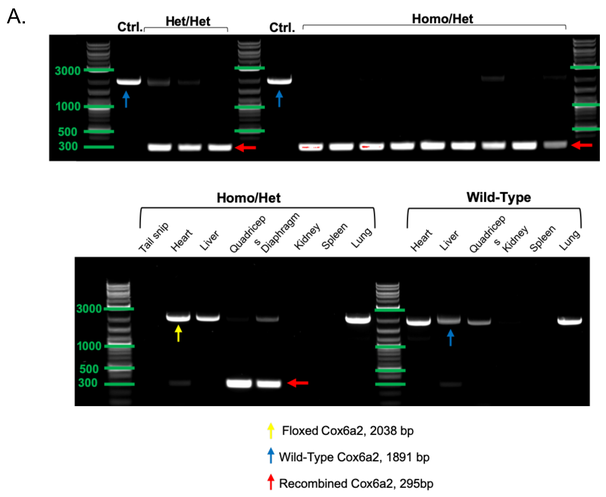


Fig 1. Breeding Scheme Our lab has generated a novel, inducible mouse model of skeletal muscle specific Cox6a2 knockdown. (**A**) Schematic of the Cox6a2 floxed allele, highlighting the location of the inserted LoxP sites. (**B**) Experimental mice were created by crossing hemizygous human α-skeletal actin (HSA)-Mer-Cre-Mer mice with homozygous Cox6a2$^{fl/fl}$ mice. The resulting HSA-MCM-Cox6a2$^{fl/-}$ mice were then backcrossed with homozygous Cox6a2$^{fl/fl}$ mice to create HSA-MCM;Cox6a2$^{fl/fl}$ mice. All the animals used in the breeding scheme have a BL6 background.

Fig 2. Validation of Inducible HSA-MCM;Cox6a2$^{fl/fl}$ Mice



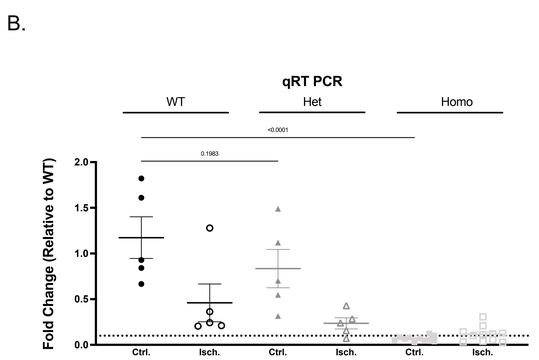
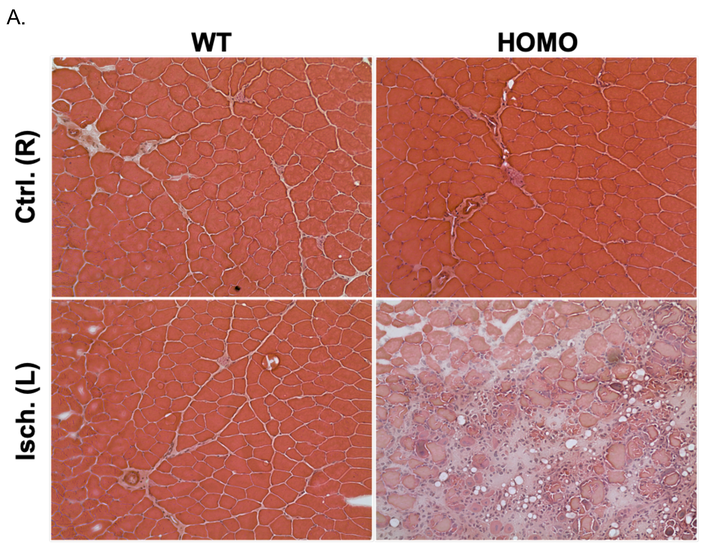
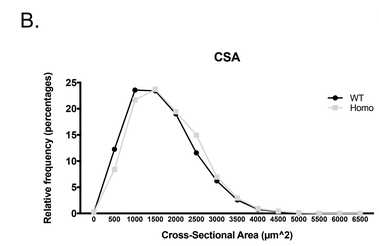
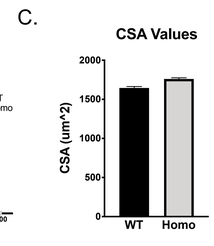


