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Future Directions in Malignant Hyperthermia Research and Patient Care

Sharon J. Hirshey Dirksen, PhD,

Malignant Hyperthermia Association of the United States, Pittsford, NY

Marilyn Green Larach, MD, FAAP,

The North American Malignant Hyperthermia Registry of MHAUS

Henry Rosenberg, MD,

St. Barnabas Medical Center, Livingston, New Jersey

Corresponding Author: Sharon J. Hirshey Dirksen, PhD, MHAUS, 35 Locke Dr. Pittsford, NY 14534, Phone: 585-218-0348, FAX: 585-218-0348, sharon@mhaus.org.

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Name: Henry Rosenberg, MD

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Name: Barbara W. Brandom, MD

Contribution: This author helped write the manuscript.

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Name: Jerome Parness, MD, PhD

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Name: Robert Scott Lang, MD

Contribution: This author helped write the manuscript

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Name: Meera Gangadharan, MD

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Name: Tyler Pezalski, MD

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Attestation: Tyler Pezalski approved the final manuscript.

Barbara W. Brandom, MD,

Department of Anesthesiology, Children's Hospital of Pittsburgh, University of Pennsylvania Medical Center, Pittsburgh, Pennsylvania

Jerome Parness, MD, PhD,

Department of Anesthesiology, Children's Hospital of Pittsburgh, University of Pennsylvania Medical Center, Pittsburgh, Pennsylvania

Robert Scott Lang, MD,

University of Pennsylvania Medical Center, Pittsburgh, Pennsylvania

Meera Gangadharan, MD, and

University of Texas Medical Branch at Galveston, Texas

Tyler Pezalski, MD

Uniformed Services University of the Health Sciences, Washington, DC

Abstract

Malignant hyperthermia (MH) is a complex pharmacogenetic disorder of muscle metabolism. To more closely examine the complexities of MH and other related muscle disorders, the Malignant Hyperthermia Association of the United States recently sponsored a scientific conference at which an interdisciplinary group of experts gathered to share new information and ideas. In this Special Article, we highlight key concepts and theories presented at the conference along with exciting new trends and challenges in MH research and patient care.

Introduction

Malignant hyperthermia (MH) is a pharmacogenetic disorder of muscle metabolism. Acute episodes of MH are usually precipitated by exposure to potent inhaled anesthetics and/or succinylcholine. At the cellular level, MH is characterized by abnormalities in excitation-contraction coupling and the control of myoplasmic calcium levels during muscle activation. In an effort to contribute to our understanding of these complex processes, the Malignant Hyperthermia Association of the United States recently sponsored a scientific conference (April, 2010). At the conference, an interdisciplinary group of experts including clinicians, geneticists involved in MH research and counseling, and basic scientists studying mechanisms of calcium regulation and dysregulation in normal and MH skeletal muscle were able to present and discuss new information and ideas as they relate to MH and other muscle disorders.

A broad range of topics was addressed, including: advances in understanding the pathophysiology of MH; genetic testing for MH susceptibility; the relationship between MH susceptibility and other muscle disorders; MH epidemiology; and future directions in clinical practice, educational initiatives, and MH-related research. (See Appendix for conference agenda, meeting speakers and topics.) In this Special Article, we have attempted to integrate key concepts and theories presented at the conference (Table 1) with those in the current literature. In doing so, we hoped to highlight both the trends and challenges in the field of MH research and patient care (Table 2).

Malignant Hyperthermia Pathophysiology

Malignant hyperthermia (MH) has been described as a pharmacogenetic disorder of skeletal muscle involving dysregulated myoplasmic calcium (Ca^{2+}), hypercontracture, and hypermetabolism in response to exposure to potent volatile anesthetics and depolarizing muscle relaxants. In some cases, the precipitating environmental factor may be exertional or

heat stress (without pharmacologic trigger agents). Susceptibility to MH is transmitted in an autosomal dominant fashion with incomplete penetrance and variable expressivity. The skeletal muscle ryanodine receptor (RyR1) is the Ca^{2+} release channel within the sarcoplasmic reticulum (SR) membrane whose major known function is supplying the myoplasmic Ca^{2+} for contraction. It is widely accepted that the gene for this protein (RyR1) is the primary, though not exclusive site for mutations conferring susceptibility to MH, and that the aberrant channel plays a critical role in the development of this syndrome.¹

Phenotypic Variability of RyR1 mutations

Thus far, more than 200 RyR1 variants² associated with MH susceptibility and/or central core disease (CCD) have been identified, though only 30* of these have garnered sufficient evidence to be considered causal, and hence diagnostic, for MH susceptibility. The MH susceptibility mutations in RyR1 are reported to be present in only 50–70 percent of families that are susceptible to MH.³ Of the many RyR1 variants reported by Rosenberg et al.², more than 150 are reported to be associated with MH, ~100 are reported to predispose to CCD, a rare myopathy that presents with slowly progressive weakness, and more than 20 appear to be associated with both MH and CCD. Furthermore, RyR1 mutations have also been associated with other myopathies such as multiminicore disease (MmD) and nemaline rod myopathy, though the linkage of these entities to RyR1 is far less certain than with CCD. Interestingly, mutations in RyR1 can produce a single disease state, such as MH susceptibility or CCD alone, or a combination of disease states, i.e., MH susceptibility and CCD together.⁴ The considerable degree of phenotypic variability associated with RyR1 mutations was a theme of this conference.

Regulation of Calcium Homeostasis

The regulation of intracellular Ca^{2+} is a focus for researchers attempting to better understand MH pathophysiology and the pathogenic implications of the different RyR1 phenotypes. In MH-susceptible (MHS) individuals, the release of Ca^{2+} through an abnormal RyR1 in the presence of volatile anesthetic activates an exaggerated increase in intramyoplasmic Ca^{2+} by an unknown mechanism. This increase in Ca^{2+} leads to muscle activation, often a prolonged state of muscle contracture, and more often the hypermetabolic response characteristic of MH.

Further understanding of MH pathophysiology may be provided by elucidating the mechanism of action of dantrolene, the direct-acting muscle relaxant that is the sole treatment for MH. Dantrolene has been shown to bind to RyR1 at a site distinct from the ryanodine binding site and to inhibit RyR1-dependent Ca^{2+} transients, thereby serving to decrease resting Ca^{2+} in the myoplasm, as well as decrease the Ca^{2+} transient after electrical excitation or exposure to caffeine or halothane.^{5–7} However, four decades after the discovery of dantrolene, the exact mechanism by which this compound exerts its pharmacological effects is still debated. Indeed, there is evidence that dantrolene may not have a direct effect on RyR1 channel properties per se⁸; rather, it may modulate Ca^{2+} levels indirectly by inhibiting processes which control Ca^{2+} entry into the myoplasm. Specifically, dantrolene may inhibit a RyR1-dependent variant of a process called Store-Operated Calcium Entry (SOCE), by which Ca^{2+} is brought into the cell through the plasma membrane from extracellular stores (see also Figure 1). Activation of SOCE occurs when the sustained opening of RyR1 channels depletes SR Ca^{2+} stores.^{9–13} Dantrolene appears to suppress that part of SOCE activity dependent upon RyR1.

