# Genetics of Malignant Hyperthermia: A Brief Update

**David Beebe, Vikram V. Puram[1], Srdjan Gajic, Bharat Thyagarajan[2], Kumar G. Belani**

Department of Anesthesiology, University of Minnesota, Minneapolis, MN, [1]University of Minnesota Medical School, Minneapolis, MN, [2]Department of Lab Medicine and Pathology, University of Minnesota, Minneapolis, MN, USA

## Abstract

Malignant hyperthermia susceptibility (MHS) and the associated condition malignant hyperthermia (MH) are rare but well-known disorders in the field of anesthesiology. MHS is usually determined by a history of a family member developing a positive episode during general anesthesia and then confirmed by an invasive caffeine halothane contracture test (CHCT). More recently, within the context of MH as a pharmacogenetic disorder, the question of whether or not MHS can be principally genetically determined is of high importance as knowledge of detailed pathogenesis may prevent against its largely invariable lethality if untreated. Thus, in this brief report, genetic terms, as well as updates in the genetics of MHS, will be reviewed in order to better understand both the condition and the current research.

**Keywords:** Genetic testing, malignant hyperthermia (MH), MH susceptibility

## Relevant Case

A 38-year-old female presents for a caffeine halothane contracture test (CHCT) as well as genetic testing for malignant hyperthermia susceptibility (MHS). Her reason was that her niece died due to complications related to the triggering of an MH episode during surgery. The patient's brother, the father of the deceased child, had a positive CHCT performed at a different hospital. The brother also had his remaining three children tested with CHCT, with two being negative and one positive. The patient's results were positive for MHS as determined by CHCT, and the patient had a heterozygous pathogenic mutation, rs112563513 (c. 7007G > A; p.Arg2336His) in the *RYR1* gene. This is a well-described pathogenic mutation for MHS. She requested to know if her children needed to undergo both a CHCT and genetic test.

**Address for correspondence:** Dr. Kumar G. Belani,
Department of Anesthesiology, University of Minnesota,
Minneapolis, MN, USA.
E-mail: kumarbelani@gmail.com

## The Basics

MH is a rare pharmacogenetic disorder of skeletal muscle. It causes both a dangerous rise in body temperature and severe muscle contractions after an MHS patient receives general anesthesia. Though genes that cause MH are inherited, most cases show no signs or symptoms until anesthesia exposure. Treatment involves the immediate administration of IV dantrolene the antidote, as well as other measures including the use of ice packs and cooling blankets to reduce body temperature.

## The Genetics

### Gene

The ryanodine receptor 1 (*RYR1*) gene, which codes for the skeletal muscle-type ryanodine receptor protein (also known as ryanodine receptor 1), is located on chromosome 19 and is the main gene implicated in MHS.[1] It is a very large gene, containing over 150,000 base pairs, a feature which has made identification of mutations challenging.[2]

## Allele

The *RYR1* gene containing a mutation at a specific MHS locus is the key allele. This can be passed from generation to generation as most, though not all, mutations associated with MHS are inherited in an *autosomal dominant* pattern.[1] There are more than 300 such mutations of the *RYR1* gene that have been identified to date, with 29 of these being approved as diagnostic mutations by the North American Malignant Hyperthermia Registry (NAMHR).[1]

## Penetrance

Most MHS mutations display *incomplete penetrance*, meaning that the MH will manifest in less than 100% of genetically susceptible individuals who are exposed to a triggering agent.[3] In fact, in a 2019 study of 229 genotype-positive individuals with exposure to trigger anesthetic agents, the overall penetrance for the analyzed *RYR1* mutations was approximately 40% with 93 MH cases.[4] One potential explanation for this variable MH phenotype involves the fact that *RYR1* co-localizes and interacts with numerous other serine and arginine-rich (SR) proteins. Thus, the *RYR1* function may be modulated by polymorphic gene variants in these proteins. For example, in patients with a *JSMPR1* gene variant (which encodes JP-45, another integral SR protein) in addition to an *RYR1* mutation, the overall phenotype has been reported to be less severe than if the *RyR1* mutation were expressed alone.[5] Though, overall, the exact penetrance of alleles in MHS is difficult to estimate because the phenotype of MH is dependent both on genetic susceptibility and a pharmacologic trigger (e.g., inhaled anesthetics) to which many individuals with MHS are never exposed.

