# SCREENING FOR SPECIFIC MUTATIONS IN MALIGNANT HYPERTHERMIA SUSCEPTIBLE FAMILIES

by

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TO MY PARENTS

# ABSTRACT

Malignant hyperthermia (MH) is an autosomal dominant pharmacogenetic disorder (Deufel *et al.*, 1992). It is potentially fatal and one of the leading causes of death due to anaesthesia. An MH episode is triggered when MH susceptible individuals are exposed to certain inhalation anaesthetic agents and depolarising skeletal muscle relaxants (MacLennan, 1992).

To date, twenty-three mutations have been reported in the skeletal muscle ryanodine receptor gene (RYR1), located on chromosome 19q13.1, and twenty-two of these mutations have been associated with MH (Brandt *et al.*, 1999). The I403M mutation has only been associated with central core disease (CCD). CCD and MH are phenotypically distinct allelic variants as mutations in the same gene are responsible for both these disorders (Quane *et al.*, 1993).

Seventeen MH families, four South African and thirteen North American families, were included in this study. DNA from selected MH individuals were screened for nine of the reported mutations (Cys35Arg, Arg163Cys, Gly248Arg, Gly341Arg, Ile403Met, Tyr522Ser, Arg614Cys, Gly2435Arg and Arg2436His). The interaction between polymorphisms and mutations, which might modify or contribute to the MH phenotype, is currently still unknown. For this reason the DNA from selected individuals were screened for the presence of all of the nine mutations investigated in this study.

Two mutations, Arg614Cys and Gly2435Arg, were observed in the group of MH families included in this study. The Arg614Cys mutation was identified in a large South African MH family (MH105). A total of 39 individuals of South African family MH105 were subsequently screened for the presence of this mutation. Twelve individuals, three of whom have not been tested via the *in vitro* contracture test (IVCT), were positive for the mutation. Two individuals from this family displayed phenotype-genotype discordance. The Gly2435Arg mutation was identified in a North American MH family (US2). This small family of five individuals included three individuals diagnosed via the North American IVCT protocol. The three phenotyped individuals included two MH positive and one MH negative individual. The Gly2435Arg mutation segregated with the MH phenotype observed in this North American family. This is the first report of the Gly2435Arg mutation in the North American MH population. The identification of the Arg614Cys and

Gly2435Arg mutations contributed towards establishing a molecular diagnostic service for individuals within these particular MH families.

None of the other seven mutations investigated were detected in the group of families included in this study. The DNA alterations are therefore not present as polymorphisms in these families, and can thus not contribute to the MH phenotype segregating in any of the families. If the families investigated in this study are truly representative of the South African and North American populations it is possible that these seven mutations are absent in these two populations. These mutations might, therefore, be population specific or even family specific.

# OPSOMMING

Maligne hipertermie (MH) is 'n outosomale dominante farmakogenetiese toestand (Deufel *et al.*, 1992). Dit is 'n potensieel fatale toestand en een van die hoof oorsake van sterftes as gevolg van narkose. 'n MH aanval word veroorsaak wanneer MH vatbare individue blootgestel word aan sekere inhalasie narkosegasse en depolariserende skeletspier verslappers (MacLennan, 1992).

Tot op hede is drie-en-twintig mutasies gerapporteer in die skeletale ryanodien reseptor geen (RYR1), gelokaliseer op chromosoom 19q13.1, waarvan twee-en-twintig geassosieer word met MH (Brandt *et al.*, 1999). Die Ile403Met mutasie is slegs geassosieer met "central core" siekte (CCS). CCS en MH is unieke fenotipiese alleliese variante aangesien mutasies in dieselfde geen verantwoordelik is vir beide hierdie toestande (Quane *et al.*, 1993).

Sewentien MH families, vier Suid-Afrikaanse en dertien Noord-Amerikaanse families, was ingesluit in hierdie studie. DNA van geselekteerde MH individue is ondersoek vir nege van die gerapporteerde mutasies (Cys35Arg, Arg163Cys, Gly248Arg, Gly341Arg, Ile403Met, Tyr522Ser, Arg614Cys, Gly2435Arg en Arg2436His). Die interaksie tussen polimorfismes en mutasies, wat moontlik die MH fenotiepe mag wysig of daartoe mag bydra, is tans steeds onbekend. Om hierdie rede is die DNA van geselekteerde individue ondersoek vir die teenwoordigheid van al nege die mutasies wat ingesluit was in die studie.