*See www.emhg.org for a listing of RyR1 mutations deemed causative for MH susceptibility by the European Malignant Hyperthermia Group (last accessed 3/11/11).

In addition to SOCE, dantrolene also appears to affect other Ca^{2+} entry pathways such as excitation-coupled calcium entry, a store-independent Ca^{2+} entry pathway that is activated by repetitive or prolonged depolarization¹⁴ and SOICR (store overload induced calcium release), a process triggered by SR Ca^{2+} overload that leads to spontaneous release of SR Ca^{2+} into the cytoplasm.¹⁵ Investigators postulate that MH-causing mutations in RyR1 serve to lower the channel's SOICR threshold, an effect increased by halothane and other volatile anesthetics. Dantrolene then appears to work by preventing the Ca^{2+} increase secondary to SOICR (as observed in isolated cell systems expressing mutant RyR1).¹⁵ These results suggest that dantrolene may act at multiple sites within the various physiological processes that underlie Ca^{2+} homeostasis in skeletal muscle, or that dantrolene works at a single site on RyR1 that affects coupling to all these different processes. Future research into the mechanism of action of dantrolene should lend insight into the regulation of skeletal muscle Ca^{2+} homeostasis, as well as the pathophysiology of MH and other skeletal muscle disorders.

Researchers studying the regulation of Ca^{2+} homeostasis and the role of RyR1 continue to propose and test different theories relating to the phenotypic variability observed in RyR1-related disorders. It appears that the rate of Ca^{2+} leak from the SR into the cytoplasm is one of the processes that may vary between MH phenotypes. It is theorized that RyR1 mutations causing a high leak rate may sufficiently deplete Ca^{2+} stores in the SR to produce a dearth of Ca^{2+} for sufficiently strong contractions during normal excitation-contraction coupling, thereby producing chronic myopathic weakness. How it would do this and not trigger replenishing of the SR Ca^{2+} store via SOCE is not clear. It is also postulated that at the same time these very same RyR1 mutations lower the threshold for activation of this channel by volatile anesthetics, allowing for the triggering of MH. Both of these phenomena in an affected individual would then account for both CCD and MH susceptibility in the same patient. In contrast, RyR1 mutations conferring a low leak rate might lower the threshold for triggering MH, but would not sufficiently deplete Ca^{2+} stores so as to cause a myopathy such as CCD.^{4,16} Additionally, another mechanism that has been proposed to explain the phenotype of CCD alone is excitation-contraction (EC)-uncoupling, wherein activation of RyR1 by the DHPR (dihydropyridine receptor) is inefficient, and does not result in the typical degree of SR Ca^{2+} release and subsequent muscle contraction. EC-uncoupling thus results in the phenotypic muscle weakness and hypotonia observed in patients with CCD.¹⁶ Of note, the impact of RyR1 mutations on the bidirectional signaling between DHPR and RyR1 during EC coupling is also an active area of investigation by many research groups.¹⁷

As researchers piece together the underlying pathophysiology of these disorders, new approaches to treatment may be developed, including the use of drugs to alter Ca^{2+} release or leak, as well as the methodology to directly target specific gene mutations, such as with allele-specific gene silencing. This method uses siRNA (small interfering RNA), a class of double-stranded RNA molecules that interfere with the expression of a specific gene, to "target" or 'silence' the damaging mutant allele, while sparing the normal wild type allele.¹⁸ As a result, an increased proportion of wild type RyR1 is expressed, with an expected rescue of channel and muscle function. Allele-specific gene silencing is currently being studied as a potential means of decreasing the deleterious effects resulting from a damaging mutant allele in autosomal dominant disorders.

Histological Changes in Myopathic Muscle Cells

The regulation of Ca^{2+} release is also being studied with regard to its role in the development of histological changes observed in myopathic muscle cells. Zvaritch et al.¹⁹ have postulated that the random association of normal and mutant RyR1 subunits creates a heterogeneous group of RyR1 tetramers with a range of degrees of functionality, yielding

variable responses of dysregulated Ca^{2+} release and muscle contraction. This is hypothesized to result in physical stresses among myofibrils, and tearing and shearing in focal areas. These areas expand to form minicores, cores, and nemaline rods. These core regions are the hallmark of CCD and are present in other myopathies such as MmD, reportedly (but not proven to be) associated with MH. Formation of these core regions has also been proposed to result from excessive SR Ca^{2+} leak, leading to the formation of reactive oxygen species and reactive nitrogen species.²⁰ Accumulation of these species results in oxidative damage to the cell, disruption of mitochondria and the sarcotubular system, and the eventual degeneration of contractile machinery. Ca^{2+} stores are depleted, cores and rods are formed, and a myopathic state develops.²¹

The Link to Environmental Heat Stroke

In addition to RyR1-related myopathies, MH susceptibility may also share a common pathogenic mechanism with environmental heat stroke. Using mouse models of MH, investigators are actively studying the role of Ca^{2+} leak and oxidative stress in the development of environmental heat stroke.²⁰ Mice with a specific knock-in MH mutation in RyR1 demonstrate muscle contractures, rhabdomyolysis and death in response to elevated temperatures, this in the absence of anesthetics. The proposed mechanism is similar to that described for core formation above, and involves excessive Ca^{2+} leak leading to the development of reactive nitrogen species. Subsequent S-nitrosylation of the mutant RyR1 channel occurs, leading to an increase in temperature sensitivity and the triggering of more Ca^{2+} release, resulting in prolonged muscle contracture and the hypermetabolic response typical of MH.

Indeed, the association of exertional heat illness and MH has been described in humans. There have been several case reports of individuals with histories of clinical MH who subsequently developed exertional rhabdomyolysis (ER) or exertional heat illness. In addition, as recently published by Capacchione et al.,²² a patient with a history of ER was reported to subsequently have experienced a clinical MH episode.²³ This patient was later found to have several variants in the RyR1, CACNA1S (the alpha subunit of the DHPR), and CASQ1 (calsequestrin) genes, although the pathophysiological significance of these variants is unclear. Because these genes encode proteins with central roles in the process of excitation-contraction coupling and the regulation of intracellular Ca^{2+} , the speculation is that perhaps variants from normal in these genes lead to enhanced sensitivity not only to the common triggers of MH (e.g., volatile anesthetics), but also to exercise and heat stress.

