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**LOSS OF HETEROZYGOSITY OF THE H4833Y  
MUTATION ON *RYR1* GENE CAUSING  
MALIGNANT HYPERTHERMIA**

A thesis presented in partial fulfilment of the requirements for the degree of  
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## ABSTRACT

Malignant hyperthermia is a potentially fatal pharmacological disorder and is triggered by volatile anaesthetics in predisposed individuals. Mutations in the *RYR1* gene, encoding the skeletal muscle calcium receptor channel have been linked to MH susceptibility. Over 200 point mutations have been found to date in the *RYR1* gene linked to MHS worldwide.

EBV-immortalization is regularly used worldwide as an effective procedure for inducing long-term growth of human B lymphocytes. In the current study, it was observed that immortalized lymphocytes from MHS patients heterozygous for the missense mutation H4833Y when initially cultured expressed both wild type and mutant allele but after a few weeks of culture they seemed to lose the mutant allele. High resolution melting assays and hybridization probe assays showed the loss of heterozygosity and this was confirmed using DNA sequencing. Genotyping and haplotype analysis using three intragenic RFLPs and two (CA)<sub>n</sub> repeat microsatellite markers tightly linked to the *RYR1* gene showed a definite change in the haplotype, suggesting more widespread changes in the genome upon short-term culture of EBV-immortalized B-lymphocytes.

## ABBREVIATIONS

4-CmC	4-Chloro- <i>m</i> -cresol
°C	Degree Celcius
6-FAM	6-Carboxyfluorescence
aCGH	Array comparative genomic hybridization
AM	Acetoxymethyl
ARVD2	Arrhythmogenic right ventricular dysplasia
ATP	Adenosine tri-phosphate
BSS	Balanced salt solution
bp	Basepairs
CaM	Calmodulin
CCD	Central core disease
CHCT	Caffeine halothane contracture test
CPVT	Catecholaminergic polymorphic ventricular tachycardia
DIC	Disseminated intravascular coagulation
DHPR	Dihydropyridine receptor
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
dNTPs	Dinucleotide triphosphates
dsDNA	Double-stranded DNA
ECC	Excitation-contraction coupling
EDTA	Ethylene diamine tetra-acetate
EMHG	European malignant hyperthermia group
ER	Endoplasmic reticulum
FCS	Fetal calf serum

FKBP	FK-506 binding proteins
FRET	Fluorescence resonance energy transfer
gDNA	Genomic DNA
HRM	High resolution melting
HybProbe	Hybridization probe (assay)
IVCT	<i>In vitro</i> contracture test
Kb	Kilobasepairs
LCL	Lymphoblasboid cell line
M	Molar (mol/L)
μM	Micromolar
MH	Malignant hyperthermia
MHE	Malignant hyperthermia equivocal
MHN	Malignant hyperthermia negative
MHS	Malignant hyperthermia susceptible
Min	Minute(s)
MmD	Multi-minicore disease
MQ	Milli-Q (deionized) water
mRNA	Messenger RNA
Mt	Mutant
nm	Nanomolar
NAMHG	North American malignant hyperthermia group
NTC	Non-template control
PSS	Porcine stress syndrome
RFLP	Restriction fragment length polymorphisms
RMH	Royal Melbourne hospital
RNA	Ribonucleic acids
RT	Room temperature

RyR	Ryanodine receptor protein
<i>RYR1</i>	Ryanodine receptor gene
RyR1	Ryanodine receptor type 1
RyR2	Ryanodine receptor type 2
RyR3	Ryanodine receptor type 3
Sec	Second(s)
SERCA	Sarco/endoplasmic reticulum Ca <sup>2+</sup> -ATPase
SNP	Single nucleotide polymorphisms
SR	Sarcoplasmic reticulum
TAE	Tris-acetate-EDTA-buffer
<i>Taq</i>	<i>Thermus aquaticus</i>
Taq polymerase	<i>Thermus aquaticus</i> DNA polymerase
T <sub>m</sub>	Melting temperature
T-tubule	Transverse tubule
Wt	Wildtype

