EXPLORING THE CORRELATION BETWEEN THE DEVELOPMENT OF MALIGNANT HYPERTHERMIA FROM ANESTHESIA WITH A HISTORY OF EXERCISE-INDUCED HYPERTHERMIA: A SCOPING REVIEW

by

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ABSTRACT

The purpose of this scoping review is to explore the correlation between the development of malignant hyperthermia after anesthesia with a history of exercise-induced hyperthermia. Malignant hyperthermia and exercise-induced hyperthermia are both life-threatening, hypermetabolic states related to calcium dysregulation in the sarcoplasmic reticulum of muscle cells. Literature suggests that a mutation in the ryanodine type 1 receptor (RYR1) and associated proteins might play a significant role in both the development of anesthesia-induced malignant hyperthermia and exercise-induced hyperthermia. However, the association between this mutation and the development of malignant hyperthermia has not been sufficiently examined. The purpose of this research is to address the following question: Are individuals with a history of exercise-induced hyperthermia with a mutated RYR1 gene more susceptible to the development of malignant hyperthermia during general anesthesia?

Three major themes occur in literature that suggest an association between the two events due to a mutation in the associated protein receptors. The first is that there is a necessity for additional testing in patients with a history of exertional rhabdomyolysis or similar symptoms during exercise. The second is that IVCT tests show similar mechanisms between MH and exercise induced hyperthermia. The final theme is that next-generation sequencing could be critical in furthering the association between the two events and focusing on specific RYR1 mutations. Overall, evidence confirms there is a clinical association between the development of malignant hyperthermia and exercise-induced hyperthermia. Because of this, healthcare and sports professionals should take this information seriously to ensure proper precautions are in place, past medical histories are thoroughly explored, and genetic testing/counseling is conducted.

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INTRODUCTION

Malignant hyperthermia is a life-threatening disorder resulting from exposure to volatile anesthetic agents or depolarizing muscle relaxants in certain malignant hyperthermia susceptible populations (van den Bersselaar, 2022). A possible association between the development of malignant hyperthermia and exercise-induced hyperthermia has been suggested, thus implying that certain populations might be at an increased risk of developing both these conditions. Literature has suggested that a mutation in the ryanodine type 1 receptor (RYR1) gene might play a significant role in the development of anesthesia-induced malignant hyperthermia after a patient has had exercise-induced hyperthermia or rhabdomyolysis (Carsana et al., 2013; Davis et al., 2002; Fiszer et al., 2015; Gardner et al., 2020; Kraeva et al., 2017; Poels et al., 1991; Rosenberg et al., 2015; Roux- Buisson et al., 2016; Wappler et al., 2001). However, the association between this mutation and the development of malignant hyperthermia has not been sufficiently examined. The purpose of this scoping review is to address the following question: Are individuals with a history of exercise-induced hyperthermia with a mutated RYR1 gene more susceptible to the development of malignant hyperthermia during general anesthesia?

Malignant Hyperthermia

Malignant hyperthermia (MH) comes from the terms “malignant” (meaning dangerous to a person's health) and “hyperthermia” (meaning an increased body temperature or overheating) (U.S. National Library of Medicine, 2020a). Malignant hyperthermia is a hypermetabolic condition that results from the administration of anesthetic agents in patients with an autosomal dominant gene responsible for the dysfunction of the receptor proteins of calcium channels in skeletal muscle- specifically the RYR1 subunit and its related functional proteins, including the dihydropyridine receptor protein (DHPR) and the calcium voltage-gated channel subunit alpha1

C (CACNA1S). The anesthetic agents, succinylcholine and halogenated anesthetic gases, create a disturbance in the intracellular calcium regulation of skeletal muscles, thus leading to continuous muscle contractions and rigidity, rhabdomyolysis (the breakdown of muscle fibers), increased heart rate, increased respiratory rate, acidosis, and lethal increases in body temperatures (U.S. National Library of Medicine, 2020a). Without immediate recognition and proper treatment, this condition can cause multi-organ failure and result in death. If a person is known to have exercise-induced hyperthermia or rhabdomyolysis from physical exertion, the same genetic alteration- a channelopathy of the RYR1 receptor - may predispose them to the development of malignant hyperthermia when they are exposed to triggering agents during general anesthesia.

