

**To my family and pack**

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## LIST OF ABBREVIATIONS

- DHPR**- Dihydropyridine Receptor complex including all subunits
- Ca<sub>v</sub>**- voltage gated Ca<sup>2+</sup> channel
- Na<sub>v</sub>**- voltage gated Na<sup>2+</sup> channel
- Ca<sub>v</sub>1-3** - principle,  $\alpha$ -subunit of the DHPR pore-forming subunit
- Ca<sub>v</sub>1.1**- skeletal muscle specific isoform of Ca<sub>v</sub>1
- Ca<sub>v</sub> $\beta$** - auxiliary  $\beta$ -subunit of the DHPR complex
- Ca<sub>v</sub> $\beta$ 1a**- skeletal muscle specific splice isoform of Ca<sub>v</sub> $\beta$ 1
- Ca<sub>v</sub> $\alpha$ 2 $\delta$** - auxiliary  $\alpha$ 2 $\delta$ -subunit of the DHPR complex
- Ca<sub>v</sub> $\gamma$** - auxiliary  $\gamma$ -subunit of the DHPR complex
- 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<sub>v</sub>1
- 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<sub>v</sub>1.1 null
- MH**- malignant hyperthermia
- Mi<sup>34</sup>**- 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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  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.