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Ryanodine Receptor Type 1 Gene Variants in the Malignant Hyperthermia-Susceptible Population of the United States

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Abstract

Background—Mutations in the ryanodine receptor type 1 gene (*RYR1*) that encodes the skeletal muscle-specific intracellular calcium (Ca2+) release channel are a cause of malignant hyperthermia (MH). In this study we examined *RYR1* mutations in a large number of North American MH-susceptible (MHS) subjects without prior genetic diagnosis.

Methods—*RYR1* was examined in 120 unrelated MHS subjects from the United States in a tiered manner. The alpha-1 subunit of the dihydropyridine receptor gene (*CACNA1S*) was screened for four variants in subjects in whom no abnormality was found in 100 or more exons of *RYR1.*

Results—Ten known causative MH mutations were found in 26 subjects. Variants of uncertain significance in *RYR1* were found in 36 subjects, 16 of which are novel. Novel variants in both *RYR1* and *CACNA1S* were found in the one subject who died of MH. Two *RYR1* variants were found in 4 subjects. Variants of uncertain significance were found outside and inside the hotspots of *RYR1.* Maximal contractures in the caffeine-halothane contracture test were greater in those who had a known MH mutation or variant of uncertain significance in *RYR1* than in those who did not.

Conclusions—The identification of novel *RYR1* variants and previously observed *RYR1* variants of uncertain significance in independent MHS families is necessary for demonstrating the significance of these variants for MH susceptibility and supports the need for functional studies of

these variants. Continued reporting of the clinical phenotypes of MH is necessary for interpretation of genetic findings, especially because the pathogenicity of most of these genetic variants associated with MHS remains to be elucidated.

Introduction

Dominant mutations in the ryanodine receptor type 1 gene (*RYR1*) encoding the skeletal muscle-specific intracellular calcium (Ca^{2+}) release channel are a cause of malignant hyperthermia (MH) and central core disease (CCD). *RYR1* variants associated with MH have been found in 50% to 86% of MH-susceptible (MHS) families depending on population of origin and study design.[1–7] Recessive mutations in *RYR1* are found in patients with congenital myopathies including nemaline myopathy, centronuclear myopathy and congenital fiber type disproportion.[8] Most of the subjects of these reports live in Europe or Japan. Differences in the frequency of some *RYR1* mutations causative for MH[1–4] and different variants of uncertain significance (VUS)[1, 3–5, 7] have been observed in different populations. The aim of the present study was to analyze *RYR1* in a large number of MHS subjects from the United States without prior genetic diagnosis. The overall goal of this project was to determine the heterogeneity of variants and mutations in *RYR1* in the MHS population of the United States. Such a compendium of variants is important for the genetic diagnosis of MHS and will help in differentiating *RYR1* variants that could be causative for MH from rare nonsynonymous polymorphisms.

Methods

Population

Subject entry criteria included personal history of positive caffeine-halothane contracture test (CHCT), or in cases without CHCT results, personal anesthetic history of an MH event as judged by the MH Diagnostic Center Director at the Uniformed Services University of the Health Sciences (USUHS) (S.M.M.) or the Director of the North American MH Registry (B.W.B.), or a family history that included presumed death from MH and/or a positive CHCT in a family member. Subjects with histologic diagnosis of CCD were accepted into this study without CHCT results. Subjects were actively consented through the North American Malignant Hyperthermia Registry and the MH Diagnostic Center at the USUHS. Subjects in whom no genetic variants had been found in previous studies at USUHS[3, 9–10] were included in the present study. These subjects had previously been recruited from several MH diagnostic centers in the United States. In addition, 12 anonymized specimens were obtained from one MH diagnostic center that was no longer active (Northwestern University). This study was approved by the IRB at the University of Pittsburgh, Northwestern University and USUHS.

Clinical information, including the personal and family histories of the subjects, was variably obtained from the subjects themselves, data from the North American Malignant Hyperthermia Registry, and records from the MH biopsy center directors. Acute MH episodes were described by the raw score of the Clinical Grading Scale (CGS)[11] when clinical details were available. CHCTs were performed and interpreted according to the criteria established by the North American Malignant Hyperthermia Group.[12] Individuals were diagnosed MHS if any one or more of the three muscle exposures produced a contracture exceeding the diagnostic threshold. The threshold values for a positive test were ≥ 0.7 g contracture in the presence of 3% halothane and/or ≥ 0.3 g contracture in the presence of 2 mM caffeine. The contracture with the greatest tension in each subject is reported in this study. Only one muscle strip per subject was reported in this study. CCD was diagnosed by muscle histology performed by a staff neuropathologist at the Armed Forces Institute of Pathology. The diagnosis of CCD was based on histochemical

identification of amorphous areas (cores) that lack mitochondria and oxidative enzyme activity in type 1 muscle fibers.[13]

RYR1 screening

Genetic analysis was performed in a tiered manner. Initially 30 exons in the three mutational hotspots of *RYR1* were screened for variants. Subsequently 50, 70, 100 exons or the entire *RYR1* coding region was examined as material resources allowed. In 17 of the subjects, 100 or more exons of *RYR1* were examined. Sequencing of *RYR1* was performed as described previously.[3] In brief, cDNA was synthesized using RNA extracted from the frozen muscle biopsy samples, and then amplified in 26 overlapping fragments. In the absence of muscle samples, exons were amplified using genomic DNA extracted from peripheral blood and intronic primers designed for each exon. The *RYR1* variants were determined by direct sequencing using an ABI 3100 DNA analyzer (Applied Biosystems, Foster City, CA). The newly identified *RYR1* variants were compared with the single nucleotide polymorphism database at the National Center for Biotechnology Information.[a] The variants of 2 subjects were identified by microarray technology using a Goldengate platform with VeraCode technology (Illumina, Inc., San Diego, CA) as previously described.[14] The frequency of novel *RYR1* variants identified in this study was determined in healthy unrelated population controls using restriction enzyme or DNA sequencing analysis as described previously.[3] Controls were 100 Caucasian individuals, 50 of whom were MH negative by CHCT. The others were healthy individuals from unrelated families enrolled into a genetic study for screening of a familial MH mutation in *RYR1.*