Exercise-Induced Hyperthermia

Exercise-induced hyperthermia occurs in the presence of exertional stress that causes the body to heat up more than the body can handle. Most cases occur during extraneous physical activity, long-duration physical activity and vigorous physical or cardiovascular exertion, extreme outdoor temperatures, or acute muscle contraction (Casarna, 2013). Many people will experience exertional rhabdomyolysis as a warning sign prior to developing hyperthermia quickly after. In some cases, exercise-induced hyperthermia occurs when the body breaks down skeletal muscles after muscle injury, eventually leading to the release of intracellular components like myoglobin and creatine kinase into the blood, thus leading to severe complications including acute kidney injury and other fatal metabolic complications. Despite the events leading up to the development of rhabdomyolysis, when a muscle is injured, it results in an influx of calcium ions into the intracellular space resulting in constant muscle contraction, which can result in cell death (Casarna, 2013). Exertional rhabdomyolysis diagnosis is confirmed when the creatinine kinase

(CK) value exceeds 10.000U/L and subsequently falls. Symptoms often include cramping, myalgia, myoglobinuria, muscle weakness, and muscle swelling. These symptoms coupled with the rapid increase and decrease of CK levels are often required to confirm the diagnosis of exercise-induced hyperthermia (van den Bersselaar, 2021). Because more people are developing MH at higher rates than expected, ongoing exploration of the risk factors for developing MH found that there may be a genetic link responsible for the development of MH with the RYR1 gene.

RyR1 Channelopathy and Associated Proteins

Recent studies have suggested the development of malignant hyperthermia after a history of known exercise-induced hyperthermia is more likely. The potential correlation to having at least one out of 48 RYR1 gene mutations in these patients might be expected (Carpenter et al., 2009; Casarna, 2013; Kraeva et al., 2017; Rosenberg et al., 2015). These mutations are a single amino acid change in the DNA sequence that codes for an essential part of the RYR1 gene. Patients that are more susceptible to both forms of hyperthermia typically have one of these mutations in their RYR1 gene. The RYR1 gene is essential in movement of skeletal muscles and the body as a whole. Normal contraction and relaxation of the skeletal muscles in a coordinated effort work to move the body in an appropriate way. The RYR1 gene codes for ryanodine receptor-1 proteins that create calcium channels. Through these channels, the proteins release positively charged calcium ions stored in the sarcoplasmic reticulum of the skeletal muscle cells. The sarcoplasmic reticulum surrounds muscle cells and stores calcium when the muscle cells are relaxed. Once muscles are activated by an initiating electrical signal through excitation-contraction coupling, the RYR1 receptors on the sarcoplasmic reticulum will release calcium

ions from the channels out into the muscle cell fluid resulting in an increased calcium concentration within the cells themselves facilitating skeletal muscle contraction and movement (U.S. National Library of Medicine, 2020b).

When there is a mutation in the RYR1 gene, the calcium channels become hypersensitive when activated by anesthetic stimuli, leading to an excess of myoplasmic calcium. The calcium channels open easier, take longer to close with exposure to triggering anesthesia drugs, and overwhelm the active calcium pumps that normally return calcium to the sarcoplasmic reticulum (U.S. National Library of Medicine, 2020b). The large amount of calcium creates a hypermetabolic state of contraction that generates excess metabolic heat production leading to hyperthermia and produces an acidic environment. Excessive skeletal muscle contraction produces rhabdomyolysis (Casarna, 2013). It is thought that up to 75% of MH susceptible cases are due to a RYR1 variation (van den Bersselaar, 2022). Individuals with a history of exertional hyperthermia likely have a metabolic cascade similar to the channelopathy triggered by anesthetics, especially from volatile anesthetics and succinylcholine thus suggesting a similar molecular mechanism must be present (Michelucci, 2017).

