Myostatin as a potential target for treatment of muscle contractures

Marianne E Emmert, Qingnian Goh, Kritton Shay-Winkler, & Roger Cornwall.
Department of Biomedical Sciences, University of Cincinnati College of Medicine.
Division of Orthopaedic Surgery, Cincinnati Children's Hospital Medical Center.

Overview

Neonatal Brachial Plexus Injury (NBPI)
- Most common cause of upper limb paralysis in childhood, occurring in 1.5 of every 1,000 live births.
- Leads to the secondary formation of muscle contractures, or "limb stiffness."
- Contractures severely impede range of motion of the involved limbs, thereby impairing activities of daily living, and ultimately resulting in skeletal deformity and dysfunction.
- Current treatments for contractures are ineffective in restoring muscle function and joint range of motion.
- To develop effective strategies for preventing and treating contractures, we first need to establish greater insights on contracture pathogenesis.

Contracture Pathogenesis
- Contractures following NBPI result from impaired longitudinal growth of the denervated muscle.[1]
- Deficits in longitudinal growth of denervated muscles are driven by increased levels of proteasome-mediated protein degradation, suggesting a dysregulation of protein balance in play.[2]
- Proteasome inhibitors markedly reduce contracture formation but also block protein degradation nonspecifically, and prolonged treatment results in potential cumulative toxicity.[3]

* *Hence, we need to identify safer strategies for preventing contractures by targeting muscle-specific regulators of the protein balance.*

Myostatin Inhibition
- Myostatin is skeletal muscle-specific negative regulator of protein balance, and limits excess muscle growth by inhibiting protein synthesis.
- Pharmacologic inhibition of myostatin signaling dramatically enhances muscle growth in adult mice.[4]
- In this current study, we report that neonatal myostatin inhibition reduces NBPI-induced contracture formation and preserves longitudinal growth of denervated muscles only in female mice.

* *Our findings therefore reveal a sex-dependent role of myostatin signaling in the development of muscle contractures after neonatal denervation injury.*

Experimental Design

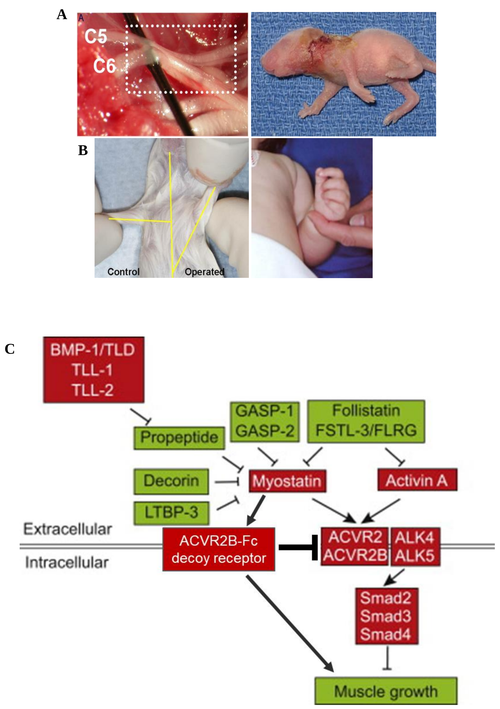

Figure 1: Mouse model of NBPI and pharmacologic inhibition of myostatin. A) To recapitulate denervation-induced contractures in infants, a mouse model of unilateral NBPI was created whereby the brachial plexus complex was surgically excised in 5-day old CD1 mice (left panel), resulting in forelimb paralysis (right panel).[1] **B)** Mice subsequently developed physiologically relevant contractures (limited shoulder rotation and elbow hyperflexion) by 4 weeks post-NBPI, which reflect that joint contractures developed in children with NBPI. **C)** Schematic depicting inhibition of the myostatin pathway (modified from Lee, 2012).[5] As a negative regulator of protein balance, myostatin binds to Activin A via its ACVR2/ACVR2B receptors, resulting in the subsequent activation of the Smad proteins which inhibits protein synthesis and muscle growth.[5] The administration of a soluble decoy receptor for Activin A, ACVR2B-Fc, pharmacologically inhibits the binding between myostatin and Activin A and leads to muscle growth.[4]

