Developing a New Gene Editing Strategy for Patients with Limb-Girdle Muscular Dystrophy Type 2A/R1

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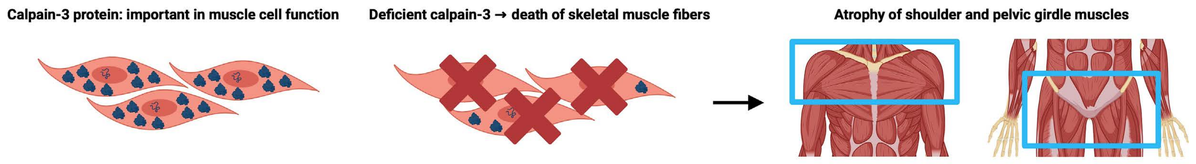
INTRODUCTION & ULTIMATE GOALS

Limb-Girdle Muscular Dystrophy Type 2A/R1 (LGMD2A/R1)

- Rare, genetic muscle disorder
- Caused by pathogenic mutations in gene called *CAPN3,* leading to loss or decrease of important muscle cell protein ("calpain-3")
- Results in degeneration of muscles surrounding shoulders and hips



Ultimate Goal #1: Determine which *CAPN3* mutations cause disease

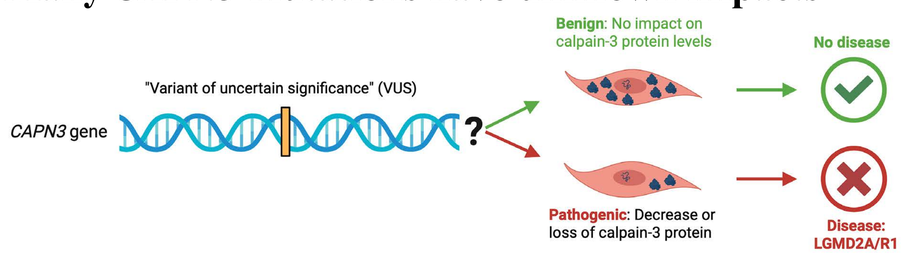
- Intermediate goal: Test method of identifying whether specific mutations increase, decrease, or do not impact amount of calpain-3 protein in human cells

Ultimate Goal #2: Use genetic therapy to fix most common gene mutation causing LGMD2A/R1, called "c.550delA"

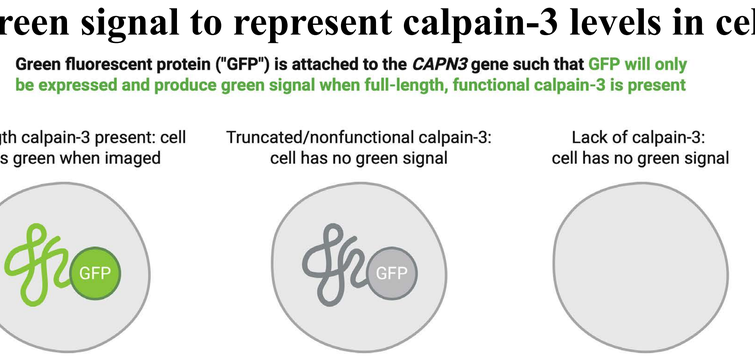
- Intermediate goal: Test gene editing tool to evaluate effective and safe mutation correction in patient cells