Future Research

Further study of the role of RyR1, CACNA1S, and CASQ1 (and the proteins they encode) in MH may be valuable because of the role their respective cardiac isoforms have in the development of CPVT (catecholaminergic polymorphic ventricular tachycardia). CPVT is an inherited syndrome manifest as sudden death related to mutations in the cardiac ryanodine receptor gene (RyR2). CPVT, like MH, occurs because of abnormal repetitive Ca^{2+} release from the SR, thereby causing cardiac arrhythmias.^{15,24,25} In fact, dantrolene has been shown to prevent CPVT in a knock-in mouse model, presumably by inhibiting Ca^{2+} leak through the cardiac RyR (RyR2). Dantrolene appears to correct the defective RyR2 interdomain interaction, illustrating again how studies of dantrolene's mechanism of action may serve to elucidate the pathophysiology of an RyR-linked disorder.²⁶

Oxidative damage and the regulation of Ca^{2+} release/ Ca^{2+} leak thus appear to be important factors in the development of the histological changes apparent in the core myopathies, as well as in the physiological response observed in environmental heat stroke and MH. At the

conference, current efforts to design new therapies for these disorders were discussed, including the evaluation of antioxidants and nitric oxide synthetase inhibitors, as well as the study of new ways to modulate RyR1 activity and Ca^{2+} homeostasis.

Genetics of MH

Identification of Gene Variants Causal for MH-Susceptibility – A Complex Matter

Very soon after the first description of the MH syndrome, investigators determined it was inherited in an autosomal dominant manner. With this observation, health care practitioners hoped it would be possible to identify MHS individuals by DNA analysis. However, it was not until 1990 that the primary gene locus for MH was identified as the RyR1 gene,²⁷ and then several years after, a second genetic locus associated with MH was identified as CACNA1S, which encodes the $\alpha 1$ subunit of the DHPR.²⁸ Subsequently, DNA testing for clinical use became available, but not until 2005, and then only for certain cases. During this time, mutations in RyR1 were also found to be causal for CCD and several other myopathies, while some cases of hypokalemic periodic paralysis were associated with mutations in DHPR.

Obtaining a diagnosis of MHS by analyzing the RyR1 and DHPR genes is both a difficult and time-consuming task. First, there is no single predominant mutation that is causal for MH in the human population. Second, RyR1 is a complex gene comprising 106 exons with a cDNA ~15,117kb,²⁹ while CACNA1S spans 46 exons³⁰ with a cDNA of ~6.16 kb²⁸. Finally, RyR1 is characterized by allelic heterogeneity; that is, there are more than 200 RyR1 variants with potential functional significance in MH and/or CCD and possibly other diseases, as well as harmless (benign) variants (polymorphisms) found in more than 1% of the population.² While some variants may ultimately be shown to be benign, not affecting amino acid sequence or function in the final protein, others may indeed be pathogenic as a result of replacement of generally conserved amino acids that lead to altered protein function. Sorting out the physiologic and clinical significance of these variants has been the focus of much of MH research over the past decade.

To determine whether a DNA variant is causally related to MH, several approaches are used. The variant must be characterized through genetic and functional studies, and meet certain requirements, as described by the European Malignant Hyperthermia Group[†]. First, the variant must be absent in at least 100 control patients. Second, the variant must be found in two unrelated families displaying clinical MH, and segregate with those who are MHS, either by clinical episode or by the caffeine-halothane contracture test (CHCT). Third, the DNA variant must represent a change in an amino acid that is ordinarily conserved across many species.

A widely accepted way to determine the significance of a DNA change or mutation is to incorporate the change into the gene for RyR1, and transfect that gene into a cell that does not normally express RyR1 (i.e., the human embryonic kidney or Chinese hamster ovary cell lines), or alternatively, to culture a myotube or immortalized lymphoblast from an affected individual. By quantitatively measuring Ca^{2+} release from the storage organelle in these cells after exposure to RyR1-agonists such as caffeine, or 4 chloro-m-cresol (4CmC), it is possible to determine whether the mutation causes enhanced Ca^{2+} flux, as found in cells from MHS individuals.¹

More recently, proof of principle of pathogenicity of RyR1 mutations has been shown by incorporating an MH-causing RyR1 mutation into the genome of a mouse (a knock-in

[†]See www.emhg.org for a listing of criteria to be used in genetic testing of MH susceptibility (last accessed 3/11/11).

mouse) and demonstrating the development of MH in these animals in response to trigger agents.³¹ Such approaches are the most accurate in determining pathogenicity, but they are time-consuming and expensive. Thus, in order to assess which DNA variants are likely to be or to not be tolerated, various computational tools have been developed. One such tool is the SIFT, or Sorting Intolerant From Tolerant, program, which evaluates the significance of a particular DNA change (and thus amino acid substitution in a protein) based on its conservation in other cell systems. A prediction is then made regarding whether a specific amino acid substitution at a specific position in the protein will have a phenotypic effect.³² Another such tool, PolyPhen, (polymorphism phenotyping) evaluates whether an amino acid change is likely to be deleterious for a protein, based on its 3 dimensional structure and multiple alignments of homologous sequences.³³

Applying these approaches to genetic testing for MH has resulted in the determination, as noted previously, that 30 DNA variants in RyR1 are indeed causal for MH, while three variants in DHPR have been shown to be causal for MH^{28,34}. Overall about 30% of known MHS patients will demonstrate one of the known RyR1 causal mutations.³⁵ However, up to 70% will demonstrate a DNA variant, whether it be a known causal mutation or a variant of unknown significance. Interpretation of genetic tests of MH susceptibility must be made carefully to differentiate those variants that are causal from those of unknown significance. It is therefore extremely important for both a trained genetic counselor and an MH expert to review the clinical findings along with the DNA results, in order to best assess the impact of these findings on the management of the patient and the diagnosis of MH susceptibility in their family.