Twee mutasies, Arg614Cys en Gly2435Arg, is waargeneem in die groep MH families wat ingesluit was in hierdie studie. Die Arg614Cys mutasie is geïdentifiseer in 'n uitgebreide Suid-Afrikaanse MH familie (MH105). 'n Totaal van 39 individue van Suid-Afrikaanse familie MH105 is gevolglik ondersoek vir die teenwoordigheid van die mutasie. Twaalf individue, drie wie nog nie voorheen getoets was via die *in vitro* kontraksie toets (IVKT) nie, is positief vir die mutasie. Twee individue van hierdie familie toon nie fenotiepe-genotiepe korrelasie nie. Die Gly2435Arg mutasie is geïdentifiseer in 'n Noord-Amerikaanse MH familie (US2). Die klein familie van vyf individue sluit drie individue in wat voorheen gediagnoseer was via die Noord-Amerikaanse IVKT protokol. Die drie gefenotipeerde individue sluit twee MH positiewe en een MH negatiewe individu in. Die Gly2435Arg mutasie segregeer saam met die MH fenotiepe wat waargeneen is in die Noord-Amerikaanse familie. Hierdie is die eerste berig van die Gly2435Arg mutasie in die

Noord-Amerikaanse MH populsie. Die identifikasie van die Arg614Cys en Gly2435Arg mutasies dra by tot die vestiging van 'n molekulêre diagnostiese diens vir individue binne hierdie bepaalde families.

Geen van die ander sewe mutasies wat ondersoek is, is geïdentifiseer in die groep families wat in hierdie studie ingesluit was nie. Hierdie DNA veranderings is dus nie teenwoordig as polimorfismes in hierdie families nie en kan dus nie bydrae tot die MH fenotiepe wat segregeer in enige van hierdie families nie. Indien die families wat in hierdie studie ondersoek is werklik verteenwoordigend is van die Suid-Afrikaanse en Noord-Amerikaanse populasies, is dit moontlik dat hierdie sewe mutasies afwesig is in die twee populasies. Hierdie mutasies mag dus populasie spesifiek of selfs familie spesifiek wees.

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# LIST OF ABBREVIATIONS AND SYMBOLS

| | |
|---|---|
| α | alpha |
| $\alpha_1$ | alpha 1 sub-unit of the DHPR, expressed in skeletal muscle |
| $\alpha_2$ | alpha 2 sub-unit of the DHPR, expressed in skeletal muscle |
| $\alpha_2/\delta$ | alpha 2 gamma sub-unit complex of the skeletal muscle dihydropyridine receptor |
| $\alpha^{32}$P-dCTP | dCTP labelled in the α position with $^{32}$P isotope |
| $\alpha^{35}$S-dATP | dATP labelled in the α position with $^{35}$S isotope |
| a | adenine |
| A or A | adenine (in DNA sequence, indicating exon sequence) |
| A or A | alanine (in amino acid sequence) |
| $A_{260}$ | absorbency at 260 nm |
| $A_{260}/A_{280}$ | ratio of absorbency measured at 260 nm and 280 nm |
| *Aci I* | restriction endonuclease isolated from *Arthrobacter citreus*, with recognition site 5'-...C↓CGC...-3' |
| ACRS | amplification created restriction site |
| ADP | adenosine diphosphate |
| Ala | alanine |
| *Alw NI* | restriction endonuclease isolated from *Acinetobacter lwoffii N*, with recognition site 5'-...CAGNNN↓CTG...-3' |
| AMP | adenosine monophosphate |
| APS | ammonium persulphate: $(NH_4)_2S_2O_8$ |
| Arg | arginine |
| Asn | asparagine |
| Asp | aspartic acid |
| ATG | initiator codon |
| ATP | adenosine triphosphate |
| ATPase | adenosine triphosphatase |
| β | beta |
| $\beta_1$ | beta sub-unit of the DHPR, expressed in skeletal muscle |
| BESS T | base excision sequence scanning mutation detection and localisation |
| boric acid | boracic acid: $H_3BO_3$ |
| bp | base pair |
| BPB | bromophenol blue: $C_{14}H_{10}Br_4O_5S$ |
| BSA | bovine serum albumin |
| *Bsh 1236I* | restriction endonuclease isolated from *Bacillus sphaericus* RFL1236, with recognition site 5'-...CG↓CG...-3' |
| *BsmA I* | restriction endonuclease isolated from *Bacillus strearothermophilus*, with recognition site 5'-...GTCTC$(N)_1$↓...-3' |
| *Bsr BI* | restriction endonuclease isolated from *Bacillus strearothermophilus cpw* 193, with recognition site 5'-...GAG↓CGG...-3' |
| *Bst UI* | restriction endonuclease isolated from *Bacillus strearothermophilus U*, with recognition site 5'-...CG↓CG...-3' |
| c | cytosine |
| C or C | cytosine (in DNA sequence, indicating exon sequence) |
| C or C | cysteine (in amino acid sequence) |
| °C | degrees centigrade |
| %C | percentage crosslinking monomer |
| ca. | circa: approximately |
| $Ca^{2+}$ | calcium ions |
| $Ca^{2+}$-ATPase | calcium adenosine triphosphatase |
| $Ca^{2+}$-release | calcium release |
| $Ca^{2+}$-channel | calcium channel |
| CACNL1A3 | $\alpha_1$ sub-unit of the skeletal muscle dihydropyridine receptor |

