APPENDIX A

Reported functional characterisation studies for RYR1 gene alterations

A summary of previously reported functional characterisation studies that have been conducted for certain RYR1 alterations observed in individuals diagnosed with either MH or CCD is provided in Table A1. The effect of each alteration on RYR1 function was determined in an experimental system in order to demonstrate if a difference occurs in the function of the mutated protein.

Table A.1: Summary of previously reported studies that have functionally characterised RYR1 alterations observed in individuals diagnosed with MH or CCD

Alteration	Test system used	Functional characterisation result/s	Reference
Hotspot one			
Cys35Arg	HEK293 cells[1]	• Displays hypersensitivity[2]	Tong *et al.*, 1997
Arg163Cys	1B5 dyspedic myotubes	• Displays hypersensitivity • Decreased sensitivity to Ca^{2+} and Mg^{2+} inhibition • Enhanced sensitivity to depolarisation	Yang *et al.*, 2003
Arg163Leu	HEK293 cells	• Displays hypersensitivity • No effect on resting Ca^{2+} concentration	Monnier *et al.*, 2005
Gly248Arg	HEK293 cells	• Displays hypersensitivity	Tong *et al.*, 1997
Arg328Trp	HEK293 cells	• Displays hypersensitivity	Loke *et al.*, 2003
Gly341Arg	1B5 dyspedic myotubes	• Displays hypersensitivity • Decreased sensitivity to Ca^{2+} and Mg^{2+} inhibition • Enhanced sensitivity to depolarisation	Yang *et al.*, 2003
Ile403Met	HEK293 cells	• Displays hypersensitivity	Tong *et al.*, 1997
Tyr522Ser	HEK293 cells	• Displays hypersensitivity	Tong *et al.*, 1997
Arg552Trp	HEK293 cells	• Displays hypersensitivity	Tong *et al.*, 1997
Arg614Leu	HEK293 cells	• Displays hypersensitivity	Tong *et al.*, 1997
Arg614Cys	1B5 dyspedic myotubes	• Displays hypersensitivity • Decreased sensitivity to Ca^{2+} and Mg^{2+} inhibition • Enhanced sensitivity to depolarisation	Yang *et al.*, 2003

Table A.1: Continued...

Alteration	Test system used	Functional characterisation results	Reference
Hotspot two			
Arg2163Cys	1B5 dyspedic myotubes	• Displays hypersensitivity[1] • Decreased sensitivity to Ca^{2+} and Mg^{2+} inhibition • Enhanced sensitivity to depolarisation	Yang *et al.*, 2003
Arg2163His	HEK293 cells	• Displays hypersensitivity	Tong *et al.*, 1997
Val2168Met	1B5 dyspedic myotubes	• Displays hypersensitivity[1] • Decreased sensitivity to Ca^{2+} and Mg^{2+} inhibition • Enhanced sensitivity to depolarisation	Yang *et al.*, 2003
Ile2182Phe[3]	1B5 dyspedic myotubes	• Affects resting Ca^{2+} concentration	Wehner *et al.*, 2003
Thr2206Met	1B5 dyspedic myotubes	• Affects resting Ca^{2+} concentration • Displays hypersensitivity	Wehner *et al.*, 2002
Thr2206Arg	HEK293 cells	• Displays hypersensitivity • No effect on resting Ca^{2+} concentration	Monnier *et al.*, 2005
Ala2350Thr	1B5 dyspedic myotubes	• Affects resting Ca^{2+} concentration • Displays hypersensitivity	Wehner *et al.*, 2004
Arg2355Trp	1B5 dyspedic myotubes	• Does not affect resting Ca^{2+} concentration • Displays hypersensitivity	Wehner *et al.*, 2004
Gly2375Ala	1B5 dyspedic myotubes	• Affects resting Ca^{2+} concentration • Displays hypersensitivity	Wehner *et al.*, 2004
Ala2428Thr	HEK293 cells	• Displays hypersensitivity • No effect on resting Ca^{2+} concentration	Monnier *et al.*, 2005
Gly2434Arg	HEK293 cells	• Displays hypersensitivity • Decreased sensitivity to inhibition	Richter *et al.*, 1997
Arg2435His	HEK293 cells	• Displays hypersensitivity	Tong *et al.*, 1997
Ile2453Thr	1B5 dyspedic myotubes	• Affects resting Ca^{2+} concentration	Wehner *et al.*, 2003
Arg2454Cys	HEK293 cells	• Displays hypersensitivity • No effect on resting Ca^{2+} concentration	Monnier *et al.*, 2005
Arg2454His	HEK293 cells	• Displays hypersensitivity • No effect on resting Ca^{2+} concentration	Monnier *et al.*, 2005
Arg2458His	1B5 dyspedic myotubes	• Displays hypersensitivity • Decreased sensitivity to inhibition • Enhanced sensitivity to depolarisation • Resistant to complete activation under physiological conditions	Yang *et al.*, 2003
Arg2458Cys	HEK293 cells	• Displays hypersensitivity	Tong *et al.*, 1997