## Prevalence

Though difficult to estimate due to the incomplete penetrance of MHS mutations, low exposure rate of the population to triggering agents, as well as the large size of the *RYR1* gene and thus many possible locations of causative mutations, the prevalence of MHS is estimated to be between 1 in 2,000 to 1 in 3,000 individuals.[6-8] Interestingly, the prevalence of MH is estimated at approximately 1 in 100,000 administered anesthesia events, a difference from MHS prevalence that shows the low penetrance of some alleles as well as the possibility of milder manifestations of MH that go clinically unrecognized.[9]

## Exon

Sequencing of all exons (coding regions) in the genome, "*exome sequencing*," is an important new tool for classifying possible MHS mutations as pathogenic or nonpathogenic.[10]

## Mutation

The majority of genomic variants currently associated with MH are *missense mutations* (single base pair alterations resulting in amino acid substitutions) located in exons.[7]

## Associated Genes and Pathogenesis

Studies have shown that mutations in *RYR1* are the most common defects present in patients who develop MH, with a range of 70–86% of MH patients having a mutation in *RYR1*.[11,12] The *CACNA1S* gene on chromosome 1 is a second gene believed to be involved in MHS, though much less common than *RYR1*.[10] *CACNA1S* is a gene that encodes the alpha-1S subunit of the voltage-dependent L-type calcium channel, also known as the dihydropyridine calcium channel, which is physically coupled with the *RYR1* channel.[13] Additionally, variants in the cysteine-rich domain 3 (STAC3) genes which code for excitation-contraction coupling machinery components in the muscle are also associated with MH. Though the overwhelming majority of variants involve *RYR1*, approximately 1% involve variants in CANCA1S or STAC3.[4,13]

The pathogenesis of these mutations is tied to the location of these channels. *RYR1* is part of a family of ryanodine receptors, all of which form channels that transport calcium ions. *RYR1* is located on the sarcoplasmic reticulum (SR) of skeletal muscle cells, which opens in response to rises in intracellular calcium ion levels that are mediated by dihydropyridine calcium channels. The opening of *RYR1* channels allows for calcium ions stored in the SR to be released into the cytoplasm and causes a further rise in intracellular calcium, ultimately allowing the binding of actin and myosin and resulting in muscle contraction. Mutations in the *RYR1* gene that are pathogenic for MHS often result in a malformed protein that has reduced affinity for $Mg^{2+}$ ions, which stimulate the closing of the channel.[14] When an individual with this mutation type is exposed to triggering agents such as volatile anesthetics, which act to greatly increase the opening of the *RYR1* channel in response to $Ca^{2+}$, the net result is unopposed calcium release from the SR causing the muscle rigidity and hypermetabolism that is characteristic of MH.[15] Indeed, the treatment for MH, dantrolene, acts via blockade of the *RYR1* channel, thus preventing this buildup of intracellular calcium.[16]

The second gene implicated in MHS the *CACNA1S* gene, codes for the dihydropyridine calcium channel which normally opens in response to depolarization through the transverse tubule system.[13] A presumed pathogenic mutation in the *CACNA1S* gene acts to increase the opening of this channel in response to triggering agents, again resulting in greatly increased intracellular calcium concentration.[17]

## Clinical Applications and Limitations

The current gold standard test for diagnosing MHS is an in-vitro contracture test, the CHCT, which involves obtaining

a generous specimen of the *vastus lateralis* muscle and exposing the freshly obtained muscle bundles to triggering agents such as 3% halothane and increasing concentrations of caffeine in a muscle bath.[18] This test is not only expensive but is too invasive to be practical as a widespread screening tool. Moreover, the exact test procedure varies between countries and the results are imperfect. While the test has a 100% sensitivity and specificity ranges of 80–97%, the ratio of result reproducibility between laboratories has only been shown to be 0.56–0.77.[19] As an alternative, numerous noninvasive tests such as nuclear magnetic resonance spectroscopy to assess ATP depletion, metabolite assays, as well as microdialysis of caffeine to examine $CO_2$ expulsion from muscle tissue have been explored as potential MHS screens.[5] However, given the clear genetic component of MHS, genetic testing has long held great promise as a potential screening tool that could result in preoperative identification of individuals with MHS. Though DNA analysis is relatively noninvasive and much less expensive than the CHCT, requiring only a blood specimen to be sent to a diagnostic laboratory, unfortunately, there are several barriers to be overcome before genetic testing for MHS can supplant the CHCT.