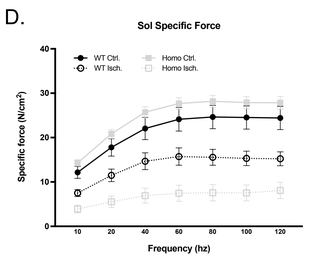
Fig 1. Validation of Inducible HSA-MCM;Cox6a2$^{fl/fl}$ Mice (A) Verification of recombination in wild-type littermate, heterozygous and homozygous knockdown animals following tamoxifen administration. The wild-type gene that encodes Cox6a2 is 1891 base pairs (blue arrows). When LoxP sites are added into the WT allele, the resulting gene is 2038 base pairs (yellow arrows). When recombined, the size of this region is 295 base pairs (red arrows). (**B**) Verification that recombination is skeletal muscle-specific recombination in homozygous knockdown mice. (**C**) Real time PCR data indicating a significant reduction in mRNA that encodes for Cox6a2 in homozygous animals, but not in heterozygous animals

Fig 3. Skeletal Muscle Morphology and Function









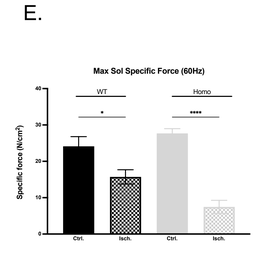
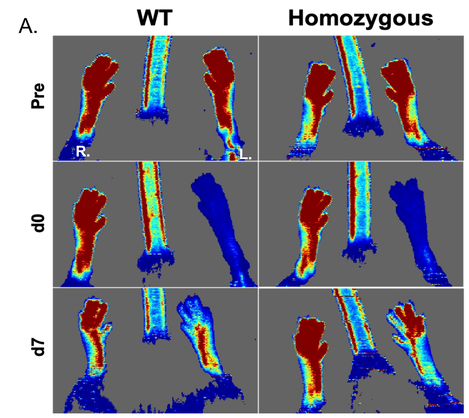


Fig 3. Skeletal Muscle Morphology and Function. Tibialis anterior (TA) muscles were collected and used for morphological measurements. (**A**) Representative 10X hematoxylin and eosin staining of the TA reveals significant damage in homozygous knockdown animals following HLI. (**B, C**) The cross-sectional area of the control limb TA muscles is not significantly altered between wild-type and knockdown animals at baseline. (**D, E**) Force frequency curves (FF) for ischemic and control Sol muscles from WT and homozygous knockdown mice after 7-days of limb ischemia (Isch. L). **P<0.05. ***P<0.0005*

Fig 3. Restoration of Paw Perfusion



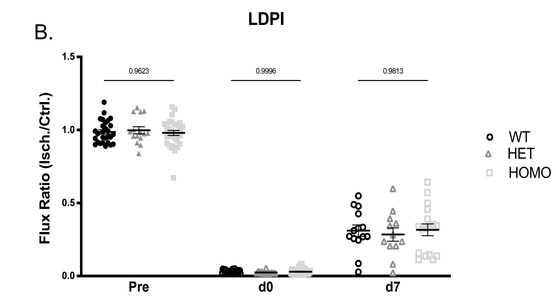


Fig 4. Restoration of Peripheral Blood Flow was Determined Using LDPI. (A) Representative laser Doppler perfusion images (LDPI) of the ischemic left (Isch. (L)) and control right (Ctrl. (R)) plantar paws in the prone position at prior to HLI (pre), immediately post-HLI (post), and at d7 following HLI. **(B)** Quantification of flux measurements pre, post, and at d7 represented as a ratio of the left/right limbs.

Summary of Findings

- The prescribed breeding scheme was effective in generating HSA-MCM;Cox6a2fl/fl mice, which was verified through genotyping
- Cox6a2 loss in ischemia-resistant C57BL/6J mice creates a muscle that is more susceptible to ischemic injury
- The phenotype of these mice is marked by functional deficits (maximum force production) and muscle damage (lesion area).

References

Ryan, T. E. *et al.* Extensive skeletal muscle cell mitochondriopathy distinguishes critical limb ischemia patients from claudicants. *JCI Insight* **3**, (2018).