Table A.1: Continued...

Alteration	Test system used	Functional characterisation results	Reference
Hotspot three			
Pro4668Ser	Chinese hamster ovary cells	• Exhibited no significant hypersensitivity	Oyamada *et al.*, 2002
Tyr4796Cys	HEK293 Cells	• Displays hypersensitivity • Causes the RyR1 channel to become leaky	Monnier *et al.*, 2000
Thr4826Ile	1B5 dyspedic myotubes	• Displays hypersensitivity • Decreased sensitivity to inhibition • Enhanced sensitivity to depolarisation • Resistant to complete activation under physiological conditions	Yang *et al.*, 2003
Leu4838Val	Chinese hamster ovary cells	• Displays hypersensitivity	Oyamada *et al.*, 2002
Arg4861His	Immortalised human B-lymphocytes	• Causes the RyR1 channel to become leaky • Sensitivity to dantrolene not impaired	Tilgen *et al.*, 2001
Gly4891Arg	Immortalised human B-lymphocytes	• Causes the RyR1 channel to become leaky • Sensitivity to dantrolene not impaired	Tilgen *et al.*, 2001
Ile4898Thr	Immortalised human B-lymphocytes	• Causes the RyR1 channel to become leaky • Sensitivity to dantrolene not impaired	Tilgen *et al.*, 2001
Gly4899Arg	Immortalised human B-lymphocytes	• Causes the RyR1 channel to become leaky • Sensitivity to dantrolene not impaired	Tilgen *et al.*, 2001
Ala4906Val	Immortalised human B-lymphocytes	• Causes the RyR1 channel to become leaky • Sensitivity to dantrolene not impaired	Tilgen *et al.*, 2001
Alterations outside of the hotspots			
Gly2060Cys	HEK293 cells	• Exhibited no significant hypersensitivity • Exhibited low levels of RyR1	Zhou *et al.*, 2006a
Pro3527Ser[3]	Immortalised human B-lymphocytes	• Affects resting Ca^{2+} concentration • Hypersensitive • Does not rest in leaky channel	Ducreux *et al.*, 2006

[1] HEK 293 refers to human embryonic kidney line 293 cells; [2] Hypersensitivity indicates that Ca^{2+} release is affected following *pharmacological action*; [3] *The Pro3527Ser alteration only affected intracellular Ca^{2+} homeostasis in the homozygous state*; Ca^{2+} = calcium ion; RyR1 = skeletal muscle ryanodine channel protein; Ala = alanine; Arg = arginine; Cys = cysteine; Gly = glycine; His = histidine; Ile = isoleucine; Leu = leucine; Met = methionine; Phe = phenylalanine; Pro = proline; Ser = serine; Thr = threonine; Trp = tryptophan; Tyr = tyrosine and Val = valine.

