To Aunt Brenda and Uncle Daryl

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Abstract

From an ENU mutagenesis screen an embryonic lethal mutant, mi34, was isolated with progressive loss of motility during early development. *In vivo* electrophysiology demonstrated that input from the nervous system to the muscle was normal, implicating a defect in muscle. Indeed, the contraction of mutant skeletal muscle in response to depolarization was significantly reduced compared to siblings. In-vivo calcium imaging demonstrated that evoked activity in mutant muscle produced significantly smaller calcium transients. These findings indicated that the functional defect in *mi34* was in excitation-contraction (EC) coupling, the conversion of electrical to chemical signals in muscle. Positional cloning identified the gene responsible for the mi34 phenotype, *stac3*. The *stac3* gene encodes a novel adapter-like protein, with no known function. We found the Stac3 protein is specifically expressed in muscle and is co-localized with known EC coupling proteins within triads. Rescue by wildtype stac3 confirmed that it is responsible for the mi34 phenotype. Based on the myopathic features of mi34 mutant skeletal muscle, such as SR swelling and sarcomere breakdown, we investigated whether mutations in human STAC3 could have a role in disease. Human *STAC3* maps within the chromosome locus 12q13-14, a region reported to contain the gene responsible for a rare congenital myopathy known as Native American

Myopathy (NAM). Analysis of seven NAM families, including 5 affected and 16 unaffected individuals identified a G>C base substitution causing a missense mutation within a conserved region of *STAC3*. The missense mutation segregated with the disease families, and was not observed in more than 200 control individuals, indicating that it is responsible for NAM. The homologous trytophan to serine substitution in zebrafish Stac3 failed to rescue the abnormal behavior of *mi34* mutant embryos, or fully restore protein localization to triads. Furthermore, *in vivo* calcium imaging of $Stac3^{NAM}$ expressing muscle fibers showed reduced calcium transients compared to $Stac3^{WT}$ rescued skeletal muscles. These data suggest that $Stac3^{NAM}$ is a partial loss of function protein incapable of supporting normal EC coupling.