Associated proteins to RYR1, including DHPR and CACNA1S, also have suggested causal mutations for MH susceptibility due to their role as calcium channels. While MH has a greater association with the RYR1 gene, some variants in the DHPR and CACNA1C receptors have confirmed associations with MH (van den Bersselaar, 2022). With this information, it is critical to evaluate the similar biological processes and determine the best way to proceed with procedures requiring triggering anesthetic agents and evaluate if more testing and precautions should occur before anesthesia is initiated.

Malignant Hyperthermia Susceptibility Diagnosis

RYR1 pathogenicity can be classified based on the European Malignant Hyperthermia group scoring matrix that has confirmed pathogenic RYR1 mutations related to malignant hyperthermia as well as the ClinGen Curation Expert Panel recommendations. If a studied RYR1 gene does not meet the confirmed list of pathogenic variants, then MH susceptibility can be determined through a caffeine-halothane contracture test (CHCT) or an in vitro contracture test. Muscle bundles are surgically cured after being exposed to halothane or caffeine. If the tissues have increased contractures, they are susceptible to MH (van den Bersselaar et al., 2022). These tests are still relied on heavily as only a select few variants have been confirmed pathogenic. The issue with these tests is that they involve unnecessary invasive muscle biopsies. Because of the extreme nature of these tests, new sequencing tests are being been created.

METHODOLOGY

Design

This scoping review examines the association between malignant hyperthermia and exercise-induced hyperthermia in the presence of calcium channelopathy. A scoping review was appropriate for this study due to the lack of published evidence linking the alteration of the RYR1 gene and the development of malignant hyperthermia after a past medical history of exercise-induced hyperthermia thus suggesting why these findings are important to recognize in clinical practice.

Search Strategies

A literature search was conducted through the databases EMBASE, PubMed, CINAHL Complete, Cochrane Library and Medline Complete by using the terms: “malignant hyperthermia,” “exercise-induced hyperthermia,” “exertional rhabdomyolysis” and “RyR1”.

Inclusion Criteria

Inclusion criteria for this study included full texts, English language, all age groups, all sexes, and all study designs within the last 30 years. The inclusion criteria were not limited in order to capture as much information possible on this topic.

Exclusion Criteria

The exclusion criteria were texts, literature not written in English, and studies published over 30 years ago.

Evidence Appraisal

The Johns Hopkins Nursing Evidence-Based Practice Research Evidence Appraisal Tool Appendix E was utilized to determine research credibility and validity of selected literature. See Table ___ for appraisals.

RESULTS/REVIEW OF LITERATURE

Necessity for Additional Testing with history of Exertional Rhabdomyolysis

In a general consensus, a large amount of literature, including work from van den Bersselaar et al. (2021), Wappler et al. (2001), and Kruijt et al. (2020), have suggested an association between exertional rhabdomyolysis and the development of malignant hyperthermia after exposure to volatile anesthetics yet advise that more variants in the RYR1 gene must be identified, classified and analyzed at the genomic level. Wappler et al. (2001) performed a study

that evaluated the MH status of individuals with previous episodes of MH-like events with physical exertion. They found that 10 out of 12 patients who experienced confirmed exertional rhabdomyolysis had positive IVCT tests and expressed significant contractures after encountering halothane. Exertional rhabdomyolysis in these cases were determined by presenting symptomology and CK levels. Three of those 12 patients also had RYR1 variations. This number could possibly have been even larger with the expanded knowledge that is now understood about the RYR1 gene. Wappler et al. (2001) suggest that a full genetic analysis at the cDNA and genomic level is required to fully understand the intricate relationship between exertional rhabdomyolysis and the RYR1 gene. Their recommendations include histologic muscle tests, IVCT, and genetic screenings for patients who have had encountered episodes of exertional rhabdomyolysis to determine MH susceptibility.

