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Clinical Significance of Ryanodine Receptor 1 Gene (*RYR1*) Variants: Proceedings of the 2013 MHAUS Scientific Conference

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Abstract

The Malignant Hyperthermia Association of the United States (MHAUS) and the Department of Anesthesia at the University of Toronto sponsored a Scientific Conference on November 1–2, 2013 in Toronto, Canada. The multidisciplinary group of experts, including clinicians, geneticists and physiologists involved in research related to malignant hyperthermia (MH), shared new insights into the pathophysiology of type-1 ryanodine receptor gene (*RYR1*)-linked diseases, as well as the relationship between MH and "awake MH" conditions, such as exertional rhabdomyolysis (ER) and exertional heat illness (EHI). In addition, the molecular genetics of MH, and clinical issues related to the diagnosis and management of *RYR1*-linked disorders, were presented. The conference also honored Dr. David H. MacLennan for his contributions to our understanding of the genetics, pathogenesis and treatment of MH and other *RYR1*-related myopathies. This report represents a summary of the proceedings of this conference.

Introduction

Malignant hyperthermia (MH) is an autosomal dominant pharmacogenetic disorder triggered by exposure to volatile anesthetics and/or depolarizing muscle relaxants.[1, 2] MH manifests as a potentially lethal hypermetabolic crisis associated with a rapid and uncontrolled increase in myoplasmic Ca^{2+} in skeletal muscle cells.[3] Since MH was first recognized as an inherited condition in 1960[4], six potential genetic loci for MH susceptibility have been reported[5], but specific MH-associated mutations have been identified in only two genes thus far, *RYR1* (MIM 180901) encoding the Ca^{2+} release channel of the sarcoplasmic reticulum and *CACNA1S* (MIM 114208), encoding the α-subunit of the voltage dependent L-type calcium channel of the transverse tubule. Products of these genes play key roles in the process of excitation-contraction (EC) coupling and in the maintenance of Ca^{2+} homeostasis in skeletal muscle cells. MH genetic research has shown that *RYR1* is the primary causal gene for MH; *RYR1* mutations are found in 60–86% of MH families with diverse ethnicity.[6, 7]

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Genetic testing for MH plays an important role in MH diagnostics today. Genetic testing has proven to be especially useful in early diagnosis of children and patients who cannot undergo the caffeine-halothane contracture test (CHCT) for MH susceptibility, because the CHCT requires an invasive muscle biopsy. However, the genetic testing exhibits low sensitivity due to the limited number of mutations that have fulfilled the criteria for MH causation as determined by the European Malignant Hyperthermia Group (www.emhg.org) and the Malignant Hyperthermia Association of the USA (www.mhaus.org).

In an effort to advance our understanding of MH and other RYR1-related diseases and to promote new insights into MH pathophysiology, diagnosis and treatment, the MH Association of the United States (MHAUS), along with the University of Toronto, Department of Anesthesia, co-sponsored the MHAUS Scientific Conference held on November 1–2, 2013 in Toronto, Canada. The multidisciplinary group of experts, including clinicians, geneticists and physiologists involved in research related to MH, shared new insights into the pathophysiology of type-1 ryanodine receptor gene (*RYR1)*-linked diseases, the relationship between MH and "awake MH" conditions, such as exertional rhabdomyolysis (ER) and exertional heat illness (EHI), the molecular genetics of MH, as well as other clinical issues related to the diagnosis and management of *RYR1*-linked disorders. A strong emphasis was focused on the clinical implications of recent advances. The conference honored Dr. David H. MacLennan for his contributions to our understanding of the genetics, pathogenesis and treatment of MH and other *RYR1*-related myopathies.[7]

The objectives of the 2013 MHAUS conference were to share the latest research on:

- Type-1 ryanodine receptor (RyR1) structure/function and assessment of *RYR1*-related myopathies
- Molecular genetic diagnosis of MH
- Relationship of MH with EHI, ER, and statin myopathies
- Role of rare diseases and genetic variation databases
- Improved education in MH diagnosis and treatment

The key concepts and theories presented at the conference, as well as the trends and future challenges in the field of MH research and patient care are summarized in Tables 1 and 2.

