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Characterisation of C-terminal RyR1 variants linked to neuromuscular disorders

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Abstract

Intracellular calcium influences a large array of cellular processes in skeletal muscle cells and as a result the movement of free calcium is tightly-regulated by a diverse set of calcium channels and accessory proteins. The main store of calcium in skeletal muscle cells is the sarcoplasmic reticulum, from which the ryanodine receptor one calcium channel (RyR1) controls calcium release. Changes in calcium homeostasis often result in the manifestation of neuromuscular disorders, most notably central core disease (CCD) and malignant hyperthermia (MH). While CCD is usually apparent from the presence of certain physical characteristics, MH is typically asymptomatic unless exposed to a trigger, at which point the disease rapidly manifests as a crisis event which is potentially fatal. Currently, the diagnosis of these disorders requires the testing of a muscle biopsy, which is an expensive and invasive procedure, and thus a genetic test would be an ideal diagnostic alternative.

For the most part, CCD and MH cases are linked to the inappropriate release of calcium by defective RyR1 channels – located in the calcium storage organelle membrane – but both are complex disorders with variable penetrance and genetic heterogeneity. A hypoactive RyR1 is thought to cause CCD while a hyperactive RyR1 is thought to cause MH, and yet individuals have been observed to be carriers of both diseases. Most of these instances have been linked to variants in the C-terminal region of RyR1, corresponding to the transmembrane portion of the channel.

This research described in this thesis focused on the functional analysis of five C-terminal domain RyR1 variants identified in patients with neuromuscular disorders. The ability of the variant RyR1 channels to release calcium in response to a stimulus in a heterologous system was measured and compared with that of the wild type channel. Moreover, one of these variants was also examined in several B-lymphoblastoid cell lines taken from carriers of the variant. Of the five variants tested in the heterologous system, four different phenotypes were observed, reinforcing the theory that these disorders are caused by a variety of factors that combine to produce a complex phenotype.

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Abbreviations

-(d/dT)	Decrease (of fluorescence) over time
α-tubulin	Alpha-tubulin
µg	Microgram
µL	Microlitre
34C	Ryanodine receptor antibody
4-C*m*C	4-chloro-*m*-cresol
A260	Absorbance at 260 nm
A280	Absorbance at 280 nm
APS	Ammonium persulfate
ATP	Adenosine triphosphate
BSA	Bovine serum albumin
BSS	Buffered salt solution
CACNA1S	Gene encoding α1 subunit of DHPR
CASQ1	Calsequestrin in skeletal muscle
Casq1	Gene encoding skeletal muscle form of calsequestrin
Cat. No.	Catalogue number
CCD	Central core disease
cDNA	Complementary DNA
CHCT	Caffeine-halothane contracture test
C-terminal	Carboxyl-terminal

DAPI	4’,6-diamidino-2-phenylindole
DHPR	Dihydropyridine receptor
DMEM	Dulbecco’s modified Eagle medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
Dyspedic	Lacking the ryanodine receptor 1 gene
E. coli	*Escherichia coli*
EC	Excitation-contraction
EC_{50}	Half maximal effective concentration
ECCE	Excitation-coupled calcium entry
EDTA	Ethylenediaminetetraacetic acid
EHS	Exertional heat stroke
EMHG	European malignant hyperthermia group
ER	Endoplasmic reticulum
FBS	Foetal bovine serum
FITC	Fluorescein isothiocyanate
FKBP12	FK506-binding protein of skeletal muscle
gDNA	Genomic DNA
HEK-293	Human embryonic kidney 293 cell line
HEK-293T	HEK-293 cell line containing the *Simian virus 40* large T antigen
HRM	High resolution melting
IP3R	Inositol triphosphate receptor

IVCT	*In vitro* contracture test
kb	Kilobase
kDa	Kilodalton
KDS	King-Denborough syndrome
LB broth	Luria Bertani broth
L-type	Long lasting activation
mg	Milligram
MH	Malignant hyperthermia
MHN	Malignant hyperthermia negative
MHS	Malignant hyperthermia susceptible
MHS(c)	MHS in response only to caffeine in IVCT
MHS(h)	MHS response only to halothane in IVCT
mL	Millilitre
MmD	Multiminicore disease
nm	Nanometres
N-terminal	Amino-terminal
Opti-MEM	Opti-minimum essential medium
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PDI	Protein disulfide isomerase
PolyPhen-2	Polymorphism phenotyping version two
P-value	Statistic denoting statistical significance

PVDF	Polyvinylidene fluoride
RYR1	Gene encoding skeletal muscle form of RyR1 protein
RyR1	Type one ryanodine receptor found in skeletal muscle
RyR2	Type two ryanodine receptor found in cardiac muscle
RyR3	Type three ryanodine receptor found in the brain
SDM	Site-directed mutagenesis
SDS	Sodium dodecyl sulfate
SDS-PAGE	SDS polyacrylamide gel electrophoresis
SEM	Standard error of the mean
SERCA	Sarco/endoplasmic reticulum calcium ATPase
SOCE	Store-operated calcium entry
SR	Sarcoplasmic reticulum
Stim1	Stromal interaction molecule one
TAE	Tris-acetate-EDTA
TBST	Tris-buffered saline containing Tween 20
TE	Tris-EDTA
TEMED	Tetramethylethylenediamine
TRITC	Tetramethylrhodamine isothiocyanate
TRPC	Transient receptor potential channel
T-tubule	Transverse tubule
UV	Ultraviolet

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