Van den Bersselaar et al. (2022) furthered this research by conducting a retrospective multicenter cohort study, which evaluated people with and without personal or family histories of adverse anesthetic events, exertional/recurrent rhabdomyolysis, RYR1 variations and exertional heat stroke. More than 40% of people that had histories of exertional or recurrent rhabdomyolysis events were confirmed MH-susceptible and often contained RYR1 variations thus suggesting MH indications. Overall, they suggested the importance of identifying and classifying more variants as well as increased testing for MH susceptibility in patients with exertional rhabdomyolysis. An additional study by van den Bersselaar et al. (2021) expounded upon this conclusion that a study should be conducted that focuses on the neuromuscular and multisystem involvements of related RYR1 myopathy diseases in patients that present with MH and exertional rhabdomyolysis to enhance diagnostic workups and counselling.

Kruijt et al. (2020) found similar results in a retrospective single-center study looking at over 1302 patients that presented with CK levels above 2000 IU/l or a confirmed diagnosis of rhabdomyolysis and included patients with heat illness or genetic myopathies. Out of these patients,193 had clinical characteristics related to genetic susceptibilities. Almost half of those patients had a confirmed mutation in their rhabdomyolysis-associated gene. Twenty-two genes were identified with 56 different variants, including in the RYR1 gene. Overall, Kruijt et al. (2020) suggest that awareness of these genotypes is crucial to determine rhabdomyolysis susceptibility and for personalized counseling regarding other life-threating events like MH.

IVCT Testing to Show Similar Mechanisms

A retrospective cohort study and systematic review by Kraeva et al. (2017) examined 17 MHS patients that experienced exertional rhabdomyolysis based on their symptomology and CK levels. Out of 17 patients, 10 had a variation in either their RYR1 or CACNA1S genes that were determined MH causative or potentially pathogenic mutations. The systematic review section found that in 20 different articles, 78% of patients with ER presented with either MH causative/associated variants including the CACNA1S and RYR1 genes. Everything signaled to an association between MH susceptibility and ER. Kraeva et al. (2017) concluded that variants in RYR1 and other MH causative mutations could increase a patient's risk of developing MH.

The role of RYR1 was confirmed in the heritability in EHI and MH from a study by Gardner et al. (2020) in which they looked at 38 different genes that were related to skeletal muscle calcium regulation. This study included 64 people that had a history of either exertional heat intolerance or exertional rhabdomyolysis and had IVCT tests completed. They found 51 potential pathogenic variations in 38 of the patients. Some of those variations were in the RYR1 gene p.T3711M and p.I3253T, which were both previously shown to be either likely pathogenic

for MH or EHI. Additional variations were also found in the CACNA1S gene. Overall, they confirmed that there is a role in RYR1 in the heritability of EHI and MH through clinical similarities and IVCT phenotypes between both conditions.

Roux- Buisson et al. (2016) looked at 23 different EHI crises in military personnel. They all responded positively to IVCT, thus suggesting MH susceptibility and a defect in muscle calcium homeostasis. Each person's body core temperature ranged from 39.5-41.6 and had elevated CK levels. It was confirmed that 6 RYR1 mutations and 1 CACNA1S mutations were found, meaning that 13% of the positive IVCT had pathogenetic variations in RYR1, thus suggesting that MH susceptible patients are at an increased risk of developing EHI in strenuous conditions. The researchers concluded that although they are at risk for developing EHI, more studies need to be completed to determine if EHS patients are at an increased risk for developing MH in anesthesia procedures because of RYR1 mutations.

