HHS Public Access

Author manuscript

Anesthesiology. Author manuscript; available in PMC 2019 January 01.

Published in final edited form as:

Anesthesiology. 2018 January ; 128(1): 168–180. doi:10.1097/ALN.0000000000001878.

Malignant Hyperthermia in the Post-Genomics Era: New Perspectives on an Old Concept

Sheila Riazi, MSc, MD[1,2], **Natalia Kraeva, PhD**[2], and **Philip M. Hopkins, MD, FRCA**[3]

[1]Associate Professor of Anesthesia, Director MH Investigation unit, Department of Anesthesia, University of Toronto

[2]MH investigation unit, University of Toronto

[3]Professor of Anaesthesia, University of Leeds, Leeds, United Kingdom

Introduction

Malignant hyperthermia (MH) is a pharmacogenetic disorder of skeletal muscle triggered by volatile anesthetics or succinylcholine. It manifests as a potentially lethal hypermetabolic crisis associated with a rapid and uncontrolled increase in myoplasmic Ca^{2+} in skeletal muscle cells.[1, 2] Advances in anesthesia monitoring and the discovery of the therapeutic efficacy of dantrolene have reduced the mortality and morbidity of MH substantially.[3] However, over the past decade studies have reported evidence that deaths associated with MH still occur, despite treatment.[4–6]

In parallel, our knowledge of the molecular and genetic etiology of MH has been advanced over the last three decades. Three genes, *RYR1*[7, 8], *CACNA1S*[9–11], and *STAC3*[12] have been definitively associated with MH susceptibility and the severe dysregulation of skeletal muscle Ca^{2+} homeostasis that results in the clinical features of an MH reaction under anesthesia. A recent report of a bleeding disorder associated with an *RYR1* variant implicated in MH susceptibility[13] expands the range of clinical defects, already including myopathies[14, 15] and exertional rhabdomyolysis,[16–19] that may be present in MH susceptible individuals. These findings emphasize that the phenotypes associated with genetic defects predisposing to MH are not confined to reactions to volatile anesthetics and imply a common or overlapping pathophysiology of these disorders.

In this review, we summarize the latest evidence on the genetics of MH susceptibility, and its connection to non-anesthesia-related disorders. We review the guidelines for genetic diagnosis of MH susceptibility and discuss the limitations of current genetic screening. We also discuss the non-anesthetic phenotypes associated with *RYR1*-related disorders.

Corresponding Author: Sheila Riazi, MSc, MD, FRCPC, 323-200 Elizabeth St, Toronto, ON, Canada M5G 2C4, Sheila.riazi@uhn.ca.

Authors declared no conflict of interest.

A- MH, a pharmacogenetic disorder

MH susceptibility is commonly stated to be a monogenic disorder with locus and allelic heterogeneity. The prevalence of the genetic trait has been estimated to be between 1:2000 and 1:3000.[20] Interestingly, the combined prevalence in the ExAC Browser database (http://exac.broadinstitute.org/gene/ENSG00000196218 accessed 4.02.2017) of functionally characterized genetic variants that have been associated with MH is 1:2750. These prevalence rates are considerably greater than the reported incidence of clinical MH episodes[21] and the discrepancy is interesting to consider. Monnier *et al*[20] suggested that the penetrance of the MH genetic trait was incomplete, which indeed was the genetic model proposed by Denborough and colleagues when they reported the first case[22]. Incomplete penetrance of a genetic trait implies that the genetic defect either requires additional factors for the phenotype to occur or other factors can prevent the occurrence of the phenotype. It is recognized that the lack of penetrance of the clinical MH phenotype can be for non-genetic reasons, as some people who develop MH are known to have had previous exposure to triggering anesthetics with no apparent problem. Unlike the Australian family reported by Denborough *et al*, however, there are relatively few MH families where the number of clinical episodes is sufficient to draw any inference about the mode of inheritance. In most families, evidence of a dominant pattern of inheritance is derived from results of laboratory testing using pharmacological challenge of excised skeletal muscle strips in an *in vitro* contracture test. These tests are known as the caffeine-halothane contracture test (CHCT) in North America and the *in vitro* contracture test in Europe. However, because these tests are invasive and costly, family studies are limited in some countries, while in others the testing strategy assumes an autosomal dominant pattern of inheritance with an inevitable bias in the resultant family structure.

Lack of penetrance of a genetic trait may also arise for genetic reasons with defects in more than one gene operating together to produce a phenotype or indeed opposing each other to modify or even obscure a phenotype. There is reproducible evidence for the presence of more than one genetic factor influencing the MH susceptibility phenotype,[23] leading Carpenter et al to propose a threshold genetic model for MH susceptibility[24]. Such a model, in which the relatively weak pathogenic effect of the more prevalent MH associated variants is subject to modifying effects of other genetic variants, provides a compelling explanation for the apparent discrepancy between the genetic prevalence and clinical incidence of MH.

RYR1, encoding the ryanodine receptor – Ca^{2+}release channel of skeletal muscle sarcoplasmic reticulum (RyR1), has been established as the major gene implicated in MH. Since the report[7] of the first human MH-associated *RYR1* variant, hundreds of MH probands and thousands of members of their families have been screened for *RYR1* variants and MH associated variants have been found in more than half of the MH families studied from different populations.[8, 25, 26] A small number of MH susceptible families carry a variant in the second MH gene, *CACNA1S*, encoding the alpha-1S subunit of the T-tubular voltage-gated Ca^{2+} channel $Ca_v1.1$, also known as the dihydropyridine receptor.[9–11,27] The alpha-1S subunit is important for the voltage sensing and Ca^{2+} conduction of the dihydropyridine receptor.

Yet, up to 50% of MH probands, who survived an MH event and whose MH susceptibility status was confirmed by a positive *in vitro* contracture test, do not carry any *RYR1* or *CACNA1S* variants and the genetic basis of their MH susceptibility remains unresolved.[21,27,28] Four additional MH loci have been implicated by linkage analysis in several European and North American families but no MH-associated gene has been confirmed within those loci yet.[29] Recently, a homozygous *STAC3* mutation has been linked to Native American Myopathy associated with MH susceptibility in one Native American family.[12] Normal functioning of Stac3 protein, encoded by *STAC3*, is thought to be required for effective co-location of dihydropyridine receptors and RyR1s.

RyR, dihydropyridine receptor and Stac3 protein are all essential components of the skeletal muscle excitation-contraction coupling complex.[30] The underlying mechanism of MH is disruption of excitation-contraction coupling resulting in abnormally enhanced Ca^{2+} release from the sarcoplasmic reticulum via RyR1 in response to either endogenous (e.g., voltage) or exogenous (e.g., halogenated anesthetics) stimuli.

Variants in *RYR1* associated with MH susceptibility are heterozygous missense changes that are shown to impact the RyR1 channel function as gain-of-function mutations, making mutant RyR1 channels more sensitive to activation. Functional analysis of the MH-associated *CACNA1S* variants showed that their effect on excitation-contraction coupling was similar to that shown for *RYR1* mutations, i.e. expression of the mutant alpha-1S in dysgenic myotubes (lacking alpha-1S) resulted in an enhanced sensitivity of RyR1 to stimuli compared with the effect of wild-type alpha-1S.[31] It was suggested that alpha-1S functions as a negative allosteric modulator of RyR1 activation and the *CACNA1S* mutations result in suppression of this negative modulatory effect.[2,31, 32] Currently, more than 200 *RYR1* variants are found in association with MH, but only 35 *RYR1* variants and 2 *CACNA1S* variants are recognized as being sufficiently functionally characterized (www.emhg.org) to be used in diagnostic genetic testing for MH. Although these variants are frequently referred to as causative mutations, we will refer to them as pathogenic variants as the functional studies demonstrate an effect that is qualitatively consistent with our understanding of MH but, in the context of a possible threshold genetic model, do not prove that the effect would translate into a clinical MH reaction. An individual carrying one of the MH pathogenic variants is considered MH susceptible, i.e. at increased risk of developing MH under anesthesia. When a familial pathogenic variant is identified, genetic testing can be extended to family members and all members of the family carrying the variant should be considered MH susceptible.[33] However, MH susceptibility cannot be ruled out for individuals who do not carry the familial variant because of the possibility of more than one pathogenic variant being present in the same family[20,23,34] and they should be offered contracture testing to confirm their MH negative status.[33]

B- New genetic technology and MH

Next-generation sequencing enables fast and cost-efficient sequencing of all protein coding regions – exons – in the human genome. The 1000 Genomes Project (2008–2015)[35] has created the largest public catalogue of human genetic variation through analysis of whole-genome sequencing data of thousands of individuals from multiple populations.

Prevalence of RYR1 and CACNA1S variants in the general population

Analysis of next-generation sequencing variation databases for MH genes by two recent studies has corroborated previous observations of high levels of allelic heterogeneity within *RYR1* and *CACNA1S* compared to other genes of the genome. Based on the Exome Sequencing Project dataset, that includes variation data from 6500 exomes (http://evs.gs.washington.edu/EVS/), it was estimated that *CACNA1S* and *RYR1* are more genetically diverse than the average gene in either African-American or in European-American populations, i.e. both these genes have a high level of natural variation.[36] Comparably high levels of variation in both *RYR1* and *CACNA1S* were detected by a second study where the authors evaluated exome sequencing data from the ClinSeq dataset on a cohort of 870 volunteers not selected for MH susceptibility.[37] Furthermore, using *RYR1* and *CACNA1S* variation data for this unselected cohort, the authors assessed the prevalence of MH susceptibility in the general population. Based on an allele frequency of less than 1%, genotype–phenotype data, and the primary literature, they found that only 19% of the *RYR1* variants identified in this cohort unselected for MH were probably benign whereas 6% were pathogenic, and 75% were variants of unknown significance. Of the pathogenic *RYR1* variants, three have been previously reported in association with MH: such a high prevalence of MH-associated *RYR1* variants has not been replicated in the much larger sample presented in the ExAC Browser. For *CACNA1S*, 20% of the variants were defined as benign and 80% as variants of unknown significance.

These studies showed that a large fraction of the *RYR1* variants found in databases were rare, with a frequency of 0.00001 or less. It is noteworthy that an abundance of rare variants is a feature common to other genetic disorders. The exome sequencing of thousands of patients with monogenic disorders has revealed that about 80% of the identified variants are unique, seen only in one proband and 96% of the variants were found three times or less.[38] Similar to variation data for *RYR1* and *CACNA1S*, variants of unknown significance comprised about 70% of the variants identified in Mendelian genes.