**List of abbreviations and symbols (continued ...)**

| | |
|---|---|
| CACNL2A | $\alpha_2/\delta$ sub-unit of the skeletal muscle dihydropyridine receptor |
| CACNLB1 | $\beta$ sub-unit of the dihydropyridine receptor |
| CACNLG | $\gamma$ sub-unit of the dihydropyridine receptor |
| caffeine | 3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione: $C_8H_{10}N_4O_2$ |
| CAG | 3' acceptor site of splice junction boundaries |
| CaM | calmodulin |
| CCAAT | regulatory DNA sequence in the 5' region of eukaryotic genes; transcription factors binding site |
| CCD | central core disease |
| cDNA | complementary DNA |
| CHCT | caffeine halothane contracture test |
| Ci | curie: quantity of any radioactive nuclide in which there are $3.7 \times 10^{10}$ disintegrations per second |
| CK | creatine kinase |
| cm | centimetre |
| cM | centimorgan |
| $CO_2$ | carbon dioxide |
| COOH | carboxyl group, indicating the C-terminal of a protein molecule |
| CpG | indicates a C covalently linked to a neighboring G on the same DNA stand |
| CPK | creatine phosphokinase |
| CsCl | cesium chloride |
| C-terminal | carboxyl terminal |
| Cys | cysteine |
| $\delta$ | delta |
| $\Delta G$ | free energy: indicating nucleic acid duplex stability |
| D or D | aspartic acid |
| dATP | 2'-deoxyadenosine 5'-triphosphate |
| dCTP | 2'-deoxycytidine 5'-triphosphate |
| ddA | dideoxyadenosine |
| ddATP | 2',3'-dideoxyadenosine 5'-triphosphate |
| ddC | dideoxycytidine |
| ddCTP | 2',3'-dideoxycytidine 5'-triphosphate |
| *Dde I* | restriction endonuclease isolated from an *E. coli* strain that carries the cloned *Dde I* gene from *Desulfovibrio desulfuricans*, with recognition site 5'-...C↓TNAG...-3' |
| ddG | dideoxyguanosine |
| ddGTP | 2',3'-dideoxyguanosine 5'-triphosphate |
| $ddH_2O$ | double distilled water |
| ddN | dideoxynucleotide |
| ddNTP | 2',3'-dideoxynucleotide |
| ddNTPs | 2',3'-dideoxynucleotides |
| ddT | dideoxythymidine |
| ddTTP | 2',3'-dideoxythymidine 5'-triphosphate |
| 7-deaza-dGTP | 7-deaza-2'-deoxyguanosine 5'-triphosphate |
| dGTP | 2'-deoxyguanosine 5'-triphosphate |
| DHP | dihydropyridine |
| DHPR | dihydropyridine receptor |
| DHPR$\beta$ | dihydropyridine receptor $\beta$ sub-unit |
| DHPR$\gamma$ | dihydropyridine receptor $\gamma$ sub-unit |
| DMD | Duchenne muscular dystrophy |
| *D. melanogaster* | *Drosophila melanogaster* |
| DNA | deoxyribonucleic acid |
| dNTP | 2'-deoxynucleotide |
| dsDNA | double stranded DNA |
| DTT | dithiothreitol: threo-1,4-dimercapto-2,3-butanediol:$C_4H_{10}O_2S_2$ |