Carpenter et al. (2009) conducted a study to assess if variations in RYR1 could cause differences in MH phenotypes by looking at IVCT tests and CK levels to create a link between MH phenotypes and genotypes. They found a new correlation between the degree of IVCT responses and the onset of MH. They also found a correlation between the baseline CK levels and MH onset set. Each phenotype severity was determined by each individual RYR1 variation, which could suggest the variability in MH cases during anesthesia and why some variations are subsequently occurring with exercise-induced rhabdomyolysis and heat stroke. This variability suggests why IVCT tests and genetic screening of the RYR1 gene are so important to determine what MH phenotype the patient might be at risk for. This study found that some RYR1 variants have increased Ca2+ cycling in different conditions including heat and exercise compared to their cycling responses under volatile anesthetics. For example, the variant p.R163C related to

high CK concentrations has been located in patients with exertional rhabdomyolysis and heat stroke. These results show how there could be a potential association in the dysregulation of the RYR1 gene and similar responses due to exercise and anesthesia.

To further this association, Carsana (2013) conducted a study that reviewed cases of ER and stress-induced MH with RYR1 variations associated with MHS. She looked at one study that found four different RYR1 variations that are confirmed MHS causative mutations. One of those variations was found in two unrelated pediatric patients who had fatal, non-anesthetic awake episodes related to febrile illness and heat stress and tested positive in IVCT tests. One of those patients who had the variant p.R3983 also had an MH attack while under general anesthesia using halothane. These clinical events show that some RYR1 variants are associated with both phenotypes of MH and exercise/stress induced rhabdomyolysis, thus suggesting priority of IVCT tests and RYR1 testing in patients with either MH events or exercise/heat events.

A profound study by Capacchione et al. (2009) corroborates these findings by presenting evidence that there is an association but not a causal relationship between exercise-induced rhabdomyolysis and MH from volatile anesthetics. There have been confirmed clinical findings that support this association based on clinical episodes of MH from anesthesia thus suggesting implications for MHS patients and exercise regimens. Because of this association, it is suggested that healthcare providers and those administering anesthesia to patients with histories of heat/exercise illness should express caution regarding MH susceptibility. To expound upon this recommendation, a clinical case of a six-year-old was presented. They experienced severe muscle rigidity during an anesthesia procedure and subsequently experienced over 100 episodes of general body rigidity years after anesthesia. Between the years of 12-15 years old, he experiences episodes of difficulty breathing and calf spasms during exercise and was labeled

positive for CHCT tests and had a confirmed RYR1 variant.

Another case of a 12-year-old had a similar clinical episode that labeled them MHS. This patient had an exertional heat stroke and passed away while playing football. The study revealed that this patient had an MH association in their RYR1 gene and that the patient's father also had the same mutation. These examples confirm the association between MH and exercise rhabdomyolysis and warn people that there is an increased risk of developing heat/exercise intolerance if they are MHS. With this conclusion, the opposite is not confirmed. There have not been confirmed cases of a patient experiencing ER then developing MH during anesthesia. Although patients with ER could test positive for MHS with IVCT, it is not a causal relationship (Capacchione et al. 2009). Because of this knowledge, more clinical and genomic studies are necessary.

Next-Generation Sequencing

One of the biggest limitations in determining a direct answer to the correlation between the development of MH after rhabdomyolysis events is due to the lack of genetic analysis with only using IVCT. Next generation sequencing has started to play a vital role in determining more genetic variations that might contribute to the development of MH. A study by Fiszer et al. (2015) extracted DNA from blood in order to conduct PCR testing. Their goal was to assess for pathogenicity and variants in RYR1 genes in 29 patients through bioinformatic approaches. Overall, they found three pathogenic and four novel RYR1 variations and confirmed an additional five variations that have previously been reported to be associated with MH. They also extracted three variants from an exertional heat illness cohort of 28 people and confirmed two previously examined variants. Additionally, they located four rare variants in the CACNA1s gene in both people with MH and EHI. Another study by Kim et al. (2013) found another

pathogenic RYR1 variation and CACNA1S variation that had not previously been determined without next gen sequencing. Because next gen sequencing allows for more sensitive recognition, less mutations are missed with conventional DNA sequencing and allows for a more powerful look at the associations between MH and EHI.

