To my family and pack

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DHPR- Dihydropyridine Receptor complex including all subunits

Ca_v- voltage gated Ca^{2+} channel

Na_v- voltage gated Na^{2+} channel

Ca_v1-3 - principle, α-subunit of the DHPR pore-forming subunit

Ca_v1.1- skeletetal muscle specific isoform of Ca_v1

Ca_vβ- auxiliary β-subunit of the DHPR complex

Ca_vβ1a- skeletal muscle specific splice isoform of $Ca_v\beta1$

Ca_vα2δ- auxiliary α2δ-subunit of the DHPR complex

Ca_vγ- auxiliary γ-subunit of the DHPR conplex

RyR1- Skeletal muscle specific, Ryanodine Receptor1 isoform

Stac- Family of proteins consisting of a Src homology three and a cysteine-rich domain

NAM- Native American Myopathy

EC coupling- excitation contraction coupling

t-tubule- Transverse Tubule

L1-2, L2-3, L3-4- transmembrane, cytoplasmic loops of the Ca_v1

C1 domain- a domain with homology to the first conserved PKC cysteine rich domain

SH3 domain- Src homology three domain

***Dyspedic* myotubes**- Ryr1 null

***Dysgenic* myotubes**- $Ca_v1.1$ null

MH- malignant hyperthermia

Mi^{34}- zebrafish *stac3* mutant

hpf- hours post fertilization

RB- Rohon Beard Neurons

CPG- central pattern generator

KA- Kolmer-Agdur interneuron

MO- Morpholino antisense oligonucleotide

ABSTRACT

Excitation-contraction (EC) coupling is the mechanism by which muscle translates depolarization of the sarcolemma into Ca^{2+} release from the sarcoplasmic reticulum (SR) required for muscle contraction. EC coupling occurs at the junctions of transverse (t) tubules and SR called triads and is dependent on interactions between the dihydropyridine receptor (DHPR) in t-tubules that is the voltage detector, and the ryanodine receptor (RyR1) in the SR that is the Ca^{2+} release channel. Despite the well-studied role in EC coupling of DHPR and RyR1, other components of the molecular complex are less understood. A mutagenesis screen of zebrafish identified an autosomal, recessive mutation that causes poor mobility and reduced Ca^{2+} release in skeletal muscle, yet exhibits normal output from the central nervous system to muscles. Through meiotic mapping, a null mutation in *stac3,* a skeletal muscle-specific gene encoding a putative adaptor protein, was identified. As *stac3* mutants display myopathic features, we explored whether *stac3* mutations might cause human myopathies and found that a mutation in *STAC3* is the basis of Native American Myopathy, which is characterized by muscle weakness and susceptibility to malignant hyperthermia. The Stac3 protein was further characterized and found to directly interact with the EC coupling complex, and to function in normal trafficking and arrangement of the DHPR in arrays of four called tetrads that are essential for Ca^{2+} release.

The neuronally expressed Stac1 protein is a homolog of Stac3, but its function is unknown. Informed by our research on Stac3 in skeletal muscle, we probed the function of Stac1 in neurons. We find *stac1* is expressed in a subset of spinal cord neurons in zebrafish embryos

called Kolmer-Agdur (KA) interneurons that are likely involved in the neuronal circuit that generates swim behaviors. To determine the function of Stac1, we knocked down expression of Stac1 protein with an antisense morpholino oligonucleotide (MO), which resulted in a motility defect in embryos, indicating KA interneurons are involved in the neuronal circuit underlying swim behavior, and Stac1 is required swimming. These results indicate that members of the previously uncharacterized *stac* family of genes are important in normal muscle and neuronal physiology.