Using Exome Data to Identify Malignant Hyperthermia Susceptibility Mutations

Stephen G. Gonsalves, M.S.N.,* David Ng, M.D.,† Jennifer J. Johnston, Ph.D.,‡ Jamie K. Teer, Ph.D.,§ NISC Comparative Sequencing Program,|| Peter D. Stenson, Ph.D.,# David N. Cooper, Ph.D.,** James C. Mullikin, Ph.D.,†† Leslie G. Biesecker, M.D.‡‡

ABSTRACT

Background: Malignant hyperthermia susceptibility (MHS) is a life-threatening, inherited disorder of muscle calcium metabolism, triggered by anesthetics and depolarizing muscle relaxants. An unselected cohort was screened for MHS mutations using exome sequencing. The aim of this study was to pilot a strategy for the RYR1 and CACNA1S genes.

Methods: Exome sequencing was performed on 870 volunteers not ascertained for MHS. Variants in RYR1 and CACNA1S were annotated using an algorithm that filtered results based on mutation type, frequency, and information in mutation databases. Variants were scored on a six-point pathogenicity scale. Medical histories and pedigrees were reviewed for malignant hyperthermia and related disorders.

Results: The authors identified 70 RYR1 and 53 CACNA1S variants among 870 exomes. Sixty-three RYR1 and 41 CACNA1S variants passed the quality and frequency metrics but the authors excluded synonymous variants. In RYR1, the authors identified 65 missense mutations, one nonsense, two that affected splicing, and one non–frameshift indel. In CACNA1S, 48 missense, one frameshift deletion, one splicing, and one non–frameshift indel were identified. RYR1 variants predicted to be pathogenic for MHS were found in three participants without medical or family histories of MHS. Numerous variants, previously described as pathogenic in mutation databases, were reclassified by the authors as being of unknown pathogenicity.

Conclusions: Exome sequencing can identify asymptomatic patients at risk for MHS, although the interpretation of exome variants can be challenging. The use of exome sequencing in unselected cohorts is an important tool to understand the prevalence and penetrance of MHS, a critical challenge for the field.

What We Already Know about This Topic
- Exome sequencing is likely to become more common in the movement toward personalized medicine
- A more thorough description of variants in genes associated with malignant hyperthermia may aid in interpreting the results of exome sequencing

What This Article Tells Us That Is New
- In 870 volunteers not ascertained for malignant hyperthermia susceptibility, numerous variants in RYR1 and CACNA1S genes were observed, some consistent and others inconsistent with presumed pathogenicity in current databases

ALIGNANT hyperthermia susceptibility (MHS) is a rare disorder of calcium dysregulation triggered by volatile anesthetics and the depolarizing muscle relaxant succinylcholine. It is an important cause of morbidity and mortality, and in its fulminant form manifests nearly always as metabolic and/or respiratory acidosis, rhabdomyolysis and

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hyperkalemia, as well some or all of the following symptoms: tachycardia, tachypnea, arrhythmias, skeletal muscle rigidity, and lethal hyperthermia. It is inherited in a predominately autosomal dominant pattern and associated with \( \text{Ryr1} \) or \( \text{Cacna1s} \) mutations, with other mapped loci. Seventy to 86% of patients with MHS have \( \text{Ryr1} \) mutations\(^5\–^7\) and 1% have \( \text{Cacna1s} \) mutations.\(^6\) The prevalence and penetrance of MHS mutations are difficult to determine because the pharmacologic exposure rate is low and it is an inconsistently manifesting gene–environment interaction; that is, when a susceptible patient is exposed to a triggering agent, the probability of malignant hyperthermia (MH) is less than 100%.

Most MHS gene and variant studies have been performed on families with multiple generations affected with typical MHS. Studying these families made possible the discovery of the two implicated genes. However, these studies had ascertainment biases for those with severe reactions to the drugs. This has complicated efforts to establish the true prevalence and penetrance of MHS mutations.