APPENDIX B

SNPs identified in the coding region of the RYR1 gene

All the synonymous SNPs that were observed in the coding region of the RYR1 gene in the fifteen South African MH probands investigated are listed in Table B1. The results listed below are a summary of the synonymous SNPs observed in the coding region in the Phase 3 study. A summary of SNPs observed in the intron sequence of the RYR1 gene is provided in Appendix C.

Table B.1: Summary of synonymous SNPs detected in the coding region of the RYR1 gene in the South African MH population

Proband[a]	Identification number[b]	Exon	Sequence variation	Reported /novel	Heterozygous/ homozygous	db SNP ID[c]
MH101-6	MH00242	7	A11541G	reported	heterozygous	rs12985668
		11	T15669C	reported	homozygous	rs10406027
		15	G22443A	reported	heterozygous	rs2288888
		19	C26165T	reported	heterozygous	rs3745847
		24	G33064A	reported	heterozygous	rs2228069
		24	C33100T	reported	heterozygous	rs2228070
		45	C66854T	reported	heterozygous	rs12973632
		50	G71171A	reported	heterozygous	rs2915951
		51	T71699C	reported	heterozygous	rs2960340
		51	T71771C	reported	heterozygous	rs2915951
		53	G72236A	reported	heterozygous	rs2915952
		55	T73251C	reported	heterozygous	rs2229146
		62	A78986G	reported	heterozygous	rs2071089
MH102-125	MH00278	7	A11541G	reported	homozygous	rs12985668
		11	T15669C	reported	homozygous	rs10406027
		15	G22443A	reported	homozygous	rs2288888
		19	C26165T	reported	homozygous	rs3745847
		24	G33064A	reported	homozygous	rs2228069
		45	C66875T	novel	heterozygous	---
		47	C67861T	reported	heterozygous	rs1465698
		62	A78986G	reported	heterozygous	rs2071089

Table B.1: Continued...

Proband[a]	Identification number[b]	Exon	Sequence variation	Reported /novel	Heterozygous/ homozygous	db SNP ID
MH103-4	MH00286	7	A11541G	reported	heterozygous	rs12985668
		11	T15669C	reported	homozygous	rs10406027
		15	G22443A	reported	heterozygous	rs2288888
		19	C26165T	reported	heterozygous	rs3745847
		24	G33064A	reported	heterozygous	rs2228069
		24	C33100T	reported	heterozygous	rs2228070
		26	C35941T	reported	heterozygous	rs11083462
MH104-26	MH00294	7	A11541G	reported	heterozygous	rs12985668
		11	T15669C	reported	heterozygous	rs10406027
		15	G22443A	reported	heterozygous	rs2288888
		19	C26165T	reported	heterozygous	rs3745847
		24	G33064A	reported	heterozygous	rs2228069
		26	C35941T	reported	heterozygous	rs11083462
		37	A57545G	reported	heterozygous	rs2228068
MH105-38	MH00324	7	A11541G	reported	homozygous	rs12985668
		11	T15669C	reported	homozygous	rs10406027
		19	C26165T	reported	heterozygous	rs3745847
		24	G33064A	reported	heterozygous	rs2228069
		26	C35941T	reported	heterozygous	rs11083462
		47	G67777A	reported	heterozygous	rs2228072
		47	G67804A	reported	heterozygous	rs2071088
		51	T71699C	reported	heterozygous	rs2960340
		51	T71771C	reported	heterozygous	rs2915951
		53	G72236A	reported	heterozygous	rs2915952
		55	T73251C	reported	heterozygous	rs2229146
		62	A78986G	reported	homozygous	rs2071089
		66	G84264A	reported	heterozygous	rs2304151
		91	C13317T	reported	heterozygous	Galli *et al.*, 2006
		94	C13759G	reported	heterozygous	rs35959206
MH113-14	MH00381	7	A11541G	reported	homozygous	rs12985668
		11	T15669C	reported	homozygous	rs10406027
		15	G22443A	reported	homozygous	rs2288888
		19	C26165T	reported	homozygous	rs3745847
		24	G33064A	reported	homozygous	rs2228069
–	MH00630	11	T15669C	reported	heterozygous	rs10406027
		24	G33064A	reported	heterozygous	rs2228069
		24	C33100T	reported	heterozygous	rs2228070
		26	C35941T	reported	heterozygous	rs11083462

Table B.1: Continued...

