INTRODUCTION: Neonatal Brachial Plexus Injury (NBPI) is the most common cause of upper extremity paralysis in children. Although the paralysis can recur, secondary muscle contractures that limit functional use of the upper limb cannot be prevented or cured with existing therapies. Previous work in animal models has shown that contractures form due to denervation in muscles that normally contribute to elbow extension. This group aimed to examine the role of myostatin and its noncanonical signaling pathways in the denervated elbow extensor muscles. We hypothesized that the noncanonical myostatin signaling pathways, including Extracellular Receptor Kinase (ERK) and c-Jun N-Terminal Kinase (JNK), may have a role in mediating contracture formation following NBPI.

METHODS: We performed a 2-way Analysis of Variance (ANOVA) tests with a Bonferroni correction of multiple comparisons using GraphPad Prism 8. A total of 29 mice were used in this study.

RESULTS: We began by characterizing ERK and JNK gene and protein expression in denervated muscles. Gene analysis via qPCR reveals a 5-fold increase in relative ERK2 gene expression in muscles of male mice after NBPI compared to contralateral controls (6.73±4.16 vs. 1.325±0.2591) (Fig. 2A). These results are mirrored in relative ERK2 expression in denervated male muscles, and JNK1 expression in denervated male muscles (Fig. 2A), though data is underpowered in this preliminary analysis. No differences were observed with protein expression of ERK and JNK in denervated muscles of either sex, nor with the ratio of phosphorylated to total ERK and JNK. We then manipulated ERK and JNK signaling via pharmacologic inhibitors. In comparison to vehicle controls, JNK inhibition reduces elbow flexion contractures in male mice only (63.41.5±6.702 vs. 30.23±14.19) (Fig. 2B-C). No changes in contracture severity were observed with ERK inhibition in either sex (Fig. 1B-C).

DISCUSSION: Our current preliminary findings reveal that the MSTN noncanonical pathway JNK may have a role in mediating contracture formation following NBPI, potentially in a sex-specific manner, as JNK inhibition reduces elbow flexion contracture formation in male mice, corresponding to a potential, though currently underpowered, increase in JNK1 mRNA levels in male denervated muscle following NBPI. Conversely, the increased ERK2 expression was not reflected in a rescue of contractures with ERK inhibition, although these preliminary analyses may have failed to find more subtle differences. Increasing sample size in ongoing experiments in additional litters will be able to detect or rule out more subtle differences in gene expression, protein levels/activity, and contracture phenotype for both ERK and JNK signaling. Additionally, future experiments will examine ERK and JNK signaling after myostatin inhibition and after genetic manipulation of myostatin signaling. Nonetheless, our preliminary data suggest a potential role for noncanonical myostatin signaling pathways in muscle growth and contractures. Furthermore, the sex dimorphisms seen here underscore the need to consider sex as a biological variable while dissecting molecular mechanisms of contracture pathophysiology.

SIGNIFICANCE/CLINICAL RELEVANCE: This study identifies further clues to the molecular basis of neuromuscular contracture formation, potentially leading to additional targets for medical contracture prevention and treatment strategies.