Search for MH-associated novel genes and variants using next generation sequencing

When applied to studies of rare monogenic or oligogenic diseases, whole exome sequencing allows unbiased, not based on any *a priori* hypothesis, screening of the coding sequence of all of a patient's genes. On the other hand, whole exome sequencing generates a large number of variants in multiple genes whose relevance to a specific phenotype is often difficult to ascertain. At present, the use of next-generation sequencing -based targeted sequencing of a restricted panel of genes associated with a disease phenotype seems to be a more practical approach. Targeted sequencing has a higher coverage (up to 99%) and accuracy and, therefore, higher sensitivity than whole exome sequencing. Targeted sequencing panels can be also supplemented with Sanger sequencing for regions poorly covered by next-generation sequencing. This gives an added level of coverage and reduces the potential for false negative results.

Targeted sequencing of panels of genes implicated in excitation-contraction coupling, alongside whole exome sequencing, holds great promise for finding a genetic cause of MH in cases where no *RYR1* and *CACNA1S* variants were found. Four recently published

studies used next-generation sequencing -based whole exome sequencing and targeted sequencing of panels of genes with a potential involvement in excitation-contraction coupling, skeletal muscle calcium homeostasis, or immune response as well as targeted *RYR1* and *CACNA1S* gene sequencing to search for MH associated variants and novel MH genes in cohorts of unrelated MH susceptible patients.[34,36,39,40] However, these first studies using next-generation sequencing for identification of MH-associated variants did not result in the discovery of novel candidate genes. They confirmed the findings of previous studies, where Sanger sequencing was used for MH variant screening, namely, that variants in *RYR1* and, to a lesser degree in *CACNA1S*, are associated with MH in the majority of MH cases. Known MH pathogenic variants comprised about 30% of the identified variants and the remaining variants were variants of unknown significance. These studies also found that up to 50% of MH susceptible individuals do not carry potentially pathogenic variants in either MH gene, corroborating previous evidence.

Rare variants in several additional genes (*CACNB1, CASQ1, SERCA1, CASQ2, KCNA1*) encoding proteins involved in calcium homeostasis in skeletal muscle have been identified using next-generation sequencing [40] and Sanger sequencing.[41,42] However, these variants will remain variants of unknown significance until functional assays are developed to validate their role in MH susceptibility.

Current approaches to characterization of potentially pathogenic RYR1 and CACNA1S variants

To increase the sensitivity and specificity of genetic testing for MH and expand the number of pathogenic variants that can be used in clinical genetic testing, all MH-associated variants have to be validated at the genetic level as well as functionally.[33] Advances in our current knowledge about genetic variation within MH genes have prompted changes in the approach to genetic characterization of the variants. Two approaches, genetic and functional characterization, are used in combination to assess the pathogenic effect of novel variants.

Genetic characterization

Today, instead of genotyping hundreds of control individuals to exclude common variants or neutral polymorphisms, estimation of the variant allele population frequency can be done by searching publicly available variation databases containing data from sequencing more than 67,000 of human exomes. [e.g., the Short Genetic Variations database (http://www.ncbi.nlm.nih.gov/SNP), the 1000 Genomes project (http://browser.1000genomes.org), NHLBI Grand Opportunity Exome Sequencing Project (https://esp.gs.washington.edu/drupal/), exome variant server (http://evs.gs.washington.edu/EVS/), the Exome Aggregation Consortium Browser (http://exac.broadinstitute.org)]. Pathogenic variants are likely to have a minor allele frequency not higher than 0.001.[34] However, since the majority of identified variants of unknown significance in *RYR1* are rare and have frequencies lower than 0.001, a low frequency of a variant cannot serve as a predictor of its pathogenicity.

The effect of a variant on protein function and/or stability can be assessed using bioinformatics prediction software tools, such as SIFT,[43] PolyPhen-2[44] and CADD.[45] These prediction tools use protein sequence information and annotations to protein functional

domains to compute predictions with relatively low false-positive and false-negative error rates.[46] However, such predictions should be taken with caution. Different prediction tools use different prediction algorithms and different input datasets (disease-associated mutation sets and neutral variation sets identified in the same protein and available from variation databases, such as the Human Gene Mutation database, Online Mendelian Inheritance in Man database and the Short Genetic Variations database), and their predictions might be discordant. Besides, the bioinformatics tools are based on imperfect algorithms and on imperfect databases.[47] A recent study compared the predicted and actual consequences of missense mutations and found that half of the *de novo* or low-frequency missense mutations found by genome sequencing and inferred as deleterious, correspond to nearly neutral variants that have little impact on the clinical phenotype of individual cases.[48] Similarly, a significant proportion of *RYR1* sequence variants in the human gene mutation database classified as "disease-causing mutations" was found to be benign, probably benign, or as being of unknown pathogenicity.[37] The sensitivity of commonly used bioinformatics prediction tools for *RYR1* and *CACNA1S* has been estimated[46] at 84%–100% with specificity of 25%–83%. Therefore, other approaches such as segregation analysis and functional studies are necessary to accurately differentiate clinically relevant variants from neutral variants.

Another challenge in genetic characterization of a variant of unknown significance is the small size of the families. It is not always possible to perform a meaningful analysis of segregation of the variant with the disease (MHS phenotype) and to generate sufficient statistical power even when combining data from several families carrying the same variant.[33,34]

Functional characterization

Functional characterization of candidate *RYR1* variants remains a key component of their validation. MH-associated *RYR1* variants are dominant gain-of function variants. They render the RyR1 channels hypersensitive to depolarization and pharmacological agonists or lead to greater depolarization-induced Ca^{2+} influx into the muscle cell.[2, 32] The effect of each MH candidate variant on RyR1 function should be assayed in one of the recombinant *in vitro* expression systems, HEK293 cells or myotubes of the dyspedic/dysgenic mouse (*RYR1/CACNA1S* knock-out).[49,50] These systems use expression of a rabbit or human *RYR1* cDNA construct with incorporated variants and measure the properties of expressed channels. The advantage of *in vitro* systems is the defined cDNA and the standardized genetic background of the recipient cell line. In view of a large number of private familial variants found to date, the revised European Malignant Hyperthermia Group guidelines have removed the need for mandatory description of the variant in more than one family, if functional characterization is done using the more rigorous genetic manipulation of heterologous or homologous expression systems.[33]

Systems using *ex vivo* expression utilize tissues from MHS patients with characterized *RYR1* variants such as myotubes, microsomal sarcoplasmic reticulum preparations from muscle biopsies, or lymphoblasts.[33] Assays of RyR1 function in *ex vivo* systems are controversial, because they assume that the identified gene variant is the only variant

present, when this may not be the case. The compromise presented in the European Malignant Hyperthermia Group guideline is the stipulation that *ex vivo* analyses should be done on samples from at least two unrelated patients with the same variant to reduce the likelihood of confounding genetic factors.

The first knock-in mouse models of MH carrying *RYR1* variants analogous to the MH pathogenic human variants Y522S[51] and R163C[52] and a mouse model of central core disease carrying an equivalent of the human uncoupling central core disease mutation I4898T[53,54] allowed *in vitro* and *in vivo* functional studies of these mutations in fully differentiated adult muscle fibers. However, generation of mouse models for validation of each of more than 150 MH associated variants is not realistic. To circumvent this obstacle, a promising novel approach has been developed to study the function of RyR1 mutant channels.[55] Using localized *in vivo* electroporation, Lefebvre and colleagues have expressed EGFP-RyR1(RyR1 N-terminally tagged with Enhanced Green Fluorescent Protein) constructs carrying MH variants in fully differentiated normal mouse muscle fibers and found that the results were consistent with those obtained for MH variants in previous studies.[55] They showed that expression of the RyR1 channels carrying MH mutations, Tyr523Ser, Arg615Cys or Arg2163His, was associated with an increased Ca^{2+} release in response to depolarization, whereas expression of the central core disease mutant, Ile4897Thr, resulted in a reduction of Ca^{2+} release compared to non-expressing regions of the same muscle cell. These results indicate that *in vivo* expression in adult mouse muscles might serve as a novel technique for assessment of functional properties of mutant RyR1s.

Some of the *RYR1* variants have been already functionally and genetically characterized and found to be likely or very likely pathogenic. The revised European Malignant Hyperthermia Group guidelines recommend an individual carrying a potentially MHS-associated variant to be considered as being at risk for MH until contracture testing can be performed.

Cryo-electron microscopy and X-ray crystallography contribution to functional assessment of RyR1 variants

Recent determination of crystal structures for the N-terminal domains together with the development of cryo-EM maps of the full-length RyR at nanometer resolution allowed elucidation of the three-dimensional architecture and domain organization of RyR1 and facilitated modeling interactions between its N-terminal, central and C-terminal domains. These studies revealed how small conformational changes in the cytoplasmic domains, induced by the binding of RyR regulators, are transmitted to the C-terminal domain regulating the channel opening.[56–58] They showed that RyR1 channel opening coincides with subtle changes in the cytoplasmic domain that affect interfaces between individual RyR1 subunits. Mapping the MH/central core disease variant hot spots and individual disease related variants onto the high resolution structure of RyR1 domains helped reveal mechanisms by which disease related *RYR1* variants might disrupt RyR1 function.[59,60] Since most of the N-terminal MH-associated variants are mapped onto the interfaces between N-terminal domains or at interfaces between subunits in the tetrameric channel it is probable that those variants weaken the interdomain interactions, thus lowering the energetic barrier to channel opening.[61–63]

The high resolution (near 3Å) cryo-EM images of the transmembrane region that contains the ion conducting pore revealed the presence of the 6 membrane-spanning helices (S1–S6) of each RyR1subunit and allowed mapping of the majority of central core disease-associated variants to the pore forming domain[64,65] (Figure 1).

Ramachandran and colleagues[65] by using homology modeling and high resolution cryo-EM data, succeeded in identification a novel interface between the pore-lining helix (amino acids 4912-4948) and a S4-S5 linker helix (amino acids 4830-4841) and showed that this interface controls RyR gating. They built structural models for the RyR1 membrane-spanning domains based on the alignment between RyR1 and two other ion channels with known crystal structures, then docked the structural models onto the cryo-map and showed the close fit between them, indicating that their structural model is suited to model interactions involved in RyR1 gating. Using the models of membrane-spanning domains in the open and closed state, they showed the S4-S5 linker helix interacts with the S6 helix and thus plays a role in gating. Based on this model they computationally predicted the effect of several variants within S4-S5 linker on RyR1 gating. They further expressed in HEK-293 cells recombinant wild-type and mutant RyR1 channels carrying the same variants and used single channel experiments to characterize the channels. The effect of each of the variants on channel gating (activating for some and deactivating for other variants) was similar to that predicted by their structural models, thus confirming the role of the S4-S5 linker helix in RyR1 gating.