Screening of other genes

In subjects in whom no abnormalities were found in 100 or more exons of *RYR1*, the alpha-1 subunit of the dihydropyridine receptor gene (*CACNA1S*) was screened for four variants, Arg174Trp in exon 4, Arg1086His and Arg1086Ser in exon 26, and Thr1354Ser in exon 44 using genomic DNA extracted from peripheral blood. Primers specific to these exons were designed; primer sequences are available upon request. Restriction enzyme MspI was used to screen Arg174Trp, and enzyme HhaI was used to screen Arg1086 and Arg1086Ser mutations. The Thr1354Ser was screened using direct sequencing of exon 44 polymerase chain reaction product. In one case of fatal MH, the entire *CACNA1S* was examined. In one case with histologic diagnosis of McArdle disease (glycogen storage disease type 5) and no findings in *RYR1,* the most common mutation, Arg50X, in the myophosphorylase gene (*PYGM*) was screened using NIaIII restriction enzyme analysis followed by sequencing.

A variant was defined as any change to the wild type sequence, other than common polymorphisms previously found in one in 100 control subjects, whether it was found in MHS or control subjects. To be considered a candidate MH causative mutation, the variant must be nonsynonymous (alter the amino acid), be present in MHS subjects only, be absent in at least 100 control subjects, and be absent in all published databases listing *RYR1* polymorphisms. The pathogenic MHS mutation should segregate with MHS in 2 unrelated families, and be shown to induce biophysical changes consistent with MHS.[b] The concomitant presence of nonsynonymous amino acid polymorphisms was not counted as a second or multiple variant in MHS subjects in this study. Mutations and variants identified in the *RYR1* were denoted according to Human Genome Variation guidelines for

[a] http://www.ncbi.nlm.nih.gov/projects/SNP
[b] http://www.emhg.org

nomenclature.[c] Variants are numbered by amino acid position according to the *RYR1* protein sequence NP_000531.2.

Statistical analysis

Data are summarized using means and standard deviations and point estimates with 95% confidence intervals. Mean values of contractures in groups of subjects with different genetic findings were compared by analysis of variance followed by Student-Newman-Keuls tests using PASW Statistics 18.0.0 (SPSS).

Results

DNA analysis was performed on 120 unrelated subjects (Figure 1) (individual numbered subjects are described in the Web Supplement). CHCT were reported for 108 subjects, one of whom was found to have CCD diagnosed by histopathology (subject #52). In subjects who did not undergo CHCT, CGS scores of their suspected MH episodes, when calculable, ranged from 23 to 76. These seven subjects were numbered 6, 15, 25, 31, 51, 53 and 63 in the Web Supplement. Subject #53 died of MH (CGS 76). Subject #51 had progressive CCD, diagnosed by histopathology, after a severe MH episode during general anesthesia (CGS 53). Subjects #17 and #27 had CCD diagnosed after a suspected MH episode. Two subjects, #8 and #49, had no CHCT results because they underwent muscle contracture tests before standardization of the CHCT, due to their history suggestive of MH susceptibility (Web Supplement), with abnormal results. One subject, #50, had a very strong family history of MH, but was unable to undergo CHCT.

Ryanodine receptor gene findings

Ten known *RYR1* mutations causative for MH were identified in 26 subjects (Table 1). A previously reported variant of unknown significance or a novel variant in *RYR1* was identified in an additional 36 subjects (Table 2).[3–7,15–35] Thus 62 of 120 or 52% (95% CI 43% to 61%) of all the subjects had a causative mutation or other variant in *RYR1.*

Eleven of the 16 novel variants reported here were found in one of the three previously defined hotspots of *RYR1*[27] in exons 2, 14, 41, 44, 90, 91, 100 and 102, and five were found outside of these hotspots, in exons 24, 66, and 76. Four novel variants were at amino acid residues, Gly40 in exon 2, Arg1043 in exon 24, Arg2248 in exon 41 and Ala4906 in exon 102, where different variants had been previously reported.[5, 7, 16, 17, 30] These novel variants were not present in any of the 100 control subjects.

Contracture test results are described in Table 3. There were no significant differences between the maximum contractures in subjects with MH causative mutations and subjects with VUS in *RYR1.* The maximum number of contractures in subjects with MH mutations or VUS in *RYR1* were significantly more than the maximum number of contractures in those with no abnormality, which includes polymorphisms, found in *RYR1.*

Known MH-causative *RYR1* mutations were found in 7 of the 12 subjects (#6, 8, 15, 27, 31, 49 and 50 in the Web Supplement) who had not undergone CHCT. Previously reported VUS in *RYR1* were found in 2 other subjects in this group (#51 and 63), one of whom also had histologically diagnosed CCD. Novel variants in *RYR1* were found in 2 subjects (#17 and 53), one of whom was the anesthetic-induced fatality and the other had CCD with no history of MH. Only one of these 12 subjects (#25) who had not undergone CHCT had no findings in the 106 exons of *RYR1* other than a known polymorphism. This subject experienced a

[c] http://www.hgvs.org/rec.html

MH event with CGS 38 (very likely MH). Clinical details of these cases are described further in the Web Supplement.

RYR1 was not examined to the same extent in all 58 subjects (one of whom did not undergo CHCT) in whom no variants were found because of the limited availability of DNA. However, there was no significant difference in maximum contractures between groups with more or fewer exons examined (Table 3).

Previously described polymorphisms (variants that are *not* expected to be pathologic that are found in at least 1 in 100 normal subjects) in *RYR1* were found in 9 of these 58 subjects and in 2 subjects who had *RYR1* VUS and 1 who had a novel *RYR1* variant. Contracture test results for 8 of these 9 subjects are summarized in Table 3. The observed polymorphisms included Val974Met, Arg1109Leu, and Ile2321Val (each in one subject), Lys1393Arg, Pro1787Leu and Gly2060Cys (each in 3 subjects). In one subject both Pro1787Leu and Gly2060Cys were found. The clinical details of these subjects are presented in the Web Supplement.

