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Screening of the Entire Ryanodine Receptor Type 1 Coding Region for Sequence Variants Associated with Malignant Hyperthermia Susceptibility in the North American Population

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Background: **Malignant hyperthermia (MH) is a life-threatening and frequently fatal disorder triggered by commonly used anesthetics. MH susceptibility is a genetically determined predisposition to the development of MH. Mutations in the ryanodine receptor type 1 (*RYR1*) gene are the major cause of MH susceptibility. The authors sought to develop a reliable genetic screening strategy based on efficient and relatively inexpensive mutation-detection procedures.**

Methods: **A cohort (n = 30) of North American MH patients and MH-susceptible individuals was studied. RNA and DNA extracted from muscle tissue or blood lymphocytes were used for analysis. The entire *RYR1* coding region was amplified in 57 overlapping fragments and subjected to denaturing high-performance liquid chromatography analysis followed by direct nucleotide sequencing to characterize *RYR1* alterations.**

Results: **Nine previously reported and nine unknown *RYR1* mutations were identified in 21 of 30 studied patients (70%). Some of the new mutations were located outside of known mutational "hot spots," suggesting that *RYR1* contains previously unknown mutation-prone areas requiring analysis. The North American MH/MH-susceptible population is characterized by a high *RYR1* allelic heterogeneity.**

Conclusions: **Denaturing high-performance liquid chromatography analysis of RNA samples extracted from the biopsied skeletal muscle followed by DNA sequencing is a highly efficient methodology for *RYR1* mutation detection. This approach allows increasing the rate of mutation detection to 70% and identifying mutations in the entire *RYR1* coding region.**

MALIGNANT hyperthermia (MH) manifests as a hypermetabolic crisis in susceptible individuals after exposure to inhalational anesthetics or succinylcholine, a depolarizing muscle relaxant. This life-threatening reaction is caused by abnormally high release of myoplasmic Ca^{2+} from the sarcoplasmic reticulum. The prevalence of MH is estimated at 1 in 8,500.[1] Susceptibility to MH (MHS) is viewed as a genetic predisposition to MH and most commonly is inherited as an autosomal dominant trait. In the absence of the triggering agents, MH-susceptible individuals are usually asymptomatic. The diagnosis of MHS can be made by caffeine–halothane contracture testing (CHCT),[2] which is based on measurement of isometric tension changes of freshly biopsied skeletal muscle in response to the ryanodine receptor agonists, caffeine and halothane. CHCT has been successfully applied for establishing the diagnosis of MHS, but it is not suitable for massive testing of the population at large or members of the MH-affected families who may be under increased risk of the development of MH if subjected to triggering anesthetics.

Molecular genetic investigations have shown considerable locus and allelic heterogeneity with six genetic loci implicated in MH.[3,4] However, accumulated data have clearly established that mutations in the *RYR1* gene on chromosome 19 are the major cause of MHS.[3,5] The *RYR1* is one of the largest genes described in humans, spanning more than 159,000-nucleotides of genomic DNA; the coding sequence contains 106 exons (of which 2 are alternatively spliced) and transcribes a 15,117-nucleotide-long RNA molecule.[6] The encoded 563-kD RYR1 protein forms a homotetrameric structure and functions as a calcium-release channel regulating Ca^{2+} content in skeletal muscle during excitation–contraction coupling.[7] Multiple *RYR1* mutations known to cause MH are dispersed throughout the gene, and to complicate the situation further, at least 16 silent DNA polymorphisms are present in the coding region.[5,8] Because of the length and complexity of the *RYR1* gene, efficient routine screening for mutations has been difficult. In addition, screening of the genetically heterogeneous North American population requires extensive knowledge of the expected spectrum of MHS-associated mutations and data on allele frequency in the background population.