Proband[a]	Identification number[b]	Exon	Sequence variation	Reported /novel	Heterozygous/ homozygous	db SNP ID
MH115-1	MH01394	24	G33064A	reported	homozygous	rs2228069
		47	G67777A	reported	heterozygous	rs2228072
MH123-1	MH00484	11	T15669C	reported	heterozygous	rs10406027
		24	C33100T	reported	heterozygous	rs2228070
		26	C35941T	reported	heterozygous	rs11083462
		47	G67777A	reported	heterozygous	rs2228072
		47	G67804A	reported	heterozygous	rs2071088
		50	G71171A	reported	heterozygous	rs2915951
		51	T71699C	reported	heterozygous	rs2960340
		51	T71771C	reported	heterozygous	rs2915951
		53	G72236A	reported	heterozygous	rs2915952
		62	A78986G	reported	heterozygous	rs2071089
		67	C10218T	reported	heterozygous	Galli *et al.*, 2006
MH125-1	MH01626	11	T15669C	reported	heterozygous	rs10406027
		24	G33064A	reported	heterozygous	rs2228069
		26	C35941T	reported	heterozygous	rs11083462
—	MH00654	7	A11541G	reported	homozygous	rs12985668
		11	T15669C	reported	homozygous	rs10406027
		15	G22443A	reported	homozygous	rs2288888
		19	C26165T	reported	homozygous	rs3745847
		24	G33064A	reported	homozygous	rs2228069
		47	C67861T	reported	heterozygous	rs1465698

[a] = indicates family number of MH proband used in this study; [b] = indicates individual number of MH proband used in this study; [c] = indicates SNP identification (ID), which refers to the reference sequence identifier in the SNP database (dbSNP). (—) indicates only individual number given to individual; SNP = single nucleotide polymorphism.

Table B.1: Continued...

Proband[a]	Identification number[b]	Exon	Sequence variation	Reported /novel	Heterozygous/ homozygous	db SNP ID
MH111-1	MH00361	11	T15669C	reported	homozygous	rs10406027
		15	G22443A	reported	heterozygous	rs2288888
		19	C26165T	reported	heterozygous	rs3745847
		24	G33064A	reported	heterozygous	rs2228069
		24	C33100T	reported	heterozygous	rs2228070
		26	C35941T	reported	heterozygous	rs11083462
		45	C66854T	reported	heterozygous	rs12973632
		51	T71771C	reported	heterozygous	rs2915951
		55	T73251C	reported	heterozygous	rs2229146
MH108-1	MH00470	11	T15669C	reported	heterozygous	rs10406027
		24	C33100T	reported	heterozygous	rs2228070
		26	C35941T	reported	homozygous	rs11083462
		37	A57545G	reported	heterozygous	rs2228068
MH122-1	MH00478	7	A11541G	reported	heterozygous	rs12985668
		11	T15669C	reported	heterozygous	rs10406027
		15	G22443A	reported	heterozygous	rs2288888
		19	C26165T	reported	heterozygous	rs3745847
		24	G33064A	reported	heterozygous	rs2228069
		26	C35941T	reported	heterozygous	rs11083462
		37	A57545G	reported	heterozygous	rs2228068
		50	G71171A	reported	heterozygous	rs2915951
		51	T71699C	reported	heterozygous	rs2960340
		51	T71771C	reported	heterozygous	rs2915951
		53	G72236A	reported	heterozygous	rs2915952
		55	T73251C	reported	heterozygous	rs2229146
		62	A78986G	reported	heterozygous	rs2071089
		66	G84264A	reported	heterozygous	rs2304151
		82	A11547G	reported	heterozygous	Galli *et al.*, 2006
MH114-1	MH00695	7	A11541G	reported	homozygous	rs12985668
		11	T15669C	reported	homozygous	rs10406027
		15	G22443A	reported	heterozygous	rs2288888
		19	C26165T	reported	heterozygous	rs3745847
		24	G33064A	reported	homozygous	rs2228069
		37	A57545G	reported	heterozygous	rs2228068
MH115-1	MH01394	7	A11541G	reported	homozygous	rs12985668
		11	T15669C	reported	homozygous	rs10406027
		15	G22443A	reported	homozygous	rs2288888
		19	C26165T	reported	homozygous	rs3745847