In addition, assigning pathogenicity to \( \text{Ryr1} \) and \( \text{Cacna1s} \) variants is challenging for several reasons. First is the issue of locus heterogeneity. With several mapped loci without identified genes, some \( \text{Ryr1} \) and \( \text{Cacna1s} \) variants may have been erroneously determined to be pathogenic when there was a causative variant in another (untested) gene. In addition, \( \text{Ryr1} \) and \( \text{Cacna1s} \) are large genes with 106 and 44 exons, respectively, making mutation screening challenging. Thus, some \( \text{Ryr1} \) and \( \text{Cacna1s} \) variants previously determined to be pathogenic may be benign, as has been shown for other genes.\(^7\)

New sequencing technologies, including exome sequencing (ES), have made sequencing of the human exome (exons of known genes) feasible. This provides the opportunity to detect mutations in MHS genes in a less biased manner. Using this approach, we can improve our understanding of the mutational spectra of the \( \text{Ryr1} \) and \( \text{Cacna1s} \) genes, and estimate their penetrance. Our objective was to identify mutations in \( \text{Ryr1} \) and \( \text{Cacna1s} \) in a population not ascertained for MHS, as a pilot for the use of exome data for predictive medicine.

Materials and Methods

To pilot the identification of MHS in an unselected population (mostly from the metropolitan areas of Washington D.C. and Baltimore in the United States), we evaluated ES data from the ClinSeq\(* \) study\(^9\) (\( n = 870 \))—a longitudinal cohort design to study the technical, medical, and genetic counseling issues associated with medical sequencing on large scale (\( i.e., \) exome or genome sequencing). The ClinSeq\(* \)

study was reviewed and approved by the National Human Genome Research Institute’s Institutional Review Board (Bethesda, MD) and all subjects provided informed consent to publish results and deposit sequence data in databases. Participants were 45–65 yr of age at enrollment with a median age of 57 yr. These volunteers were unselected for MHS because they were ascertained for a spectrum of coronary artery disease, which is not associated with MHS. This sample of 870 participants was 89% Caucasian, 96.3% not of Hispanic or Latino background, and 49.7% female. Details of family history, race, ethnicity, current medical status, and clinical data were collected at enrollment, although a personal or family history of MHS was not specifically solicited. Race and ethnicity were determined by self-report on an intake questionnaire. First-degree relatives of another participant were excluded but one dyad of participants were first cousins and one dyad were first cousins once removed.

During their initial visit, participants underwent an electrocardiogram, echocardiogram, and computed tomography scan for coronary calcium, clinical chemistries, and blood sample collection for genomic analysis. Sequence variants deemed clinically relevant were validated in a Clinical Laboratory Improvement Amendments–certified laboratory and the results returned to the participant.

The sequence data were generated at the National Institutes of Health’s Intramural Sequencing Center. The sequencing method used solution–hybridization exome capture, performed with the SureSelect All Exon System (Version 1.0) from Agilent Technologies (Santa Clara, CA). The sequencing of 101 base-pair (paired-end reads) was performed with the GAIIx sequencer from Illumina, Inc. (San Diego, CA). One or two 101 base-pair, paired-end flow-cell lanes were sufficient to generate more than 85% coverage of the targeted exome with high-quality variant detection.\(^9\) Filters were applied with the VarSifter Next-Gen variation analysis software.\(^10\) DNA isolation, library preparation, capture, sequencing, and alignment and base calling were performed as described.\(^11\)