According to genetic linkage studies, MHS is linked to the *RYR1* locus in more than 50% of studied affected families,[9] whereas *RYR1* mutations are found in only approximately 22–25% of individuals with positive CHCT results.[10,11] Improvements of the screening techniques increase the rate of mutation detection,[12] suggesting that the discrepancy reflects methodologic problems of finding mutations in this large gene. Thus far,

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RYR1 screening studies targeted only three mutation-clustered regions: the N-terminal region between codons 34 and 614, the central region between codons 2163 and 2458, and the C-terminal region between codons 4136 and 4973.[3,5,13–17] The combined length of all these mutational "hot spot" areas accounts for only approximately a third of the entire coding region of the *RYR1* gene. The presence of MHS-causing mutations in other parts of the gene has not been systematically investigated.

The aim of this study was to develop an efficient methodology for identification of most, if not all, mutations in the *RYR1* gene and to devise a strategy for molecular diagnosis of MHS in the North American population.

Materials and Methods

MH Patients, MH-susceptible Individuals, and Population Controls

We performed genetic analysis of 30 MH/MH-susceptible individuals. The study was approved by the institutional review boards of all collaborating institutions. Patients had been referred for CHCT because of development of signs of MH during anesthesia or a positive family history of MH. In 28 at-risk family members, the diagnosis of MHS was established according to the North American CHCT protocol, which requires exposure of muscle strips to caffeine and halothane.[2] Testing with each drug was performed on at least 3 muscle fascicles, and individuals were considered MH positive if any one of the three exposures exceeded the diagnostic threshold. The threshold values for a positive test were greater than 0.7 g contracture response to 3% halothane or greater than 0.2 g in response to 2 mM caffeine. CHCT testing was performed at the Department of Anesthesiology, Uniformed Services University of the Health Sciences, Bethesda, Maryland, and the Wake Forest University, Winston-Salem, North Carolina. A single individual was diagnosed with MHS on the basis of abnormal muscle contracture results before standardization of the CHCT in North America. Two patients who did not undergo CHCT had clinical episodes of MH, one of them fatal.

The frequency of each *RYR1* mutation identified in the North American MH/MH-susceptible cohort was estimated in 100 healthy unrelated American population controls. All of these 100 samples were previously collected for other studies and were made available for this study without personal information.

RNA and DNA Samples

To facilitate the screening and to capture the effect of splicing mutations, we used muscle tissue because *RYR1* is most abundantly expressed in skeletal muscle. Total RNA was extracted from skeletal muscle biopsy tissue using the RNA-wiz reagent (Ambion, Austin, TX). Three micrograms of total RNA was used for reverse transcription reaction to synthesize complementary DNA. Avian Myeloblastosis Virus Reverse Transcriptase (Promega, Madison, WI) was used in the reaction as specified in the manufacturer's protocol. Complementary DNA was subsequently used for amplification reactions. Genomic DNA was extracted from muscle tissue and anticoagulated blood using the Wizard Genomic DNA Purification Kit (Promega).

Polymerase Chain Reaction

Complementary DNA (cDNA) and genomic DNA served as templates for PCR. The entire 15,117-nucleotide-long coding region of the *RYR1* gene was amplified as 57 overlapping fragments, each approximately 350 nucleotides long. Primers used for this purpose were designed using OLIGO software (Molecular Biology Insights, Cascade, CO). Intronic primers for amplification from genomic DNA were designed for each exon within the three mutational hot spots (exons 2–17, exons 39–46, and exons 90–104), and subsequently for exons 34, 51, and 67 in which novel mutations were detected during screening. All primer sequences are available on request. Amplification from genomic DNA was performed using an optimal procedure designed for each exon. Amplification was performed in a total volume of 20–25 μl with 1 μl single-stranded cDNA or 50–100 ng genomic DNA, 0.5 μM of each primer, 125 μM of each dNTP, 1.5 mM of $MgCl_2$, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, and 0.6 U *Taq*DNA polymerase (Applied Biosystems, Foster City, CA).