First, there remains a significant fraction of approximately 15–30% of patients studied after a clear MH episode in whom a causative mutation in *RYR1* or *CACNA1S* has not been discovered. Thus, further research to identify additional pathogenic mutations is crucial in order to improve the sensitivity and the negative predictive value of the test. Several new candidate loci, on chromosomes 3, 7, and 17, have indeed been proposed in recent years based on new research.[13]

Once a candidate mutation is identified, the exact criterion for classifying a mutation as pathogenic differs between MH authorities and is generally stringent, posing obstacles to the rapid incorporation of findings into clinical guidelines. For example, the European Malignant Hyperthermia Group (EMHG) criteria for pathogenicity classification require genetic characterization including a full DNA and protein description, functional characterization showing an effect of the mutation either *in vitro* or *ex vivo*, as well as the publication of these results in the scientific literature.[20]

Next-generation sequencing (NGS) technology is a novel genetic sequencing technology that holds the promise of being able to identify previously unidentified pathogenic mutations and expand the knowledge on MHS. Exome sequencing is one example of NGS that is useful when the identity of the specific genes involved in the disease has not yet been identified. The technique, which entails sequencing only 1% of the genome that contains exons, is less costly than whole-genome sequencing and is increasingly being used for the diagnosis of rare inherited disorders. A 2013 study on MHS using exome sequencing was able to identify over 100 *RYR1* and *CACNA1S* variants in an asymptomatic population, reclassify several previously classified pathogenic variants as likely non-pathogenic due to high prevalence, as well as alert several individuals with a high likelihood of MHS based on known pathogenic mutations.[7] The major use of large scale exome sequencing efforts in MHS will be to allow researchers to identify the pathogenicity of variants identified in *RYR1* and *CACNA1S* genes. In addition, the technique can be used in combination with functional studies to identify new mutations in families for whom traditional methods have not identified a mutation. A limitation of this technology, however, is that many variants of unknown significance (VUS) are often identified, complicating the interpretation, and large-scale clinical application of the results.

A further limitation of genetic testing is the apparent incomplete penetrance of MHS. Studies have revealed a proportion of individuals who are positive for a known pathogenic mutation but exhibit a negative muscle contracture test.[2] These findings along with the large gap in prevalence between MHS and known cases of MH indicate that there may be more complex gene-environment interactions underlying MHS and MH development.[6] However, while this characteristic of MHS would increase false positives in genetic screening, this increased risk is more tolerable than false negatives given the presence of alternative anesthetic options and lethality of untreated MH. Thus, due to the heterogeneity of MH, the high possibility of finding only VUS as well as inconsistencies within family phenotypes, it is difficult to rule out MHS with a negative genetic testing result.

Currently, genetic screening for MHS is most useful as an adjunct to CHCT in patients with a known family history of MH. According to recommendations from the EMHG, family members of a patient with a known pathogenic MHS mutation should undergo genetic screening for MHS. This can be done using targeted methods for a particular mutation such as Sanger sequencing. If the test is positive for the same known pathogenic mutation, these individuals can be classified to have MHS and need no invasive muscle biopsy testing. If the test is negative for the causative mutation, however, individuals are still recommended to undergo CHCT. This is because some pedigrees studied have shown discordance between genetic test and CHCT results, possibly due to additional yet-undiscovered susceptibility alleles being present in these families.[18]

Genetic counseling is a crucial component of care in families affected by this condition, as it is important for patients to understand the nuances of MHS genetic testing and have

questions about the interpretation of their test results answered thoroughly. Thus, currently, it is best to test only for variants that are the known causative factors for altered $Ca^2+$ release from the SR.[5] Overall, knowledge about the genetic basis of MHS is rapidly increasing and, with further advancements in understanding, there is hope that genetic screening can one day be an effective, noninvasive, and widely available pre-op screening tool for MHS.

In the case of the 38-year-old female with a niece who died due to an MH episode who was MHS by CHCT, her children should first undergo genetic testing, particularly if they are going to undergo surgery in the near future. This is much less invasive than muscle biopsy, does not require an anesthetic and can be performed in young children. If the genetic test indicates they have the same genetic variant as the mother that causes susceptibility to MH, they should be considered MHS and only be anesthetized with non-triggering agents. However, if a VUS or no variant is found, MHS follow-up should be conducted with muscle biopsy and CHCT when they are old enough in case they have an unknown genetic variant that causes MHS but not yet identified by the current genetic testing. If one of her children requires surgery before CHCT can be done, they should be considered MH susceptible and only be administered non-triggering anesthetic agents.

In summary, genetic testing shows promise as a means to determine if a patient is MHS and, if positive for a variant that is associated with MH, allow them to forgo a muscle biopsy. However, more study needs to be done on the genetic variants associated with MH and how they cause this syndrome before CHCT can be abandoned altogether.

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## Conflicts of interest
There are no conflicts of interest.

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