\( \text{Ryr1} \) and \( \text{Cacna1s} \) variants were filtered for mutation type, frequency, and information in locus-specific mutation databases (LSDBs). The complementary DNA variants and their predicted protein changes are referred to by their protein designations in the text (see variant tables, Supplemental Digital Content 1, http://links.lww.com/ALN/A976, and Supplemental Digital Content 2, http://links.lww.com/ALN/A977, which are tables of the \( \text{Ryr1} \) and \( \text{Cacna1s} \) variants identified in this study, respectively). \( \text{Ryr1} \) nucleotide numbering is based on transcript \( \text{NM}_000540.2 \), and \( \text{Cacna1s} \) \( \text{NM}_000069.2 \), according to the Human Genome Variation Society nomenclature.\(^11\) Variants with low genotype quality were designated class 0; the remainder were scored 1–5 using an adaptation of published criteria.\(^12\–^17\) Briefly, class 1 variants were definitely benign, class 2 probably benign, class 3 of uncertain pathogenicity, class 4 probably pathogenic, and class 5 definitely pathogenic.
Further evaluation of the variants was performed using the Human Gene Mutation Database (HGMD)** and the LSDB, Leiden Open Variation Database (LOVD)*** and for potentially pathogenic variants, review of the medical literature (table 1). We elected not to use amino acid substitution mutation analysis tools because their predictive power is variable.

Medical histories of the probands and their pedigrees were reviewed for diagnoses or symptoms of MHS and related disorders. We learned retrospectively that one participant self-referred to the study because of a clinical diagnosis of MHS (subsequently found to have RYR1 p.Asp3986Glu). Clinically relevant results were returned to participants for management. For the family with a history of MH, we used standard linkage methods, typing short tandem repeat polymorphism markers and polymerase chain reaction amplification and Sanger sequencing of exons not covered by exome data.

Results

The sequencing coverage (defined as the number of coding base-pairs with quality calls/total number of targeted base-pairs) of the coding exons was 83% (RYR1) and 93% (CACNA1S) (fig. 1), and there exists an inherent risk of false negatives. Sequence coverage is dependent on many factors including DNA quality, capture efficiency, percent GC (guanine-cytosine) content, and repeat elements. Our average depth of coverage in the target region for each sample was 89x.

One CACNA1S variant was a false positive, recognized by its marginal most probable genotype score and confirmed by manual review of sequence reads. We identified 123 total variants, 70 in RYR1 and 53 in CACNA1S, among 870 exomes. These variants were identified in 1–419 participants, each. Seventeen of the 122 variants (7 RYR1 and 11 CACNA1S) were excluded because they were too common (fig. 2, and see the Supplemental Digital Content 1, http://links.lww.com/ALN/A976, and Supplemental Digital Content 2, http://links.lww.com/ALN/A977). The National Heart, Lung, and Blood Institute, Exome Variant Server††† frequency threshold was set to 0.5% as this was approximately 10-fold higher than the higher end of the MHS prevalence estimate,1 and the ClinSeq* frequency was set to 1% because it includes approximately 1/10 as many exomes as the Exome Variant Server and therefore chance variation could inadvertently exclude a variant. Our focus was on the identification of highly penetrant alleles that cause autosomal dominant MHS, though we did detect some recessive myopathy alleles.

The remaining 104 variants (63 in RYR1 and 41 in CACNA1S) were considered rare variants. Seventeen of the 63 RYR1 rare variants were listed in the HGMD as “disease-causing” for either MHS, central core disease, multiminicore disease, atypical periodic paralysis, or congenital myopathy. Three of the 63 RYR1 variants were not present in HGMD but were listed in the LSDB as pathogenic. One of the 41 CACNA1S variants (p.Thr1354Ser) was listed in HGMD as pathogenic for MHS, and one (p.Arg498His) was listed in the LSDB as pathogenic but without any supporting evidence. Of the 20 RYR1 variants (present in HGMD or LSDBs, and with an allele frequency <1%), only four met our criteria (table 1) for class 5 pathogenicity; the remaining 16 were scored as a 3 (variants of unknown significance) or class 2 (likely not pathogenic).

Four class 5 RYR1 variants were identified in 870 exomes. The p.Arg614Cys variant was found in one participant and listed in HGMD as pathogenic based on three publications25–27 and reported 37 times in LOVD. All the submitting authors of these entries had concluded that it was pathogenic. This p.Arg614Cys variant is one of the 31 RYR1 mutations on the European Malignant Hyperthermia Group‡‡‡ list of pathogenic mutations and is also included in the 2002 North American MH consensus list of 17 causative mutations.23 We designated this mutation as class 5, pathogenic. It is interesting to note that the 62-yr-old female participant with this variant had no family or personal history of MHS, despite having surgery with general anesthesia thrice.