Mutation Screening with Denaturing High-performance Liquid Chromatography

Denaturing high-performance liquid chromatography (DHPLC) detects sequence mismatches by separation of heteroduplexes and homoduplexes of double-stranded DNA molecules, based on a difference in denaturing characteristics.[18,19] DHPLC analysis was performed on an automated WAVE Nucleic Acid Fragment Analysis System (Transgenomic, Omaha, NE) according to the manufacturer's protocol. Briefly, 5–7 μl of each PCR product was denatured at 95°C for 5 min and then gradually reannealed by decreasing the sample temperature from 95° to 25°C over a period of 45 min to promote the formation of heteroduplexes. To identify homozygous mutation, the patient's sample was mixed with a control sample. The samples were subsequently eluted by acetonitrile gradient consisting of a mixture of buffer B (0.1 M triethylamine acetate) and buffer A (25% acetonitrile). Temperature for heteroduplex molecules was determined by using the DHPLC melting algorithm (Wavemaker Software; Transgenomic). The 57 overlapping PCR fragments of each MH/MH-susceptible individual covering the complete *RYR1* coding sequence were

Table 1. Phenotype and Genotype Data of North American MH/MH-susceptible Individuals

Sample*	CHCT Results†		Assigned Phenotype (Diagnosis)	*RYR1* Mutations‡		
	Caffeine (2 mM)	Halothane (3%)		Exon Number	Nucleotide Change	Amino Acid Substitution
US502	2.20	5.78	MHS	6	487C>T	Arg163Cys
US848	ND	ND	MH	6	487C>T	Arg163Cys
BG101	0.53	2.93	MHS	11	1021G>A	Gly341Arg
BG74	0.49	2.67	MHS	34	**5182T>C**	**Ser1728Phe**
BG100	0.78	7.04	MHS	39	6487C>T	Arg2163His
BG113	0.66	3.0	MHS	39	**6599C>T**	**Ala2200Val**
US903	1.60	5.60	MHS	39	6617C>T	Thr2206Met
AC	ND	ND	MH	40	**2228G>T**	**Val2210Phe**
US602	2.20	7.50	MHS	44	2348G>A	Ala2350Thr
US202	3.40	5.60	MHS	45	7303G>A	Gly2434Arg
BG33	1.49	3.63	MHS	45	7303G>A	Gly2434Arg
BG106	0.21	3.44	MHS	45	7303G>A	Gly2434Arg
US80	1.30	6.50	MHS	46	7373C>A	Arg2458Cys
BG30	0.35	2.87	MHS	51	**8198G>A**	**Gly2733Asp**
BG96	1.06	4.83	MHS	67	**10043G>A**	**Arg3348His**
BG31	0.45	1.36	MHS	90	**12355A>T**	**Asn4119Tyr**
BG75	0.16	1.71	MHS	98	**14197T>G**	**Tyr4733Asp**
BG16	0.60	5.00	MHS	101	14545G>A	Val4849Ile
B2§	Positive	Positive	MHS	101	**14627A>G**	**Lys4876Arg**
BG73	0.39	1.57	MHS	101	**14639T>C**	**Met4880Thr**
BG93	1.10	4.80	MHS	103	14818G>A	Ala4940Thr
BG51	0.13	1.67	MHS	NM	NM	NM
BG81	1.48	1.08	MHS	NM	NM	NM
US43	0.00	0.78	MHS	NM	NM	NM
US46	1.20	7.97	MHS	NM	NM	NM
US56	0.30	0.87	MHS	NM	NM	NM
US79	0.23	0.87	MHS	NM	NM	NM
US97	0.00	0.80	MHS	NM	NM	NM
US101	0.50	1.50	MHS	NM	NM	NM
US203	0.00	0.80	MHS	NM	NM	NM

* Samples coded according to the biopsy centers: US and AC (Uniformed Services University of the Health Sciences) and BG (Wake Forest University of Medicine). † The values given for each subject are an average of the caffeine–halothane contracture test (CHCT) responses (see Materials and Methods). ‡ Newly identified mutations are shown in bold. § This patient was diagnosed in 1975 by CHCT and the results were positive: The concentration of caffeine required to produce 1 g contracture was 1.05 mM (normal, 9–17.5 mM) in this individual, and, in the presence of 4% halothane, 0.12 mM caffeine produced 1 g contracture (normal, 1.4–2.4 mM).