Optimization of neonatal myostatin inhibition

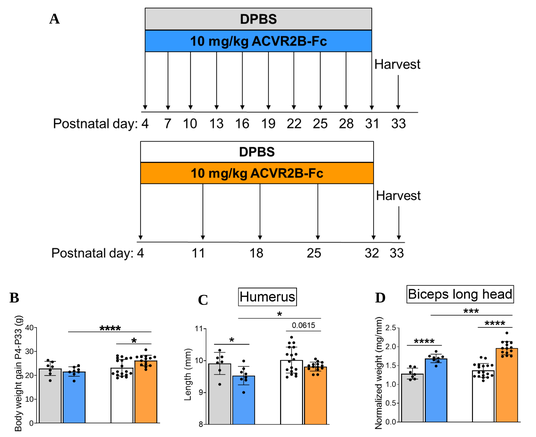

Figure 2: Neonatal myostatin inhibition enhances muscle growth. A) Schematic comparing different dosages of ACVR2B-Fc. The top schematic depicts administration of ACVR2B-Fc every three days, the dosage used in a previous study conducted on adult mice.[4] The bottom schematic depicts weekly administration of ACVR2B-Fc. **B)** Treatment with ACVR2B-Fc every three days was less effective in promoting body weight gain than weekly treatment, and **C)** even reduced skeletal growth of the humerus. **D)** While both dosages increased the weight of biceps muscles, weekly treatment of ACVR2B-Fc resulted in greater skeletal muscle growth. All data are presented as mean ± s.d. n = 7-18. Statistical analyses: **(B)**, **(C)**, **(D)** unpaired two-tailed Student's t-tests. The degree of significance between data sets is depicted as follows: *****P<0.05, *******P<0.001, ********P<0.0001.

Sex-Specific Response to Neonatal Myostatin Inhibition – Developmental Growth

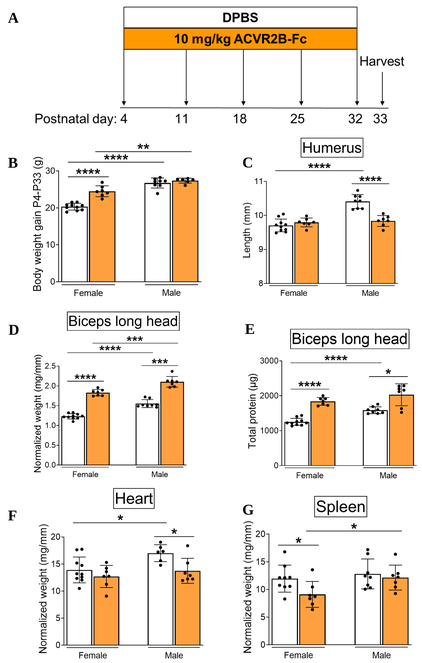

Figure 3: Sexual dimorphisms in developmental growth with neonatal myostatin inhibition. A) Schematic depicting weekly administration of ACVR2B-Fc in neonatal mice. **B)** Myostatin inhibition increased body weight in female mice but **C)** decreased humerus length in male mice. **D)** Myostatin inhibition increased the weight and **E)** total protein content of biceps muscles in both sexes. However, this increase in skeletal muscle growth was associated with sex-specific perturbations in non-skeletal muscle tissues, specifically reductions in **F)** hearts of male mice and **G)** spleens of female mice. All data are presented as mean ± s.d. n = 6-10. Statistical analyses: **(B)**, **(C)**, **(D)**, **(E)**, **(F)**, **(G)** unpaired two-tailed Student's t-tests. The degree of significance between data sets is depicted as follows: *****P<0.05, ******P<0.01, *******P<0.001 ********P<0.0001.

Sex-Specific Response to Neonatal Myostatin Inhibition – Elbow Contractures

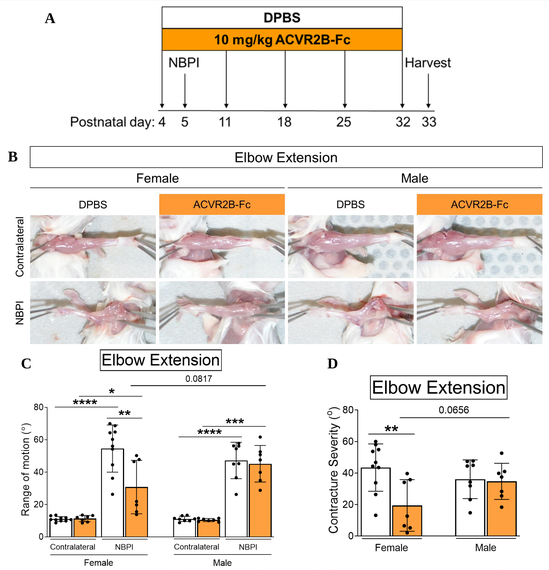

Figure 4: Neonatal myostatin inhibition reduces formation of elbow contractures in a sex-specific manner. A) Schematic depicting weekly administration of ACVR2B-Fc in neonatal mice prior to and after unilateral NBPI. **B)** Representative images of denervated (NBPI) and contralateral forelimbs reveal an improvement in elbow extension only in female mice treated with ACVR2B-Fc. Quantitative analyses further demonstrate **C)** a rescue in elbow joint range of motion in the NBPI forelimb and **D)** a reduction in elbow flexion contracture severity of female, but not male mice. Contracture severity is calculated as the difference in passive elbow extension between the denervated (NBPI) side and the contralateral control side. All data are presented as mean ± s.d. n = 7-10. Statistical analyses: **(C)** unpaired, two-tailed Student's t-test between groups and paired, two-tailed Student's t-tests between limbs of mice in each group, **(D)** unpaired two-tailed Student's t-tests. The degree of significance between data sets is depicted as follows: *****P<0.05, ******P<0.01, *******P<0.001, ********P<0.0001.