The second class 5 RYR1 pathogenic variant, p.Arg2241X, was detected in two participants. It was described as pathogenic in HGMD, based on a single patient with congenital myopathy, episodes of generalized, atypical normokalemic paralysis, and multiminicore disease with external ophthalmoplegia and episodes of atypical periodic paralysis.24 The molecular data in this published report were complex. The patient had, in addition to p.Arg2241X, p.Asp708Asn in cis and p.Arg2939Lys in trans to p.Arg2241X with apparent nonsense-mediated messenger RNA decay of the p.Arg2241X-bearing allele. In another study of 37 patients with dominant or recessive RYR1-related myopathies, the p.Arg2241X variant was described in three patients with recessive myopathies and ophthalmoplegias.25 In two siblings, 7 and 5 yr old, the p.Arg2241X variant cooccurred with the previously described putatively pathogenic variant p.Arg109Trp,26,27 and in a third patient the p.Arg2241X variant cooccurred with two missense variants, the putatively pathogenic p.Arg2939Lys27 and p.Asp708Asn (these three variants were likely from the same patient reported in two case series by this same group).24,27 The RYR1 variant p.Arg2241X was also categorized as a pathogenic recessive mutation in a patient with a congenital myopathy and muscle biopsy finding of an RYR1-related myopathy from a study.
Table 1. Variant Pathogenicity Classification System

<table>
<thead>
<tr>
<th>Database Literature Designation</th>
<th>Novel (Not Published)</th>
<th>Published as Pathogenic</th>
<th>Published as VUS</th>
<th>Published as Benign</th>
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<tbody>
<tr>
<td>Mutation Type</td>
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<tr>
<td>Class 5 (pathogenic)</td>
<td></td>
<td>Missense</td>
<td>Nonsense Frameshift Splice</td>
<td>Any</td>
</tr>
<tr>
<td>Similar to disease-causing mutation and consistent family history</td>
<td>On EMHG list of 31 approved diagnostic (causative) mutations, and/or NAMH group’s mutation panel, or two or more reports as pathogenic and no evidence against</td>
<td>On EMHG list of 31 approved diagnostic (causative) mutations, and/or NAMH group’s mutation panel, or single report as pathogenic with supporting evidence</td>
<td>Two or more reports as pathogenic with single evidence against, or single report as pathogenic without supporting evidence</td>
<td></td>
</tr>
</tbody>
</table>

Class 4 (likely pathogenic)      | Similar to disease-causing mutation and inconsistent family history | Two or more reports as pathogenic with single evidence against, or single report as pathogenic without supporting evidence |

Class 3 (uncertain)              | All novel missense or in-frame insertions/deletions without supporting publications | Two or more reports as pathogenic with multiple evidence against, or single report as pathogenic with single evidence against | Reported as VUS (no convincing evidence that they have a causative effect, no evidence to support polymorphism), or single case reported as pathogenic with insufficient supporting evidence |

Class 2 (likely not pathogenic) | No similar disease-causing mutation reported as pathogenic, or inconsistent family history | Single report as pathogenic with multiple evidence against | Single report as benign with insufficient supporting evidence |

Class 1 (not pathogenic)         |                      |                        | Some evidence to support as polymorphism, or multiple evidence against pathogenicity | On EMHG list of 156 nonpathogenic variants, or multiple cases reported as benign with insufficient evidence, or multiple report as benign with supporting evidence |

Variant pathogenicity classification system, criteria for assignment of pathogenicity class 1–5 for MH gene variants. RYR1 and CACNA1S variants were filtered for quality and frequency, and then assigned to pathogenicity classes based on data available in the HGMD, LSDBs, and family history, as well as from the EMHG’s list of diagnostic and nonpathogenic variants and the NAMH mutation panel. Variants that did not pass quality filters were defined as class 0, variants that did not pass frequency filters were defined as class 1, all other variants were assessed according to the criteria presented in the table.