Diagnostic Testing

For more than 40 years, the primary laboratory diagnostic test for MH continues to be the CHCT in North America. In Europe, the diagnostic test is known as the in vitro contracture test. The two tests use the same general approach of exposure of freshly biopsied skeletal muscle to caffeine and halothane, but they differ slightly in methodology and interpretation.³⁶

After the introduction of DNA analysis, researchers began to compare findings of the contracture tests and DNA changes in the two genes that are associated with MH. In general the agreement is excellent, though there are reports of discordance.³⁷⁻³⁹ Such cases suggest there are as yet unknown factors responsible for the clinical expressivity of MH. Two approaches have been followed since then, first to refine the analysis of the contracture test in relation to the DNA test, and the second to further characterize the molecular genetics of MH in order to be able to apply DNA analysis as the primary test for MH susceptibility. In addition, Ginz et al. have recently described a new approach to resolving the differences between the contracture test and the genetic test results; the area under the curve of the contracture response to halothane or caffeine is analyzed, rather than the peak contracture response alone. By this approach, the sensitivity and specificity of predicting MH mutations has been reportedly improved to 93% and 95% respectively.⁴⁰

Challenges Remain

Although much progress has been made in clarifying the molecular genetics of MH, there are still many challenges to overcome. At issue is the need to confirm a patient's phenotype before DNA analysis is performed, because genetic testing undertaken in patients or family members with questionable clinical episodes is generally unproductive. There are several methods used to assist with phenotype confirmation; one tool is the Clinical Grading Scale, a scoring system relating the clinical signs of MH to the likelihood of an MH event.⁴¹ A second method is to investigate family history in detail for clinical MH crises expressed over

several generations. Ultimately, in the absence of a definitive clinical event, the most useful evaluation tool in confirming phenotype is the CHCT in North America and the in vitro contracture test in Europe.⁴²

Adding to the complexity of analyzing DNA variants is the observation that DNA mutation profiles (and possibly the functional effect of specific variants) may differ between human populations. For example, Sambuughin et al. have reported that a specific RyR1 variant associated with MH susceptibility in the Caucasian population may be a neutral polymorphic change in individuals of African descent.⁴³ On the other hand, in some cases, the search for disease-causing mutations may be simplified because certain mutations are more prevalent in specific populations. For example, the G2434R mutation appears to be the most common in the United Kingdom, followed by T2206M and then G341R; whereas in Germany, the R163C, R614C, T2206M, G2434R and R2454H mutations are common.²⁹ Such population-based variations are valuable for rapid screening of those at risk and their family members.

As noted previously, the penetrance and expression of the MH clinical phenotype appears to be quite variable among individuals who harbor RyR1 variants. A recent study by Carpenter et al.⁴⁴ suggests that the severity of the MH phenotype may be correlated with the type of RyR1 variant, and mutations within highly conserved regions of the gene may, in fact, be associated with a more severe MH phenotype. It is also possible that there are other factors not related to DNA sequence changes (i.e., epigenetic factors), which may affect the expression of RyR1 variants. For example, Robinson et al.⁴⁵ recently examined whether epigenetic allele silencing of RyR1 mutations was a contributing factor to the variable penetrance of MH susceptibility.

Although these investigators concluded that allele silencing of RyR1 was not likely to play a role in the inheritance of MH susceptibility, they did not discount the possibility that allelic silencing may be a contributing factor with respect to other genetic loci. Furthermore, reports of MHS patients and families who harbor more than one RyR1 variant^{39,43,46} lead us to question how the clinical expression of MH may be altered by the presence of more than one RyR1 variant. Is there a synergistic effect of these variants, leading to the preferential expression of MH susceptibility in certain individuals? Certainly, the discrepancy between the incidence estimates of MH events (discussed later in this article) and the much higher prevalence estimates of RyR1 variants within certain populations (1:2000 individuals by Monnier et al.⁴⁷ and 1:3,000 individuals by Ibarra et al.⁴⁸) lends further support to unknown, possibly epigenetic, factors at work.

As we enter the era of molecular medicine, rapid progress is being made in DNA analysis. It is noteworthy that DNA sequencing costs have declined 15,000-fold over the past decade and are likely to decline further in the next decade. In the very near future it will be economically possible to sequence the entire coding region of a gene and then the entire genome through use of such techniques as exome sequencing. This will lead to the detection of many more DNA variants whose significance will need to be determined, and, if phenotype information is available, this should also greatly enhance our understanding of the relationship between genotype and phenotype. In the case of MH susceptibility, it is hoped that such techniques will lead to the identification of other DNA changes that account for cases of phenotypic MH without either RyR1 or DHPR mutations.

Genetic testing for MH susceptibility has certainly led to greater appreciation of the clinical presentation of MH. However, we are far from the goal of using genetic testing alone for determining whether a specific individual is or is not at risk for developing a clinical episode of MH.

Malignant Hyperthermia Epidemiology

Conference speakers presented data on the incidence of MH, the mortality and morbidity from MH events, the prevalence of MH susceptibility, and the demographics of patients experiencing MH events.

The existence of the MH clinical grading scale defining MH events and MH susceptibility⁴¹ and the international classification of disease (ICD) code for MH due to anesthetics were fundamental to recent progress. The MH clinical grading scale has facilitated analysis of cases reported to the North American Malignant Hyperthermia Registry and comparison of cases from diverse sources. The MH ICD code has permitted analysis of cause-of-death-files from the National Vital Statistics System⁴⁹ and administrative databases such as the Nationwide Inpatient Sample⁵⁰, the Healthcare Cost and Utilization Project State Inpatient Database⁵¹ and the Kids' Inpatient Database.

Brady et al. have estimated the MH prevalence rate for the years 2001–2005 at 1.1/100,000 New York State surgical discharges with probable anesthetic exposures.⁵¹ However, as cited previously, Monnier et al. and Ibarra et al.'s analyses of individuals with homozygous or compound heterozygous mutations yielded an estimated MH susceptibility prevalence as high as 1 in 2,000–3,000 in the French and Japanese populations.^{47,48} These different prevalence estimates are a product of the fact that not all people are exposed to anesthetics and MH has variable expressivity.

Recent mortality estimates have been disparate partially as a function of the characteristics of the database used for analysis. Li et al. recently estimated the risk of MH-related mortality at 0.0082/100,000 United States surgical inpatients encompassing approximately 1% of all anesthesia-related deaths for the years 1999–2005.⁴⁹ In contrast, Rosero et al.⁵⁰ estimated the risk of MH-related mortality at 0.1310/100,000 United States inpatients. Li et al.'s lower estimate of MH-related mortality is more likely than Rosero et al.'s since the latter study used a less rigorously collected administrative database and it did not isolate medical from surgical patients.

While MH mortality rates are low, the rate of complications in acute MH episodes is much higher. Larach et al. found a 34.8% morbidity rate in 181 cases reported to the North American MH Registry between 1987 and 2006. Frequently reported complications included: change in consciousness level/coma, cardiac dysfunction, pulmonary edema, renal dysfunction, disseminated intravascular coagulation and hepatic dysfunction.⁵² The high complication rate emphasizes the need to continue to educate practitioners on the most effective way to diagnose and treat MH events and to study whether these efforts subsequently decrease the morbidity rate.