GOAL #1: IDENTIFY PATHOGENIC MUTATIONS

Problem: Many *CAPN3* mutations have unknown impacts



Solution: Use green signal to represent calpain-3 levels in cells



Results: Progress in developing a method of variant interpretation

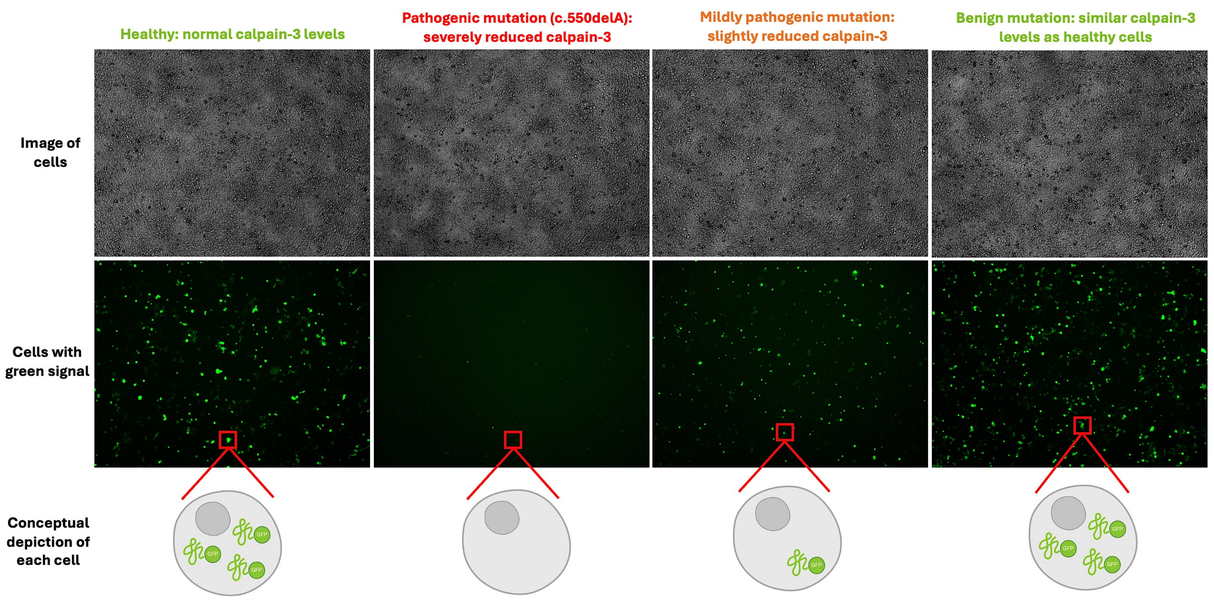
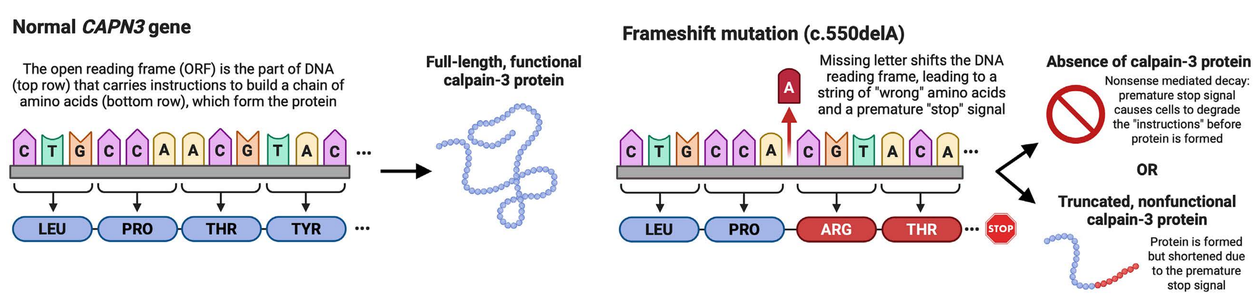


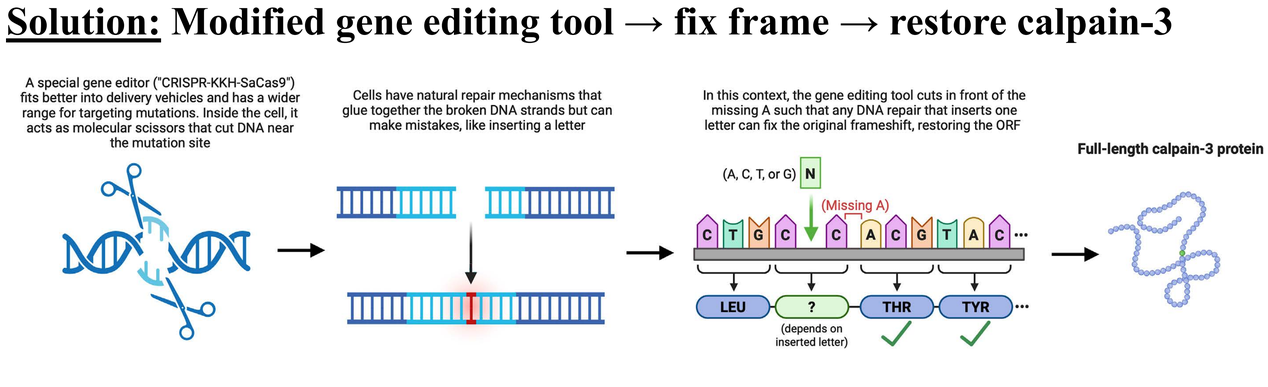
Figure 1. Fluorescent images of cells with different CAPN3 variants and calpain-3 levels. *Known CAPN3 variants (three different mutations) were delivered to a human cell line (HEK293T cells). Images confirmed pathogenic variants decreased calpain-3 protein (indicated by green signal), whereas a benign variant demonstrated similar calpain-3 as healthy cells (no mutation).*