## LIST OF FIGURES

Figure 1. The mutational hot spot regions on the RyR1 protein .....	5
Figure 2. Arrangement of the RyR1, DHPR and associated proteins involved in SR calcium release .....	7
Figure 3. E-C coupling of the skeletal muscle .....	8
Figure 4. Regions of divergence between RyR1 and RyR2 .....	11
Figure 5. Side view of the surface representation of the RyR1 protein .....	12
Figure 6. Solid body representations of the three isoforms of the ryanodine receptor .....	13
Figure 7. Central cores within the muscle fibre .....	16
Figure 8. HRM assay using LightCycler® 480 gene scanning software .....	24
Figure 9. The principle of mutation detection using LightCycler 1.2 system.....	26
Figure 10. HybProbe assay .....	27
Figure 11. Relative positions of the markers on chromosome 19 .....	31
Figure 12. Microsatellite marker analysis.....	32
Figure 13. Fluorescence excitation spectra of Fura-2AM.....	38
Figure 14. Typical signals obtained when a cell loaded with Fura-2AM is excited at 340 and 380 nm (Modified from [131] .....	39
Figure 15. HRM assay at 4 weeks culture .....	42
Figure 16. HRM assay at 9 weeks culture .....	43
Figure 17. HRM assay for 1042 at 4 and 9 weeks culture .....	45
Figure 18. Melting peaks for 1261 DNA at 4 and 12 weeks culture.....	46
Figure 19. Melting peaks for 1052 DNA at 4 and 12 weeks culture.....	47
Figure 20. HybProbe assay for 1051 DNA.....	48
Figure 21. PCR of exon 100-103.....	50
Figure 22. Chromatogram of the sequencing of 1051 DNA at 4 and 9 weeks of culture.....	51
Figure 23. HRM assay for the three RFLPs using the cell line 1051.....	55
Figure 24. HRM assay for the three RFLPs using the cell line 1051 .....	56



Figure 25 PCR of D19S47 with FastStart Taq polymerase .....	57
Figure 26. Chromatogram for D19S47 and D19S220 with 4 week cultured 1051 cell line .....	58
Figure 27. Chromatogram for D19S47 and D19S220 with 1051 DNA from fresh leucocytes .	59
Figure 28. Chromatogram for D19S47 and D19S220 with 9 week cultured 1051 cell lines....	60
Figure 29 . Calcium release stimulated by 4-CmC in human B-lymphoblastoid cell lines ....	64

## LIST OF TABLES

Table 1. Reaction components for the HRM protocol using LightCycler® 480 HRM Master .	24
Table 2. High resolution melting program.....	25
Table 3 HybProbe assay using the FastStart DNA MasterPLUS HybProbe .....	28
Table 4. HybProbe LightCycler program.....	29
Table 5. Reaction components for the microsatellite D19S47 using FastStart Taq polymerase .....	33
Table 6. FastStart PCR programme .....	33
Table 7. Reaction components for PCR for the microsatellite D19S220 Phusion Polymerase .....	34
Table 8. Phusion Polymerase PCR protocol.....	35
Table 9. D19S47 alleles.....	36
Table 10. D19S220 alleles.....	36
Table 11. Properties of RFLPs .....	37
Table 12. Summary of the genotyping results for 1051 and 1042 cell lines.....	61
Table 13. Haplotypes for 1051 and 1042 cell lines .....	62