Generation of structural models of the open and closed states of RyR1 facilitated comparison of computational impact predictions of C-terminal variants with the results of single channel experiments and identification of amino acid residues in the predicted pore-lining helix and a linker helix that are important for channel gating.[65,66]

Future studies will further refine the three-dimensional RyR1 structure and elucidate the complex molecular mechanisms involved in RyR1 channel regulation and function, thus allowing a more reliable computational prediction of the functional impact of newly discovered variants.

Incidental findings

Whole exome sequencing generates a large number of variants in multiple genes, and some of those variants might be of clinical relevance to a condition that is different from the original clinical condition for which whole exome sequencing was offered. Such variants are called incidental findings. The American College of Medical Genetics and Genomics (ACMG) has included MH genes *RYR1* and *CACNA1S* among the list of clinically relevant genes, whose potentially pathogenic variants should be reported as incidental findings.[67] Reporting of incidental findings, however, has created clinical and ethical challenges.[47,67–69] The ACMG guidelines on reporting the incidental findings emphasize the need for accurate assessment of clinical and research evidence supporting a variant's pathogenicity before reporting it to a patient. The guidelines also caution against excessive reliance on *in silico* predictions of pathogenicity in the diagnostic context. In light of the prevalence of rare variants in *RYR1* and *CACNA1S* and the difficulty in assessing their pathogenicity it is likely that a significant number of patients undergoing whole exome sequencing for non-MH

indications will be labelled as potentially susceptible to MH when only a small minority will be at risk.

C- RYR1-related disorders

Besides MH, variants in *RYR1* have been previously associated with several other skeletal muscle conditions and congenital myopathies, namely, central core disease, multiminicore disease, congenital myopathy with central or internalized nuclei and congenital fiber-type disproportion.[14,15,70,71] To this list, King–Denborough syndrome,[72] benign Samaritan congenital myopathy,[73] heat/exercise-induced exertional rhabdomyolysis,[16, 74] atypical periodic paralysis,[75] and statin myopathies[76,77] were recently added.

RYR1-related congenital myopathies show both dominant (central core disease) and recessive (multiminicore disease, centronuclear myopathy, congenital fiber-type disproportion) modes of inheritance. Moreover, some *RYR1* variants may act as dominant with regard to the MH phenotype but as recessive with regard to the congenital myopathy phenotype.[78, 79]

These myopathies present a challenge for clinical molecular diagnosis due to their strong phenotypic and genetic heterogeneity. A recent study applied an integrated strategy combining whole exome sequencing with clinical and histopathological investigations to reach an accurate diagnosis for several patients with congenital myopathies. Different sets of recessive *RYR1* variants were found in 4 patients, whose phenotypes ranged from a severe lethal neonatal myopathy to a mild adult-onset muscle weakness, underscoring the phenotypic variability of *RYR1*–related disorders.[80]

Next-generation sequencing panel-based analysis of neonatal hypotonia in a Chinese cohort found several *RYR1* variants in this genetically heterogeneous condition, although in the majority of cases these were heterozygous changes involving variants for which a dominant pathogenic effect is not established.[81]

In another study, whole exome sequencing allowed identification of a *de novo RYR1* variant in a patient who was originally diagnosed with limb girdle muscular dystrophy on the basis of clinical and histological presentations: the histological features were in fact myopathic rather than dystrophic, emphasizing the importance of establishing a genetic diagnosis in order to exclude an *RYR1* etiology.[82]

The spectrum of *RYR1*-related diseases was expanded further to include a myasthenic-like component of muscle weakness with partial response to pyridostigimine: direct sequencing of the *RYR1* gene in this case revealed compound heterozygous RYR1 variants: c.6721C>T (p.Arg2241X) nonsense variant and novel c.8888T>C (p.Leu2963Pro) missense variant.[83]

The role of RyR1 is not limited to skeletal muscle: a mouse model of central core disease, homozygous for a dominant *RYR1* variant that causes a loss of function of the RyR1 channel, exhibited embryonic developmental delay and neonatal lethality with multisystem developmental defects, including atrial septal defect: it was hypothesized that RyR1 plays an important role in early cardiac development.[53] In favor of this hypothesis, exome sequencing

revealed two rare, potentially deleterious missense *RYR1* variants in a patient with atrioventricular septal defect who had no potentially pathogenic variants in other candidate genes.[84]

RYR1 variants have been previously associated with fetal akinesia.[70] Several recent studies used whole exome sequencing to expand the phenotypes associated with recessive *RYR1* variants to include arthrogryposis multiplex congenital fetal akinesia,[85,86] and lethal multiple pterygium syndrome.[87] Lethal multiple pterygium syndrome is a fatal disorder associated with prenatal growth failure with pterygium present in multiple areas, akinesia, and severe arthrogryposis. Lethal multiple pterygium syndrome has been associated with variants in genes encoding components of the neuromuscular junction. Identification of *RYR1* variants in fetuses affected by lethal multiple pterygium syndrome, together with variants in genes encoding proteins at the neuromuscular junction (*CHRNA1, CHRND, CHRNG* and *RAPSN*), might indicate that lethal multiple pterygium syndrome is caused by defects in the excitation-contraction coupling mechanism.[87] Interestingly, lethal multiple pterygium syndrome in association with MH has been described before.[88]

Whole exome sequencing analysis revealed the first case of severe congenital myopathy with ophthalmoplegia caused by a variant in the *CACNA1S* gene,[89] pathogenic variants in which have been associated with hypokalemic periodic paralysis type 1.[90,91] The authors hypothesize that the p.Gln1265His variant results in disruption of coupling between dihydropyridine receptor and RyR1, causing *CACNA1S*- related myopathy. Interestingly, another patient from this study with similar myopathic symptoms was found to carry an in-frame insertion in *RYR1*.[89] The authors hypothesized that since this variant showed dominant inheritance, it likely had a dominant negative effect on RyR1 tetramer formation and function.

Whole exome sequencing of patients presenting with severe congenital ophthalmoplegia and facial weakness in association with malignant hyperthermia revealed the presence of missense variants resulting in two homozygous *RYR1* amino acid substitutions and two compound heterozygous *RYR1* substitutions in a consanguineous and a non-consanguineous pedigree, respectively.[92] While ophthalmoplegia may occur in *RYR1*-related myopathies, these children were atypical because they lacked significant muscle weakness, respiratory insufficiency, or scoliosis. The common *RYR1* variant in these cases, p.R3772Q, was previously reported to be associated with MH susceptibility in the heterozygous state and MH susceptibility with myopathy in the homozygous state.[79]

Another interesting case is of congenital ptosis, scoliosis, and MH susceptibility in siblings who are homozygous for the MH-pathogenic *RYR1* variant, p.T2206M.[93] This variant in heterozygous carriers was previously reported in association with mild clinical and histopathological features.[94] The last two studies emphasize the notion that *RYR1*-associated myopathies should be included in the differential diagnosis of congenital ptosis with scoliosis, and of congenital ophthalmoplegia and facial weakness without scoliosis, especially because a risk of MH can be high in these patients.

This wide spectrum of clinico-pathological conditions reflects the distinct effects of different *RYR1* variants on skeletal muscle Ca^{2+} homeostasis and excitation-contraction coupling.[95] Functional studies showed that different central core disease variants exhibited varying degrees of excitation-contraction uncoupling with impaired Ca^{2+} release. Certain dominant variants displayed dual functional characteristics accounting for both, MH (hypersensitivity to voltage activation and to agonists) and myopathy (reduced SR Ca^{2+} content and voltage-gated Ca^{2+} release) phenotypes.[96] Additionally, some recessive *RYR1* variants led to a reduction in RyR1 protein levels.[2, 32]

Complexity of functional effects of *RYR1* variants together with clinical overlap between different *RYR1*-related myopathies complicates MH susceptibility counseling in patients with *RYR1*- related myopathies. Certainly, patients with myopathies carrying MH-associated *RYR1*variants as well as potentially pathogenic variants of unknown significance should avoid triggering anesthetics. However, patients carrying uncoupling, loss-of-function *RYR1* variants may be considered as being at a low risk of developing MH.

Counseling in *RYR1*- related myopathic patients as for MH susceptibility requires a combined approach, integrating clinical, histopathological, *in vitro* contracture testing, MRI and genetic findings.[3]

RYR1 in non-skeletal muscle cells

Lopez and colleagues reported that some MH susceptible patients, carrying specific gain-of-function *RYR1* variants, give a history of mild bleeding abnormalities.[13] They demonstrated that $RyR1^{Y522S}$ mice carrying the MH gain-of-function variant had abnormalities of vascular smooth muscle cell Ca^{2+} homeostasis consistent with a bleeding phenotype.[13] Indeed, although RyR1 is predominantly found in skeletal muscle, it is also present at lower levels in immune and smooth muscle cells. The study found that primary vascular smooth muscle cells from $RyR1^{Y522S}$ mice had an increased frequency of Ca^{2+} spark events and were significantly more hyperpolarized than those from wild-type mice. In contrast to skeletal muscle cells where gain-of function *RYR1* variants led to an increased sensitivity to activating stimuli and to sustained muscle contractions, primary vascular smooth muscle cells from $RyR1^{Y522S}$ mice showed a decreased Ca^{2+} influx through the dihydropyridine receptor and smooth muscle relaxation, causing prolonged rather than shorter bleeding times. Administration of the specific RyR1 antagonist dantrolene, which is clinically approved for the treatment of MH reactions, reversed the bleeding phenotype by decreasing spark activity in murine vascular smooth muscle cells. Thus, this study suggested that *RYR1* variants may be responsible for certain cases of mild bleeding abnormalities. If the clinical findings of Lopez *et al* of prolonged bleeding in MH patients carrying *RYR1* variants are confirmed, their animal studies offer a pathological mechanism and indicate a potential therapeutic use of dantrolene for such cases.