GOAL #2: FIX SPECIFIC *CAPN3* MUTATION

Problem: *CAPN3* mutation → "frameshift" → lack calpain-3 protein



Solution: Modified gene editing tool → fix frame → restore calpain-3



Results: Successful editing to restore reading frame in patient cells

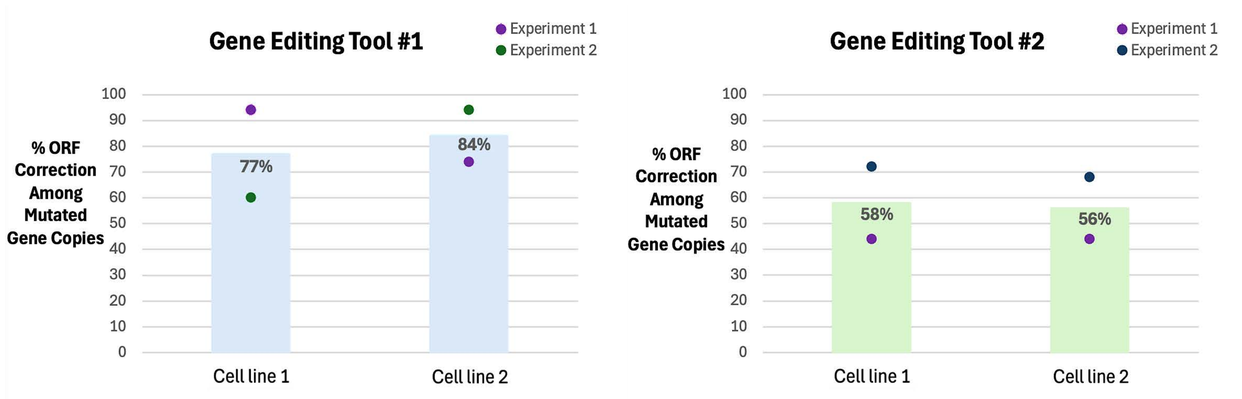


Figure 2. Open reading frame (ORF) correction achieved by editing in patient muscle cells. *Two versions of the gene editing tool (KKH-SaCas9) were delivered to two muscle cell lines from LGMD2A/R1 patients. Because the patient cells carried only one gene copy with the mutation ("heterozygous"), editing was assessed based on ORF correction among mutated genes. Tool #1 and Tool #2 demonstrated average editing efficiencies of ~80.5% and ~57%, respectively.*

CONCLUSIONS & PATIENT IMPACT

Study Conclusion 1:
Made progress in designing a framework to identify which *CAPN3* mutations impact calpain-3 protein levels and therefore cause disease

Implications for Patient Outcomes:

- Diagnosis: identifying which mutations impact calpain-3 levels can enable LGMD2A/R1 diagnosis among patients with variants of uncertain significance (VUS)
- Treatment specificity: patients with mutations identified as disease-causing can seek care and clinical trials targeted for calpain-3 disorders
- Family planning: can conduct prenatal screening for disease mutations

Study Conclusion 2:
Successfully used new gene editing approach to correct the c.550delA mutation in LGMD2A/R1 patient muscle cells

Implications for Patient Outcomes:

- Potential treatment: can expand this work to animal models/clinical trials to develop therapies capable of fixing c.550delA and other mutations, thus restoring calpain-3 protein and muscle cell function
- Treatment delivery: the specific editing tool, KKH-SaCas9, can enable easier packaging/delivery of the therapy to cells and offer more flexibility for precise targeting of mutations