In the same study, Larach et al. also reported the demographic characteristics of 286 patients experiencing an MH event. They were predominantly male (75%) and young (median age of 22.0 years with 45% ≤ 19 years). Twenty-nine percent of patients had a muscular body build and 7% had a history of increased muscle tone. Before experiencing their MH event, 75% of these patients had undergone at least one general anesthetic and 51% had undergone 2 or more general anesthetics in which no signs of MH were observed.⁵² The experience of multiple prior unremarkable anesthetics illustrates the difficulty in determining the prevalence of MH susceptibility in the general population without an inexpensive, noninvasive MH diagnostic test. Furthermore, in a presentation based on a Kids' Inpatient Database study for the years 2000, 2003, and 2006, Li et al. found that children with MH were 69% male and had co-morbid muscle ligament disease, muscular dystrophies, and strabismus.‡ Larach et al.'s and Brady et al.'s studies confirm earlier epidemiologic studies by Britt et al.⁵³, Ordning⁵⁴, and Mauritz et al.⁵⁵ which reported that this autosomal

dominantly inherited disease with variable penetrance is expressed predominantly in the young and in males. Further molecular biologic and biochemical studies of the effects of age and gender on Ca^{2+} regulation and channel function are needed to elucidate the reasons for MH preferential expression.

Conference participants agreed that the success of future epidemiologic studies will depend upon continued reporting of adverse anesthetic events to the North American MH Registry, the creation of a separate ICD code for MH susceptibility history, and evaluation of current MH ICD coding accuracy by reviewing medical records at multiple institutions. Furthermore, future epidemiologic studies should focus on defining the clinical factors responsible for precipitating MH expression, including determining whether these factors behave differently with varying MH genotypes.

MH Susceptibility and Relationship to other Muscle Disorders

Assessment of Risk—The risk of MH or MH-like reactions in patients with known muscular disorders is not easy to determine. When such patients are scheduled for surgical procedures, this question becomes acute. Avoiding inhaled anesthetics may not make a general anesthetic safer because it is difficult to predict clinical perioperative events based on knowledge of the pathophysiology of the muscular disease. There have been many case reports and even small series that have discussed patients with myotonias, muscular dystrophies, core myopathies, and enzyme defects who developed one or more of the following symptoms: rigidity, increased temperature, arrhythmia, rhabdomyolysis, and hyperkalemia, during or after anesthesia. These reports were reviewed at the 2008 symposium sponsored by the Society for Pediatric Anesthesia and Malignant Hyperthermia Association of the United States (MHAUS), and subsequently published in a special series of articles.^{56–60} It is important to note that these reports are based on patients with the disease who had complications, not on the complete population with that disease; therefore, the incidence of serious postanesthetic complications in myopathic patients with these muscle diseases remains unknown.

RyR1-Related Myopathies - Closely Linked to MH Susceptibility

There is general agreement that certain rare myopathies, presenting with varying degrees of muscle weakness as well as differing clinical, genetic, and histologic findings are closely linked to MH susceptibility; specifically, individuals affected with King-Denborough syndrome, CCD or MmD are considered to be MHS.^{56,57} While a syndrome is defined by gross features of clinical presentation, myopathies such as CCD or MmD have been defined on the basis of histologic changes. Nevertheless, both syndromes and histologic findings may be associated with a variety of defects in genes and proteins. Current literature suggests that RyR1 variants are found in the majority of CCD cases. RyR1 variants are also associated with MmD, although the predominant form of MmD is most frequently associated with mutations in the selenoprotein N1 gene.⁵⁷ In King-Denborough syndrome, inheritance is unclear and most cases appear to be sporadic; however, RyR1 mutations have been associated with this syndrome.⁵⁶ It is thought that individuals who harbor a RyR1 variant may be at higher risk of MH than those with other gene variants; however, little has been published about anesthetic risk with regard to genotype in these disorders. Nonetheless, drugs that have caused MH are strongly contraindicated in individuals with either King-Denborough Syndrome, CCD or MmD.^{56,57,61}

[‡]Li G, Brady JE, Rosenberg H, and Sun LS. Excess comorbidities associated with malignant hyperthermia in children. Presented at the International Anesthesia Research Society's Annual Meeting, Honolulu, Hawaii, March 20–23, 2010.

Where the Risk for MH-Susceptibility is Unclear

Other muscle disorders in which the risk of MH-like reactions is less clear include those caused by enzyme deficiencies such as carnitine palmitoyltransferase type II (CPTII) deficiency, McArdle's disease and Myoadenylate Deaminase Deficiency; as well as those caused by abnormalities in other proteins, such as in Duchenne Muscular Dystrophy (DMD) and Becker Dystrophy (BD), and those in which the etiology is unknown such as Asymptomatic HyperCKemia.

Enzyme deficiencies compromise the energy supply of muscle. When muscle affected by enzyme deficiency is exercised, rhabdomyolysis (increase of the muscular form of serum creatine kinase more than 5 times the upper limit of normal) often occurs. In dystrophinopathies such as DMD and BD, there is continual muscle breakdown, rhabdomyolysis, and muscle regeneration until the muscle becomes fibrotic. While no causative RyR1 mutation has been identified in these dystrophic diseases, there may be alteration of normal RyR1 channel function contributing to abnormal Ca^{2+} homeostasis. In fact, investigators studying the mechanism of altered Ca^{2+} homeostasis in a mouse model of DMD, recently reported that RyR1 Ca^{2+} release channels are leaky (due to hypernitrosylation of the channel protein) in dystrophic muscle, thus contributing to muscle weakness and damage.⁶² Thus, for many reasons, individuals with these enzyme deficiencies or dystrophinopathies may be at an increased risk of rhabdomyolysis during and after anesthesia.

There is debate on recommendations regarding anesthetics in all of these conditions, myopathies, enzyme deficiencies and myotonias. Succinylcholine should be avoided because it causes depolarization of the muscle and some degree of increased resting tension in the process of inducing neuromuscular block.⁶³ Muscle metabolism in normal muscle increases as repolarization occurs after removal of succinylcholine.⁶⁴ Thus, succinylcholine causes muscle injury in people with normal muscle and significant rhabdomyolysis in myopathic patients.⁶⁵ Furthermore, there have been reports of hyperkalemic cardiac arrest in patients with subclinical myopathies.⁶⁶ In contrast, inhaled anesthetics less frequently produce clinically significant increases in rhabdomyolysis in patients with dystrophinopathy or muscle enzyme deficiency.