EMHG = European Malignant Hyperthermia Group; HGMD = Human Gene Mutation Database; LSDBs = locus-specific databases; MH = malignant hyperthermia; NAMH = North American Malignant Hyperthermia; NHLBI ESP = National Heart, Lung, and Blood Institutes, exome sequencing project; VUS = variant of unknown significance.
of 71 families with RYR1 mutations. The patient had two additional recessive pathogenic variants, p.Asp708Asn and p.Met485Val, and a synonymous variant of unknown significance c.11547G>A (p(=)). The p.Arg2241X variant was not detected in the Exome Variant Server. We categorized p.Arg2241X as class 5 because it was described in affected patients and of the category of variants (nonsense) strongly predictive of an autosomal recessive RYR1-related myopathy.

The third class 5 RYR1 variant, c.5183C>T; p.Ser1728Phe, was listed in HGMD with references to two studies as pathogenic. We found this variant in a 47-yr-old (Irish/British ancestry) female (1/1,740 alleles) without a personal or family history of MHS. The p.Ser1728Phe variant was reported in three independent families from an analysis of the U.K. MH patients. In a subsequent genotype-phenotype correlation study, the p.Ser1728Phe variant was found in seven individuals and two families—six with a weaker in vitro contraction test phenotype compared with the known pathogenic p.Gly2434Arg mutation, suggesting a lesser effect on channel function as compared with their control. Because the rare p.Ser1728Phe variant (1/10,757 alleles in the Exome Variant Server) was reported multiple times as pathogenic, with no evidence against, it was scored as a class 5, pathogenic variant.

The fourth class 5 RYR1 variant, c.11958C>G; p.Asp3986Glu, was listed in HGMD with references to the same two U.K. studies cited above. The variant was seen in five MH patients with MH disease status and associated with more severe static caffeine contractures and higher creatine kinase levels than the p.Gly2434Arg control or other variants. It was also identified in one 45-yr-old (Irish/German ancestry) male, ClinSeq™ volunteer (1/1,740 alleles) with a history of MH. The volunteer had a history of multiple fulminant MH events—symptoms of myopathy, myotonia (dysphagia), proximal muscle weakness, and a positive in vitro contraction test contact and a serum creatine kinase value of 1,271 U/l and lactate dehydrogenase level of 238 U/l (see participant description table, Supplemental Digital Content 3, http://links.lww.com/ALN/A980, containing the characteristics of the ClinSeq® volunteers with RYR1 and CACNA1S variants, respectively).

One patient was found to have a novel RYR1 missense variant p.Arg3498Gly and a three-generation family history of MHS with an in vitro contraction test diagnostic for MHS. To assess the potential pathogenicity of this variant, we performed a segregation analysis of the variant in the family. The variant did not segregate with the MHS phenotype (see Supplemental Digital Content 6, http://links.lww.com/ALN/A981, a pedigree of the MH family). We ruled out an error in phenotyping, after acquiring muscle biopsy and caffeine halothane contracture test results for seven of the family members from The North American Malignant Hyperthermia Registry. We next performed a candidate linkage analysis of the RYR1 locus. Genotyping and manual haplotyping showed that an RYR1 haplotype cosegregated with the phenotype, but this haplotype was in trans to p.Arg3498Gly. We concluded that p.Arg3498Gly was not pathogenic and hypothesized that this family most likely had MHS attributable to an undetected RYR1 variant in trans to p.Arg3498Gly in the proband. We next evaluated the exon coverage of RYR1 in this proband and found that he had 91.9% sequence coverage. We performed a Sanger sequencing of exons with poor exome sequence read-depth but found no mutations. We concluded that the ES of RYR1 generated both a false-negative and a false-positive result in that the p.Arg3498Gly is not pathogenic and the participant likely has a mutation in RYR1 not captured by ES or Sanger sequencing.

