Myotonic dystrophy type 2 (DM2) is characterized by many symptoms including progressive muscle wasting, myotonia, cataracts, and cardiac problems [1], and there is currently no cure. DM2 is caused by a repeating nucleotide motif that is found within the first intron of the *CNBP* gene, thereby resulting in up to 11,000 nucleotide repeats for individuals with DM2 [2]. These excessive repeats result in mutant, double stranded, *CNBP* pre-mRNA transcripts that sequester and bind to critical RNA-binding proteins (RBPs), thereby causing changes in skeletal muscle function [3]. In addition, reduced translation of functional CNBP protein—which is suspected to be an RBP and DNA binding protein itself—is believed to contribute to many of the detrimental skeletal muscle changes associated with DM2 [4]. These two disease mechanisms likely work in conjunction with one another, *but the specifics of how decreases in functional CNBP protein contributes to DM2 muscle weakness and wasting has yet to be determined.*

The objective of this study is to study how low levels of CNBP contributes to muscle wasting and weakness. I **hypothesize** that CNBP loss contributes to muscle weakness and wasting by affecting pathways not associated with the *CNBP* pre-mRNA-gain-of-function mechanism. The **long-term goal** of this research is to better characterize the specific skeletal muscle genes and pathways affected by CNBP loss, so that therapies aimed at restoring their function in skeletal muscle can be developed. *Mus musculus* will be used as a model organism because they have a highly conserved CNBP homolog with humans and because muscle weakness and wasting are easy to study in mice [5].

Aim 1- Identify conserved sites of CNBP important to proper skeletal muscle function

Approach: Using protein sequence data from the NCBI's BLAST database, I will search for CNBP homologs in various species and identify their protein domains using the SMART database. Using *Mus musculus* as a model organism, I will individually make targeted amino acid substitutions using CRISPR/Cas9 within amino acids conserved only in muscular organisms near or within these domains. Following this, I will screen for mice that exhibit muscle wasting and weakness DM2 phenotypes to determine which conserved sites of CNBP are most critical to the proper functioning of skeletal muscle tissue. **Rationale:** The conserved regions of CNBP that are most vital to the regulation of skeletal muscle can be identified using individual CRISPR/Cas9 analyses. Amino acids conserved only in muscular organisms are likely related to muscle function. **Hypothesis:** I hypothesize that specific sites conserved only within muscular organisms will be critical in regulating muscle function.

Aim 2- Identify differentially expressed transcripts in CNBP mutants important for skeletal muscle function.

Approach: I will use RNA-seq on wild-type (WT) mice and the CNBP mouse mutants from Aim 1 to identify RNA transcript profiles in muscle tissue and determine any differentially expressed genes. Using Gene Ontology (GO), I will sort expressed genes by function and identify any differences in transcript levels between the normal and disease states. I will then knock out these genes using CRISPR to determine if they show the same muscle wasting and weakness phenotypes as the CNBP mutants. **Rationale:** This screen will determine genes that may be transcriptionally regulated by CNBP. This screen will also identify which genes specific to skeletal muscle function are up-regulated and down-regulated in the WT vs. mutant state. **Hypothesis:** I hypothesize that CNBP mutants will have differentially expressed genes due to changes in CNBP-DNA binding and that these genes are critical to regulating muscle function.

Aim 3- Quantify dysregulation of proteins involved in skeletal muscle function

Approach: Using an iTRAQ method of proteomic analysis, I will isolate proteins found within the skeletal muscle of WT mice and the mice mutants from Aim 1 in order to quantify up-regulated and down-regulated proteins in each state. Using PANTHER, I will classify these proteins by their GO terms to determine what protein classes and biological processes are affected in the disease state. I will then knock out dysregulated proteins using CRISPR and screen for muscle weakness and wasting phenotypes. **Rationale:** This screen will determine any dysregulation in skeletal muscle genes at the post-transcriptional level caused by decreased CNBP levels. This screen will also identify what protein classes are most affected via GO analysis. **Hypothesis:** I hypothesize that CNBP mutants will have dysregulated proteins levels and that these proteins will be critical to skeletal muscle function.

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