D- MH, a metabolic disorder

Since RyR1 plays an essential role in maintenance of Ca^{2+} homeostasis and in excitation-contraction coupling in skeletal muscle cells[2], MH susceptible individuals carrying *RYR1* variants may have skeletal muscle metabolism abnormalities even in the absence of

triggering anesthetics.[97,98] Studies on animal models of MH[51,99,100] have shown that MH-associated *RYR1* variants result in a significant increase in mitochondrial matrix Ca^{2+}, increased reactive oxygen species (ROS) production, and lower expression of mitochondrial proteins, which in conjunction with lower myoglobin and glycogen contents and lower glucose utilization suggested a compromised bioenergetics state. Furthermore, the elevation in resting myoplasmic Ca^{2+} may lead to an enhanced oxidation of RyR1 which in turn may increase open channel probability, enhanced Ca^{2+}-induced Ca^{2+} release,[101] thus increasing muscle sensitivity to heat and other stimuli.[102] Such studies might explain the connection between MH and exertional rhabdomyolysis (ER) and/or exercise-related or exertional heat illness (EHI). The most severe form of EHI, exertional heat stroke (EHS), is characterized by a rapid increase in body temperature and neurological impairment, with rhabdomyolysis as a common feature.[75,103] It occurs during sustained exercise frequently in physically fit young adults and children, especially under hot or/and humid ambient conditions but it may occur in temperate climates.[16, 34,104]

Exertional rhabdomyolysis (ER) often presents with severe muscle pain and is diagnosed by elevated serum CK levels five times higher than the upper limit of normal values.[16,103] ER is one of the frequent signs of EHI but often does not involve a drastic increase in body temperature. EHS and MH share clinical features such as hyperthermia, muscle rigidity, tachycardia, tachypnea, elevated serum creatine kinase and disseminated intravascular coagulation; skeletal muscle breakdown may cause hyperkalemia, myoglobinuria and acute kidney injury. On the basis of abnormal *in vitro* contracture test results in survivors of EHS and their first degree relatives, it was postulated that there may be a familial skeletal muscle abnormality in some EHS patients similar to that in MH, i.e. uncontrolled increase in intracellular calcium and hypermetabolism.[105]

Dantrolene, the only drug available for treating MH, has been shown to reduce clinical symptoms in patients with ER[74] supporting a notion of a common pathological pathway of these potentially fatal conditions.

Recurrent ER as an inherited condition has been linked to defects in the genes known to be associated with a number of neuromuscular disorders, such as metabolic myopathies and muscular dystrophies, many of which are autosomal-recessive or X-linked.[106,107]

Recent genetic studies have suggested that *RYR1* variants may be implicated in EHI/ER.[16, 17] Moreover, identification of MH-associated *RYR1* variants in up to 30% of cases of recurrent ER[16, 98,108–110] has strengthened the possibility of a link between MH and EHI/ER. This possible link, important to MH researchers, clinicians and, especially, patients with MHS and EHI, has been discussed in several publications.[16, 103,111] We have considered two questions in summarizing the clinical and genetic evidence regarding the relationship between MH and EHI/ER.

The first question: Are MH susceptible patients at a higher risk of EHI/ER?

Based on the clinical data available on MH susceptible patients, there seems to be no strong correlation between MH susceptibility and predisposition to EHI. There are numerous MH families with at least one family member who survived an MH crisis under general

anesthesia. Hundreds of individuals from these families have been diagnosed as MH susceptible by the *in vitro* contracture testing and additionally have been found to carry one of the known MH pathogenic variants.[112] These MH susceptible individuals show no apparent predisposition to EHI and are reported to be clinically healthy.[2, 8] Moreover, none of the MH susceptible individuals who are homozygous for MH pathogenic variants – p.R614C, the analog of the p.R615C porcine stress syndrome mutation,[20,113–115] and p.C35R,[116] nor individuals who are compound heterozygous for two MH variants have been reported to present with any clinical symptoms suggestive of EHI.[8,20,26,115] Indeed, there have been only a few documented cases of patients with a previous personal or family history of MH, who later in life experienced an EHS/EHI episode.[103,109,117,118] There is perhaps a greater risk of MH susceptible individuals developing ER than EHI. In addition to reported cases[17] the authors have been contacted by several patients tested MH susceptible in their units who have developed ER. Nevertheless, these observations do not support a notion of all MH susceptible patients being at an increased risk of EHI/ER.

Second question: Are EHI/ER patients at risk of MH?

A notion that EHI patients may be predisposed to MH mostly stems from the reports of positive *in vitro* contracture testing results, i.e. MHS diagnoses in a substantial number of EHI patients.[18,34,98,105,108,119–121]

It is important to emphasize that the *in vitro* contracture tests have been validated only for patients with a suspected anesthetic-induced MH reaction, and the sensitivity and specificity of the *in vitro* contracture testing in patients with EHI/ER are unknown. Patients with certain myopathies, such as muscular dystrophies and muscle channelopathies, may have abnormal *in vitro* contracture test results due to their persistent muscle cell abnormalities not necessarily related to MH.[122–124]

The genetic connection between EHI/ER and MH also remains inconclusive.[17,19,34,97,120] An increasing number of reported EHI/ER cases with MH-associated *RYR1* variants favor this connection and, undoubtedly, EHI/ER patients carrying MH associated variants should be considered MH susceptible until demonstrated otherwise. However, in more than 70% of the EHI/ER patients the identified *RYR1* variants are rare variants of unknown functional significance.[16,34,110] The relevance of these variants of unknown significance to either MH susceptibility or EHI/ER remains unclear, especially since some rare, potentially deleterious variants of unknown significance have been identified in EHI patients who have had normal *in vitro* contracture responses, while others are found in EHI patients with abnormal *in vitro* contracture responses. Remarkably, among the numerous EHI/ER cases with identified *RYR1* variants there have been no reports of personal or familial history of MH; only one episode of MH in a patient who had a previous ER event has been reported so far.[74]

Thus, although current studies cannot rule out a possible connection between MH and EHI/ER, the extent of the overlap between EHI/ER and MH remains unknown. Both conditions have a complex etiology and in the majority of cases probably result from the interplay between genetic and environmental factors and indeed, in some cases, possibly multiple genetic factors. Importantly, in patients with ER or EHI differential diagnoses, such

as muscular dystrophies, metabolic and mitochondrial disorders, should be considered. To make an efficient and definitive diagnosis in a clinically and genetically heterogeneous condition, such as ER, targeted parallel sequencing of a panel of candidate genes using next-generation sequencing seems to be an especially appropriate approach. Currently some laboratories offer screening of panels of genes using next-generation sequencing technologies for patients with myopathy/rhabdomyolysis, among those in North America are Baylor Miraca Genetics Laboratories, Baylor College of Medicine (http://bmgl.com/) and Fulgent Therapeutics LLC (https://fulgentgenetics.com/).

The existence of a possible link between ER and disorders of muscle calcium metabolism warrant an expansion of the next-generation sequencing gene panel for myopathy/rhabdomyolysis to include the genes involved in excitation-contracture coupling, such as *RYR1* and *CACNA1S*. Indeed, several centers have already included the *RYR1* gene in their expanded panels, e.g. Next-generation sequencing Rhabdomyolysis and Metabolic Myopathies Panel (the Greenwood Genetic Center Diagnostic Laboratories http://www.ggc.org/diagnostic.html) and Metabolic Myopathy and Rhabdomyolysis Panel (Blueprint Genetics http://blueprintgenetics.com).

E- Summary and Implication for Anesthesiologists

With the discovery of genes associated with MH (*RYR1, CACNA1S*, and *STAC3)* and developments in genetic screening tools, such as next-generation sequencing, our understanding of the genetics of MH has improved dramatically. The cost-effectiveness of next-generation sequencing has enabled genetic testing for MH susceptibility to be a viable first-line diagnostic test for patients suspected of having an MH reaction under anesthesia and for relatives of known MH cases in many countries.

However, initial experience with next-generation sequencing -based whole exome sequencing studies has not brought about an anticipated increase in the sensitivity and specificity of MH genetic testing. Whole exome sequencing applied to MH cohorts has revealed that up to 50% of MH susceptible individuals do not carry potentially pathogenic variants in known MH-associated genes, corroborating previous evidence for the genetic heterogeneity of MH susceptibility. Additionally, due to the possible presence of more than one pathogenic variant in the same family, MH susceptibility cannot be ruled out for individuals who do not carry a familial variant, leaving muscle contracture testing as the only reliable diagnostic test for MH susceptibility for such patients. The specificity of genetic testing (true negative rate) is limited by the fact that out of more than 200 *RYR1* variants identified in MH families, only 35 *RYR1* and 2 *CACNA1S* variants are sufficiently characterized to be regarded as pathogenic for MH. The majority of newly identified *RYR1* and *CACNA1S* variants remain to be functionally evaluated as to their role in MH. Individuals carrying variants of unknown significance in these genes should therefore be considered as being at risk of developing MH under anesthesia and should be offered contracture testing to ascertain their MH status.

The relatively low cost of whole exome sequencing, the prevalence of rare variants of unknown significance in *RYR1* and *CACNA1S*, and the designation of these variants of

unknown significance as reportable incidental findings has already led to many people under investigation for other diseases or even those just curious about their genetic heritage, being labeled as potentially at risk of developing MH. Such labeling is not risk-neutral for anesthesia and can also have implications for the individuals and their family with respect to eligibility for certain occupations, ease of access to insurance policies and concerns regarding some overseas travel. We would therefore question the ethics of offering sequencing of *RYR1* and *CACNA1S* to individuals at low *a priori* risk without appropriate counseling and funded access to definitive *in vitro* contracture testing for MH susceptibility diagnosis should variants of unknown significance be found.

The phenotypic variability exposed in recent studies of *RYR1*-related disorders has taught us that abnormalities in this gene may confer not only a potentially life-threatening reaction to anesthesia, but may predispose individuals to myopathies, metabolic derangements, EHI/ER and even possibly bleeding disorders. Specifically, anesthesiologists should insist on a genetic work-up for *RYR1* variants in patients with a previous history of recurrent rhabdomyolysis or those with congenital myopathies without a genetic diagnosis, before administration of triggering anesthetics. Such patients in whom a *RYR1* variant is found or indeed patients with a known *RYR1*- related myopathy should be referred to a specialized MH center for assessment of their MH risk and advice on further investigation.

Acknowledgments

The authors are grateful to Dr. Alexander Kraev for his assistance in the design of the figure.