RYR1 compound heterozygotes and variants in CACNA1S and PYGM

Two variants, novel or previously observed VUS in *RYR1,* were found in 4 of the 120 subjects (Table 4 and the Web Supplement). This count does not include polymorphisms. One subject listed in Table 4 was not included in Table 2, or in the total count of subjects in this study, because this subject had an MH episode after which a sibling underwent CHCT. Both siblings were entered into this study before it was recognized that they were related. The proband in this family had a known *RYR1* mutation causative for MH and a novel variant. The sibling of this proband, who had strongly positive results on the contracture test (Table 2), had only the novel variant in *RYR1.* In the subject who died of MH, a novel variant, Val875Met, was found in exon 20 of *CACNA1S* in addition to a novel *RYR1* variant, Arg3283Gln.

In the 17 subjects in whom no variants were found in more than 100 exons of *RYR1*, the *CACNA1S* was examined for four variants. None of the four *CACNA1S* variants associated with MHS in previous studies was found. Two of the 17 subjects reported that a first-degree relative died during anesthesia and 1 who reported that a more distant relative died during anesthesia. In contrast, in the families of 18 different subjects in whom *RYR1* variants were found (8 causative MH mutations and 10 VUS), 13 subjects reported perioperative deaths in first-degree relatives and 5 reported perioperative deaths in more distant relatives. Unfortunately, the records documenting the cause of death were not available in most of these cases. It is certainly possible that the cause of death was not MH. Nevertheless these unexpected deaths motivated family members to undergo CHCT. The clinical data available for these cases are presented in the Web Supplement.

A homozygous Arg50X mutation was found in *PYGM* in one subject who had repeated episodes of muscle weakness and pain after exercise. His basal creatine kinase was 3000 to 6000 IU. After moderate exercise creatine kinase increased to >100,000 IU. He had never had anesthesia, but a second-degree relative was reported to have had anesthetic complications; therefore, CHCT was performed. The maximum contracture was 0.28 g in the presence of 2 mM caffeine and 2.68 g in the presence of 3% halothane. Examination of the entire *RYR1* gene did not identify a variant. An additional muscle biopsy was performed and McArdle disease was diagnosed histologically.

Clinical history of subjects in whom no RYR1 variants or polymorphisms and none of 4 CACNA1S variants were found

All 49 of these subjects had CHCT results (Web Supplement). In this group, 17 underwent CHCT because of a family history of an MH event. In 3 families between 3 and 5 positive CHCTs per family unit were documented. In one of these 3 families there was a perioperative death. In another family there was a perioperative cardiac arrest with death and subsequently positive CHCT in a first-degree relative who is a subject in this study. The clinical presentation of the 7 subjects who were reported to have had a MH event, with no family history of MH, in whom no *RYR1* or *CACNA1S* variants were observed, included masseter muscle rigidity in 2 and 1 with CGS of 38 (very likely MH).[11] Another had a CGS of 18 and the rest had CGS of 15 or less. Rhabdomyolysis without more evidence for MH was the indication for CHCT in 3 subjects. There was no documented indication for CHCT in 22 of the subjects in whom there were no genetic findings. There were no deaths attributed to MH in the 49 subjects in whom no genetic variants were found in *RYR1* or *CACNA1S* or in the 9 subjects in whom only a polymorphism in *RYR1* was observed.

Discussion

In this sample of 120 subjects with the diagnosis of MHS, we found 10 MH causative mutations, 16 novel *RYR1* variants, one novel *CACNA1S* variant and 18 previously reported VUS in *RYR1*. A 2005 study of 30 subjects from North America, using direct sequencing of the entire coding region of *RYR1*,[3] found 7 previously reported known MH mutations in 10 subjects, 2 VUS in 2 subjects, and 9 novel *RYR1* variants in 9 other subjects. Seven of these previously observed mutations and variants were also found in this study of 120 subjects, but none of the 9 novel variants reported in 2005 was observed again. Gly2423Arg was the most frequent MH mutation reported in 2001, 2005 and in the current study. This mutation was also the most frequently observed MH mutation in Great Britain[36] and Canada,[6] but not in France,[2] Japan,[4] Australia,[27] Switzerland,[5] or Italy.[7] Numerous cohort and case studies have reported *RYR1* variants in subjects with clinical evidence of MH, including positive contracture tests. When these reports were comprehensively reviewed by Robinson et al. in 2006, 178 missense *RYR1* variants were identified.[17] As of November 2012, 414 unique variants in *RYR1* had been reported in the Leiden Open Variation Database.[d] This report adds more *RYR1* variants to this list. Thus, the number of described *RYR1* variants is increasing rapidly, but the number proven to meet the strict criteria of a causative mutation is an order of magnitude lower.

Previous studies reported a high percentage of *RYR1* variants thought to be unique or private to one family. This report supports the reassignment of variants from the private to the recurrent category. In 8 subjects in this study a variant of uncertain significance was found that had been published only once before.[6, 17, 18, 28, 31] Other VUS observed in this study have been reported at least twice previously, but not yet widely accepted as MH-causative mutations.[4, 5, 7, 17, 19, 20–27, 30, 32–35] Thus, this report provides data to support the potential MH-causative nature of many VUS in *RYR1*. Many variants listed in Table 2 have been observed previously in association with MHS, but the functional tests needed to prove that these variants cause the MH syndrome have not yet been performed.

The novel variants and VUS in *RYR1* documented in this study support the need for examination of the entire *RYR1* gene in MHS patients when searching for the genetic marker of this condition. Nine of the VUS observed in this study were outside of the *RYR1* hotspots as these were defined.[27] Fatal episodes of MH, MH death in a sibling, CCD,

[d] http://www.dmd.nl/nmdb2/home.php?select_db=RYR1

positive CHCT results and observation of the same variant in other studies[4] were reported in these nine subjects.

In contrast to earlier claims that *RYR1* variants associated with CCD are found in the C terminal exons 85–103,[17] we observed *RYR1* variants in exons 24, 47 and 66 in subjects with CCD. The same variant in exon 47 was observed in Japanese subjects with MH and CCD.[4] The other 2 are novel *RYR1* variants.