MH = malignant hyperthermia; MHS = malignant hyperthermia susceptible; ND = not determined; NM = no mutation is detected.

screened by DHPLC. Five of the 57 PCR fragments had abundant GC nucleotide content that prevented reliable use of DHPLC methodology; these fragments were analyzed by sequencing alone. Two researchers interpreted DHPLC results independently. Samples with abnormal peaks or peaks at different retention times were further analyzed by sequencing.

Mutation Identification by DNA Sequencing

Each sample showing an abnormal DHPLC peak pattern was sequenced in both directions using a fluorescent Big DyeTerminator Cycle Sequencing protocol on an automated ABI 310 Genetic Analyzer (Applied Biosystems, Foster City, CA). The samples were purified with QIAquick PCR Purification Kit (Qiagen, Valencia, CA) before sequencing. To further verify the detected *RYR1* sequence variants, appropriate exons were amplified from the same patient's genomic DNA, and nucleotide sequences were determined. All nucleotide and codon numbers used in this analysis refer to the wild-type cDNA sequence of the *RYR1* gene.[6] *RYR1* mutation numbering is based on the same reference sequence. To establish population frequency of *RYR1* mutations we encountered in the North American MH/MH-susceptible cohort, we studied the appropriate *RYR1* segments by DHPLC testing or restriction enzyme analysis or both in 100 unrelated controls.

Results

RYR1 Mutations Identified in the North American MH/MH-susceptible Cohort

Of 30 individuals enrolled in the study, two were diagnosed with MH after a clinical episode (US848 and AC), and in 28, the MHS status was confirmed by unequivocal CHCT results (table 1). Each MH/MH-susceptible individual was characterized by DHPLC screening of muscle biopsy tissue–derived RNA. Twenty-one of these individuals revealed unusual elution profiles in one of the PCR produced fragments that were distinct from those of the wild type samples: They showed double peaks or atypical configurations suggesting heterodu-

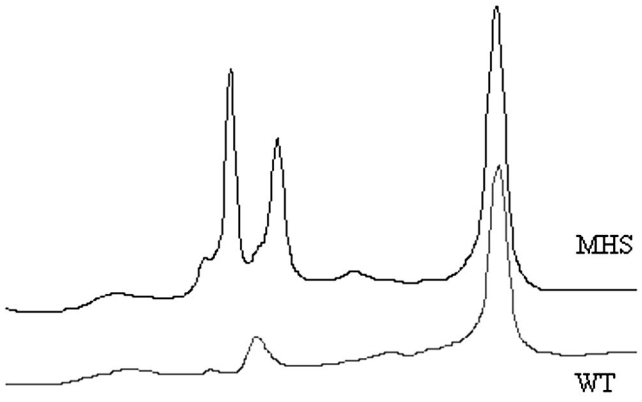

Fig. 1. Denaturing high-performance liquid chromatography results. Additional peaks indicate heteroduplex formation due to sequence variations in the DNA sample under examination as compared with the wild-type DNA profile of a control subject. MHS = malignant hyperthermia–susceptible individual; WT = control.

plex formation (fig. 1). Subsequent sequencing of these abnormal fragments determined the exact sequence alteration and led to the identification of the underlying *RYR1* mutation. We identified nine previously reported mutations in 12 individuals (table 1). The Arg163Cys substitution was detected in two individuals, and the Gly2434Arg substitution was detected in three individuals.