This work is supported by Merit award to Sheila Riazi from Department of Anesthesia, University of Toronto, Canada and a grant from the National Institutes of Health (NIH): National Institute of Arthritis, Musculoskeletal and Skin Diseases (P01 AR052354) to Dr. Hopkins

References

1. MacLennan DH, Phillips MS. Malignant hyperthermia. Science. 1992; 256:789–94. [PubMed: 1589759]
2. Maclennan DH, Zvaritch E. Mechanistic models for muscle diseases and disorders originating in the sarcoplasmic reticulum. Biochim Biophys Acta. 2011; 1813:948–64. [PubMed: 21118704]
3. Riazi S, Kraeva N, Muldoon SM, Dowling J, Ho C, Petre MA, Parness J, Dirksen RT, Rosenberg H. Malignant hyperthermia and the clinical significance of type-1 ryanodine receptor gene (RYR1) variants: Proceedings of the 2013 MHAUS scientific conference. Can J Anaesth. 2014; 6:1040–9.
4. Rosero EB, Adesanya AO, Timaran CH, Joshi GP. Trends and outcomes of malignant hyperthermia in the United States, 2000 to 2005. Anesthesiology. 2009; 110:89–94. [PubMed: 19104175]
5. Larach MG, Brandom BW, Allen GC, Gronert BA, Lehman EB. Cardiac arrests and deaths associated with malignant hyperthermia in North America from 1987 to 2006: A report from The North American Malignant Hyperthermia Registry of the United States. Anesthesiology. 2008; 108:603–11. [PubMed: 18362591]
6. Larach MG, Brandom BW, Allen GC, Gronert GA, Lehman EB. Malignant hyperthermia deaths related to inadequate temperature monitoring, 2007–2012: A report from the North American malignant hyperthermia registry of the malignant hyperthermia association of the United States. Anesth Analg. 2014; 119:1359–66. [PubMed: 25268394]
7. Gillard EF, Otsu K, Fujii J, Khanna VK, de Leon S, Derdemezi J, Britt BA, Duff CL, Worton RG, MacLennan DH. A substitution of cysteine for arginine 614 in the ryanodine receptor is potentially causative of human malignant hyperthermia. Genomics. 1991; 11:751–5. [PubMed: 1774074]

8. Robinson R, Carpenter D, Shaw MA, Halsall J, Hopkins P. Mutations in RYR1 in malignant hyperthermia and central core disease. Hum Mutat. 2006; 27:977–89. [PubMed: 16917943]
9. Stewart SL, Hogan K, Rosenberg H, Fletcher JE. Identification of the Arg1086His mutation in the alpha subunit of the voltage-dependent calcium channel (CACNA1S) in a North American family with malignant hyperthermia. Clin Genet. 2001; 59:178–84. [PubMed: 11260227]
10. Carpenter D, Ringrose C, Leo V, Morris A, Robinson RL, Halsall PJ, Hopkins PM, Shaw MA. The role of CACNA1S in predisposition to malignant hyperthermia. BMC Med Genet. 2009; 10:104. [PubMed: 19825159]
11. Toppin PJ, Chandy TT, Ghanekar A, Kraeva N, Beattie WS, Riazi S. A report of fulminant malignant hyperthermia in a patient with a novel mutation of the CACNA1S gene. Can J Anaesth. 2010; 57:689–93. [PubMed: 20431982]
12. Horstick EJ, Linsley JW, Dowling JJ, Hauser MA, McDonald KK, Ashley-Koch A, Saint-Amant L, Satish A, Cui WW, Zhou W, Sprague SM, Stamm DS, Powell CM, Speer MC, Franzini-Armstrong C, Hirata H, Kuwada JY. Stac3 is a component of the excitation-contraction coupling machinery and mutated in Native American myopathy. Nat Commun. 2013; 4:1952. [PubMed: 23736855]
13. Lopez RJ, Byrne S, Vukcevic M, Sekulic-Jablanovic M, Xu L, Brink M, Alamelu J, Voermans N, Snoeck M, Clement E, Muntoni F, Zhou H, Radunovic A, Mohammed S, Wraige E, Zorzato F, Treves S, Jungbluth H. An RYR1 mutation associated with malignant hyperthermia is also associated with bleeding abnormalities. Sci Signal. 2016; 9:ra68. [PubMed: 27382027]
14. Klein A, Lillis S, Munteanu I, Scoto M, Zhou H, Quinlivan R, Straub V, Manzur AY, Roper H, Jeannet PY, Rakowicz W, Jones DH, Jensen UB, Wraige E, Trump N, Schara U, Lochmuller H, Sarkozy A, Kingston H, Norwood F, Damian M, Kirschner J, Longman C, Roberts M, Auer-Grumbach M, Hughes I, Bushby K, Sewry C, Robb S, Abbs S, Jungbluth H, Muntoni F. Clinical and genetic findings in a large cohort of patients with ryanodine receptor 1 gene-associated myopathies. Hum Mutat. 2012; 33:981–8. [PubMed: 22473935]
15. Amburgey K, Bailey A, Hwang JH, Tarnopolsky MA, Bonnemann CG, Medne L, Medne L, Mathews KD, Collins J, Daube JR, Wellman GP, Callaghan B, Clarke NF, Dowling JJ. Genotype-phenotype correlations in recessive RYR1-related myopathies. Orphanet J Rare Dis. 2013; 8:117. [PubMed: 23919265]
16. Voermans NC, Snoeck M, Jungbluth H. RYR1-related rhabdomyolysis: A common but probably underdiagnosed manifestation of skeletal muscle ryanodine receptor dysfunction. Rev Neurol (Paris). 2016; 172:546–58. [PubMed: 27663056]
17. Dlamini N, Voermans NC, Lillis S, Stewart K, Kamsteeg EJ, Drost G, Quinlivan R, Snoeck M, Norwood F, Radunovic A, Straub V, Roberts M, Vrancken AF, van der Pol WL, de Coo RI, Manzur AY, Yau S, Abbs S, King A, Lammens M, Hopkins PM, Mohammed S, Treves S, Muntoni F, Wraige E, Davis MR, van Engelen B, Jungbluth H. Mutations in RYR1 are a common cause of exertional myalgia and rhabdomyolysis. Neuromuscul Disord. 2013; 23:540–8. [PubMed: 23628358]
18. Roux-Buisson N, Monnier N, Sagui E, Abriat A, Brosset C, Bendahan D, Kozak-Ribbens G, Gazzola S, Quesada JL, Foutrier-Morello C, Rendu J, Figarella-Branger D, Cozonne P, Aubert M, Bourdon L, Lunardi J, Fauré J. Identification of variants of the ryanodine receptor type 1 in patients with exertional heat stroke and positive response to the malignant hyperthermia in vitro contracture test. Br J Anaesth. 2016; 116:566–8. [PubMed: 26994242]
19. Snoeck M, Treves S, Molenaar JP, Kamsteeg EJ, Jungbluth H, Voermans NC. “Human stress syndrome” and the expanding spectrum of RYR1-related myopathies. Cell Biochem Biophys. 2016; 74:85–7. [PubMed: 26972305]
20. 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; 97:1067–74. [PubMed: 12411788]
21. Rosenberg H, Pollock N, Schiemann A, Bulger T, Stowell K. Malignant hyperthermia: A review. Orphanet J Rare Dis. 2015; 10:93. [PubMed: 26238698]
22. Denborough MA, Forster JF, Lovell RR, Maplestone PA, Villiers JD. Anaesthetic deaths in a family. Br J Anaesth. 1962; 34:395–6. [PubMed: 13885389]

23. Robinson RL, Curran JL, Ellis FR, Halsall PJ, Hall WJ, Hopkins PM, Iles DE, West SP, Shaw MA. Multiple interacting gene products may influence susceptibility to malignant hyperthermia. Ann Hum Genet. 2000; 64:307–20. [PubMed: 11415515]
24. Carpenter D, Robinson RL, Quinnell RJ, Ringrose C, Hogg M, Casson F, Booms P, Iles DE, Halsall PJ, Steele DS, Shaw MA, Hopkins PM. Genetic variation in RYR1 and malignant hyperthermia phenotypes. Br J Anaesth. 2009; 103:538–48. [PubMed: 19648156]
25. 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; 79:438–47. [PubMed: 20681998]
26. 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; 58:504–13. [PubMed: 21455645]
27. Gillies RL, Bjorksten AR, Du Sart D, Hockey BM. Analysis of the entire ryanodine receptor type 1 and alpha 1 subunit of the dihydropyridine receptor (CACNA1S) coding regions for variants associated with malignant hyperthermia in Australian families. Anaesth Intensive Care. 2015; 43:157–66. [PubMed: 25735680]
28. Klingler W, Heiderich S, Girard T, Gravino E, Heffron JJ, Johannsen S, Jurkat-Rott K, Rüffert H, Schuster F, Snoeck M, Sorrentino V, Tegazzin V, Lehmann-Horn F. Functional and genetic characterization of clinical malignant hyperthermia crises: a multi-centre study. Orphanet J Rare Dis. 2014; 9:8. [PubMed: 24433488]
29. Robinson RL, Monnier N, Wolz W, Jung M, Reis A, Nuernberg G, Curran JL, Monsieurs K, Stieglitz P, Heytens L, Fricker R, van Broeckhoven C, Deufel T, Hopkins PM, Lunardi J, Mueller CR. A genome wide search for susceptibility loci in three European malignant hyperthermia pedigrees. Hum Mol Genet. 1997; 6:953–61. [PubMed: 9175745]
30. Bannister RA. Bridging the myoplasmic gap II: More recent advances in skeletal muscle excitation-contraction coupling. J Exp Biol. 2016; 219:175–82. [PubMed: 26792328]
31. Treves S, Jungbluth H, Voermans N, Muntoni F, Zorzato F. Ca^{2+} handling abnormalities in early-onset muscle diseases: Novel concepts and perspectives. Semin Cell Dev Biol. 2017; 64:201–12. [PubMed: 27427513]
32. Weiss RG, O'Connell KM, Flucher BE, Allen PD, Grabner M, Dirksen RT. Functional analysis of the R1086H malignant hyperthermia mutation in the DHPR reveals an unexpected influence of the III-IV loop on skeletal muscle EC coupling. Am J Physiol Cell Physiol. 2004; 287:C1094–102. [PubMed: 15201141]
33. Hopkins PM, Ruffert H, Snoeck MM, Girard T, Glahn KP, Ellis FR, Müller CR, Urwyler A, European Malignant Hyperthermia Group. European Malignant Hyperthermia Group guidelines for investigation of malignant hyperthermia susceptibility. Br J Anaesth. 2015; 115:531–9. [PubMed: 26188342]
34. Fiszer D, Shaw MA, Fisher NA, Carr IM, Gupta PK, Watkins EJ, Roiz de Sa D, Kim JH, Hopkins PM. Next-generation sequencing of RYR1 and CACNA1S in malignant hyperthermia and exertional heat illness. Anesthesiology. 2015; 122:1033–46. [PubMed: 25658027]
35. Genomes Project Consortium. Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, Korbel JO, Marchini JL, McCarthy S, McVean GA, Abecasis GR. A global reference for human genetic variation. Nature. 2015; 526:68–74. [PubMed: 26432245]
36. Kim JH, Jarvik GP, Browning BL, Rajagopalan R, Gordon AS, Rieder MJ, Robertson PD, Nickerson DA, Fisher NA, Hopkins PM. Exome sequencing reveals novel rare variants in the ryanodine receptor and calcium channel genes in malignant hyperthermia families. Anesthesiology. 2013; 119:1054–65. [PubMed: 24013571]
37. Gonsalves SG, Ng D, Johnston JJ, Teer JK, Stenson PD, Cooper DN, Mullikin JC, Biesecker LG, NISC Comparative Sequencing Program. Using exome data to identify malignant hyperthermia susceptibility mutations. Anesthesiology. 2013; 119:1043–53. [PubMed: 24195946]
38. Rehm HL, Berg JS, Brooks LD, Bustamante CD, Evans JP, Landrum MJ, Ledbetter DH, Maglott DR, Martin CL, Nussbaum RL, Plon SE, Ramos EM, Sherry ST, Watson MS, ClinGen. Clingen–the clinical genome resource. N Engl J Med. 2015; 372:2235–42. [PubMed: 26014595]