The subjects in this report who underwent CHCT were evaluated by anesthesiologists at one of several MH diagnostic centers. Review of personal medical and anesthetic history and family history are part of these evaluations. These anesthesiologists may consult with a neurologist before proceeding with CHCT so that maximum diagnostic use can be made of the patient's muscle biopsy. Not all patients who present for CHCT are accepted for this procedure. If there is not a high prior probability of MH being present, the CHCT is not warranted.[37]

Individuals who had not undergone CHCT were subject to similar scrutiny before entry into this study. The high yield of *RYR1* findings in this subset without a personal history of CHCT is likely due to the rigor of that review. Review of medical records before testing an individual for MHS includes detailed review of the anesthetic record, including minute ventilation and exhaled gas concentrations. These details can be clearly provided by the electronic anesthetic record.[38] Other clinical and laboratory data should also be part of this assessment.[39] Often, however, medical records, especially paper records, are incomplete. Additionally, because anesthesia providers should intervene to treat MH before it becomes fulminant, severe hypercarbia and rhabdomyolysis are averted. Thus, in many cases a CGS associated with "very likely MH" (CGS of 35 to 49) or "almost certain MH," (CGS of 50 or more),[11] will not be observed. This was the case in the cohort reported here.

The observation of subjects with 2 variants is consistent with the previous claim that the frequency of an MH-causative genetic variant may be as much as 1 in 2,000 to 3,000 people in the general population.[4,35]

The percentage of subjects in this study in whom variants in *RYR1* were identified is less than in several previously published studies. This is likely due in part to our inability to examine the entire *RYR1* gene in all subjects due to the limited amount of genetic material available from MH diagnostic centers that have closed. Nevertheless it is of interest that in those subjects in whom 100 or more *RYR1* exons were examined without identification of any variant, the average maximum contractures were less than in the groups in which either known MH-causative mutations or VUS were found in the *RYR1* gene. Because of the high sensitivity and low specificity of the CHCT there can be false positive CHCT results in as many as 22% of those tested.[40] Therefore the personal anesthetic and medical histories and family histories have to be re-examined in order to diagnose the underlying condition. It may be that the low specificity of the CHCT produces clinically positive results in individuals who are not really at increased risk of experiencing MH. For example, the subject who was homozygous for a mutation causing McArdle disease had a maximum caffeine contracture less than 0.3 g, but was enlisted in this study because he had a maximum halothane contracture of more than 2 g and suspicion of an adverse anesthetic event in a relative. The medical history of the individual should guide testing so that the differential diagnosis of exercise-induced rhabdomyolysis, the chief complaint of this individual, includes more than *RYR1* variants.

CHCT may have positive results, diagnostic of MHS, in myopathic conditions with elevated calcium in muscle that do not include genetic abnormalities of *RYR1*. Appropriately focused blood testing could provide a diagnosis without performing muscle biopsy. When muscle

biopsy is performed, comprehensive pathologic examination should be carefully planned prospectively to maximize diagnostic yield. If muscle contracture testing is needed to evaluate MHS, the biopsy should be performed at a MH diagnostic center (see www.mhaus.org for a list of active MH diagnostic centers in North America). CHCT remains the only method available in North America to confirm that the diagnosis is *not* MHS.

Previous work has demonstrated that greater halothane contractures are more likely to be found in individuals with MH-causative mutations in *RYR1*.[41] Yet our subjects without abnormalities in *RYR1* had been diagnosed as MHS by the CHCT. They or their first degree relatives experienced adverse anesthetic events. It is necessary to continue to collect detailed anesthetic records, family histories and biologic specimens from such people to discover the genetic factors associated with these adverse events. It may be that a new genetic locus for MH can be identified in subjects with strong family histories of adverse anesthetic events without *RYR1* variants. Variants in regulatory regions and deep intronic variants of *RYR1*, or in areas of *CACNA1S* not examined in this study, may be responsible for the MHS diagnosis in this group. Given the dispersion of the population and potential difficulty confirming anesthetic details, it is necessary to continue collection of data in a central repository such as the North American MH Registry, so that future studies of this potentially life-threatening syndrome can be supported.

In summary, these results contribute to increasing the utility of genetic testing of MHS by documenting the presence of *RYR1* variants in independent families and by describing novel variants. These results are consistent with previous work that identified MH-causative mutations more often in those with greater muscle contractures on in vitro testing.[41] Technologic improvements in electronic health records and genetic analysis should lead to a more comprehensive view of both the phenotype and the genetic basis of MHS. Continued detailed reporting of the phenotypes of MHS is necessary to support interpretation of genetic results.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Rueffert H, Olthoff D, Deutrich C, Meinecke CD, Froster UG. Mutation screening in the ryanodine receptor 1 gene (RYR1) in patients susceptible to malignant hyperthermia who show definite IVCT results: identification of three novel mutations. Acta Anaesthesiol Scand. 2002; 46:692–698. [PubMed: 12059893]
2. Monnier N, Kozak-Ribbens G, Krivosic-Horber R, Nivoche Y, Qi D, Kraev N, Loke J, Sharma P, Tegazzin V, Figarella-Branger D, Romero N, Mezin P, Bendahan D, Payen JF, Depret T, Maclennan DH, Lunardi J. Correlations between genotype and pharmacological, histological, functional, and clinical phenotypes in malignant hyperthermia susceptibility. Hum Mutat. 2005; 26:413–425. [PubMed: 16163667]
3. Sambuughin N, Holley H, Muldoon S, Brandom BW, de Bantel AM, Tobin JR, Nelson TE, Goldfarb LG. Screening of the entire ryanodine receptor type 1 coding region for sequence variants

associated with malignant hyperthermia susceptibility in the North American population. Anesthesiology. 2005; 102:515–521. [PubMed: 15731587]