APPENDIX C

SNPs identified in the intron sequence of the RYR1 gene

All the synonymous SNPs that were observed in the intron sequence of the RYR1 gene in fifteen South African MH probands are listed in Table C1. A summary of SNPs observed in the coding region of the RYR1 gene is provided in Appendix B.

Table C.1: Summary of synonymous SNPs detected in the intron sequence of the RYR1 in the South African MH population

Proband[a]	Identification number[b]	Region	Sequence variation	Reported /novel	Heterozygous /homozygous	db SNP ID[c]
MH101-6	MH00242	12-13	C19691T	reported	heterozygous	rs10406027
		13-14	G19989A	reported	heterozygous	rs12460768
		15-16	G22476C	reported	heterozygous	rs2288889
		17-18	T24617C	reported	heterozygous	rs2071085
		18-19	T25990G	reported	heterozygous	rs4802474
		19-20	C27208T	reported	heterozygous	rs2304147
		47-48	C67901G	reported	heterozygous	rs2960323
		50-51	G71413A	reported	heterozygous	rs2915949
		50-51	A71494G	reported	heterozygous	rs2915950
		55-56	C73337T	reported	heterozygous	rs2960344
		56-57	T73475G	reported	heterozygous	rs2960345
		56-57	T73584C	reported	heterozygous	rs2915957
		56-57	G73720C	reported	heterozygous	rs2915958
MH102-125	MH00278	13-14	G19989A	reported	homozygous	rs12460768
		15-16	G22476C	reported	homozygous	rs2288889
		17-18	T24617C	reported	heterozygous	rs2071085
		18-19	T25990G	reported	homozygous	rs4802474
		19-20	C27208T	reported	homozygous	rs2304147
MH103-4	MH00286	13-14	G19989A	reported	heterozygous	rs12460768
		15-16	G22476C	reported	heterozygous	rs2288889
		17-18	T24617C	reported	heterozygous	rs2071085
		17-18	C24681G	**novel**	heterozygous	---
		18-19	T25990G	reported	heterozygous	rs4802474
		19-20	C27208T	reported	heterozygous	rs2304147

Table C.1: Continued...