**List of abbreviations and symbols (continued ...)**

| | |
|---|---|
| dTTP | 2'-deoxythymidine 5'-triphosphate |
| dUTP | 2'-deoxyuracil 5'-triphosphate |
| E or E | glutamic acid |
| EC-coupling | excitation-contraction coupling |
| EDTA | ethylenediamine tetra-acetic acid: $C_{10}H_{16}N_2O_8$ |
| EF | EF hand domain |
| EMG | electromyograph |
| EtBr | ethidium bromide: $C_{21}H_{20}BrN_3$ |
| EtOH | ethanol: $CH_3CH_2OH$ |
| F or F | phenylalanine |
| FFA | free fatty acids |
| FKBP12 | FK-506 binding protein |
| FMC | FMC Corporation |
| Formamide | carbamaldehyde: $CH_3NO$ |
| γ | gamma |
| $\gamma^{32}$P-dATP | dATP labelled in the γ position with $^{32}$P isotope |
| $\gamma^{32}$P-dCTP | dCTP labelled in the γ position with $^{32}$P isotope |
| g | guanine |
| g | gram |
| G or G | guanine (in DNA sequence, indicating exon sequence) |
| G or G | glycine (in amino acid sequence) |
| GC | indicates a G on one strand that is hydrogen-bonded to a C on the opposite strand |
| g/v | gram per volume |
| gDNA | genomic DNA |
| Genbank | GenBank®[1] : United States repository of DNA sequence information |
| Gln | glutamine |
| Glu | glutamic acid |
| Gly | glycine |
| GPI | glucose phosphate isomerase |
| GT | 5' donor site of splice junction boundaries |
| H or H | histidine |
| *Hae III* | restriction endonuclease isolated from an *E. coli* strain that carries the cloned *Hae III* gene from *Heamophilus aegyptius*, with recognition site 5'-...GG↓CC...-3' |
| halothane | 2-bromo-2-chloro-1,1,1-trifluoroethane: $C_2HBrClF_3$ |
| HCl | hydrochloric acid |
| $HCO_3$ | bicarbonate |
| *Hga I* | restriction endonuclease isolated from an *E. coli* strain that carries the cloned *Hga I* gene from *Heamophilus gallinarum*, with recognition site 5'-...GACGC$(N)_5$↓...-3' |
| Hg | mercury |
| His | histidine |
| HLA | human leukocyte antigen |
| $H_2O$ | water |
| I or I | isoleucine |
| ICU | intensive care unit |
| Ile | isoleucine |
| IU | international units |
| IU.$l^{-1}$ | international units per litre |
| IV | *intra venous* |
| IVCT | *in vitro* contracture test |
| K or K | lysine |
| kb | kilo base pair |

[1] GenBank® is a registered trademark of the National Institutes of Health, U.S.A.

**List of abbreviations and symbols (continued ...)**

| | |
|---|---|
| kcal.mol$^{-1}$ | kilo calorie per mole |
| KCl | potassium chloride |
| kDa | kilo dalton |
| kinase | polynucleotide kinase: ATP: 5'-dephosphopolynucleotide 5'-phospho-transferase |
| L or L | leucine |
| Leu | leucine |
| LDH | lactate dehydrogenase |
| 2X loading buffer | 95% formamide, 10 mM EDTA, 0.1% XC and 0.1% BPB [pH 11.0] |
| 10X loading buffer | 0.25% bromophenol blue (BPB), 0.25% xylene cyanol FF (XC), 0.4% Orange G (OG) and 50% glycerol |
| Ltd. | limited |
| Lys | lysine |
| lysis buffer | 0.32 M sucrose; 10 mM Tris-HCl [pH 8.0]; 5 mM $MgCl_2$; 1% Triton X-100® [1] |
| µ | micro: $10^{-6}$ |
| µCi | micro Curie |
| µg | microgram |
| µg.l$^{-1}$ | microgram per litre |
| µg.µl$^{-1}$ | microgram per microlitre |
| µg.ml$^{-1}$ | microgram per millilitre |
| µl | microlitre |
| µM | micromolar |
| m | milli: $10^{-3}$ |
| M | molar: moles per litre |
| M or M | methionine |
| M' – M" | transmembrane regions |
| M1 - M10 | transmembrane regions, numbers 1 to 10 |
| *Mbo I* | restriction endonuclease isolated from an *E. coli* strain that carries the cloned *Mbo I* gene from *Moraxella bovis*, with recognition site 5'-...↓GATC...-3' |
| 5meC | 5-methylcytosine |
| mEq | milliequivalents |
| mEq.kg$^{-1}$ | milliequivalents per kilogram |
| mEq.l$^{-1}$ | milliequivalents per litre |
| Met | methionine |
| MeV | mega electron volt |
| mg | milligram |
| mg.kg$^{-1}$ | milligram per kilogram |
| $Mg^{2+}$ | magnesium ion |
| $MgCl_2$ | magnesium chloride |
| MH | malignant hyperthermia |
| MHE | MH equivocal |
| MHEc | MH equivocal: positive only for caffeine |
| MHEh | MH equivocal: positive only for halothane |
| MHN | MH normal |
| MHS | MH susceptible |
| min | minutes |
| ml | millilitre |
| mm | millimetre |
| mmHg | millimetre mercury |
| mM | millimolar |
| MMR | masseter muscle rigidity |