Evaluating Rhabdomyolysis

The need for a comprehensive evaluation of unexplained episodes of rhabdomyolysis was emphasized during the conference (Figure 2). The cause of rhabdomyolysis should be diagnosed because it has significant implications for relatives of the patient as well as for the medical management of the index case. All potential enzyme defects in muscle which may mimic one or another symptom of MH must be considered before jumping to a diagnosis of MH susceptibility. Evaluation of family history, the presence of other medical conditions, and a complete medical and neurological examination should guide the physician in ordering diagnostic tests. The neurologist may detect signs of occult myopathy, such as a preclinical dystrophinopathy, which were not detected by other physicians. The Exercise Intolerance Panel (DNA tests which screen for common mutations in the myophosphorylase [PYGM], myoadenylate deaminase [AMPD1], and carnitine palmitoyltransferase [CPTII] genes) and other enzyme analyses where deficiencies may underlie other possible metabolic myopathies may be useful. Genetic testing is available for many structural myopathies. If these assays are not diagnostic, it is recommended that a muscle biopsy and myoglobinuria panel be performed to analyze muscle tissue for the presence of other metabolic diseases which cannot be identified using only blood samples (e.g., mitochondrial myopathy). In addition, the bioassay diagnostic for MH susceptibility, the CHCT, may be completed as well. Viable muscle is needed to perform a valid CHCT. If MH susceptibility is diagnosed

by CHCT, examination of RyR1 may facilitate evaluation of MH status in relatives. As noted at the conference, one must be careful because the validity of a positive CHCT in a patient with an existing myopathy is questionable.

Association of Heat- and Exercise-Induced Rhabdomyolysis with MH Susceptibility

As mentioned earlier, the link between heat and exercise-induced rhabdomyolysis and susceptibility to MH is an active area of investigation at both the mechanistic level in animal models, as well as at the clinical case level.^{20,23} It is difficult to make a strong statement regarding the incidence of ER and heat-related illness (EHI) in MHS humans because data are limited, and there are many causes of ER and EHI which are not related to MH susceptibility.²³ Interestingly, new techniques discussed at this conference, such as heat tolerance testing used in the military, may prove to be useful tools for evaluating heat tolerance in MHS individuals.^{67,68} Because ER and EHI can occur in normal people, a more extensive investigation of potential genetic risk factors for these disorders is usually delayed until a second episode of ER occurs.

Risk Factors for Metabolic Muscle Disease

A more comprehensive testing strategy may help clinicians uncover the causes of previously unexplained myopathic symptoms. In addition, new research suggests that in the future, we may also be able to predict an individual's predisposition for metabolic muscle disease based on genetic risk factors. For example, investigations into the development of myopathies in patients taking statin medications (to lower cholesterol) suggest that underlying genetic variants may play a role in determining whether a myopathy develops. In fact, Vladutiu et al. reported that in clinical trial patients taking statins, those who developed a myopathy were more likely to be carriers of CPTII deficiency and myophosphorylase deficiency (McArdle's disease) when compared to those patients who did not develop a myopathy.^{69,70} In addition, a recently published study examining the pathologic effect of statins suggests that a preexisting impairment in Ca^{2+} homeostasis, such as in MHS muscle, confers an increased risk for statin-induced myopathy, since it appears that statins may have a pathologic effect on intracellular Ca^{2+} handling.⁷¹ Furthermore, an MH-like syndrome, without documentation of increased carbon dioxide production, was reported to occur in a child heterozygous for a mutation in the CPTII gene after exposure to succinylcholine and halothane. This raises the possibility that the presence of a pathogenic mutation in an apparently normal CPTII heterozygote may be a significant risk factor upon exposure to drugs capable of producing rhabdomyolysis.⁶⁰ It will be interesting to follow future research efforts aimed at identifying genetic risk factors for metabolic muscle disease.

In addition, there has been described a MH-like syndrome with rhabdomyolysis in patients with recent onset of type-1 diabetes.⁷² Clearly, when speaking of a syndrome such as MH that presents without pathognomonic signs, assigning pathophysiological causality with certainty is fraught with difficulty, and lends itself to incorrect diagnostics. Hence, there is a need for a comprehensive approach to diagnostics, and a continued intensive research program designed to identify the various potential causes of MH and MH-like syndromes.

Future Directions in Patient Safety

Anesthesia Workstations for the MHS Patient—As anesthesia machines have evolved into complex anesthesia workstations, it has become more challenging to prepare a “clean machine” for MHS patients and to safely ventilate patients' lungs using workstations during acute MH crises. As reviewed by Kim and Nemergut⁷³, the problem is two-fold. First, the ventilator is now part of the internal gas delivery system. Second, larger amounts of volatile-absorbent plastic and rubber materials are incorporated into the anesthesia workstation. Thus, even when the vaporizers are turned off, the workstation may continue to

deliver a significant dose of potent inhaled anesthetics fueling an on-going MH event or triggering a new event in MHS individuals.

In recognition of these developments, MHAUS has suggested that anesthesiologists contact manufacturers for workstation volatile anesthetic washout procedures. Manufacturers responded by suggesting that anesthesiologists contact MHAUS. To break this cycle, future research, carefully coordinated with manufacturers, is critical. A suggested approach is the following: (1) determine the minimum concentration of anesthetic gas required to trigger a MH crisis in MHS swine; (2) evaluate the safety and efficacy of activated charcoal filters (ACF) placed on the inspiratory limb of the breathing circuit to scrub volatile anesthetics from various workstations; (3) examine each major type of workstation and determine a safe and effective method for preparing each of them for use in MH patients. These issues should be addressed by conducting both in vitro and in vivo experiments with MHS swine, the best large animal model for MH events.⁷⁴

Preliminary in vitro work in this area by Jantzen and Kleemann⁷⁵ and Gunter et al.⁷⁶ has demonstrated the utility of an ACF placed in the inspiratory circuit for decreasing inspiratory inhaled anesthetic concentrations. Recently, Birgenheier et al.⁷⁷ have also demonstrated the utility of Food and Drug Administration-approved ACFs placed in both the inspiratory and expiratory limbs of contaminated Apollo and Aestiva workstations after their anesthesia circuits and rebreathing bags were changed. The ACFs served to decrease volatile anesthetic vapor concentrations to less than 5 ppm in less than 2 minutes and maintain these low concentrations for 60 to 90 minutes.

While awaiting these future studies, a safer method for managing MHS patients may be to use the FDA-approved ACF with a clean circuit and rebreathing bag and taped off anesthetic vaporizers, while administering high gas flows. It is important to remember, however, that the filters need to be changed every 90 minutes. In the opinion of two authors (MGL and BWB) of this manuscript, for patients experiencing an acute MH event, the filter use should probably be limited to 60 minutes only; the acute MH patient should then be placed on an uncontaminated ventilator with subsequent sedation/anesthetic provided IV. In the chaos of treating an MH event, changing machines after an hour may be preferable to changing the filters every hour.