39. Schiemann AH, Durholt EM, Pollock N, Stowell KM. Sequence capture and massively parallel sequencing to detect mutations associated with malignant hyperthermia. Br J Anaesth. 2013; 110:122–7. [PubMed: 23035052]
40. Broman M, Kleinschnitz I, Bach JE, Rost S, Islander G, Muller CR. Next-generation DNA sequencing of a Swedish malignant hyperthermia cohort. Clin Genet. 2015; 88:381–5. [PubMed: 25256590]
41. Bjorksten AR, Gillies RL, Hockey BM, Du Sart D. Sequencing of genes involved in the movement of calcium across human skeletal muscle sarcoplasmic reticulum: Continuing the search for genes associated with malignant hyperthermia. Anaesth Intensive Care. 2016; 44:762–8. [PubMed: 27832566]
42. Mestre TA, Manole A, MacDonald H, Riazi S, Kraeva N, Hanna MG, Lang AE, Männikkö R, Yoon G. A novel KCNA1 mutation in a family with episodic ataxia and malignant hyperthermia. Neurogenetics. 2016; 17:245–9. [PubMed: 27271339]
43. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the sift algorithm. Nat Protoc. 2009; 4:1073–81. [PubMed: 19561590]
44. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR. A method and server for predicting damaging missense mutations. Nat Methods. 2010; 7:248–9. [PubMed: 20354512]
45. Kircher M, Witten DM, Jain P, O'Roak BJ, Cooper GM, Shendure J. A general framework for estimating the relative pathogenicity of human genetic variants. Nat Genet. 2014; 46:310–5. [PubMed: 24487276]
46. Schiemann AH, Stowell KM. Comparison of pathogenicity prediction tools on missense variants in RYR1 and CACNA1S associated with malignant hyperthermia. Br J Anaesth. 2016; 117:124–8. [PubMed: 27147545]
47. Bertier G, Hetu M, Joly Y. Unsolved challenges of clinical whole-exome sequencing: A systematic literature review of end-users' views. BMC Med Genomics. 2016; 9:52. [PubMed: 27514372]
48. Miosge LA, Field MA, Sontani Y, Cho V, Johnson S, Palkova A, Balakishnan B, Liang R, Zhang Y, Lyon S, Beutler B, Whittle B, Bertram EM, Enders A, Goodnow CC, Andrews TD. Comparison of predicted and actual consequences of missense mutations. Proc Natl Acad Sci U S A. 2015; 112:E5189–98. [PubMed: 26269570]
49. Tong J, Oyamada H, Demaurex N, Grinstein S, McCarthy TV, MacLennan DH. Caffeine and halothane sensitivity of intracellular Ca^{2+} release is altered by 15 calcium release channel (ryanodine receptor) mutations associated with malignant hyperthermia and/or central core disease. J Biol Chem. 1997; 272:26332–9. [PubMed: 9334205]
50. Yang T, Allen PD, Pessah IN, Lopez JR. Enhanced excitation-coupled calcium entry in myotubes is associated with expression of RYR1 malignant hyperthermia mutations. J Biol Chem. 2007; 282:37471–8. [PubMed: 17942409]
51. Chelu MG, Goonasekera SA, Durham WJ, Tang W, Lueck JD, Riehl J, Pessah IN, Zhang P, Bhattacharjee MB, Dirksen RT, Hamilton SL. Heat- and anesthesia-induced malignant hyperthermia in an RyR1 knock-in mouse. FASEB J. 2006; 20:329–30. [PubMed: 16284304]
52. Yang T, Riehl J, Esteve E, Matthaei KI, Goth S, Allen PD, Pessah IN, Lopez JR. Pharmacologic and functional characterization of malignant hyperthermia in the R163C RyR1 knock-in mouse. Anesthesiology. 2006; 105:1164–75. [PubMed: 17122579]
53. Zvaritch E, Depreux F, Kraeva N, Loy RE, Goonasekera SA, Boncompagni S, Kraev A, Gramolini AO, Dirksen RT, Franzini-Armstrong C, Seidman CE, Seidman JG, Maclennan DH. An Ryr1 I4895T mutation abolishes Ca^{2+} release channel function and delays development in homozygous offspring of a mutant mouse line. Proc Natl Acad Sci U S A. 2007; 104:18537–42. [PubMed: 18003898]
54. Zvaritch E, Kraeva N, Bombardier E, McCloy RA, Depreux F, Holmyard D, Kraev A, Seidman CE, Seidman JG, Tupling AR, MacLennan DH. Ca^{2+} dysregulation in Ryr1(I4895T/wt) mice causes congenital myopathy with progressive formation of minicores, cores, and nemaline rods. Proc Natl Acad Sci U S A. 2009; 106:21813–8. [PubMed: 19959667]

55. Lefebvre R, Legrand C, Gonzalez-Rodriguez E, Groom L, Dirksen RT, Jacquemond V. Defects in Ca^{2+} release associated with local expression of pathological ryanodine receptors in mouse muscle fibres. J Physiol. 2011; 589:5361–82. [PubMed: 21969454]
56. Efremov RG, Leitner A, Aebersold R, Raunser S. Architecture and conformational switch mechanism of the ryanodine receptor. Nature. 2015; 517:39–43. [PubMed: 25470059]
57. Yan Z, Bai XC, Yan C, Wu J, Li Z, Xie T, Peng W, Yin CC, Li X, Scheres SH, Shi Y, Yan N. Structure of the rabbit ryanodine receptor RyR1 at near-atomic resolution. Nature. 2015; 517:50–5. [PubMed: 25517095]
58. Zalk R, Clarke OB, des Georges A, Grassucci RA, Reiken S, Mancia F, Hendrickson WA, Frank J, Marks AR. Structure of a mammalian ryanodine receptor. Nature. 2015; 517:44–9. [PubMed: 25470061]
59. Tung CC, Lobo PA, Kimlicka L, Van Petegem F. The amino-terminal disease hotspot of ryanodine receptors forms a cytoplasmic vestibule. Nature. 2010; 468:585–8. [PubMed: 21048710]
60. Yuchi Z, Van Petegem F. Ryanodine receptors under the magnifying lens: Insights and limitations of cryo-electron microscopy and x-ray crystallography studies. Cell Calcium. 2016; 59:209–27. [PubMed: 27103405]
61. Amador FJ, Liu S, Ishiyama N, Plevin MJ, Wilson A, MacLennan DH, Ikura M. Crystal structure of type 1 ryanodine receptor amino-terminal beta-trefoil domain reveals a disease-associated mutation “hot spot” loop. Proc Natl Acad Sci U S A. 2009; 106:11040–4. [PubMed: 19541610]
62. Kimlicka L, Lau K, Tung CC, Van Petegem F. Disease mutations in the ryanodine receptor N-terminal region couple to a mobile intersubunit interface. Nat Commun. 2013; 4:1506. [PubMed: 23422674]
63. Samsó M. A guide to the 3D structure of the ryanodine receptor type 1 by cryoEM. Protein Sci. 2017; 26:52–68. [PubMed: 27671094]
64. Wei R, Wang X, Zhang Y, Mukherjee S, Zhang L, Chen Q, Huang X, Jing S, Liu C, Li S, Wang G, Xu Y, Zhu S, Williams AJ, Sun F, Yin CC. Structural insights into Ca(2+)-activated long-range allosteric channel gating of RyR1. Cell Res. 2016; 26:977–94. [PubMed: 27573175]
65. Ramachandran S, Chakraborty A, Xu L, Mei Y, Samso M, Dokholyan NV, Meissner G. Structural determinants of skeletal muscle ryanodine receptor gating. J Biol Chem. 2013; 288:6154–65. [PubMed: 23319589]
66. Mei Y, Xu L, Mowrey DD, Mendez Giraldez R, Wang Y, Pasek DA, Meissner G. Channel gating dependence on pore lining helix glycine residues in skeletal muscle ryanodine receptor. J Biol Chem. 2015; 290:17535–45. [PubMed: 25998124]
67. Green RC, Berg JS, Grody WW, Kalia SS, Korf BR, Martin CL, McGuire AL, Nussbaum RL, O’Daniel JM, Ormond KE, Rehm HL, Watson MS, Williams MS, Biesecker LG, American College of Medical Genetics and Genomics. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. Genet Med. 2013; 15:565–74. [PubMed: 23788249]
68. Hehir-Kwa JY, Claustres M, Hastings RJ, van Ravenswaaij-Arts C, Christenhusz G, Genuardi M, Melegh B, Cambon-Thomsen A, Patsalis P, Vermeesch J, Cornel MC, Searle B, Palotie A, Capoluongo E, Peterlin B, Estivill X, Robinson PN. Towards a European consensus for reporting incidental findings during clinical NGS testing. Eur J Hum Genet. 2015; 23:1601–6. [PubMed: 26036857]
69. Boycott K, Hartley T, Adam S, Bernier F, Chong K, Fernandez BA, Friedman JM, Geraghty MT, Hume S, Knoppers BM, Laberge AM, Majewski J, Mendoza-Londono R, Meyn MS, Michaud JL, Nelson TN, Richer J, Sadikovic B, Skidmore DL, Stockley T, Taylor S, van Karnebeek C, Zawati MH, Lauzon J, Armour CM, Canadian College of Medical Geneticists. The clinical application of genome-wide sequencing for monogenic diseases in Canada: Position statement of the Canadian College of Medical Geneticists. J Med Genet. 2015; 52:431–7. [PubMed: 25951830]
70. Romero NB, Monnier N, Viollet L, Cortey A, Chevallay M, Leroy JP, Lunardi J, Fardeau M. Dominant and recessive central core disease associated with RYR1 mutations and fetal akinesia. Brain. 2003; 126:2341–9. [PubMed: 12937085]
71. Clarke NF, Waddell LB, Cooper ST, Perry M, Smith RL, Kornberg AJ, Muntoni F, Lillis S, Straub V, Bushby K, Guglieri M, King MD, Farrell MA, Marty I, Lunardi J, Monnier N, North KN.