In addition, nine previously unknown single-nucleotide substitutions leading to a predicted amino acid change were identified in nine MH/MH-susceptible individuals. In each of the nine individuals with newly identified *RYR1* mutations, the MHS status was confirmed by CHCT (table 1). All newly identified amino acid substitutions were screened in 100 unrelated North Americans by DHPLC profiling or restriction analysis of the affected exons amplified from the genomic DNA. None of the control samples contained any of the nine newly identified mutations. The original residues were highly conserved through the *RYR1* evolution (fig. 2). Six of the nine affected residues were also conserved across the RYR species, RYR1, RYR2, and RYR3 (fig. 2).

While screening for mutations, numerous neutral sequence variants in the coding region of the *RYR1* gene were identified. Four of them, Pro1785Leu, Gly2059Cys, Gln3756Glu, and Val4849Ile, are alterations leading to a predicted amino acid substitution, but they were previ-

Amino acid position		1728	2200	2210	2733	3348	4119	4733	4876	4890
MHS mutations		F	V	F	D	H	Y	D	R	T
Human	RyR1	S	A	V	G	R	N	Y	K	M
Pig	RyR1	S	A	V	G	R	N	Y	K	M
Rabbit	RyR1	S	A	V	G	R	N	Y	K	M
Chicken	RyR1	S	A	V	G	R	N	Y	K	M
Human	RyR2	M	A	V	G	R	N	Y	K	M
Human	RyR3	M	V	V	G	K	N	Y	K	M

Fig. 2. Amino acid sequence alignment of ryanodine receptor type 1 (RYR1) in evolutionarily diverse species, and other human RYR proteins, type 2 (RYR2), and type 3 (RYR3). Amino acid numbering corresponds to the human RYR1 protein sequence. MHS = malignant hyperthermia susceptible individual.

ously detected and proven to be DNA polymorphisms because they were found in control subjects.[8,13,20] The Val4849Ile change in homozygous and heterozygous state was previously reported in association with central core disease in two families but was also found in 1 of 50 French controls.[13,21] Because the Val4849Ile substitution is located in the C-terminal mutational hot spot corresponding to a transmembrane region of RYR1, alters a highly conserved amino acid, and has been identified in an MH-susceptible individual (BG16) with strongly positive CHCT results (table 1), we have conducted an extensive search for the presence of this substitution among 50 North American white, 50 Asian, and 50 African-American individuals with negative results. Above data suggest that the Val4849Ile substitution may be pathogenic. On the contrary, the Pro1785Leu, Gly2059Cys, and Gln3756Glu substitutions seen in control individuals in this and other studies[8,20] are most likely nonpathogenic.

The nine remaining MH-susceptible individuals did not show any alterations of the DHPLC profiles throughout the entire *RYR1* coding sequence, although the majority had convincingly positive results in the CHCT studies (table 1). The rate of *RYR1* mutation detection in the studied MH/MH-susceptible cohort is therefore 70% (21 of 30).

Location of the Newly Identified Mutations within the RYR1 *Coding Region*

All previously identified *RYR1* mutations were located within one of three mutational hot spot areas of the gene (fig. 3). Of the newly identified mutations, two are located in the central transmembrane domain, and four are located in the C-terminal transmembrane domain (fig. 3). However, three novel mutations, Ser1728Phe, Gly2733Asp, and Arg3348His, were detected outside the mutational hot spots. The Ser1728Phe and Gly2733Asp mutations are in close proximity of the central mutational hot spot, whereas Arg3348His is at equal distances from the central and C-terminal regions (fig. 3). This suggests that RYR1 contains other critical domains that cause MHS if mutated and that the entire coding sequence must be analyzed to identify all MH-causing mutations.