Temperature Monitoring

Another patient safety issue is temperature monitoring. Larach et al. advocated for electronic monitoring of core temperature in all anesthetics expected to last 30 minutes. Reasons for this recommendation include the following: temperature abnormalities may be an early MH sign; higher maximum temperatures significantly increase the likelihood of MH complications including disseminated intravascular coagulation; and skin liquid crystal temperature probes do not trend accurately during MH events.⁵² Participants noted that safe and effective nasal electronic temperature probes should be manufactured to substitute for current “repurposed” and inaccurate surface temperature probes.

Additional Preparation for MH Needed at Free-Standing Health Care Facilities

—While all hospital-based and free-standing anesthetizing facilities, such as ambulatory surgery and office-based surgery centers, should be prepared to diagnose and treat an infrequently occurring MH event, the more thinly resourced free-standing facilities will find advanced preparation even more essential. Meeting participants recommended that free-standing facilities should: conduct MH drills, check for the ready availability of 36 vials of dantrolene§, plan to obtain critical laboratory studies, and develop transfer plans** for stabilized patients to appropriate hospital facilities. Advance preparations are required to ensure the survival of these critically ill patients. If a free-standing facility does not use

volatile anesthetics and does not depend on succinylcholine as an airway rescue drug, then such preparation is unnecessary, assuming that myopathic patients are not treated at that facility. Multiple MHAUS Hotline reports of MH deaths occurring after anesthetics delivered in outpatient surgery facilities highlight the importance of adequate preparation for MH crises.

Communication

Finally, patient safety mandates good communication and transparency between primary care providers and their consultants, physicians and their patients, and MHS individuals and their families.^{††} Clear communication coupled with education saves lives. Furthermore, continued collaboration between MH researchers and clinicians will advance the science of MH and inform educational efforts to improve the care of those who are MHS or who have related disorders.

Conclusion

In this article, we have attempted to provide the reader with an overview of the current trends in MH-related research and patient care, as highlighted at the Scientific Conference. While it was not possible to go into great detail about each topic, we hoped to communicate the need for further evaluation in such areas as:

- the physiology and regulation of Ca^{2+} homeostasis and the impact of temperature on such processes;
- factors involved in the predisposition of an individual to triggerable myopathies;
- genotype – phenotype relationships, and the reasons behind incomplete penetrance with regard to the clinical expressivity of MH.

Continued progress in our understanding of these subjects will no doubt translate into the development of new methods to diagnose, treat, and care for patients with MH and related disorders.

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[§]25% of 229 MH events required more than 36 vials of dantrolene for treatment. (Larach et al., 2010)

^{**}See www.mhaus.org for guidance for ambulatory surgical centers with regard to the development of emergent MH transfer plans for suspected MH patients (last accessed 3/11/11).

^{††}16 of 248 patients experiencing an MH event had an MH family history (Larach et al., 2010).

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Appendix I

AGENDA Day 1- Friday, April 23, 2010

Topic	Speaker(s)
Welcome and Introductions	Henry Rosenberg, MD, President, MHAUS Andrew Herlich, MD, UPMC and John P. Williams, M.D., UPMC
Pathophysiology of MH Overview and Recent Advances in Understanding	Session Chairperson: Jerome Parness, MD, PhD, UPMC
Introduction to MH Pathophysiology; fresh approaches to understanding MH at cellular level; dantrolene & mechanism of action	Jerome Parness, MD, PhD, UPMC
Lessons from a Mouse Model of Malignant Hyperthermia: Mechanisms and New Interventions	Susan Hamilton, PhD, Baylor College of Medicine (Keynote Speaker)
Mechanistic Models for the Cause of MH and for Core Formation in CCD	David MacLennan, PhD University of Toronto
New Frontiers in Personalized Medicine: RyR1 Allele-Specific Gene Silencing in Mouse Models of MH and CCD	Robert T. Dirksen, PhD University of Rochester
Changes in DHPR kinetics induced by MH-related conformational changes in RyR1	Paul Allen, MD, PhD Brigham and Women's
Genetics of MH: Results and Future Directions	Chairperson: Sheila Muldoon, MD, USUHS
Genetic testing for MH susceptibility in Europe; role of new diagnostic testing strategies in evaluating MH risk	Henrick Rueffert, MD University of Leipzig, Germany
Reassessment of the operating characteristics of IVCT given genetic RyR1 results; update on the EMHG Reference: Improve on Phenotyping	Thierry Girard, MD University Hospital of Basel, Switzerland
Results of genetic testing (RyR1 gene findings) in basic research laboratories	Khishge Sambuughin, PhD, USUHS
Assessment of RyR1 gene variants for Pathogenicity: Theory and Examples	Jeffrey Kant, MD, PhD UPMC
Results of genetic testing (RyR1 gene findings) in CLIA-approved clinical laboratories	Jim Weber, PhD Prevention Genetics

RyR1 (skeletal muscle ryanodine receptor gene); MH (malignant hyperthermia); CHCT (caffeine-halothane contracture test); IVCT (in vitro contracture test); MHS (MH-susceptible); central core disease (CCD)

AGENDA Day 2- Saturday, April 24, 2010

Topic	Speaker(s)
Welcome, Comment on Day 1	Sheila Muldoon, MD, USUHS
Relationship between MH-susceptibility and other muscle conditions/ disorders	Chairperson: Barbara Brandom, MD, UPMC
Review of 2008 SPA Meeting Recommendations (anesthetic considerations in MH-associated diseases)	Barbara Brandom, MD UPMC
Creatine Kinase and Heat Tolerance in Mice and Men	Patricia Deuster, PhD, MPH USUHS
Confounding Cases: Is this MH and How Would You Provide Anesthesia?	John Capacchione, MD USUHS
Genetic susceptibility to statin-induced myopathy	Georgirene Vladutiu, PhD SUNY at Buffalo

Topic	Speaker(s)
MH Epidemiology	Chairperson: Henry Rosenberg, President, MHAUS
Introduction to Epidemiology of MH; role of MH Hotline and North American MH Registry (NAMHR) databases as research and educational tools; implications and limitations of epidemiologic studies, Epidemiology Slide Handout	Henry Rosenberg President, MHAUS
MH Comorbidities	Joanne E. Brady, SM, Columbia University
What the Registry has Taught Us: MH Presentation, Treatment, and Outcome; Is it Time for Revisions to MHAUS Clinical Care Recommendations?	Marilyn Larach, MD Penn State College of Medicine
Future Directions/Challenges	Chairperson: Henry Rosenberg, MD, President, MHAUS
Changing Recommendations for Anesthesia Machine Preparation	David Rymer, MD UPMC
Need for building the NAMHR database as source for future clinical trials; Recent reporting trends; Current contributions from the MH Diagnostic	Barbara Brandom, MD UPMC
Welcome, Comment on Day 1	Sheila Muldoon, MD, USUHS
Centers	
Patient Safety First	Dale Micalizzi Board of Directors, MHAUS
Closing Remarks: Future directions for MH – research and non-invasive diagnostic testing; Future Directions for MHAUS – patient safety initiatives (ambulatory surgery issues, educational initiatives [simulation]); advice for MHS individuals.	Henry Rosenberg, MD MHAUS