Recessive mutations in RYR1 are a common cause of congenital fiber type disproportion. Hum Mutat. 2010; 31:E1544–50. [PubMed: 20583297]

72. Dowling JJ, Lillis S, Amburgey K, Zhou H, Al-Sarraj S, Buk SJ, Wraige E, Chow G, Abbs S, Leber S, Lachlan K, Baralle D, Taylor A, Sewry C, Muntoni F, Jungbluth H. King-Denborough syndrome with and without mutations in the skeletal muscle ryanodine receptor (RYR1) gene. Neuromuscul Disord. 2011; 21:420–7. [PubMed: 21514828]
73. Bohm J, Leshinsky-Silver E, Vassilopoulos S, Le Gras S, Lerman-Sagie T, Ginzberg M, Jost B, Lev D, Laporte J. Samaritan myopathy, an ultimately benign congenital myopathy, is caused by a RYR1 mutation. Acta Neuropathol. 2012; 124:575–81. [PubMed: 22752422]
74. Capacchione JF, Sambuughin N, Bina S, Mulligan LP, Lawson TD, Muldoon SM. Exertional rhabdomyolysis and malignant hyperthermia in a patient with ryanodine receptor type 1 gene, L-type calcium channel alpha-1 subunit gene, and calsequestrin-1 gene polymorphisms. Anesthesiology. 2010; 112:239–44. [PubMed: 20010423]
75. Zhou H, Lillis S, Loy RE, Ghassemi F, Rose MR, Norwood F, Mills K, Al-Sarraj S, Lane RJ, Feng L, Matthews E, Sewry CA, Abbs S, Buk S, Hanna M, Treves S, Dirksen RT, Meissner G, Muntoni F, Jungbluth H. Multi-minicore disease and atypical periodic paralysis associated with novel mutations in the skeletal muscle ryanodine receptor (RYR1) gene. Neuromuscul Disord. 2010; 20:166–73. [PubMed: 20080402]
76. 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; 104:167–73. [PubMed: 21795085]
77. Knoblauch M, Dagnino-Acosta A, Hamilton SL. Mice with RyR1 mutation (Y524S) undergo hypermetabolic response to simvastatin. Skelet Muscle. 2013; 3:22. [PubMed: 24004537]
78. Kraeva N, Heytens L, Jungbluth H, Treves S, Voermans N, Kamsteeg E, Ceuterick-de Groote C, Baets J, Riazi S. Compound RYR1 heterozygosity resulting in a complex phenotype of malignant hyperthermia susceptibility and a core myopathy. Neuromuscul Disord. 2015; 25:567–76. [PubMed: 25958340]
79. Carpenter D, Ismail A, Robinson RL, Ringrose C, Booms P, Iles DE, Halsall PJ, Steele D, Shaw MA, Hopkins PM. A RYR1 mutation associated with recessive congenital myopathy and dominant malignant hyperthermia in Asian families. Muscle Nerve. 2009; 40:633–9. [PubMed: 19645060]
80. Bohm J, Vasli N, Malfatti E, Le Gras S, Feger C, Jost B, Monnier N, Brocard J, Karasoy H, Gérard M, Walter MC, Reilich P, Biancalana V, Kretz C, Messaddeq N, Marty I, Lunardi J, Romero NB, Laporte J. An integrated diagnosis strategy for congenital myopathies. PLoS One. 2013; 8:e67527. [PubMed: 23826317]
81. Wang Y, Peng W, Guo HY, Li H, Tian J, Shi YJ, Yang X, Yang Y, Zhang WQ, Liu X, Liu GN, Deng T, Sun YM, Xing WL, Cheng J, Feng ZC. Next-generation sequencing-based molecular diagnosis of neonatal hypotonia in Chinese population. Sci Rep. 2016; 6:29088. [PubMed: 27353517]
82. Reddy HM, Cho KA, Lek M, Estrella E, Valkanas E, Jones MD, Mitsuhashi S, Darras BT, Amato AA, Lidov HG, Brownstein CA, Margulies DM, Yu TW, Salih MA, Kunkel LM, MacArthur DG, Kang PB. The sensitivity of exome sequencing in identifying pathogenic mutations for LGMD in the United States. J Hum Genet. 2017; 62:243–52. [PubMed: 27708273]
83. Illingworth MA, Main M, Pitt M, Feng L, Sewry CA, Gunny R, Vorstman E, Beeson D, Manzur A, Muntoni F, Robb SA. RYR1-related congenital myopathy with fatigable weakness, responding to pyridostigimine. Neuromuscul Disord. 2014; 24:707–12. [PubMed: 24951453]
84. Priest JR, Osoegawa K, Mohammed N, Nanda V, Kundu R, Schultz K, Lammer EJ, Girirajan S, Scheetz T, Waggott D, Haddad F, Reddy S, Bernstein D, Burns T, Steimle JD, Yang XH, Moskowitz IP, Hurles M, Lifton RP, Nickerson D, Bamshad M, Eichler EE, Mital S, Sheffield V, Quertermous T, Gelb BD, Portman M, Ashley EA. De novo and rare variants at multiple loci support the oligogenic origins of atrioventricular septal heart defects. PLoS Genet. 2016; 12:e1005963. [PubMed: 27058611]
85. Todd EJ, Yau KS, Ong R, Slee J, McGillivray G, Barnett CP, Haliloglu G, Talim B, Akcoren Z, Kariminejad A, Cairns A, Clarke NF, Freckmann ML, Romero NB, Williams D, Sewry CA, Colley A, Ryan MM, Kiraly-Borri C, Sivadorai P, Allcock RJ, Beeson D, Maxwell S, Davis MR,

Laing NG, Ravenscroft G. Next generation sequencing in a large cohort of patients presenting with neuromuscular disease before or at birth. Orphanet J Rare Dis. 2015; 10:148. [PubMed: 26578207]

86. Ellard S, Kivuva E, Turnpenny P, Stals K, Johnson M, Xie W, Caswell R, Lango Allen H. An exome sequencing strategy to diagnose lethal autosomal recessive disorders. Eur J Hum Genet. 2015; 23:401–4. [PubMed: 24961629]
87. Kariminejad A, Ghaderi-Sohi S, Hossein-Nejad Nedai H, Varasteh V, Moslemi AR, Tajsharghi H. Lethal multiple pterygium syndrome, the extreme end of the RYR1 spectrum. BMC Musculoskelet Disord. 2016; 17:109. [PubMed: 26932181]
88. Robinson LK, O'Brien NC, Puckett MC, Cox MA. Multiple pterygium syndrome: A case complicated by malignant hyperthermia. Clin Genet. 1987; 32:5–9. [PubMed: 3621655]
89. Hunter JM, Ahearn ME, Balak CD, Liang WS, Kurdoglu A, Corneveaux JJ, Russell M, Huentelman MJ, Craig DW, Carpten J, Coons SW, DeMello DE, Hall JG, Bernes SM, Baumbach-Reardon L. Novel pathogenic variants and genes for myopathies identified by whole exome sequencing. Mol Genet Genomic Med. 2015; 3:283–301. [PubMed: 26247046]
90. Burge JA, Hanna MG. Novel insights into the pathomechanisms of skeletal muscle channelopathies. Curr Neurol Neurosci Rep. 2012; 12:62–9. [PubMed: 22083238]
91. Hanchard NA, Murdock DR, Magoulas PL, Bainbridge M, Muzny D, Wu YQ, Wang M, McGuire AL, Lupski JR, Gibbs RA, Brown CW. Exploring the utility of whole-exome sequencing as a diagnostic tool in a child with atypical episodic muscle weakness. Clin Genet. 2013; 83:457–61. [PubMed: 22901280]
92. Shaaban S, Ramos-Platt L, Gilles FH, Chan WM, Andrews C, De Girolami U, Demer J, Engle EC. RYR1 mutations as a cause of ophthalmoplegia, facial weakness, and malignant hyperthermia. JAMA Ophthalmol. 2013; 131:1532–40. [PubMed: 24091937]
93. AlBakri A, Karaoui M, Alkuraya FS, Khan AO. Congenital ptosis, scoliosis, and malignant hyperthermia susceptibility in siblings with recessive RYR1 mutations. J AAPOS. 2015; 19:577–9. [PubMed: 26691049]
94. Rueffert H, Wehner M, Ogunlade V, Meinecke C, Schober R. Mild clinical and histopathological features in patients who carry the frequent and causative malignant hyperthermia RYR1 mutation p.Thr2206Met. Clin Neuropathol. 2009; 28:409–16. [PubMed: 19919814]
95. Jungbluth H, Dowling JJ, Ferreiro A, Muntoni F, 182nd ENMC international workshop. RYR1-related myopathies, 15–17th April 2011, Naarden, The Netherlands. Neuromuscul Disord. 2012; 22:453–62. [PubMed: 22226685]
96. Dirksen RT, Avila G. Distinct effects on Ca^{2+} handling caused by malignant hyperthermia and central core disease mutations in RYR1. Biophys J. 2004; 87:3193–204. [PubMed: 15347586]
97. Timmins MA, Rosenberg H, Larach MG, Sterling C, Kraeva N, Riazi S. Malignant hyperthermia testing in probands without adverse anesthetic reaction. Anesthesiology. 2015; 123:548–56. [PubMed: 26068069]
98. Wappler F, Fiege M, Steinfath M, Agarwal K, Scholz J, Singh S, Matschke J, Schulte Am Esch J. Evidence for susceptibility to malignant hyperthermia in patients with exercise-induced rhabdomyolysis. Anesthesiology. 2001; 94:95–100. [PubMed: 11135728]
99. Corona BT, Rouviere C, Hamilton SL, Ingalls CP. Eccentric contractions do not induce rhabdomyolysis in malignant hyperthermia susceptible mice. J Appl Physiol. 2008; 105:1542–53. [PubMed: 18787086]
100. Giulivi C, Ross-Inta C, Omanska-Klusek A, Napoli E, Sakaguchi D, Barrientos G, Allen PD, Pessah IN. Basal bioenergetic abnormalities in skeletal muscle from ryanodine receptor malignant hyperthermia-susceptible R163C knock-in mice. J Biol Chem. 2011 Jan 07; 286(1): 99–113. [PubMed: 20978128]
101. Durham WJ, Aracena-Parks P, Long C, Rossi AE, Goonasekera SA, Boncompagni S, Galvan DL, Gilman CP, Baker MR, Shirokova N, Protasi F, Dirksen R, Hamilton SL. RyR1 S-nitrosylation underlies environmental heat stroke and sudden death in Y522S RyR1 knockin mice. Cell. 2008; 133:53–65. [PubMed: 18394989]
102. Eltit JM, Bannister RA, Moua O, Altamirano F, Hopkins PM, Pessah IN, Molinski TF, López JR, Beam KG, Allen PD. Malignant hyperthermia susceptibility arising from altered resting coupling

between the skeletal muscle L-type Ca^{2+} channel and the type 1 ryanodine receptor. Proc Natl Acad Sci U S A. 2012; 109:7923–8. [PubMed: 22547813]