One of the 41 CACNA1S rare variants, p.Arg498His, identified in one exome, was listed in LOVD as pathogenic (it was not listed in HGMD). However, the pathogenicity of this entry was not supported by the primary literature, nor did LOVD provide details of the CACNA1S-associated phenotype. We contacted the LOVD curators and learned that the variant had been recategorized as “unknown pathogenicity,” although the database itself had not been updated. We therefore categorized it as a variant of uncertain significance (score 3).

The CACNA1S variant p.Thr1354Ser was identified in 9 of 870 ClinSeq™ exomes (minor allele frequency 0.7%) and in the Exome Variant Server with an allele count of 48 of 12,958 (minor allele frequency 0.4%). HGMD listed this variant as pathogenic, citing a publication showing segregation of p.Thr1354Ser in one family, its absence in 282...
controls, and functional data demonstrating abnormal Ca\(^{2+}\) flux. However, we concluded that this was more likely a benign variant in linkage disequilibrium with the (undetected) true pathogenic variant in the family described by Pirone et al. Of the remaining 39 CACNA1S rare variants, none were present in either HGMD or the LSDBs. These variants were also assigned to class 3. None of these patients had a personal or family history of MHS (see Supplemental Digital Content 5, http://links.lww.com/ALN/A980, with the characteristics of the ClinSeq\textsuperscript{®} volunteers with CACNA1S variants).

**Discussion**

Four examples of both the power and the limitations of ES for studying MHS were identified in this study. First, we detected a causative (class 5) RYR1 mutation, p.Arg614Cys, in a proband who had no clinical/phenotypic evidence of MHS and a negative family history (see participant description table, Supplemental Digital Content 3, http://links.lww.com/ALN/A978). The p.Arg614Cys variant was included in both the North American MHS and the European Malignant Hyperthermia Group causative mutation lists. We conclude that this represents a presymptomatic diagnosis of MHS in this participant, which is an example of the predictive, personalized genomic medicine in practice. We confirmed this variant in a clinical testing lab, returned it to the participant.
with medical and genetic counseling, and referred her for consideration for caffeine halothane contracture test testing and enrollment in the Malignant Hyperthermia Association of the U.S. registry. Until such testing is performed on the patient or she has a reaction to a triggering agent, we cannot claim to have proven she has MHS. However, because this variant is listed in both the North American MHS and the European Malignant Hyperthermia Group causative mutation lists, we believe that it is extremely unlikely that this variant is benign solely because it was ascertained in context of this study design. Second, the p.Thr1354Ser CACNA1S variant, previously assumed to be pathogenic, was deemed likely to be class 3, that is, of uncertain pathogenicity. The frequency of this single variant was approximately 20 times higher than the frequency of MHS attributed to all loci and all mutations (0.74–1% p.Thr1354Ser heterozygotes).

Although there are good functional data implicating this variant in MHS, we believe that the population genetic data mandate that it should be scored class 3, of unknown pathogenicity. Our findings, supported by the Exome Variant Server CACNA1S allele frequencies, suggest that other previously implicated MHS variants may be benign. Caution is warranted regarding variants claimed to be causative for MHS, especially when used for predictive individualized medicine. Third, we found a novel RYR1 p.Arg3498Gly variant that was not pathogenic in an individual positive for MH by the caffeine halothane contracture test and a family history of MHS. The variant was rare but did not segregate with the phenotype, and this family most likely had MHS attributable to an undetected RYR1 variant, or, less likely, a variant at another locus. We suggest that other previously reported rare RYR1 variants without robust genetic data may

