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A functional analysis of *RYR1* mutations causing
malignant hyperthermia

A thesis presented to Massey University in partial fulfillment of the requirements for the
degree of Doctor of Philosophy in Biochemistry

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2009

ACKNOWLEDGEMENTS

I would especially like to thank my supervisors, Associate Professor Kathryn Stowell and Associate Professor John Tweedie for their help, advice and patience in my study.

I came to New Zealand in 2000, not expecting to complete a PhD, but it was always an option that I had considered. The nine years I have spent at Massey University have not always been easy and I found the last few years, trying to complete my PhD very difficult. It has been a stressful time in very many ways and I do not think I would have completed the PhD if it had not been for the support of my supervisors and my lab mates.

I would particularly like to thank Natisha who has always been my friend as well as someone to discuss my work with. Special thanks also go to Robyn for technical support and as a travelling companion in Spain after attending a conference in Lund. Hilbert and his wife Liat made travelling in Europe an interesting experience, and William, Lili and her husband Cameron have also been my friends. It was fun to work in the lab next to Miranda and I will always remember the good times we had as a group.

I would especially like to thank my friends Dr Aiko Tanaka and Dr Daigo Takemoto working in Japan. They understand me and are always there for me over the internet or at the other end of an email.

I am grateful for the scholarship provided by Mid Central Health, without which my PhD study would not have been possible.

Finally I would like to thank my mother living in Japan. I have been away from home for a long time and it has not been easy either for me or for her as I am her only son. I am looking forward to going back to Japan and renewing those ties that are most important to me.

ABSTRACT

Malignant hyperthermia (MH) is a rare pharmacogenetic disorder in humans induced by volatile anaesthetics and depolarising muscle relaxants. An MH reaction shows abnormal calcium homeostasis in skeletal muscle leading to a hypermetabolic state and increased muscle contracture. A mutation within the skeletal muscle calcium release channel ryanodine receptor gene (*RYR1*) is associated with MH and is thought to cause functional defects in the RYR1 channel leading to abnormal calcium release to the sarcoplasm and consequent MH reactions. Mutations within *RYR1* are also associated with a rare congenital myopathy, central core disease (CCD). CCD is characterised by muscle weakness and is thought to be caused by insufficient calcium release from the RYR1 channel during excitation-contraction (EC) coupling.

To investigate functional effects of *RYR1* mutations, the entire coding region of human *RYR1* was assembled and cloned into an expression vector. Mutant clones containing *RYR1* mutations linked to MH or CCD were also constructed. Wild-type (WT) and mutant *RYR1* clones were used for transient transfection of HEK-293 cells. Western blotting was performed after harvesting and expressed WT and mutant RYR1 proteins were successfully detected. Immunofluorescence showed co-localisation of RYR1 proteins and the endoplasmic reticulum in HEK-293 cells. [³H]ryanodine binding assays showed that RYR1 mutants linked to MH were more sensitive to the agonist 4-chloro-*m*-cresol (4-*CmC*) and less sensitive to the antagonist Mg²⁺ compared with WT. Two C-terminal RYR1 mutants T4826I and H4833Y were very significantly hypersensitive to 4-*CmC* and they may also result in a leaky channel. This hypersensitivity of mutants linked to MH may result in abnormal calcium release through the RYR1 channel induced by triggering agents leading to MH reactions. RYR1 mutants linked to CCD showed no response to 4-*CmC* showing their hyposensitive characteristics to agonists.

This study showed that the human RYR1 proteins could be expressed in HEK-293 cells. Moreover, using the recombinant human RYR1 clone, a single mutation within *RYR1* resulted in a functional defect in expressed RYR1 proteins and functions of mutant RYR1 proteins varied from hypersensitive to hyposensitive depending on the mutation and whether it was linked to MH or CCD.

ABBREVIATIONS

A	absorbance
APS	ammonium peroxodisulfate
ATP	adenosine triphosphate
bp	base pair
BSA	bovine serum albumin
CCD	central core disease
cDNA	complementary DNA
CHAPS	3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate
CHCT	caffeine-halothane contracture test
4-CmC	4-chloro- <i>m</i> -cresol
cpm	counts per minute
C-terminal	carboxy terminal
DAPI	4',6-diamino-2-phenylindole dihydrochloride
DMEM	Dulbecco's modified eagle's medium
DNA	deoxyribonucleic acid
DEPC	diethylpyrocarbonate
DHPR	dihydropyridine receptor
DNase	deoxyribonuclease
DMSO	dimethyl sulphoxide
dNTPs	deoxynucleoside triphosphates
DTT	dithiothreitol
EC	excitation-contraction
EC₅₀	half maximal effective concentration
ECCE	excitation-coupled calcium entry
EDTA	ethylenediaminetetraacetic acid

EGTA	ethyleneglycol-bis(2-amino-ethylether)-N,N,N',N'-tetraacetic acid
ER	endoplasmic reticulum
FITC	fluorescein isothiocyanate
IC₅₀	half maximal inhibitory concentration
IgG	immunoglobulin G
IVCT	<i>in vitro</i> contracture test
kb	kilobase
kDa	kilo Dalton
MEGAWHOP	megaprimer PCR using whole plasmids
MH	malignant hyperthermia
MHE	malignant hyperthermia equivocal
MHN	malignant hyperthermia negative
MHS	malignant hyperthermia susceptible
MmD	multi-minicore disease
mRNA	messenger RNA
N-terminal	amino terminal
PAGE	polyacrylamide gel electrophoresis
PIPES	piperazine-1,4-bis(2-ethanesulfonic acid)
POPOP	1,4-bis(5-phenyl-2-oxazolyl)benzene
PPO	2,5-diphenyloxazole
pBS	pBlueScript
PBS	phosphate buffered saline
pc	pcDNA
PCR	polymerase chain reaction
PDI	protein disulfide isomerase

RNase	ribonuclease
RT	reverse transcriptase
RT-PCR	reverse transcription-polymerase chain reaction
RYR1	ryanodine receptor 1
SDS	sodium dodecyl sulphate
SEM	standard error of the mean
SERCA	sarco/endoplasmic reticulum Ca^{2+} -ATPase
SLB	super LB
SOCE	store-operated calcium entry
SR	sarcoplasmic reticulum
TAE	Tris-acetate-EDTA buffer
TEMED	N,N,N',N'-tetramethylethylenediamine
TBS	tris buffered saline
TBST	tris buffered saline Tween 20
TE	Tris-EDTA buffer
Tris	tris(hydroxymethyl)aminomethane
TRITC	tetramethylrhodamine isothiocyanate
TRPC	transient receptor potential channel
T-tubule	transverse tubule
UV	ultraviolet light
WT	wild-type

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