MH, *RYR1*-related myopathies and related conditions

Structural and functional studies of the ryanodine receptor

Dr. F. Van Petegem (University of British Columbia) presented novel structural mechanisms by which disease-related *RYR1* mutations disrupt RyR1 function. Analysis of pseudo-atomic models and high resolution structures of N-terminal domains of the ryanodine receptor indicate that disease mutations weaken interdomain interactions in RyR1, thus lowering the energetic barrier to channel opening and leading to enhanced calcium release.[8] Such studies, currently limited by the inability to crystallize the entire RyR1 protein, will aid in the design of new drugs for the treatment of MH and other *RYR1*-related disorders.

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Dr. C. Franzini-Armstrong (University of Pennsylvania), a well-known expert on skeletal muscle ultrastructure, presented a study elucidating a role of the type-3 ryanodine receptor (RyR3). RyR3 is expressed in neonatal skeletal muscles and, at a low levels in a variety of cell types but not adult mammalian skeletal muscle. However, the physiological role of RyR3 in non-mammalian muscle remains unclear. Dr. Franzini-Armstrong, working on zebrafish skeletal muscle, showed that RyR3s forms parajunctional structures -"feet"- adjacent to the triad junction and that they serve to enhance Ca^{2+} sparks initiated by RyR1.[9]

Molecular mechanisms of RYR-related disorders

Excitation-contraction (EC) coupling is the process whereby an electrical signal in the T-tubule membrane (an action potential) is converted into an intracellular chemical signal (a Ca2+ transient) that is used to drive muscle contraction. EC coupling is composed of a series of steps in which a conformational change in the voltage-dependent, L-type calcium channel (DHPR) induced by depolarization triggers activation of the RyR1 Ca2+ release channel in the adjacent terminal cisternae of the sarcoplasmic reticulum (SR). While the effects of MH mutations in RyR1 on EC coupling are well established, alterations in several other calcium signaling mechanisms have also been implicated in MH. In a process known as store overload-induced calcium release (SOICR), RyR1 spontaneously releases Ca^{2+} from the SR once luminal Ca^{2+} concentrations reach a critical threshold.. Recent evidence suggests that RyR1 mutations may lower the threshold for SOICR thus promoting spontaneous SR release of Ca^{2+}. HEK293 cells expressing RyR1 receptors bearing the porcine MH mutation, R615C, displayed a reduced threshold for SOICR in comparison with wild type RyR1, a response that was potentiated by halothane and inhibited by dantrolene.[10] Important parallels can be drawn between the RyR1-mediated mechanisms in MH and RyR2-mediated mechanisms in catecholaminergic polymorphic ventricular tachycardia (CPVT), a stress-triggered condition leading to bidirectional ventricular tachycardia and sudden cardiac death. The keynote speaker of the conference, Dr. W. Chen (University of Calgary) discussed similarities between MH and CPVT. Dr. Chen's studies suggest that both disorders are caused by RyR mutations that reduce the threshold for SOICR at baseline, which is then further depressed by their respective triggers to cause severe spillover of Ca^{2+} into the cytoplasm, ultimately leading to uncontrolled and sustained contraction of the myofibrils.[11]

A recent study[12] identified yet another mechanism of RyR1 regulation by $Ca_v1.1$, the pore-forming subunit of the L-type Ca^{2+} channel, encoded by *CACNA1S*. $Ca_v1.1$ functions not only to activate RyR1 during EC coupling, but also to suppress resting RyR1-mediated Ca^{2+} leak from the SR. Disruption of this $Ca_v1.1$ inhibitory regulation of RyR1 Ca^{2+} leak represents a novel molecular mechanism for increased sensitization of muscle cells to MH triggers.

In addition to mediating calcium release from the SR, RyR1 is also implicated in sarcolemmal calcium entry processes. One such Ca^{2+} entry process is known as excitation-coupled calcium entry (ECCE) in which calcium enters the cell through $Ca_v1.1$ channels in the T-tubule membrane in response to membrane depolarization. ECCE is not only significantly enhanced in myotubes expressing MH mutants of RyR1, but is also suppressed to wild type levels following exposure to dantrolene.[13,14] A second mechanism of Ca^{2+}

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entry, store-operated calcium entry (SOCE), recruits extracellular Ca^{2+} entry when intra-sarcoplasmic stores become depleted, as occurs during sustained contractions observed during an MH crisis. Myotubes from MH mouse models exhibit an increased rate of SOCE channel activation.[15]