4. Ibarra MC, Wu S, Murayama K, Minami N, Ichihara Y, Kikuchi H, Noguchi S, Hayashi YK, Ochiai R, Nishino I. Malignant hyperthermia in Japan: mutation screening of the entire ryanodine receptor type 1 gene coding region by direct sequencing. Anesthesiology. 2006; 104:1146–1154. [PubMed: 16732084]
5. Levano S, Vukcevic M, Singer M, Matter A, Treves S, Urwyler A, Girard T. Increasing the number of diagnostic mutations in malignant hyperthermia. Hum Mutat. 2009; 30:590–598. [PubMed: 19191329]
6. Kraeva N, Riazi S, Loke J, Frodis W, Crossan ML, Nolan K, Kraev A, Maclennan DH. Ryanodine receptor type 1 gene mutations found in the Canadian malignant hyperthermia population. Can J Anaesth. 2011; 58:504–513. [PubMed: 21455645]
7. Tammaro A, Di Martino A, Bracco A, Cozzolino S, Savoia G, Andria B, Cannavo A, Spagnuolo M, Piluso G, Aurino S, Nigro V. Novel missense mutations and unexpected multiple changes of RYR1 gene in 75 malignant hyperthermia families. Clin Genet. 2011; 79:438–447. [PubMed: 20681998]
8. Jungbluth H, Sewry CA, Muntoni F. Core myopathies. Semin Pediatr Neurol. 2011; 18:239–249. [PubMed: 22172419]
9. Sambuughin N, Sei Y, Gallagher KL, Wyre HW, Madsen D, Nelson TE, Fletcher JE, Rosenberg H, Muldoon SM. North American malignant hyperthermia population: screening of the ryanodine receptor gene and identification of novel mutations. Anesthesiology. 2001; 95:594–599. [PubMed: 11575529]
10. Sei Y, Sambuughin NN, Davis EJ, Sachs D, Cuenca PB, Brandom BW, Tautz T, Rosenberg H, Nelson TE, Muldoon SM. Malignant hyperthermia in North America: genetic screening of the three hotspots in the type I ryanodine receptor gene. Anesthesiology. 2004; 101:824–830. [PubMed: 15448513]
11. Larach MG, Localio AR, Allen GC, Denborough MA, Ellis FR, Gronert GA, Kaplan RF, Muldoon SM, Nelson TE, Ording H, Rosenberg H, Waud BE, Wedel DJ. A clinical grading scale to predict malignant hyperthermia susceptibility. Anesthesiology. 1994; 80:771–779. [PubMed: 8024130]
12. Larach MG. Standardization of the caffeine halothane muscle contracture test. North American Malignant Hyperthermia Group. Anesth Analg. 1989; 69:511–515. [PubMed: 2675676]
13. Monnier N, Romero NB, Lerale J, Nivoche Y, Qi D, MacLennan DH, Fardeau M, Lunardi J. An autosomal dominant congenital myopathy with cores and rods is associated with a neomutation in the RYR1 gene encoding the skeletal muscle ryanodine receptor. Hum Mol Genet. 2000; 9:2599–2608. [PubMed: 11063719]
14. Vladutiu GD, Isackson PJ, Kaufman K, Harley JB, Cobb B, Christopher-Stine L, Wortmann RL. Genetic risk for malignant hyperthermia in non-anesthesia-induced myopathies. Mol Genet Metab. 2011; 104:167–173. [PubMed: 21795085]
15. Wu S, Ibarra MC, Malicdan MC, Murayama K, Ichihara Y, Kikuchi H, Nonaka I, Noguchi S, Hayashi YK, Nishino I. Central core disease is due to RYR1 mutations in more than 90% of patients. Brain. 2006; 129:1470–1480. [PubMed: 16621918]
16. Jungbluth H, Lillis S, Zhou H, Abbs S, Sewry C, Swash M, Muntoni F. Late-onset axial myopathy with cores due to a novel heterozygous dominant mutation in the skeletal muscle ryanodine receptor (RYR1) gene. Neuromuscul Disord. 2009; 19:344–347. [PubMed: 19303294]
17. Robinson R, Carpenter D, Shaw MA, Halsall J, Hopkins P. Mutations in RYR1 in malignant hyperthermia and central core disease. Hum Mutat. 2006; 27:977–989. [PubMed: 16917943]
18. Sambuughin N, McWilliams S, de Bantel A, Sivakumar K, Nelson TE. Single-aminoacid deletion in the RYR1 gene, associated with malignant hyperthermia susceptibility and unusual contraction phenotype. Am J Hum Genet. 2001; 69:204–208. [PubMed: 11389482]
19. McWilliams S, Nelson T, Sudo RT, Zapata-Sudo G, Batti M, Sambuughin N. Novel skeletal muscle ryanodine receptor mutation in a large Brazilian family with malignant hyperthermia. Clin Genet. 2002; 62:80–83. [PubMed: 12123492]
20. Wehner M, Rueffert H, Koenig F, Olthoff D. Functional characterization of malignant hyperthermia-associated RyR1 mutations in exon 44, using the human myotube model. Neuromuscul Disord. 2004; 14:429–437. [PubMed: 15210166]