Figure 1. Schematic of the elements of skeletal muscle excitation- Ca^{2+} release coupling that functions in normal excitation-contraction coupling

Depolarization of the sarcolemma is sensed by the DHPR (dihydropyridine receptor) in the T-tubule membrane. DHPR undergoes a conformational change and directly contacts RyR1 (the skeletal muscle ryanodine receptor) in the SR (sarcoplasmic reticulum) membrane, causing release of Ca^{2+} (primarily bound to CSQ [calsequestrin]) from SR stores (small blue circles). Ca^{2+} then becomes available for stimulation of the contractile apparatus (not shown). If the requirements for myoplasmic Ca^{2+} increase beyond that which can be provided by SR stores, SOCE (Store-Operated Ca^{2+} Entry) channels composed of Orai1 and likely subtypes of the TRP channel resident in the T-tubule membrane are activated by the SR membrane luminal Ca^{2+} sensor protein, STIM1, and allow more Ca^{2+} into the cell (SOCE). Alternatively, when muscle is highly stimulated, Ca^{2+} may enter the cell through the DHPR in a SR store-independent manner in the process termed excitation-coupled Ca^{2+} entry (ECCE). It is not yet known whether one or both processes are active during normal skeletal muscle physiology, or during pathophysiological processes such as malignant hyperthermia.

Figure and modified legend from the following citation- Parness J, Bandschapp O, Girard T. The myotonias and susceptibility to malignant hyperthermia. *Anesth Analg* 2009;109:1054-reprinted with permission (granted 3/8/11) from Wolters Kluwer Health.

Figure 2.
Evaluation of Individuals with Unexplained Rhabdomyolysis or Myopathic Symptoms

Table 1**Key Concepts and Theories Presented at the Scientific Conference**

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- Intracellular Ca^{2+} elevation observed in MH may not be derived through RyR1-mediated Ca^{2+} release alone, but may also derive in large part from extracellular storage sites, and involve processes such as SOCE or ECCE.
  - Variation in MH phenotype, for example, susceptibility to MH with or without clinical signs of CCD, may be linked to the pathologic mechanism (eg, Ca^{2+} leak or uncoupling) of the RyR1 mutation.
  - Potential treatment approaches for MH and CCD include the development of drugs which alter Ca^{2+} release or leak, as well as the targeting, or silencing, of specific deleterious gene mutations through such techniques as allele-specific gene silencing.
  - Development of core regions (areas of muscle cell damage) may be associated with excessive SR Ca^{2+} leak, driving the formation of reactive oxygen species and consequent oxidative damage.
  - Random association of normal and mutant RyR1 subunits may contribute to the heterogeneity of muscle contraction, causing physical stresses and damage to the muscle fibers.
  - Presence of a RyR1 mutation or defect in Ca^{2+} regulation may be associated with increased risk for MH-like reactions in response to nonanesthetic triggers, such as heat and/or exercise stress. Increased temperature appears to exacerbate Ca^{2+} leak, the development of reactive oxygen species, and consequent oxidative damage.
  - In the absence of functional studies, computational tools such as SIFT and PolyPhen may be used to predict the pathogenicity of RyR1 mutations. Predictions must be interpreted cautiously along with available clinical data.
  - Data from the CHCT or the IVCT may be used to more accurately select patients for genetic studies and thus increase the probability of identifying genetic variants.
  - The mutation profile of RyR1 varies considerably amongst populations with different ethnic backgrounds.
  - Success of epidemiologic studies rests on the continued data submission to research databases, as well as the quality management and analyses of these databases. Results from these studies provide the basis for many future research efforts.
  - Individuals who present with unexplained myopathies must be given a comprehensive medical evaluation to check for underlying muscle defects which may precipitate MH-like symptoms, but for which treatment may be very different from MH.
  - Testing methodology used in the military (e.g., heat tolerance testing) may prove useful in evaluating the association of heat and exercise stress with MH susceptibility.
  - The presence of multiple underlying genetic variants may increase an individual's predisposition to development of a (triggerable) myopathy.
  - Efforts to accurately monitor temperature and to rapidly diagnose MH and administer dantrolene remain our best defense for treating MH successfully and decreasing its associated morbidities.
  - There is much confusion in the field regarding how to best prepare an anesthetic machine for MHS patients. ACFs appears to show great promise in this regard.
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MH (malignant hyperthermia); RyR1 (skeletal muscle ryanodine receptor); RyR1 (skeletal muscle ryanodine receptor gene); SOCE (Store-Operated Ca^{2+} Entry); ECCE (Excitation-Coupled Ca^{2+} Entry); CCD (Central Core Disease; SR (sarcoplasmic reticulum); SIFT (Sorting Intolerant from Tolerant); PolyPhen (Polymorphism Phenotyping); CHCT (caffeine-halothane contracture test); IVCT (in vitro contracture test); ACF (activated charcoal filter); MHS (MH-susceptible)

Table 2

Research Directions and Patient Care Issues: Selected Highlights

Basic Research	Patient Care
• How do different RyR1 mutations produce ≥ 1 disease state? • What is the relationship between specific gene mutations and MH expression? • What is the relationship between specific gene mutations and accentuated contractures in the IVCT and CHCT? • What are the factors responsible for the regulation/dysregulation of calcium homeostasis in MH and other RyR1-related myopathies; and will the study of dantrolene's mechanism of action help to uncover these factors? • How does the regulation/dysregulation of myoplasmic calcium affect skeletal muscle cellular histology? • How is the pathogenesis of MH related to that of heat stroke? • Can exercise/other pharmacologic factors lead to significant calcium release such as to precipitate an MH-like syndrome <u>without</u> genetic mutations? • How can we most effectively determine the pathogenicity of RyR1 variants? • How many genes/mutations are causal, and how can we best screen for them? • Why is the incidence of MH higher in males than females, and higher in children than in adults?	• How can we best assess a patient with unexplained rhabdomyolysis or myopathic symptoms? • What myopathies/other disorders predispose to MH? • What factors control the expression of the MH clinical phenotype (as the prevalence estimates of RyR1 variants are considerably higher than the reported incidence of MH events)? • What is the best method to prepare anesthetic machines for MHS patients and safely anesthetize those in MH crisis? • What precautions should MHS individuals take in everyday life?

RyR1 (skeletal muscle ryanodine receptor gene); MH (malignant hyperthermia); CHCT (caffeine-halothane contracture test); IVCT (in vitro contracture test); MHS (MH-susceptible)