103. Capacchione JF, Muldoon SM. The relationship between exertional heat illness, exertional rhabdomyolysis, and malignant hyperthermia. Anesth Analg. 2009; 109:1065–9. [PubMed: 19617585]
104. Jungbluth H, Ochala J, Treves S, Gautel M. Current and future therapeutic approaches to the congenital myopathies. Semin Cell Dev Biol. 2017; 64:191–200. [PubMed: 27515125]
105. Hopkins PM, Ellis FR, Halsall PJ. Evidence for related myopathies in exertional heat stroke and malignant hyperthermia. Lancet. 1991; 338:1491–2. [PubMed: 1683922]
106. Scalco RS, Snoeck M, Quinlivan R, Treves S, Laforet P, Jungbluth H, Voermans NC. Exertional rhabdomyolysis: Physiological response or manifestation of an underlying myopathy? BMJ Open Sport Exerc Med. 2016; 2:e000151.
107. Chan EK, Kornberg AJ, Ryan MM. A diagnostic approach to recurrent myalgia and rhabdomyolysis in children. Arch Dis Child. 2015; 100:793–7. [PubMed: 25633066]
108. Davis M, Brown R, Dickson A, Horton H, James D, Laing N, Marston R, Norgate M, Perlman D, Pollock N, Stowell K. Malignant hyperthermia associated with exercise-induced rhabdomyolysis or congenital abnormalities and a novel RYR1 mutation in New Zealand and Australian pedigrees. Br J Anaesth. 2002; 88:508–15. [PubMed: 12066726]
109. Tobin JR, Jason DR, Challa VR, Nelson TE, Sambuughin N. Malignant hyperthermia and apparent heat stroke. JAMA. 2001; 286:168–9. [PubMed: 11448278]
110. Kraeva N, Sapa A, Dowling JJ, Riazi S. Malignant hyperthermia susceptibility in patients with exertional rhabdomyolysis: a retrospective cohort study and updated systematic review. Can J Anaesth. 2017; doi: 10.1007/s12630-017-0865-5
111. Hopkins PM. Is there a link between malignant hyperthermia and exertional heat illness? Br J Sports Med. 2007; 41:283–4. [PubMed: 17261558]
112. Robinson RL, Anetseder MJ, Brancadoro V, van Broekhoven C, Carsana A, Censier K, Fortunato G, Girard T, Heytens L, Hopkins PM, Jurkat-Rott K, Klinger W, Kozak-Ribbens G, Krivosic R, Monnier N, Nivoche Y, Olthoff D, Rueffert H, Sorrentino V, Tegazzin V, Mueller CR. Recent advances in the diagnosis of malignant hyperthermia susceptibility: How confident can we be of genetic testing? Eur J Hum Genet. 2003; 11:342–8. [PubMed: 12700608]
113. Deufel T, Sudbrak R, Feist Y, Rubsam B, Du Chesne I, Schafer KL, Roewer N, Grimm T, Lehmann-Horn F, Hartung EJ. Discordance, in a malignant hyperthermia pedigree, between in vitro contracture-test phenotypes and haplotypes for the MHS1 region on chromosome 19q12–13.2, comprising the C1840T transition in the RYR1 gene. Am J Hum Genet. 1995; 56:1334–42. [PubMed: 7762556]
114. 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; 46:692–8. [PubMed: 12059893]
115. Wolak S, Rucker B, Kohlschmidt N, Doetsch S, Bartsch O, Zechner U, Tzanova I. Homozygous and compound heterozygous RYR1 mutations. New findings on prevalence and penetrance of malignant hyperthermia. Anaesthesist. 2014; 63:643–50. [PubMed: 25047158]
116. Lynch PJ, Krivosic-Horber R, Reyford H, Monnier N, Quane K, Adnet P, Haudecoeur G, Krivosic I, McCarthy T, Lunardi J. Identification of heterozygous and homozygous individuals with the novel RYR1 mutation Cys35Arg in a large kindred. Anesthesiology. 1997; 86:620–6. [PubMed: 9066328]
117. Ryan JF, Tedeschi LG. Sudden unexplained death in a patient with a family history of malignant hyperthermia. J Clin Anesth. 1997; 9:66–8. [PubMed: 9051549]
118. Reske-Nielsen C, Schlosser K, Pascucci RC, Feldman JA. Is it exertional heatstroke or something more? A case report. J Emerg Med. 2016; 51:e1–5. [PubMed: 27041490]
119. Figarella-Branger D, Kozak-Ribbens G, Rodet L, Aubert M, Borsarelli J, Cozzone PJ, Pellissier JF. Pathological findings in 165 patients explored for malignant hyperthermia susceptibility. Neuromuscul Disord. 1993; 3:553–6. [PubMed: 8186710]

120. Sambuughin N, Capacchione J, Blokhin A, Bayarsaikhan M, Bina S, Muldoon S. The ryanodine receptor type 1 gene variants in African American men with exertional rhabdomyolysis and malignant hyperthermia susceptibility. Clin Genet. 2009; 76:564–8. [PubMed: 19807743]

121. Sagui E, Abriat A, Kozak-Ribbens G, Foutrier-Morello C, Bernard M, Canini F, Brosset C, Bendahan D. Is muscle energy production disturbed in exertional heat stroke? Mil Med. 2014; 179:342–5. [PubMed: 24594472]

122. Heiman-Patterson TD, Rosenberg H, Fletcher JE, Tahmoush AJ. Halothane-caffeine contracture testing in neuromuscular diseases. Muscle Nerve. 1988; 11:453–7. [PubMed: 3374516]

123. Lehmann-Horn F, Iaizzo PA. Are myotonias and periodic paralyses associated with susceptibility to malignant hyperthermia? Br J Anaesth. 1990; 65:692–7. [PubMed: 2248848]

124. Hopkins PM, Ellis FR, Halsall PJ. Comparison of in vitro contracture testing with ryanodine, halothane and caffeine in malignant hyperthermia and other neuromuscular disorders. Br J Anaesth. 1993; 70:397–401. [PubMed: 8499197]

Brief Summary Statement

This review summarizes evidence on the genetics of MH, its complexity and development of new genetic techniques. It also discusses the connection of MH and *RYR1* related disorders to other morbid phenotypes.

Summary of the key points of the article

This article reviews the advancements in genetic understanding of malignant hyperthermia and new technologies and approaches for diagnosis of MH along with their existing limitations due to the complexity of the genetics of MH. It also reviews the various *RYR1* related disorders and phenotypes, such as myopathies, exertional rhabdomyolysis and bleeding disorders; and examines the connection between these disorders and malignant hyperthermia.

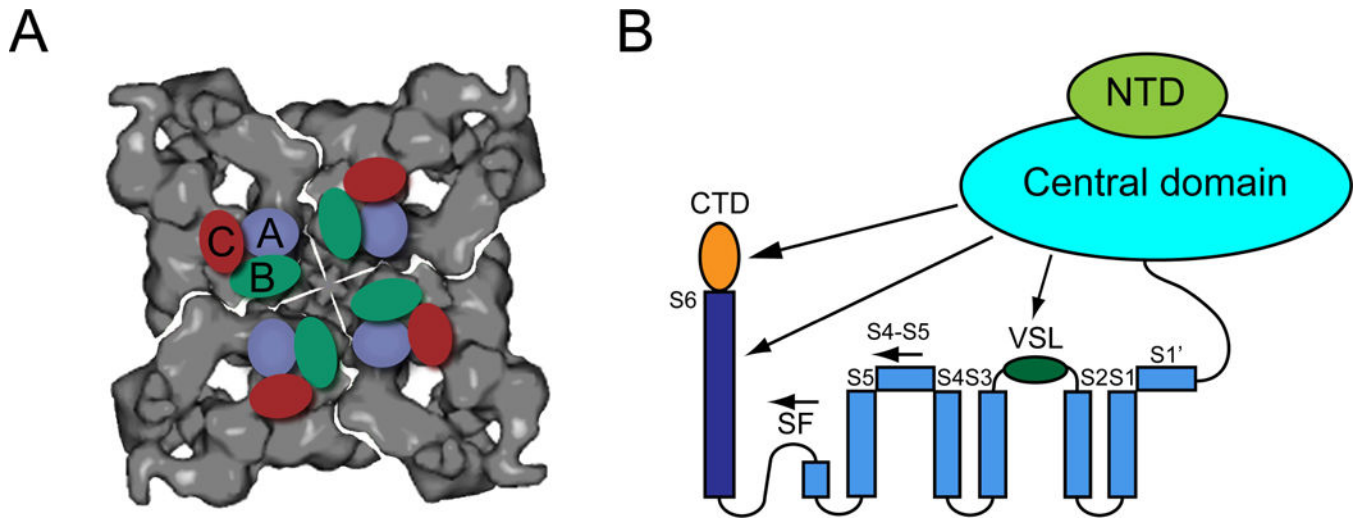

Figure-1.

A. A schematic illustration of the N-terminal domains docked in the pseudo-atomic model of the RyR1 tetramer. The RyR1 N-terminal domain (NTD), corresponding to the MH hot spot 1, is composed of three subdomains: A, B, and C. Interactions among the domains A, B, and C on the same RyR1 subunit together with the interactions between domains of the neighboring subunits are involved in the global conformational RyR1 transmissions that control effector-induced channel gating. The variants impair the domain-domain interactions and thus would cause the RyR1 channel dysfunction.

B. A schematic model of domain organization in a RyR1 monomer, composed of the N-terminal domain (NTD), the central domain and the channel domain. Each domain consists of several interconnected subdomains. The channel domain consists of the six transmembrane fragments (S1-S6), and pore helices with the selectivity filter (SF), the linker helix of S4-S5, the voltage sensor like domain (VSL), and C-terminal domain (CTD). Binding Ca^{2+} to the central domain, initiates a cascade of conformational transmissions via allosteric intradomain and interdomain interactions from the central domain to the NTD, to the VSL, the CTD and S6, ultimately inducing opening of the channel.[64] Together with the amino acids forming the ion channel – the pore helix and the selectivity filter (amino acid 4894-4900), the S4-S5 linker (amino acid 4830-4841), Gly4934 – which serves as a “hinge” for the outward movement of the helix S6, and the CTD (amino acid 4957-5033) are all critical for RyR1 channel gating (modified with permission from reference 64).