21. Barone V, Massa O, Intravaia E, Bracco A, Di Martino A, Tegazzin V, Cozzolino S, Sorrentino V. Mutation screening of the RYR1 gene and identification of two novel mutations in Italian malignant hyperthermia families. J Med Genet. 1999; 36:115–118. [PubMed: 10051009]
22. Galli L, Orrico A, Cozzolino S, Pietrini V, Tegazzin V, Sorrentino V. Mutations in the RYR1 gene in Italian patients at risk for malignant hyperthermia: evidence for a cluster of novel mutations in the C-terminal region. Cell Calcium. 2002; 32:143–151. [PubMed: 12208234]
23. Tammaro A, Bracco A, Cozzolino S, Esposito M, Di Martino A, Savoia G, Zeuli L, Piluso G, Aurino S, Nigro V. Scanning for mutations of the ryanodine receptor (RYR1) gene by denaturing HPLC: detection of three novel malignant hyperthermia alleles. Clin Chem. 2003; 49:761–768. [PubMed: 12709367]
24. Chan B, Chen SP, Wong WC, Mak CM, Wong S, Chan KY, Chan AY. RYR1-related central core myopathy in a Chinese adolescent boy. Hong Kong Med J. 2011; 17:67–70. [PubMed: 21282829]
25. Dekomien G, Gencik M, Gencikova A, Klenk Y, Epplen JT. Gene symbol: RYR1. Disease: malignant hyperthermia. Hum Genet. 2005; 118:543.
26. Monnier N, Marty I, Faure J, Castiglioni C, Desnuelle C, Sacconi S, Estournet B, Ferreiro A, Romero N, Laquerriere A, Lazaro L, Martin JJ, Morava E, Rossi A, Van der Kooi A, de Visser M, Verschuuren C, Lunardi J. Null mutations causing depletion of the type 1 ryanodine receptor (RYR1) are commonly associated with recessive structural congenital myopathies with cores. Hum Mutat. 2008; 29:670–678. [PubMed: 18253926]
27. Gillies RL, Bjorksten AR, Davis M, Du Sart D. Identification of genetic mutations in Australian malignant hyperthermia families using sequencing of RYR1 hotspots. Anaesth Intensive Care. 2008; 36:391–403. [PubMed: 18564801]
28. Kaufmann A, Kraft B, Michalek-Sauberer A, Weigl LG. Novel ryanodine receptor mutation that may cause malignant hyperthermia. Anesthesiology. 2008; 109:457–464. [PubMed: 18719443]
29. Jungbluth H, Muller CR, Halliger-Keller B, Brockington M, Brown SC, Feng L, Chattopadhyay A, Mercuri E, Manzur AY, Ferreiro A, Laing NG, Davis MR, Roper HP, Dubowitz V, Bydder G, Sewry CA, Muntoni F. Autosomal recessive inheritance of RYR1 mutations in a congenital myopathy with cores. Neurology. 2002; 59:284–287. [PubMed: 12136074]
30. Tilgen N, Zorzato F, Halliger-Keller B, Muntoni F, Sewry C, Palmucci LM, Schneider C, Hauser E, Lehmann-Horn F, Muller CR, Treves S. Identification of four novel mutations in the C-terminal membrane spanning domain of the ryanodine receptor 1: association with central core disease and alteration of calcium homeostasis. Hum Mol Genet. 2001; 10:2879–2887. [PubMed: 11741831]
31. Shepherd S, Ellis F, Halsall J, Hopkins P, Robinson R. RYR1 mutations in UK central core disease patients: more than just the C-terminal transmembrane region of the RYR1 gene. J Med Genet. 2004; 41:e33. [PubMed: 14985404]
32. Sewry CA, Muller C, Davis M, Dwyer JS, Dove J, Evans G, Schroder R, Furst D, Helliwell T, Laing N, Quinlivan RC. The spectrum of pathology in central core disease. Neuromuscul Disord. 2002; 12:930–938. [PubMed: 12467748]
33. Quinlivan RM, Muller CR, Davis M, Laing NG, Evans GA, Dwyer J, Dove J, Roberts AP, Sewry CA. Central core disease: clinical, pathological, and genetic features. Arch Dis Child. 2003; 88:1051–1055. [PubMed: 14670767]
34. Davis MR, Haan E, Jungbluth H, Sewry C, North K, Muntoni F, Kuntzer T, Lamont P, Bankier A, Tomlinson P, Sanchez A, Walsh P, Nagarajan L, Oley C, Colley A, Gedeon A, Quinlivan R, Dixon J, James D, Muller CR, Laing NG. Principal mutation hotspot for central core disease and related myopathies in the C-terminal transmembrane region of the RYR1 gene. Neuromuscul Disord. 2003; 13:151–157. [PubMed: 12565913]
35. Monnier N, Krivosic-Horber R, Payen JF, Kozak-Ribbens G, Nivoche Y, Adnet P, Reyford H, Lunardi J. Presence of two different genetic traits in malignant hyperthermia families: implication for genetic analysis, diagnosis, and incidence of malignant hyperthermia susceptibility. Anesthesiology. 2002; 97:1067–1074. [PubMed: 12411788]
36. Carpenter D, Robinson RL, Quinell RJ, Ringrose G, Hogg M, Casson F, Booms P, Eles DE Halsall PJ, Steele DS, Shaw MA, Hopkins PM. Genetic variation in. *RYR1* and malignant hyperthermia phenotypes. Br J Anaesth. 2009; 103:538–548. [PubMed: 19648156]

37. Loke JC, MacLennan DH. Bayesian modeling of muscle biopsy contracture testing for malignant hyperthermia susceptibility. Anesthesiology. 1998; 88:589–600. [PubMed: 9523800]

38. Maile MD, Patel RA, Blum JM, Tremper KK. A case of malignant hyperthermia captured by an anesthesia information management system. J Clin Monit Comput. 2011; 25:109–111. [PubMed: 21638068]

39. Larach MG, Gronert GA, Allen GC, Brandom BW, Lehman EB. Clinical presentation, treatment, and complications of malignant hyperthermia in North America from 1987 to 2006. Anesth Analg. 2010; 110:498–507. [PubMed: 20081135]

40. Allen GC, Larach MG, Kunselman AR. The sensitivity and specificity of the caffeine-halothane contracture test, a report from the North American Malignant Hyperthermia Registry. Anesthesiology. 1998; 88:579–588. [PubMed: 9523799]

41. Ginz HF, Girard T, Censier K, Urwyler A. Similar susceptibility to halothane, caffeine and ryanodine in vitro reflects pharmacogenetic variability of malignant hyperthermia. Eur J Anaesthesiol. 2004; 21:151–157. [PubMed: 14977348]