DISCCUSSION

There is enough evidence to stress the importance of increased knowledge that an association exists between the development of malignant hyperthermia and exercise-induced rhabdomyolysis. Thus, this information should be escalated within the healthcare profession and sports medicine industries to prevent future complications. Although there is not a confirmed causative correlation, there is a significant association that must be evaluated further through deeper genetic analysis and next generation sequencing. There is strong evidence that IVCT is a good steppingstone to acknowledge some of the shared genetic variations in MH and exercise-induced rhabdomyolysis, but it is not extensive enough and lacks many crucial elements in which MH susceptibility may go unrecognized. The evolution of next generation sequencing can help advance the future of research on this topic and has proven to increase the knowledge of different genetic mutations that could be potentially dangerous.

Deeper genetic testing should be completed on patients who have had a history of exercise-induced hyperthermia or with presenting symptoms, including increased CK levels, cramping, myalgia, myoglobinuria, muscle weakness, and muscle swelling prior to anesthesia based on the fact that there are confirmed similar channelopathies in the RYR1 genes causing both exercise-induced rhabdomyolysis and malignant hyperthermia. One case is one too many if this a preventable condition. The knowledge of MH has increased expansively yet still has much room for improvement on preventative measures rather than reactive measures.

Limitations

The greatest limitation of this research is that there has not been much published literature on this topic, thus there is not much data to confirm a statistically significant correlation between the development of MH in anesthesia after a history of exercise-induced rhabdomyolysis and heat stroke. Only a few confirmed cases have been identified, thus an association can be confirmed but not a correlation. Additionally, no research has confirmed a history of MH in anesthesia causes an increased risk of developing exercise-induced rhabdomyolysis, thus there is still a vast amount of genetic testing that must be done to determine why the reverse is not applicable. The available literature during this time was used in this scoping review so the most up-to-date information was utilized.

CONCLUSION

Overall, it can be confirmed that with just IVCT tests alone, people with past medical histories of exercise-induced hyperthermia could have an increased risk of being MH-susceptible based on a RYR1 channelopathy as they both follow similar calcium regulation mechanisms in the same affected receptors. What that risk is and how serious it could be are still unknown, although there is enough evidence to suggest that a similar channelopathy is noted in both circumstances and should be taken seriously by medical professionals in all fields. Subsequently, patients who have had malignant hyperthermia events under anesthesia should be closely monitored during extensive exercise because of the risk of developing exercise-induced rhabdomyolysis if they have the mutated RYR1 gene.

It is important to increase interprofessional communication between these two fields so that these patients are closely monitored and educated about potential risks. If patients were screened before anesthesia for any of these receptor channelopathies if they had a history of

exercise-induced rhabdomyolysis or similar symptoms or screened after a malignant hyperthermia event, healthcare professionals may decrease the number of patients that are experience fatal outcomes from these conditions by implementing preventative care either while under anesthesia or while exercising.