Proband[a]	Identification number[b]	Region	Sequence variation	Reported /novel	Heterozygous /homozygous	db SNP ID
MH104-26	MH00294	13-14	G19989A	reported	heterozygous	rs12460768
		15-16	G22476C	reported	heterozygous	rs2288889
		17-18	T24617C	reported	heterozygous	rs2071085
		18-19	T25990G	reported	heterozygous	rs4802474
		19-20	C27208T	reported	heterozygous	rs2304147
		36-37	C57264T	reported	heterozygous	rs17708009
MH105-38	MH00324	13-14	G19989A	reported	homozygous	rs12460768
		17-18	T24617C	reported	heterozygous	rs2071085
		17-18	C24681G	**novel**	heterozygous	---
		18-19	T25990G	reported	heterozygous	rs4802474
		19-20	C27208T	reported	heterozygous	rs2304147
		47-48	C67901G	reported	heterozygous	rs2960323
		55-56	C73337T	reported	heterozygous	rs2960344
		56-57	T73475G	reported	heterozygous	rs2960345
		56-57	T73584C	reported	heterozygous	rs2915957
		56-57	G73720C	reported	heterozygous	rs2915958
		57-58	T73870A	reported	heterozygous	rs2915959
MH113-14	MH00381	13-14	G19989A	reported	homozygous	rs12460768
		15-16	G22476C	reported	homozygous	rs2288889
		17-18	T24617C	reported	homozygous	rs2071085
		17-18	C24681G	**novel**	heterozygous	---
		18-19	T25990G	reported	homozygous	rs4802474
		19-20	C27208T	reported	homozygous	rs2304147
—	MH00630	---	---	---	---	---
MH111-1	MH00361	13-14	G19989A	reported	heterozygous	rs12460768
		15-16	G22476C	reported	heterozygous	rs2288889
		17-18	T24617C	reported	heterozygous	rs2071085
		18-19	T25990G	reported	heterozygous	rs4802474
		19-20	C27208T	reported	heterozygous	rs2304147
		47-48	C67901G	reported	heterozygous	rs2960323
		50-51	A71494G	reported	heterozygous	rs2915950
MH108-1	MH00470	36-37	C57264T	reported	heterozygous	rs17708009
MH122-1	MH00478	13-14	G19989A	reported	heterozygous	rs12460768
		15-16	G22476C	reported	heterozygous	rs2288889
		17-18	T24617C	reported	heterozygous	rs2071085
		18-19	T25990G	reported	heterozygous	rs4802474
		19-20	C27208T	reported	heterozygous	rs2304147
		36-37	C57264T	reported	heterozygous	rs17708009

Table C.1: Continued...

Proband[a]	Identification number[b]	Region	Sequence variation	Reported /novel	Heterozygous /homozygous	db SNP ID
MH122-1	MH00478	47-48	C67901G	reported	heterozygous	rs2960323
		50-51	G71413A	reported	heterozygous	rs2915949
		50-51	A71494G	reported	heterozygous	rs2915950
		55-56	C73337T	reported	heterozygous	rs2960344
		56-57	T73475G	reported	heterozygous	rs2960345
		56-57	T73584C	reported	heterozygous	rs2915957
		56-57	G73720C	reported	heterozygous	rs2915958
		57-58	T73870A	reported	heterozygous	rs2915959
MH114-1	MH00695	13-14	G19989A	reported	homozygous	rs12460768
		15-16	G22476C	reported	heterozygous	rs2288889
		17-18	T24617C	reported	heterozygous	rs2071085
		17-18	C24681G	**novel**	heterozygous	---
		18-19	T25990G	reported	heterozygous	rs4802474
		19-20	C27208T	reported	heterozygous	rs2304147
		36-37	C57264T	reported	heterozygous	rs17708009
MH115-1	MH01394	13-14	G19989A	reported	homozygous	rs12460768
		15-16	G22476C	reported	homozygous	rs2288889
		17-18	T24617C	reported	homozygous	rs2071085
		17-18	C24681G	**novel**	heterozygous	---
		18-19	T25990G	reported	homozygous	rs4802474
		19-20	C27208T	reported	homozygous	rs2304147
MH123-1	MH00484	50-51	G71413A	reported	heterozygous	rs2915949
		56-57	G73720C	reported	heterozygous	rs2915958
		57-58	T73870A	reported	heterozygous	rs2915959
MH125-1	MH01626	50-51	A71494G	reported	heterozygous	rs2915950
—	MH00654	13-14	G19989A	reported	homozygous	rs12460768
		15-16	G22476C	reported	homozygous	rs2288889
		17-18	T24617C	reported	homozygous	rs2071085
		18-19	T25990G	reported	homozygous	rs4802474
		19-20	C27208T	reported	homozygous	rs2304147
		50-51	A71494G	reported	heterozygous	rs2915950
		63-64	C81823T	reported	heterozygous	rs12461853

[a] = indicates family number of MH proband used in this study; [b] = indicates individual number of MH proband used in this study; [c] = indicates SNP identification (ID), which refers to the reference sequence identifier in the SNP database (dbSNP); (—) indicates only individual number given to individual. (---) indicates information not available; SNP = single nucleotide polymorphism.