Dr. J. Parness (Children's Hospital of Pittsburgh) focused on the question of potential explanations for incomplete penetrance in the presentation of MH, i.e., occurring after several uneventful exposures to volatile anesthetics. Two mechanisms were proposed: 1) involvement of a prion-like structure in a volatile-anesthetic-sensitive metabolic response element; 2) an inducible metabolic genetic element that is normally silenced, but capable of being activated by MH-triggering agents and/or elevated Ca^{2+}. Dr. Parness suggested that engagement of metabolomics approaches, through detection of uniquely elevated metabolites during an MH episode, could reveal MH target proteins, genes, and metabolic pathway(s) involved in MH hypermetabolism.

MH and other RYR1-related myopathies

Dr. J. Dowling (University of Toronto) brought attention to the expanding spectrum of *RYR1*-related myopathies that, in addition to MH, include congenital core myopathies (central core disease and multiminicore myopathy) and a growing collection of recessive non-core myopathies. Analysis of 106 cases with recessive *RYR1* mutations revealed their histologic heterogeneity - from classic core myopathies to non-core myopathies.[16] In pediatric populations, *RYR1* mutations are associated with extremity and axial weakness, and often include ophthalmoplegia and facial weakness. Unlike dominantly-inherited MH and Central Core Disease (CCD), where mutations are often located in "hot spots" of *RYR1*, recessive mutations are spread throughout the *RYR1* gene. Cases with severe clinical phenotype were significantly associated with hypomorphic *RYR1* mutations, which result in decreased RyR1 protein expression.[16] It was suggested that increasing expression of the RyR1 protein might ameliorate the disease severity in such cases. The risk for MH in patients with those mutations is uncertain.[17]

Dr. Dowling discussed two recently identified causes of non-*RYR1* core myopathies. One was caused by a mutation in *MYH7* (a gene encoding myosin heavy chain beta (MHC-β) isoform),[18] and another by a splice site mutation in *CCDC78* (a gene encoding the coiled-coil domain-containing 78 protein that plays a role in skeletal muscle contraction) validated in a zebrafish model. Dr. Dowling also presented a study that identified a recessive mutation in *STAC3* (a gene encoding the SH3 and cysteine rich domain 3 protein), encoding a newly identified component of the EC-coupling machinery,[19] in patients with Native American Myopathy, a rare myopathy associated with dysmorphic features and malignant hyperthermia susceptibility. Based on studies on *RYR* zebrafish, as well as on myotubes from patients with *RYR1* mutations, Dr. Dowling showed that antioxidant treatment reduces oxidative stress and improves motor function. Thus, the use of antioxidants may represent a viable therapeutic strategy for patients with *RYR1*-related myopathies.[20]

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Evaluation and anesthetic management of patients with myopathy

To minimize the risk of an adverse reaction in patients with myopathies, a preoperative patient evaluation should ideally be conducted in conjunction with a neurologist. Anesthesiologists, aware of an increased risk for MH in patients with *RYR1*-related congenital myopathies, should consider MH susceptibility in myopathic patients who develop fever, tachycardia, hypercapnia, and/or hyperkalemia perioperatively very high on their differential diagnostic list and manage accordingly.

The presence of skeletal muscle weakness is a major perioperative concern, especially if it involves the respiratory and/or bulbar muscles. Although congenital myopathies are not usually associated with primary myocardial involvement, scoliosis-related restrictive lung disease can lead to heart failure. A formal clinical evaluation of respiratory and bulbar muscle function, and of cardiac reserve, is a mandatory aspect of the preoperative evaluation of patients with congenital myopathies; the availability of postoperative ventilatory support and a suitable postoperative intensive care unit should also be planned for.