A Study of a Large MH-affected Family

We analyzed a previously described[22] North American family with fatal MH episodes in three family members (fig. 4). A surviving family member (B2) underwent contracture testing and was diagnosed with MHS (table 1). Screening for *RYR1* mutations in this individual was performed with the use of genomic DNA extracted from blood lymphocytes. Sequencing of all exons within the mutational hot spot regions of the *RYR1* gene revealed the Lys4876Arg mutation. Ten additional members of this family were available for genetic evaluation. In these individuals, DNA was extracted from blood lympho-

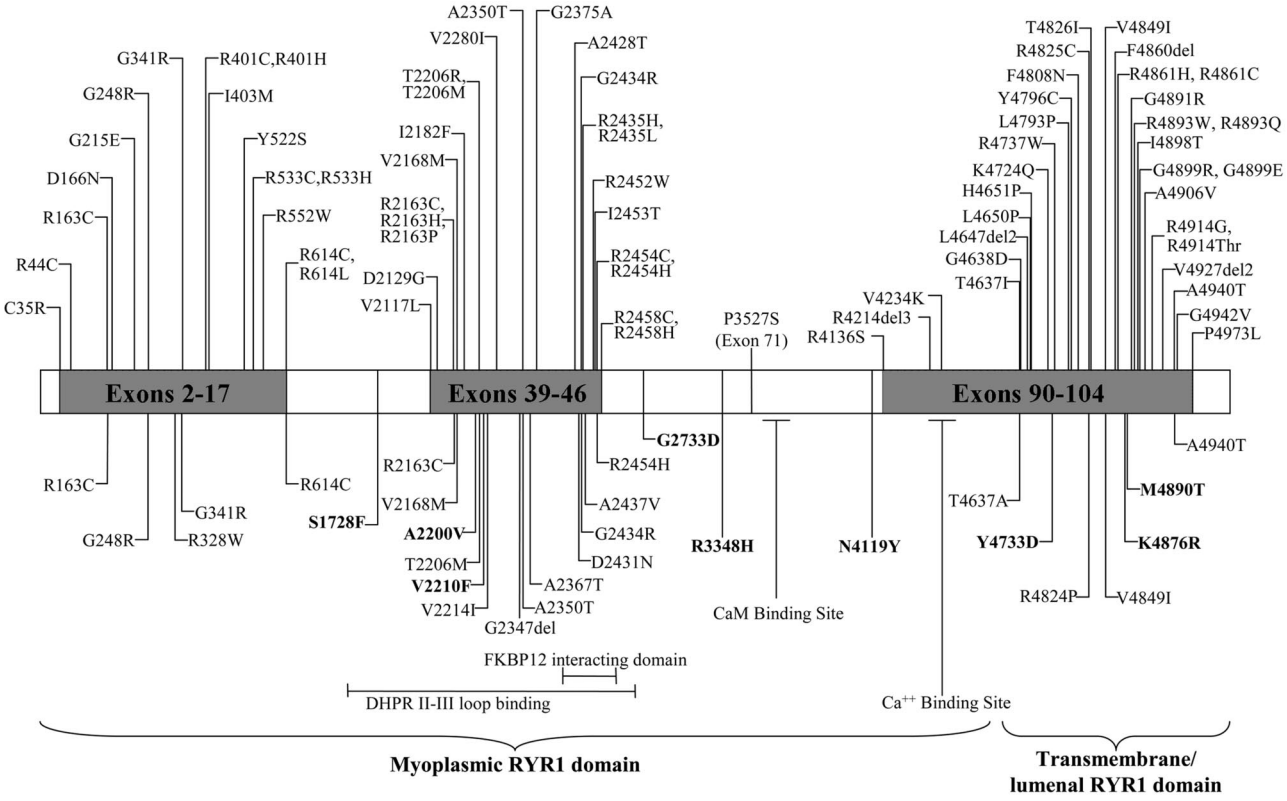

Fig. 3. Location of ryanodine receptor type 1 (RYR1) mutations[32–36] associated with malignant hyperthermia susceptibility and central core disease. Mutations found in European and Australian malignant hyperthermia–susceptible/central core disease families are shown at the *top*; mutations found in North American malignant hyperthermia–susceptible/central core disease families are shown at the *bottom* of the diagram. The novel mutations identified in this study are shown in *bold*. The three mutational hot spot areas are *shadowed*. CaM = calmodulin; DHPR = dihydropyridine receptor; FKBP12 = FK506-binding protein 12.

