Characterisation RyR1 variants linked to malignant hyperthermia

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Abstract

Malignant hyperthermia is a potentially fatal disorder of skeletal muscle manifesting as a rise in body temperature in response inhalational anaesthetics and muscle relaxants. Further clinical signs include muscle rigidity and increased oxygen consumption. The increased metabolism is induced by alterations to Ca^{2+} homeostasis resulting from the dysregulation of the sarcoplasmic reticulum protein the ryanodine receptor type 1 (RyR1). A large proportion of known malignant hyperthermia linked genetic variants reside within the gene encoding the type 1 ryanodine receptor, *RYR1*. Malignant hyperthermia can be diagnosed by *in vitro* contracture testing of biopsied muscle tissue. The use of DNA diagnostic testing is advantageous, however it is limited to only 35 of the proposed 400 *RYR1* linked variants known to be associated with malignant hyperthermia.

The research described in this thesis reports the functional characterisation of two *RYR1* variants linked to malignant hyperthermia, c.641C>T and c.7042_7044delCAG resulting in the amino acid changes p.T214M and p.ΔE2348. The ability of each variant to release Ca^{2+} in response to a stimulus was examined in a heterologous system. The variant p.ΔE2348 was shown to be hyperactive in response to agonists indicating the variant is the cause of malignant hyperthermia, while the p.T214M variant does not appear to have an effect ryanodine receptor function.

To understand the relationship between RyR1 function and any structural alterations induced by the p.T214M and p.ΔE2348 variants, the domain housing each variant was cloned for bacterial expression. Subsequent purification and structural characterisation could be used to explain the role each variant plays with respect to the onset of MH. The RyR1 N-terminal domain, amino acids 1-558, and helical domain, amino acids 2091-2525, were expressed in *E. coli* and partially purified. The domains were shown to be soluble and stable following expression.

Abbreviations

A280	Absorbance at 280 nm
ABC	Ammonium bicarbonate
ACN	Acetonitrile
AM	Acetoxymethyl
ADP	Adenosine di-phosphate
ATP	Adenosine tri-phosphate
Bp	Base pairs
Casq	Calsequestrin
CCD	Central core disease
cDNA	Complementry DNA
DAPI	4',6'-diamidino-2-phenylindole
DHPR	Dihydropyrudine receptor
DMEM	Dulbecco's modified eagle's medium
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
DP4	Domain peptide 4
DTT	Dithiothreitol
Dyspedic	Lack of the ryanodine receptor type 1 gene
E. coli	*Escherichia coli*
EC	Excitation-contraction
EC_{50}	Half maximal effective concentration
EDTA	Ethlenediaminetetraacetic acid
EGTA	Ethylene glycol tetraacectic acid
EM	Electron microscopy
ER	Endoplasmic reticulum
FCS	Foetal calf serum
FITC	Fluorescein isothiocyanate
FKBP12	12-kDa FK506 binding protein
GST	Glutathione s transferase
HEK239T	Human embryonic kidney cells
HEPES	4-(2-hydroxethyl)-1-piperazineethanesulfonic acid

IP3R	Inositol tri phosphate receptor
IP3	Inositol tri phosphate
IPTG	Isopropyl-β-D-1-thiogalactopyranoside
IVCT	*In vitro* contracture test
kDa	Kilo Dalton
LB	lysogeny broth
MBP	Maltose binding protein
MH	Malignant hyperthermia
MHN	Malignant hyperthermia negative
MHS	Malignant hyperthermia susceptible
MS	Mass spectrometry
NTD	N-terminal domain
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PDI	Protein disulphide isomerase
PVDF	Polyvinylidene fluoride
RIH	Ryanodine receptor, Inositol triphosphate receptor homology
RyR1	Ryanodine receptor protein
RYR1	Ryanodine receptor cDNA
SDS	Sodiumdodecysulfate
SDS-PAGE	Sodiumdodecysulfate-polyacrylamide gel electrophoresis
SEM	Standard error of the mean
SERCA	Sarco- and- endoplasmic reticulum ATP-ase
SR	Sarcoplasmic reticulum
TAE	Tris acetate EDTA buffer
TBST	Tris buffered saline Tween 20
TE	TE
TEMED	Tetramethylethylenediamine
Tris	Trisaminomethane
TRITC	Tetramethyl rhodamine isothiocyanate
T-tubule	Transverse tubule

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