Dr. M. Tarnopolsky (McMaster University) recommended evaluation of baseline serum potassium and creatine kinase (CK) levels, and levels of aspartate aminotransferase (AST) and alanine transaminase (ALT) as key indicators of propensity for profound perioperative rhabdomyolysis.[21] Interpretation of CK levels requires consideration of the variability of normal CK values with CK of 800U/L being the upper limit for African Americans but only 200U/L being the upper limit for Caucasians.[22] Dr. Tarnopolsky outlined a diagnostic algorithm for evaluating patients with myopathies and suggested that patients presenting with an abnormal neurological exam/unexplained chronic high CK/any CK over 1000/ positive family history of high CK, NMD or non-hypertensive cardiomyopathy, should be sent for further testing (i.e. muscle MRI and/or muscle biopsy, as well as genetic testing). He also pointed out that special considerations are warranted for myotonic muscular dystrophy type 1 patients with cardiac conduction defects and slower drug metabolism rates, and for Duchenne and other severe muscular dystrophy patients who may have cardiomyopathy and may be particularly susceptible to electrolyte disturbances such as hyperkalemia and sudden cardiac death.

One important discussion point was whether myopathic children should automatically be administered non-triggering anesthetic, i.e. total intravenous anesthesia (TIVA). Participants agreed that patients with multi-minicore or central core disease should avoid triggering anesthetics, while children and adults with significant muscle weakness due to primary myopathies should have their functional status assessed before operation, and that transfer to specialty centers with intensive care facilities should be considered. While all agreed that succinylcholine should be avoided in such patients, a blanket prohibition of volatile anesthetics in any and all myopathies was not considered appropriate. In conclusion, a fully informed decision concerning the use of volatile anesthetics in a patient with myopathy includes a thorough analysis of family and personal history, and requires an understanding of the underlying molecular defect, as different underlying pathophysiological mechanisms would have different implications for anesthetic management of patients and their families.

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Relationship of MH with EHI, ER, and statin myopathies

The link to heat/exercise-induced rhabdomyolysis

Similar to *RYR1* related myopathies, exertional heat illness (EHI) and exertional rhabdomyolysis (ER) may share a common pathogenic mechanism with MH susceptibility. The relationship between EHI/ER and MH was explored in studies presented by Drs. F. O'Connor, J. Capacchione and N. Sambuughin of the Uniformed Services University of the Health Sciences (USUHS). One military study showed that 15 out of 26 unrelated active-duty males diagnosed with recurrent or unexplained ER had a positive CHCT, and that 9 of 13 who underwent genetic screening carried an *RYR1* variant.[23] Dr. P. Hopkins (Leeds Malignant Hyperthermia Unit, UK) found that many patients, recovering from heat stroke or ER and whose symptoms responded to dantrolene, carried pathogenic *RYR1* mutations and tested positive on the halothane-caffeine *in vitro* contracture test (IVCT). Dr. F. O'Connor described studies from Dr. P. Deuster's lab at USUHS showing that some patients tolerated heat stress less than others and had a more prominent rise in post-exercise CK. Questions emerging from these studies include: 1) what percentage of individuals suffering from EHI/ER will exhibit pathologic *RYR1* variants, 2) what precautions should be taken with regards to heat/exercise in MH susceptible individuals, and 3) what is the potential utility of dantrolene in the treatment of EHI/ER?

A recent case of "awake MH", reported by Dr. Capacchione, supported a link between EHI and MH.[24] An otherwise healthy 6-year-old boy developed lower extremity rigidity, trismus, and fever after playing in a splash pool. On arrival in the emergency department, he appeared to be seizing. Use of succinylcholine led to cardiac arrest, and death. Postmortem genetic analysis revealed a novel *RYR1* variant that was later found in his father who tested positive by CHCT and was diagnosed histologically with CCD.[24] Similar cases of "awake MH" in children, with and without recognized myopathies, have been described previously.[25,26]

Health care providers, especially first responders who use succinylcholine (e.g., Emergency Department physicians), should be aware of the risks of succinylcholine administration in patients, particularly children, presenting with muscle rigidity and hyperthermia, even in the absence of exposure to anesthetic drugs.