cytes, and exon 101 containing Lys4876 was amplified and analyzed by DHPLC. Four of the 10 at-risk members of this family showed the presence of the Lys4876Arg mutation (fig. 4, subjects B1, B4, C1, and C3).

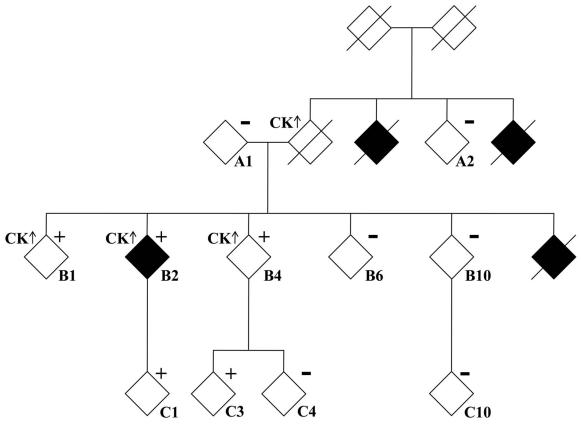

Fig. 4. Pedigree of the North American malignant hyperthermia–affected family. The gender of family members is unspecified for the protection of personal identity. *Open symbol with line through* = deceased individual; *filled symbol with line through* = individual who died of malignant hyperthermia crisis; *filled symbol* = diagnosed as malignant hyperthermia susceptible based on contracture tests; *open symbol* = contracture testing not performed; *plus* = mutation positive; *minus* = mutation negative; *CK with an arrow* = increased resting concentration of creatine kinase.

Discussion

Although defects in the *RYR1* gene are considered the primary cause of MHS, genetic linkage studies indicate that only 50% of studied affected families show linkage to the *RYR1* locus,[9] whereas *RYR1* mutations are found in only approximately 22–25% of the individuals with positive CHCT results.[10,11] There are significant technical difficulties in establishing linkage and finding mutations in a gene spanning a 159,000-nucleotide genomic DNA segment and transcribing a 15,117-nucleotide-long RNA molecule.[6] To complicate the matter further, the majority of *RYR1* mutations are rare and unique to a particular family,[5] and in addition, multiple silent DNA polymorphisms are interspersed with disease-associated mutations.[5,8] Most *RYR1* mutations known to cause MH/MHS are clustered in three mutational hot spots. Because of the enormous size of the gene, the current mutation-detection methodology settles for screening the mutational hot spots only by sequencing or restriction analysis, which leaves the possibility for the presence of nonrecognized mutations in other areas of the gene.

The above circumstances determined the strategy of this study. We performed a complete screening of the *RYR1* RNA derived from the biopsied muscle tissue in 30 MH/MH-susceptible individuals using DHPLC, a highly

sensitive mutation-detection methodology.[23] Muscle tissue is the optimal source of RNA because *RYR1* is preferentially expressed in the skeletal muscle. Analysis of RNA extracted from muscle but not lymphocytes proved to be a successful strategy of mutation identification in dystrophin.[24] Most importantly, RNA analysis allows to significantly reduce the number of DHPLC runs, in the case of *RYR1* from 106 to 57 per patient. With the approach based on screening of the entire *RYR1* gene, we hoped to obtain direct and complete estimate of mutations associated with MH/MHS in the North American population and to observe the distribution of the mutations within the gene. Although the number of MH and MSH individuals we studied is modest, our results indicate that screening of the entire *RYR1* coding sequence with DHPLC followed by sequencing is highly efficient: This methodology significantly increased the mutation-detection rate from 22–25% to 70%. Based on our experience, we concur with other investigators involved in studies of large genes such as dystrophin and *RYR2* in that DHPLC is a highly sensitive, rapid, and efficient method for mutation screening.[18,19,23] With all these improvements, there are nine MH/MH-susceptible individuals in which *RYR1* mutations have not been found. There may be several explanations for this: (1) involvement of other genes; (2) possible defects in the regulatory and untranslated regions that have not been screened in the mutation-negative individuals; (3) incorrect results of contracture testing; and (4) several additional mutations could be missed because of less-than-ideal DHPLC sensitivity: Most stringent assessments indicate that the lower limit of sensitivity may be between 90 and 98%.[19,23,25]