Fig. 3. Frequency histogram of RYR1 variants. Frequency histogram of the 69 RYR1 variants with predicted protein changes from the ClinSeq® 870 cohort. The three RYR1 hotspot regions (Region 1/N-terminal, Region 2/central, and Region 3/C-terminal) are emphasized for purposes of orientation. Blue arrows in the figure point to variants referenced in the text. (ClinSeq® trademark held by National Institutes of Health, Bethesda, MD.)
have been misclassified as pathogenic. Fourth, we identified the class 5 variant, p.Arg2241X, which has been associated with phenotypes inherited in a recessive pattern, but recent publications have questioned the pathogenicity of this variant.15,24–26,28 The risk of MHS in most recessive myopathies is uncertain, and has only been proven for central core disease.31 This example shows that even when one can identify pathogenic variants, it can be challenging to associate them unequivocally with specific phenotypes.

Using ES, we identified 123 distinct variants (70 RYR1 and 53 CACNA1S) among 870 participants (figs. 3 and 4). Our analyses yielded a spectrum of pathogenicity scores from benign to pathogenic (figs. 5 and 6). All but two of the RYR1 variants classified as “disease causing mutations” in HGMD were reclassified by us as benign, probably benign, or variant of unknown significance, scores 1–3. We
reclassified these variants based on the criteria in table 1, under the assumption that a variant was benign, unless a critical review of the data supported a higher pathogenicity category. It is critical to recognize that our assessment of “benign” or “probably benign” is limited to the specific context of using such a variant for individualized predictive medicine and that it is certainly not our intention for it to be interpreted to mean that the variant has no role in the pathogenicity of MHS, myopathy, or other phenotypes. In addition, more than half of the RYR1 variants (43 of 69, 62%) we identified were not listed by HGMD or the LSDB databases, or in biomedical literature citations. Because we screened a cohort unselected for MHS, we predicted that most of the novel variants would be benign. More than half (40 of 69) of the RYR1 variants were rare and not found in the Exome Variant Server. A fifth (10 of 51) of the CACNA1S variants were common polymorphisms, which we assigned to class 1 (benign), with the remaining assigned to class 3 (unknown; fig. 6). Four individuals (three males, one female) had more than one RYR1 variant and two of the four participants with two RYR1 variants had benign CACNA1S variants as well (see Supplemental Digital Content 3, http://links.lww.com/ALN/A978, and Supplemental Digital Content 5, http://links.lww.com/ALN/A980, containing the characteristics of the ClinSeq® volunteers with RYR1 and CACNA1S variants).

The purpose of this study was to identify high-penetrance variants associated with MHS. As noted above, that we conclude a variant is class 1–3 does not automatically mean that the variant has no physiological effects. Moreover, the data did not allow us to evaluate whether interactions could have occurred among variants in a given individual, although this should be specifically addressed in future studies. We deliberately set our threshold for pathogenicity high to avoid the error of wrongly diagnosing an individual as susceptible in an ascertainment mode where the previous probability that they are affected was low. The risk of false negatives in ES will diminish as future ES and follow-up studies generate additional data.

The filtering process for analysis of MHS variants from ES requires a manual method of evaluating variants to extract meaningful information. We used allele frequency, genotype–phenotype databases, and the primary literature to identify pathogenic variants. Unfortunately, there is at present no single information source that allows one to reliably ascertain whether a variant is benign or pathogenic. Many sequence databases (e.g., the Exome Variant Server and The Single Nucleotide Polymorphism Database) include pathogenic, potentially pathogenic and nonpathogenic variants and do not include phenotype data. Furthermore, there is often no indication as to whether some individuals harbor multiple variants within a single gene, which limited our ability to evaluate these data. Our evaluation of 870 exomes using HGMD and LSDBs indicated likely significant levels of misclassification and variability in the pathogenicity determination not only in HGMD and the LSDBs, which is primarily attributable to the source literature.

Exome sequencing has some limitations: the method can miss pathogenic variants such as structural variation, or copy-number variants, in the genome—larger insertions and deletions, duplications, and inversions. Although technology has improved target coverage over the years, it will most likely never reach 100%. In view of the distribution of variants and the complexity of the genome, ES remains an efficient way to identify most mutations altering protein sequence in any single DNA sample. However, to our knowledge, to date the only genomic variants associated with MH are missense variants in coding exons, so most of these limitations do not pertain, given our current knowledge of the disorder.

