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

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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 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 $(CA)_n$ 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-C*m*C	4-Chloro-*m*-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	*In vitro* 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
RYR1	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^{2+}-ATPase
SNP	Single nucleotide polymorphisms
SR	Sarcoplasmic reticulum
TAE	Tris-acetate-EDTA-buffer
Taq	*Thermus aquaticus*
Taq polymerase	*Thermus aquaticus* DNA polymerase
T_m	Melting temperature
T-tubule	Transverse tubule
Wt	Wildtype

LIST OF FIGURES

LIST OF TABLES

TABLE OF CONTENTS