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APPENDIX

Article (author/date)	Evidence Type	Level	Quality	Number of Subjects	Symptoms	Why They Were Chosen	Main Findings
Antonio Michelcci et al	Quasi-experimental	II	High	unknown	Whole body contractures, difficulty breathing, impaired/spasmodic movements, increased core temperatures (5.4-degree increase), increased CK, K+ and Ca2+ levels,	Male mice, RYR1 and CASQ1-null	There is a similar mutation in ryanodine receptortype-1 (RYR1Y522S/WT) and calsequestrin-1 knockout (CASQ1-null), 2 proteins that control Ca2+release in skeletal muscle in mice. Thus shows common molecular mechanisms underlie MH crises and exertional HS in mice
Carsana, Antonella	Review article	n/a	High	n/a	n/a	n/a	Some sequence variants of RYR1 are associated with both ER and stress induced MH events so IVCT and screenings are important. Suggests that exercise-induced muscle injury can decrease severity of stress induced MH if caught quickly.
Capacchione, John F et al	Literature Review	n/a	Good	n/a	n/a	n/a	EHI, ER, and MH have similar pathophysiology as hypermetabolic states with high demand for ATP, accelerated oxidative, chemical and mechanical stress of muscle and uncontrolled increase in intracellular calcium. IVCT confirm anesthesia MH associations yet not conclusive for EHI and ER. EHI and MH relationship established for MHS by ICVT, but no RYR1 testing. ER and MH relationship established with RYR1 mutations. May have a relationship but not causal relationship.
D Carpenter et al	Quasi-experimental	III	High	504	Increased CK concentrations, shorter ICVT test time	In UK MH Investigation unity for determination of susceptibility to MH. Either suspected clinical MH reaction, close relative to one who was unable to be tested or related to someone who was MH susceptible.	There is a significant difference in MH phenotypes with different RYR1 variations. Some variants lead to more severe MH phenotypes and are in relatively conserved sites in the proteins. This variation in MH phenotype shows why some people might be more susceptible to ER and anesthesia depending on the area of RYR1 affected.
Dorota Fiszer	Quasi-experimental	II	High	57 (29 MH susceptible and 28 EHI)	Clinical episodes of heat illness, inability to thermoregulate, failed 2 separate heat intolerance tests	Patients in MH unit for IVCT test for diagnosis of MH-susceptibility	Thee pathogenic and four novel RYR1 variants were found in 29 patients, 5 RYR1 variants previously found were reported again. Thee novel RYR1 variants were found in exertional heat illness and two others in association with MH. 4 rare variants were found in the CACNA1s gene in the exertional heat illness and

							MH groups. Next gen sequencing is successful in identifying diagnostically useful variants in RYR1 and CACNA1S in MH and exertional heat.
Gardner, Lois et al.	Quasi-experimental	II	High	68	CNS disturbance, organ damage, rhabdomyolysis, myalgia, cramps, fatigue, CK > 1200 IU/L, acute kidney injury, metabolic acidosis, disseminated intravascular coagulation, seizure, cardiac arrest	Military personnel (male, young, fit) with history of EHI or ER and phenotyped with IVCT	51 non-polymorphic pathogenic variants in 20 genes in 38 patients. Two RYR1 genes pathogenic for EHI and PYGM gene pathogenic for 3 patients. One CACNA1S gene pathogenic. Confirms role of RYR1 in heritability of EHI and ER.
Luuk. R can den Bersselaar, MD et al.	Prospective, cross-sectional cohort study	II	High	N/A	Muscle weakness, hypermobility, respiratory weakness, resting CK level	Patients from MH and ERM cohorts at national MH investigation unit	Currently in process, trying to improve recognition of RYR1 related symptoms and more personal approaches to those with this mutation. MH and exertional rhabdomyolysis are episodic phenotypes that respond to external triggers and stimuli in people that have RYR1 variants.
Luuk R. van den Bersselaar, MD., et al.	Retrospective multicenter cohort study	III	High	520	Positive IVCT test, rhabdomyolysis	Patients in MH investigation units	3 most frequent referral indications to MH units included personal history of adverse anesthetic event (40/6%), family history of adverse anesthetic event (22.1%) and exertional and/or recurrent rhabdomyolysis (8.8%). 39.2% of referred patients without personal or family history of anesthetic adverse events were suspected to be MH susceptible with history of exertional rhabdo, exertional heat stroke, RYR1 myopathies
M. Davis et al.	Nonexperimental	III	High	3 unrelated families	Increased HR, muscle rigidity, temp 38.2, myoglobinuria, increased CK, muscle cramps,	Non=specific myopathies associated with EIR or congenital musculoskeletal abnormalities with MH susceptibility.	Two of the three cases had exercise-induced rhabdomyolysis. IN each case, MHS was confirmed with IVCT. DNA sequence analysis testing showed mutation in the arginine401-cysteine substitution in RYR1 thus confirming a mutation hotspot in both MH and ER.