# TABLE OF CONTENTS

CHAPTER 1 INTRODUCTION.....	1
1.1 Malignant Hyperthermia.....	1
1.1.1 History.....	2
1.1.2 Clinical symptoms.....	2
1.1.3 Treatment.....	3
1.1.3.1 <i>Dantrolene</i> .....	3
1.1.4 Molecular genetics.....	4
1.1.4.1 <i>Introduction</i> .....	4
1.1.4.2 <i>Mutations</i> .....	4
1.1.4.3 <i>Other loci associated with MH</i> .....	5
1.1.5 Pathophysiology of MH.....	6
1.1.5.1 <i>Calcium homeostasis</i> .....	6
1.1.5.2 <i>Excitation-Contraction coupling</i> .....	7
1.1.6 Diagnostic Testing.....	9
1.1.6.1 <i>In Vitro Contracture Testing</i> .....	9
1.1.6.2 <i>Limitations of IVCT</i> .....	10
1.1.6.3 <i>Functional assays</i> .....	10
1.1.6.4 <i>Genetic testing</i> .....	10
1.2 Ryanodine receptors.....	11
1.2.1 RyR1.....	12
1.2.2 RyR modulators.....	13

1.2.2.1 <i>Endogenous modulators</i> .....	13
1.2.2.2 <i>Modulation by associated proteins</i> .....	14
1.3 Associated myopathies.....	16
1.3.1 CCD.....	16
1.3.2 Multi-mini core disease.....	17
1.4 Research question and objective.....	18
1.4.1 Background.....	18
1.4.2 Research objective.....	18
1.4.3 Significance.....	18
CHAPTER 2. MATERIALS AND METHODS.....	19
2.1 Materials.....	19
2.2 Methods.....	20
2.2.1 Mammalian Cell Culture.....	20
2.2.1.1 <i>Reactivation of lymphoblastoid cell lines from liquid</i> <i>nitrogen stocks</i> .....	20
2.2.1.2 <i>Freezing down BLCL for storage</i> .....	20
2.2.2 Isolation of genomic DNA from lymphoblastoid cell lines.....	21
2.2.3 Quantification of genomic DNA.....	21
2.2.4 High Resolution Melting Assays.....	21
2.2.4.1 <i>Melting curve analysis</i> .....	22
2.2.5 Hybridization Probe assay.....	25
2.2.6 Sequencing.....	29
2.2.6.1 <i>Polymerase chain reaction</i> .....	29

2.2.6.2 Agarose gel electrophoresis.....	29
2.2.6.2 Purification of PCR products.....	30
2.2.6.3 DNA sequencing.....	30
2.2.7 Genotyping.....	30
2.2.7.1 Microsatellite analysis.....	31
2.2.7.2 Restriction fragment length polymorphisms.....	36
2.2.8 Functional assays.....	37
2.2.8.1 Calcium release assay.....	37
CHAPTER 3. RESULTS.....	41
3.1 Mutation detection using high resolution melting assay.....	41
3.2 Mutation detection using hybridization probe assay.....	49
3.3 Mutation screening using DNA sequencing.....	52
3.4 Genotyping by haplotype analysis.....	52
3.4.1 Genotyping with intragenic restriction fragment length polymorphism.....	52
3.5.1.1 RFLP Ile <sup>1151</sup> .....	52
3.5.1.2 RFLP Asp <sup>2729</sup> .....	53
3.5.1.3 RFLP Ser <sup>2862</sup> .....	53
3.4.2 Microsatellite analysis.....	55
3.4.2.1 D19S220.....	55
3.4.2.2 D19S47.....	56
3.6 Calcium release assays.....	63

CHAPTER 4. DISCUSSION.....	65
4.1 SNP genotyping.....	66
4.2 Haplotype analysis.....	67
4.2.1 <i>Restriction fragment length polymorphisms</i> .....	67
4.2.2 <i>Microsatellite markers</i> .....	69
4.3 Functional assays.....	70
CHAPTER 5. REFERENCES.....	73
CHAPTER 6. APPENDICES.....	A1
Appendix I   HybProbe assay for 1042 cell line.....	A1
Appendix II  HRM assay for the three RFLPs using the 1042 cell line. ....	A2
Appendix III Sequencing results of exon 100.....	A4
Appendix IV  Microsatellite analysis with D19S47 and D19S220 using 1042 cell line.....	A8
Appendix V   Primer sequences.....	A12
Appendix VI  Buffer composition.....	A13