Although functional studies are needed to conclusively define their exact pathogenic effects, the nine newly identified *RYR1* missense mutations induce structural and functional damage as suggested by their cosegregation with the MH/MHS status, absence among 100 healthy controls, and their location in areas of high degree of evolutionary conservation. We also consider Val4849Ile substitution as a candidate mutation for MHS because it was associated with central core disease and MHS in three independent studies, including ours, and absent among 250 control individuals of different ethnic backgrounds that we and others have studied.[21] However, we emphasize that caution must be taken in determining whether newly discovered amino acid changes in the *RYR1* gene are candidate mutations for MH/MHS or simply polymorphic codons. Ultimately, functional studies in animal or cell culture models will provide evidence for the pathogenic role of these mutations.

Analysis of the *RYR1* coding sequence in its entirety allowed us to identify three novel candidate mutations located outside of the known mutational hot spots that otherwise would not have been detected. The Arg3348His mutation is markedly distant from the mutational hot spots but close to the putative calmodulin binding site.[15] A recent study described a calmodulin binding site–linked Pro3527Ser *RYR1* mutation that caused central core disease.[26] Our data indicate that *RYR1* mutations are distributed throughout the gene, and therefore, it is important to screen the entire coding sequence, particularly the region between the central and C-terminal mutational hot spots, to reliably determine the cause of MHS.

Genetic testing of a large MH-affected family illustrates the effectiveness of the proposed strategy of screening for MHS. The strategy consists of using the remnants of muscle tissue of individuals with positive CHCT results for RNA extraction or using genomic DNA for screening for *RYR1* mutations. When the mutation is found and identified, detection-mutation testing based on DHPLC alone, sequencing of a single *RYR1* exon amplified from genomic DNA, or restriction analysis alone can be offered to each family member. Almost half of the individuals with positive CHCT results had new mutations in *RYR1*, and only two were recurrent mutations. Therefore, because of heterogeneity, the North American MH-susceptible population is likely to have higher mutation diversity or allelic heterogeneity than other more stable populations. A schematic representation of the known *RYR1* mutations, including the novel mutations identified in this study (fig. 3), indicates that some mutations are preferentially present in the North American population. Regionally distributed *RYR1* mutations have previously been described: The G341R mutation is most frequent in Ireland and England, whereas R614C is more common in mainland Europe, and V2168M occurs with a high frequency in the MH-susceptible population of Switzerland.[5,27,28] Of several MH/MHS mutations previously identified in the United States, V2214I, A2367T, A2350T, D2431N, and G2347del, only A2350T was found in a single individual in Europe.[11,29–31]

In conclusion, the results of this study indicate that defects in the *RYR1* gene cause MH/MHS in at least 70% of MH/MH-susceptible individuals, and new mutations can be identified outside the known mutational hot spots. DHPLC methodology is highly accurate, rapid, and efficient in detecting mutations in the entire *RYR1* gene and may be used as primary method for mutation screening in MH patients and CHCT-confirmed MH-susceptible individuals and subsequently for mutation detection in members of MH-affected families. The ability to identify mutations in the entire *RYR1* coding region would further increase the sensitivity of a genetic test.

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