Kraeva, Natalia, PhD et al.	Systematic Review	III	High	17	Mild exercise-induced myalgia, rhabdomyolysis	Canadian MGS patients who presented with rhabdomyolysis. More than 2 episodes of ER and diagnosed with MHS.	Although the contracture tests (CHCT/ICVT) were originally designed for testing patients with suspected anesthetic-induced MH reaction, a positive CHCT in patients with rhabdomyolysis caused by non-anesthetic triggers may imply existence of a common muscle defect. Presence of MH causative mutations and putative deleterious RYR1 variants in pts with unanticipated or recurrent exertional rhabdomyolysis and without history of adverse anesthetic reactions suggested their increased risk for MH.
Kruijt, N. et al.	Retrospective single-center study	II	High	1302	Rhabdo and increased CK values	Acute CK level exceeding 2000 IU/l. Diagnosed with rhabdomyolysis.	Anoxia was most reported trigger of rhabdo. 72 patients had unequivocal genetic defect. 22 genes with pathogenic variants were identified with 52 different variants including RYR1.
P.J.E Poels, et al.	Quasi-experimental	II	Good	6	Increased K+, positive ICVT, muscle pain, dark urine, elevated serum CK, pigmenturia, muscle contractures/weakness	Unexplained and recurrent attacks of rhabdomyolysis	Positive IVCT in 5/6 patients thus suggesting the association between unexplained non-anesthetic-induced rhabdomyolysis and MH susceptibility is not exceptionally rare. Once the gene for MH has been isolated then the question can be answered with certainty wither patients will suffer from MH.
Frank Wappler, M.D. et al.	Quasi-experimental	III	Good	58	Myoglobinuria, muscle cramping, fever, rhabdomyolysis, increased CK	Severe ER, intense muscle cramping or aching in history were investigated in lab for MHS.	10 ER patients had positive IVCT results and showed pronounced contractures after exposition to ryanodine. 3 ER patients had mutations in RYR1 gene. All anesthesia induced MH patients had positive IVCT results. Control pt had normal IVCT results and no MH mutations. Should do muscle biopsies for histology examination and IVCT tests in patients with ER and additional genetic screening. Goal to develop standardized test protocols for exercise studies to determine MHS and trigger.

Henry Rosenberg et al	Review	III	High	N/A	N/A	N/A	Over 400 variants have been identified in theRYR1gene located on chromosome 19q13.1, and at least 34 are causal for MH. Less than 1 % of variants have been found in CACNA1S but not all of these are causal. MH occurs when exposed to volatile anesthetics and in some cases from vigorous exercise. Several reports link MH with exertional heat stroke and indicate hypersensitivity to RyR1 agonists, causing heat sensitivity.
Roux-Buisson, N. et al	Non-experimental	III	High	23	Positive IVCT, muscle hypermetabolism	Military cohort with well-documented EHS crisis and positive IVCT tests	13% of patients had RYR1 putative causative variations, thus suggesting EHI might result from RYR1 mutations. Suggests that MH patients are at risk for EHS. Harder to see reverse correlation. Share similar pathologies but might come from different causes.
Jerry H Kim et al	Quasi-expirimental	II	High	Four families, control group 5,379	N/A	Multiple MH cases lacking mutations in RYR1 and CACNA1S by sanger sequencing	Detected three rare and likely pathogenic variants in RYR1 and one in CACNA1S. Each of these variants was missed by conventional, automated cDNA Sanger sequencing methods. These were not seen in variant databases or in our control population sample of 5,379 exomes