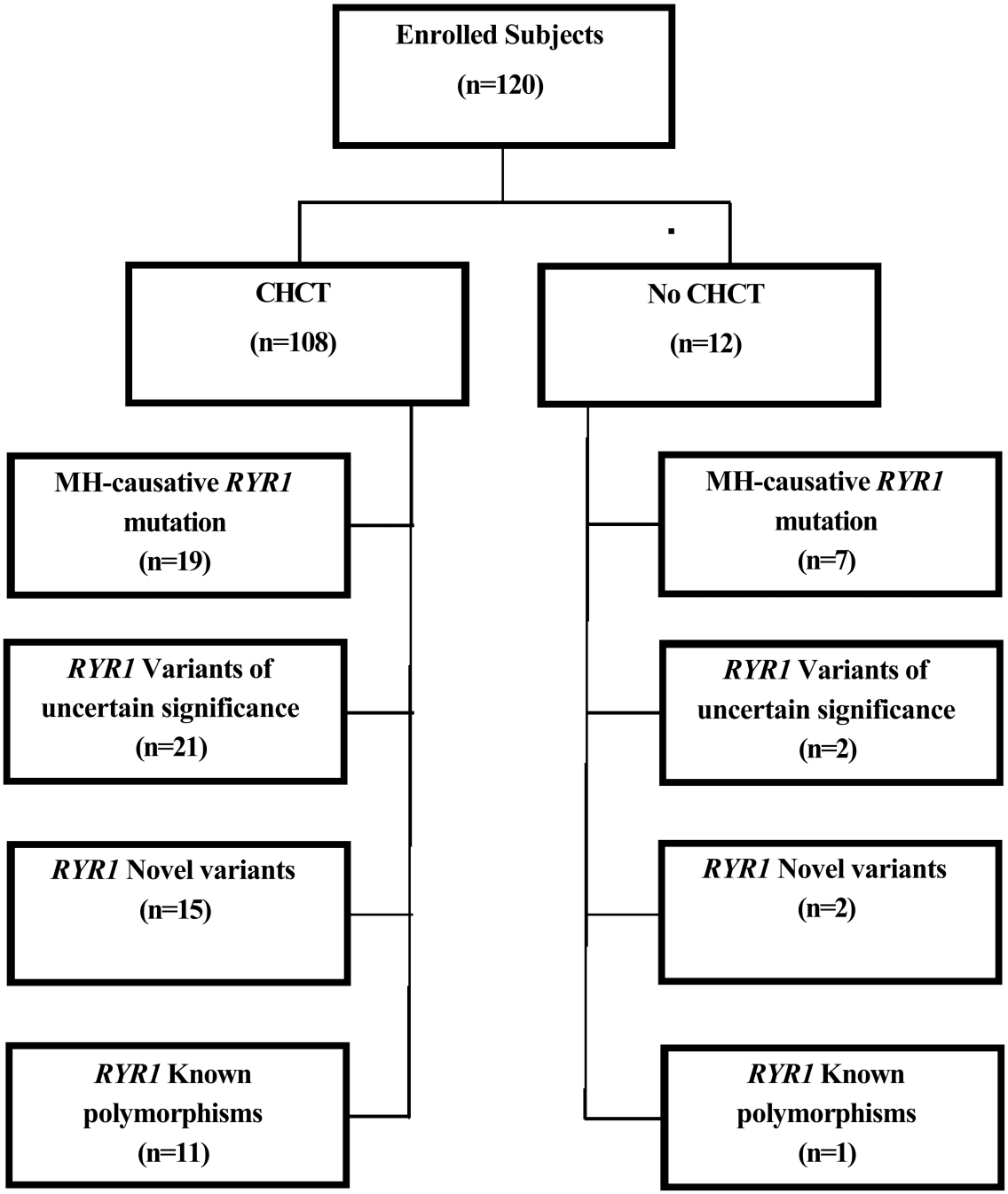

Figure 1.

This flow chart illustrates the outcomes of DNA sequencing for subjects with and without prior caffeine-halothane contracture test (CHCT). A variant of uncertain significance (VUS) is a variant that has been previously reported, but it is not yet proven to be malignant hyperthermia (MH)-causative. A novel variant is a variant that has not been previously reported, and it has not been proven to be MH-causative. A polymorphism is a change in the amino acid sequence of the gene that has been found at least once in 100 normal subjects and is not expected to be pathogenic. The numbers in parentheses are the numbers of subjects found to have this type of change in *RYR1.*

Two novel variants in the ryanodine receptor type one gene (*RYR1*) were found in one subject. A novel variant and a VUS in *RYR1* were found in one subject. Two different VUS were found in 2 subjects. A VUS and a polymorphism were found in one subject. A novel variant and a polymorphism in *RYR1* were found in another subject. One subject had 2 polymorphisms in *RYR1.* In summary, there were 7 subjects who had 2 different variants when polymorphisms in *RYR1* are included in this count. Thus, the sum of the numbers in the sub-boxes is 7 more than the number of subjects in this study reported to have mutations, VUS, novel variants and/or polymorphisms. (see Table 4 and the Web Supplement)

Table 1

Known Malignant Hyperthermia (MH) Causative Mutations

Amino Acid Substitution	Exon	Cases (n)
Arg163Cys	6	2
Gly248Arg	9	2
Gly341Arg	11	2
Arg614Cys	17	5
Arg2163His	39	1
Val2168Met	39	1
Thr2206Met	40	4
Gly2434Arg	45	7
Arg2454His	45	1
Arg4861His	101	1

Known MH-causative mutations were found in 26 subjects, 21% of the total in this study (95% CI 5%–37%).
There is no statistically significant difference in the frequency of these mutations in this sample.
The subject with Val2168Met had central core disease (CCD) diagnosed by histopathology.
The anesthetic details that are available about each of these cases are presented in the Web Supplement.

Table 2

RYR1 Variants of Uncertain Significance in Malignant Hyperthermia-Susceptible Subjects

***RYR1* Variants Amino Acid Substitution**	**Exon**	**Caffeine-Halothane Contracture Test (g)**		**Inclusion criteria**	**Citation**
		Caffeine 2mM	**Halothane 3%**		
Leu13Arg	1	0.34	2.55	MH	4, 5
Gly40Ala	2	0.83	2.7	FH	Current study (16)
Leu487Pro	14	2.4	3.6	FH	Current study
Val518Ala (a)	14	1.0	1.3	MH	Current study
Arg975Trp	24	na	na	CCD	Current study
Arg1043Cys (d)	24	3.3	5.7	MH, FH	5, 17
Arg1043His	24	0.95	1.8	FH	Current study (5,17)
Arg2248His (b)	41	0.3	2.3	MH	Current study (7)
Arg2336His (d)	43	3.3	5.7	MH, FH	5, 17
Asn2342Ser	43	0.2	1.5	MH	17
del Glu2347	44	1.36	5.9	FH	18
Asn2351His	44	0.5	5.0	MH	Current study
Val2354Met	44	1.25	3.4	MH	Current study
Arg2355Trp	44	2.6	9.5	MH	17, 19, 20
Arg2355Trp	44	1.82	1.6	MH	17, 19, 20
Arg2355Trp	44	0.3	5.1	MH	17, 19, 20
Ile2358Leu (b)	44	0.3	2.3	MH	Current study
Arg2435Leu	45	1.9	1.1	FH	7, 17, 21–23
Arg2508His	47	na	na	MH, CCD	15, 24
Arg3238Gly	66	0	0	CCD	Current study
Arg3283Gln*	66	na	na	MH Death	Current study
Arg3539His	71	0.8	5.2	MH	25, 26
Glu3583Gln (c)	72	1.0	2.6	MH, FH	17
Thr3711Arg	76	0.85	7.5	FH	Current study
Tyr3933Cys (a)	86	1.0	1.3	MH	7, 27