Sex-Specific Response to Neonatal Myostatin Inhibition – Shoulder Contractures

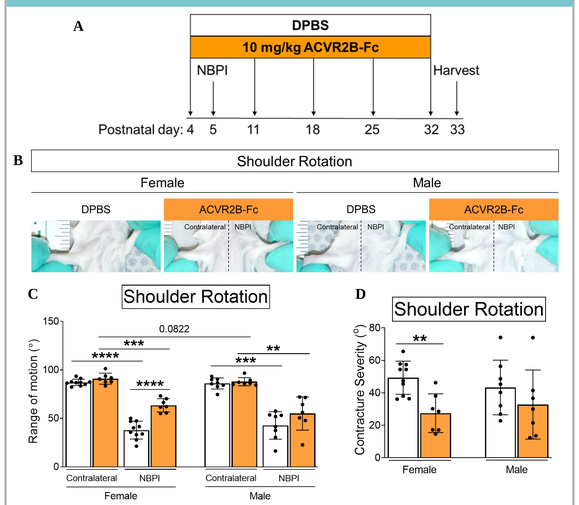

Figure 5: Neonatal myostatin inhibition reduces formation of shoulder contractures in a sex-specific manner. A) Schematic depicting weekly administration of ACVR2B-Fc in neonatal mice prior to and after unilateral NBPI. **B)** Representative images of denervated (NBPI) and contralateral forelimbs reveal an improvement in shoulder rotation in female mice treated with ACVR2B-Fc. Quantitative analyses further demonstrate **C)** a rescue in shoulder joint range of motion in the NBPI forelimb and **D)** a reduction in shoulder rotation contracture severity of female, but not male mice. Contracture severity is calculated as the difference in passive shoulder rotation between the denervated (NBPI) side and the contralateral control side. All data are presented as mean ± s.d. n = 7-10. Statistical analyses: **(C)** unpaired, two-tailed Student's t-test between groups and paired, two-tailed Student's t-tests between limbs of mice in each group, **(D)** unpaired two-tailed Student's t-tests. The degree of significance between data sets is depicted as follows: ******P<0.01, *******P<0.001, ********P<0.0001.

Sex-Specific Response to Neonatal Myostatin Inhibition – Denervated Muscle

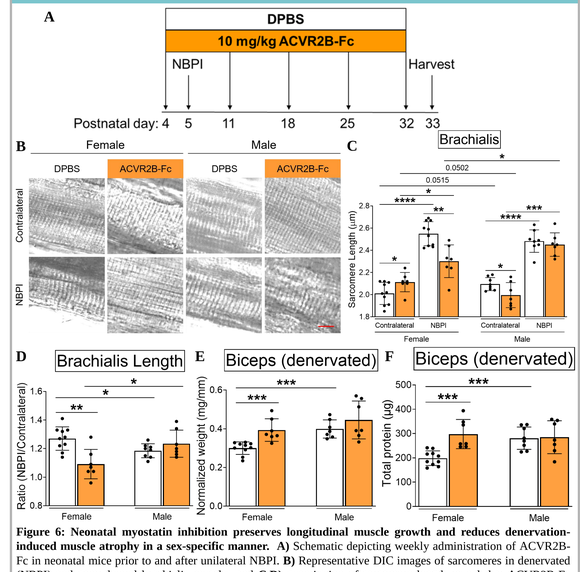

Figure 6: Neonatal myostatin inhibition preserves longitudinal muscle growth and reduces denervation-induced muscle atrophy in a sex-specific manner. A) Schematic depicting weekly administration of ACVR2B-Fc in neonatal mice prior to and after unilateral NBPI. **B)** Representative DIC images of sarcomeres in denervated (NBPI) and contralateral brachialis muscles and **C,D)** quantitation of sarcomere length reveal that ACVR2B-Fc treatment reduced sarcomere overstretch after NBPI in female mice, indicating an improvement in functional length of denervated muscles. However, the sarcomeres of denervated muscles in male mice remain elongated following ACVR2B-Fc treatment, indicating functional muscle shortening. The restoration in longitudinal muscle growth with myostatin inhibition in female mice is accompanied by an increase in **E)** muscle weight and **F)** total protein content of denervated biceps. In **(D)**, sarcomere length on the NBPI side is normalized to the contralateral side, with a normal sarcomere length ratio of 1.0, and any value over 1.0 indicating sarcomere overstretch on the NBPI side, and thus, functional muscle shortening. All data are presented as mean ± s.d. n = 7-10. Statistical analyses: **(C)** unpaired, two-tailed Student's t-test between groups and paired, two-tailed Student's t-tests between limbs of mice in each group, **(D)**, **(E)**, **(F)** unpaired two-tailed Student's t-tests. The degree of significance between data sets is depicted as follows: *****P<0.05, ******P<0.01, *******P<0.001, ********P<0.0001. Scale bar: 100 pixels.

Conclusion/Future Directions

- Neonatal inhibition of myostatin enhances skeletal muscle growth.
- Following neonatal muscle denervation, myostatin inhibition is effective in preserving longitudinal muscle growth and preventing contractures only in female mice.
- This posits a sex-specific role for myostatin in mediating denervation-induced contractures.
- To further elucidate sexual dimorphisms in myostatin-mediated contracture formation, future studies will investigate the role of sex hormones in neonatal muscle denervation.

Acknowledgements

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