One is enough: RyR1 allele-specific gene silencing in mouse models of central core disease (CCD) and malignant hyperthermia (MH)

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Central Core Disease (CCD) and Malignant Hyperthermia (MH) are linked to single amino acid substitutions in the skeletal muscle Ca^{2+} release channel, the type 1 ryanodine receptor (RyR1). We focus on two autosomal dominant (AD) RyR1 mutations, Y522S (YS) and I4898T (IT), which cause MH and CCD, respectively. The AD mode of inheritance and data indicating knock-out of one RyR1 allele is well-tolerated in mice led us to hypothesize that allele-specific gene silencing (ASGS) of the mutant allele would rescue RyR1 functional defects in skeletal muscle cells from YS and IT knock-in mice.

We evaluated the functional consequences of allele-specific silencing in YS and IT muscle cells using short interfering RNAs (siRNAs). To screen potential siRNAs for relative knockdown efficacy and allele specificity, we generated cDNAs encoding fusion proteins derived from wild type (WT) (Venus-Exons-3XFLAG) and either YS or IT mutation-containing (Cherry-Exons-3XHA) exons. Simultaneous transfection of these cDNAs and siRNAs into HEK293 cells and subsequent evaluation of mRNA (semi-quantitative RT-PCR) and protein levels (fluorescence microscopy and western blotting) was used to determine knockdown efficacy and allele-specificity prior to functional rescue experiments. Myotubes derived from heterozygous YS mice (YS/+) exhibit ~4-fold increase in caffeine sensitivity (EC50 values were 0.5mM and 2.3mM for YS/+ and WT, respectively). Treatment of YS/+ myotubes with a YS-selective siRNA, normalized caffeine sensitivity (EC50 = 2.5mM) without decreasing peak caffeine-induced release. Similarly, YS-selective siRNA treatment rescued the increased voltage sensitivity of Ca^{2+} release in YS/+ myotubes determined in perforated-patch clamp experiments (VF1/2: WT = -18mV, YS/+ scrambled = -35mV, YS/+ YS-selective = -18mV). These results indicate that ASGS represents a promising approach for normalization of RyR1 function in MH and CCD. Similar functional rescue experiments in adult skeletal muscle fibres are currently underway.