RYR1 Variants Amino Acid Substitution	Exon	Caffeine-Halothane Contracture Test (g)		Inclusion criteria	Citation
		Caffeine 2mM	Halothane 3%		
Trp3985Arg	87	1.2	2.2	MH	28
Asp3986Glu	87	1.9	8.8	MH	17
Asp3986Glu	87	2.5	10.8	MH	17
Gly4178Val	90	1.77	6.95	FH	Current study
Met4230Arg	91	3.3**	6.3**	MH, FH	Current study
Gln4837Glu	100	0.5	9.9	FH	Current study
Val4847Leu	101	na	na	MH	6
Val4849Ile	101	1.9	9.8	MH	2, 3, 29
Val4849Ile	101	0.3	3.6	FH	2, 3, 29
Val4849Ile	101	0.9	5.0	MH, FH	2, 3, 29
Val4849Ile (c)	101	1.0	2.6	MH, FH	2, 3, 29
Ala4906Gly	102	0.4	2.1	FH	Current study (30)
Asp4939Glu	103	2.0	11.3	MH, FH	31
Ala4940Thr	103	0.84	8.42	FH	3, 32–34
Pro4973Leu	104	0.85	2.1		22, 35

na = not done; MH indicates that a personal episode of malignant hyperthermia was reported; FH indicates that a family history of MH was reported; CCD = central core disease; del = deletion.

When no data were available regarding an anonymized subject the cell remains empty. The letters (a), (b), (c) and (d) indicate the variants were both observed in a single subject, (a), (b), (c) and (d) respectively. Therefore there are 36 subjects represented in the 40 rows of this Table. The number appearing in parentheses below "current study" refers to the citation in which a different amino acid variant was reported at the same location. This was the case in 4 subjects in Table 2.

*One RYR1 variant and a novel variant in CACNA1S found in this subject.

**Caffeine-halothane contracture test (CHCT) was from soleus muscle; the parent had positive CHCT results from deltoid muscle.

Further details that are available regarding these subjects are presented in the Web Supplement.

Table 3

Caffeine-Halothane Contractures are greater in Malignant Hyperthermia-Susceptible Subjects with *RYR1* MH Mutations and Variants than in those with no abnormalities found in *RYR1*

	Genetic Result	N	CHCT (g)	
			Caffeine 2 mM	Halothane 3%
Group 1	Known causative malignant hyperthermia mutations	19	1.3 (0.8)	5.6 (2.2)
Group 2	VUS in *RYR1*	32	1.3 (0.9)	4.9 (3.2)
Group 3	Known polymorphisms in *RYR1*	8	0.6 (0.5)	2.5 (1.9)
Group 4	No variants in 100 or more exons of *RYR1*[*]	17	0.6 (0.5)	1.7 (0.7)
Group 5	No variants in *RYR1* after screening 70 or fewer exons[*]	32	0.6 (0.7)	2.2 (1.4)
	ANOVA for difference among groups		$P < 0.001$	$P < 0.001$

Data are mean (standard deviation).

VUS is variant of uncertain significance.

A polymorphism is a DNA change that is commonly observed and has not been associated with disease.

There was no significant difference between Groups 1 and 2 in either caffeine or halothane contracture tests. There were no significant differences between Groups 3, 4 and 5 in caffeine or halothane contracture tests. (Student-Newman-Keuls test). There was a significant difference between Groups 1 and 2 and Groups 3, 4 and 5, $P < 0.001$.

[*] In 4 subjects in whom 100 or more exons were screened a previously reported polymorphism was in *RYR1* was found. In 3 subjects in whom 70 or fewer exons were screened a known polymorphism in *RYR1* was found, thus the numbers reported in this table differ slightly from those in the text. When both a polymorphism and a variant of uncertain significance were found, the contracture results of that subject were included in Group 2.

CHCT = Caffeine-halothane contracture test.

Table 4

Individuals with Two Genetic Variants

RYR1	***RYR1***	***CACNA1S***	**CHCT (g)**		**Clinical History**
Amino Acid Substitution			**Caffeine 2mM**	**Halothane 3%**	
Arg2248Gln	Ile2358Leu	—	0.3	2.3	MH episode CGS 15
Glu3583Gln	Val4849Ile	—	1.0	2.6	MH episode CGS 18, sibling was reported to have died of MH
Arg614Cys	Thr3711Arg	—	na	na	MH episode, no details available muscle cramps & rhabdomyolysis after exercise sibling had positive CHCT
Arg3283Gln	—	Val875Met	na	na	MH death, CGS 76
Val518Ala	Tyr3933Cys	—	1.0	1.3	possible MH episode with postop rhabdomyolysis CGS 5 *
Arg1043Cys	Arg2336His	—	3.3	5.7	MH episode CGS 18, FH possible MH death, sibling had positive CHCT

Of 120 subjects, 4 were found to have 2 variants in *RYR1* that could be associated with MH and one had a variant in both *RYR1* and *CACNA1S*. The compound heterozygote with R614C and T3711R was found in a subject, who after study entry was found to be related to the subject with only the novel T3711R finding. Therefore this compound heterozygote was not included in the count of total cases in Tables 1 or 2, but it was included here.

* Indicates the subject described in McKenney KA, Holman SJ. Delayed postoperative rhabdomyolysis in a patient subsequently diagnosed as malignant hyperthermia susceptible. Anesthesiology 2002; 96:764–5. For more clinical details see Web Supplement.

CHCT = caffeine-halothane contracture test; na = not available; MH = malignant hyperthermia; CGS = Clinical Grading Scale; FH = Family History.