[1] Triton X-100® is a registered trademark of Rohm & Haas Company, Philadelphia, PA, U.S.A.

**List of abbreviations and symbols (continued ...)**

| | |
|---|---|
| MMS | masseter muscle spasm |
| *Mnl I* | restriction endonuclease isolated from an *E. coli* strain that carries the cloned *Mnl I* gene from *Moraxella nonliquefaciens*, with recognition site 5'-...CCTC$(N)_7$↓...-3' |
| mRNA | messenger RNA |
| *Msp I* | restriction endonuclease isolated from an *E. coli* strain that carries the cloned *Msp1* gene from *Moraxella* species, with recognition site 5'-...C↓CGG...-3' |
| mV | millivolt |
| n | nano: $10^{-9}$ |
| N | A or C or G or T nucleotide |
| N or N | asparagine |
| Na | sodium |
| NA | not available |
| NaCH | sodium channel |
| NaCl | sodium chloride |
| $NaClO_4$ | sodium perchlorate |
| $Na_2EDTA$ | disodium EDTA: $C_{10}H_{14}N_2Na_2O_8.2H_2O$ |
| NaOH | sodium hydroxide |
| ng | nanogram |
| $NH_2$ | amino group, indicating the N-terminal of a protein molecule |
| nm | nanometer |
| nM | nanomolar |
| NR | no response at |
| NT | not tested with the *in vitro* contracture test |
| N-terminal | amino terminal |
| $O_2$ | oxygen |
| OD | optical density |
| OG | orange G: $C_{16}H_{10}N_2O_7S_2Na_2$ |
| OI | osteogenesis imperfecta |
| p | short arm of chromosome |
| p | pico: $10^{-12}$ |
| P or P | proline |
| $^{32}P$ | phosphorus isotope: maximum β emission energy 1.71 MeV: half-life 14.3 days |
| $P_aCO_2$ | partial pressure of arterial $CO_2$ |
| PAGE | polyacrylamide gel electrophoresis |
| pBR322 | *E. coli* plasmid cloning vector |
| pBR322/*Hae III* | pBR322 vector DNA digestion with restriction endonuclease *Hae III* |
| $pCO_2$ | carbon dioxide partial pressure |
| PCR | polymerase chain reaction |
| $P_{ET}CO_2$ | partial pressure of end-tidal $CO_2$ |
| pH | indicates acidity: numerically equal to the negative logarithm of $H^+$ concentration expressed in molarity |
| Phe | phenylalanine |
| $P_i$ | inorganic phosphate |
| PIC | polymorphism information content |
| $PLA_2$ | phospholipase $A_2$ |
| pmol | pico mole |
| $pmol.\mu l^{-1}$ | pico mole per microlitre |
| PMRS | PCR-modified restriction site |
| Pro | proline |
| q | long arm of chromosome |
| Q or Q | glutamine |
| R or R | arginine |
| RFLP | restriction fragment length polymorphism |