The published prevalence of MHS mutations varies widely from 1 in 2,0001–3 1 to 1 in 10,0004 but the penetrance has been difficult to determine. Our study of 870 exomes, although it represents a prodigious amount of data, is still too small to estimate the prevalence of MHS. The ES of patients not ascertained for a personal or family history of MHS allows, in principle, an unbiased approach to genotype–phenotype correlation that has not been feasible with previous technologies. We conclude that some RYR1 and CACNA1S variants may have been misclassified as pathogenic without adequate genetic (e.g., cosegregation) or functional data. It is important to stress that in addition to robust genetic analysis, there is a critical need for a robust and noninvasive functional test for MHS, which together with genetic data could allow accurate determination of the prevalence and penetrance of this trait. Presently, ES cannot replace clinical investigations, but rather assists clinicians in determining which patients should undergo further genetic and/or functional analyses. This approach to variant identification in MHS should be extended to other cohorts undergoing ES, and may be useful as a first screening approach, before more invasive and time-consuming investigations. Analysis of thousands of exomes has the potential to provide the MHS field with an exhaustive catalog of variants to determine the true prevalence, penetrance, and expressivity of this life-threatening disorder. Although the assessment of the pathogenicity of both known and novel variants remains challenging, we demonstrate that causative mutations can be identified from ES data. These data suggest that clinically relevant mutations can be identified as incidental findings in exomes sequenced for clinical care and clinical research. This should inform the debate on the return of such secondary results to research participants. Furthermore, the application of ES technology to large and diverse cohorts has the potential to accelerate the pace of MHS gene mutation discovery. We speculate that the results of these studies will allow the development of clinical genomic screening for MHS, which should reduce the incidence of life-threatening events and increase life expectancy for those individuals who harbor pathogenic variants in these genes.
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References
20. A Genomic Approach to Malignant Hyperthermia


Appendix. The NIH Intramural Sequencing Center
Group (5625 Fishers Ln, Rockville, MD 20892-9400)

Center
Director Jim Mullikin, Ph.D.
Deputy director Jim Thomas, Ph.D.
Sequencing group
Group director Robert Blakesley, Ph.D.
Group deputy director Alice Young, B.A.
Robotic specialist Sean Lovett, B.Sc.
Library construction
Coeleader Joel Han, B.Sc.
Coeleader Richelle Legaspi, M.Sc.
Library technician Christina Sison, B.Sc.
Library technician Casandra Montemayor, M.Sc.
Sequence production
Leader Michael Gregory, M.Sc.
Production technician April Hargrove, B.Sc.
Production technician Taccara Johnson, B.Sc.
Production technician Nancy Riebow, B.Sc.
NextGen production
Leader Brian Schmidt, B.Sc.
Sequence finishing
Leader Jyoti Gupta, M.Sc.
Finishing technician Betty Benjamin, B.Sc.
Finishing technician Shelise Brooks, B.Sc.
Finishing technician Holly Coleman, M.Sc.
Finishing technician Shi-ling Ho, B.Sc.
Finishing technician Karen Schandler, M.Sc.
Finishing technician Mal Stantripop, B.Sc.
Instrumentation
Leader Quino Maduro, B.Sc.
Bioinformatics group
Group director Dr. Gerry Bouffard, Ph.D.
Staff bioinformatician Mila Dekhtyar, M.Sc.
Staff bioinformatician Dr. Xiaobin Guan, Ph.D.
Staff bioinformatician Cathy Masiello, M.Sc.
Staff bioinformatician Baishali Maskeri, Ph.D.
Staff bioinformatician Jenny McDowell, Ph.D.
Staff bioinformatician Morgan Park, Ph.D.
Staff bioinformatician Meg Vemulapalli, M.Sc.

NIH = National Institutes of Health.