Visceral Myopathy-Associated ACTG2 Mutants Decrease Stiffness in Intestinal Smooth Muscle Cells

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Visceral myopathy is a life-threatening myopathic disorder primarily caused by heterozygous mutations in smooth muscle γ-actin (ACTG2). Individuals with ACTG2 mutations suffer from weakness of bowel, bladder, and uterine smooth muscle, which often culminates in childhood death. Currently, we do not know how disease-causing mutations impair visceral smooth muscle function. This study sought to investigate the effects of two prevailing ACTG2 mutations (R257C and R40C) on the mechanical properties of primary human intestinal smooth muscle cells. Adherent cells were overexpressed with wild-type (WT), R257C, and R40C plasmids for 48 hours, after which RGD-coated ferrimagnetic beads were functionalized to the cytoskeleton (CSK) through cell surface integrin receptors. We first visualized *spontaneous* bead motions and found no substantial differences in the rate of cytoskeletal remodeling (as computed by mean square displacements of beads over time) between ACTG2 mutants and WT overexpressing cells. Interestingly, after imposing large oscillatory mechanical force to the cells, R257C cells exhibited a markedly increased rate of CSK remodeling while R40C cells showed decreased CSK remodeling dynamics. Compared to WT cells, R257C and R40C cells produced notably reduced cellular stiffness as measured by *forced* bead motions with optical magnetic twisting cytometry. These findings characterize previously undefined genotype-specific mechanical properties of disease-causing ACTG2 mutants. Future studies include utilization of traction force microscopy and live-cell microscopy to evaluate the effects of ACTG2 mutants on the contractile stress and migratory behavior of intestinal smooth muscle cells. Supported as a Grover Scholar by Dr. and Mrs. Gary Grover, NIH R01HL164404, and NIH P01HL180318.