**List of abbreviations and symbols (continued ...)**

| | |
|---|---|
| RNA | ribonucleic acid |
| rpm | revolutions per minute |
| RT | room temperature |
| *Rsa 1* | restriction endonuclease isolated from *Rhodopseudomonas sphaeroides,* with recognition site 5'-...GT↓ AC...-3' |
| RYR | ryanodine receptor |
| RYR1 | RYR gene expressed in skeletal muscle |
| RYR2 | RYR gene expressed in cardiac muscle and brain |
| RYR3 | RYR gene expressed by the β4 gene |
| RYRs | ryanodine receptors |
| S or S | serine |
| $^{35}S$ | sulphur isotope: maximum β emission energy 0.167 MeV: half-life 87.4 days |
| S1 - S6 | transmembrane segments numbers 1 to 6 |
| SA | South African |
| SAP | shrimp alkaline phosphatase |
| *Sau* 3AI | restriction endonuclease isolated from *Staphylococcus aureus* 3A, with recognition site 5'-...↓GATC...-3' |
| SCN4A | α sub-unit of the skeletal muscle sodium channel |
| SDS | sodium dodecyl sulphate: $C_{12}H_{25}NaSO_4$ |
| SDS-EDTA | 0.5% SDS; 50 mM EDTA |
| sec | seconds |
| Sequenase | Sequenase® [1] Version 2.0 T7 DNA Polymerase |
| Sequenase buffer | 200 mM Tris-HCl [pH 7.5]; 100 mM $MgCl_2$; 250 mM NaCl |
| Ser | serine |
| SLS | single lane sequencing |
| Sp1 | GC box sequences usually clustered near the initiation site of a gene |
| SR | sarcoplasmic reticulum |
| SSCP | single stranded conformational polymorphism |
| ssDNA | single stranded DNA |
| stop buffer | 95% formamide; 0.05% xylene cyanol FF; 0.05% bromophenol blue; 20 mM EDTA |
| STRP | short tandem repeat polymorphism |
| suspension buffer | 50 mM Tris-HCl [pH 8.0]; 100 mM EDTA; 150 mM NaCl |
| t | thymine |
| T or T | thymine (in DNA sequence, indicating exon sequence) |
| T or T | threonine (in amino acid sequence) |
| $T_a$ | annealing temperature |
| $T_c$ | calculated annealing temperature |
| $T_m$ | melting temperature |
| TAG | termination codon |
| Taq DNA Polymerase | deoxynucleosidetriphosphate: DNA deoxynucleotidyltransferase from *Thermus aquaticus* |
| TATA | conserved non-coding DNA sequence in the 5' region of most eukaryotic genes |
| TBE | 89.15 mM Tris® [2] [pH 8.0], 88.95 mM boric acid, 2.498 mM $Na_2EDTA$ |
| TE | 10 mM Tris-HCl [pH 7.5]; 1 mM EDTA |
| TEMED | N,N,N',N'-tetramethylethylenediamine: $C_6H_{16}N_2$ |
| temp | temperature |
| Thr | threonine |
| Tris | Tris®: tris(hydroxymethyl)aminomethan: 2-Amino-2-(hydroxymethyl)-1,3-propanediol: $C_4H_{11}NO_3$ |

[1] Sequenase ® is a registered trademark of United States Biochemical Corporation, Cleveland, Ohio, U.S.A.

[2] Tris® is a registered trademark of Rohm & Haas Company, Philadelphia, PA, U.S.A.

**List of abbreviations and symbols (continued ...)**

| | |
|---|---|
| Tris-HCl | 2-amino-2-(hydroxymethyl)-1,3-propanediol hydrochloride: $C_4H_{11}NO_3.H_2O$ |
| Triton X-100 | Triton X-100®: octylphenolpoly(ethylene-glycolether)$_n$ : $C_{34}H_{62}O_{11}$, for n = 10 |
| Trp | tryptophan |
| T-tubule | transverse tubule |
| Tyr | tyrosine |
| U | uracil |
| U | units |
| U.ml$^{-1}$ | units per millilitre |
| UK | United Kingdom |
| Urea | $H_2NCONH_2$ |
| US | United States |
| USA | United States of America |
| USB | United States Biochemical |
| UV | ultraviolet |
| V | volts |
| V or v | valine |
| Val | valine |
| W | watts |
| W or w | tryptophan |
| XC | xylene cyanole FF: $C_{25}H_{27}N_2O_6S_2Na$ |
| xg | gravitational acceleration |
| Y or y | tyrosine |
| □ / ○ | male/female: tested normal for malignant hyperthermia susceptibility with the IVCT |
| ■ / ● | male/female: tested susceptible to malignant hyperthermia with the IVCT |
| ▩ / ◍ | male/female: never tested, malignant hyperthermia status unknown |
| ▩ / ◍ | male/female: obligate malignant hyperthermia susceptible carrier |
| ▨ / ⊘ | male/female: malignant hyperthermia equivocal, positive for halothane |
| ☒ / ∅ | male/female: deceased |
| ◊ | sex unknown |
| ——// | divorced |
| ↓ | spontaneous abortion |
| ↖ | proband |
| ↓ | indicates restriction enzyme cutting site |

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