The observation that many individuals with EHI/ER are positive in either the CHCT or IVCT indicates that a subset of EHI/ER might represent a variant clinical expression of MH. It is not clear, however, whether individuals with documented MH episodes are more susceptible to EHI. There are yet no biochemical, genetic, or functional tests to diagnose susceptibility to EHI/ER or to clearly differentiate between MH and EHI/ER. Unexplained, persistently elevated serum CK levels in the absence of neuromuscular disease should alert anesthesiologists and sports medicine physicians to a potentially greater than normal risk of MH and EHI/ER.[23–28]

The link between MH and statin myopathies

Dr. G. Vladutiu (University of Buffalo) discussed statin-induced myopathies (SIM). She pointed out that although serious adverse myopathic reactions to statin therapy are rare,

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about 200,000 people in the U.S. (i.e. 0.5% of 40 million who are taking statins) are at risk for life-disabling myopathy and 4–6 million (10–15%) develop muscle pain/weakness. In some cases these symptoms become chronic.[29,30] A study by Dr. Vladutiu's team revealed that predisposition to SIM is a genetically complex condition, associated with mutations in at least 8 muscle disease genes, including *RYR1* and *CACNA1S*. Carrier status for certain autosomal recessively inherited myopathies is increased as much as 20-fold in severe statin myopathy cases.[31]

Dr. S. Hamilton (Baylor College of Medicine) showed that some statins, such as simvastatin, interfere with intramyofiber Ca^{2+} homeostasis. Simvastatin triggered hypermetabolic responses in mice carrying a MH-associated Y524S mutation in RyR1, and this response was prevented by pre-treatment with AICAR, possibly through its direct effect on RyR1.[32]

The potential role of statins as a risk factor for increasing the likelihood of an MH reaction in an MHS individual during anesthesia remains unknown.

Clinical diagnosis of MH

The clinical presentation of MH is highly variable. Common early signs include hypercarbia ($ETCO_2$>60 mmHg), sinus tachycardia, masseter muscle rigidity and generalized muscle rigidity. Hyperthermia was an early sign in only 8.2% of cases but was the first to third sign in 63.5% of cases.[33,34] Most commonly, MH presents intraoperatively within an hour of anesthetic induction, but may also present in the early post-operative period and may recrudesce following treatment. Other signs of MH include hyperkalemia-related ECG changes, ventricular ectopy or fibrillation, bigeminy, tachycardia, myoglobinuria, and disseminated intravascular coagulation (DIC). The likelihood of development of MH complications increases 1.6-fold for every 30-minute increase in time period between the first MH sign and the first dantrolene dose, and 2.9-fold for every 2°C increase in maximum temperature (T_{max}).[33]

Dr. M. Larach (North American MH Registry) presented data on MH deaths collected in the North American Registry (NAMHR) from 2007–2012 and compared the results with the analysis of similar cases reported between 1987 and 2006.[25] The NAMHR database examination for the earlier time period revealed 291 MH episodes with 8 cardiac arrests (2.7%), including 4 fatalities (1.4%). In analyzing 84 cases with 7 cardiac arrests and 8 deaths (9.5%) cases from the AMRA (adverse metabolic/musculoskeletal reaction to anesthesia) reports filed with the Registry from 2007–2012, important differences were found between MH death and survival groups: (1) Use of a skin temperature probe or absence of any temperature monitoring increases the likelihood of death 14-fold and every 1°C increase in T_{max} increases the likelihood of death 4.2-fold, underscoring the importance of accurate, real-time temperature monitoring during anesthesia. (2) Every 1 mEq of potassium rise increases the likelihood of death 4.5-fold. (3) DIC greatly increases the likelihood of death (odds ratio, 101.5). Compared to the earlier NAMHR study, there was an increased failure to rescue from MH even though these patients were, for the most part, young, healthy, and undergoing low to intermediate risk surgery. In an effort to improve our understanding of this potentially lethal condition, healthcare providers are encouraged to obtain blood specimens appropriate for molecular genetic analysis on all patients suffering a

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possible MH-related arrest or death. Whenever possible, muscle biopsy contracture studies should be performed on survivors of cardiac arrest potentially associated with MH.[6,25]

Update on caffeine-halothane contracture testing

The caffeine-halothane contracture test, known as CHCT in North America[35] and as IVCT[36] in Europe, remains the gold standard for MH diagnosis, being at present the most sensitive diagnostic test for MH Susceptibility. As reported by Dr. S. Muldoon (Uniformed Services University), 60–80 CHCT tests are performed annually in five MH testing centers in North America using a standardized protocol[1]. Anesthesiologists at these centers review each referral's medical, family, and anesthetic history to determine the patient's prior probability of having an MH event. A Clinical Grading Scale (CGS) generated by a consensus of MH experts often aids in evaluating the likelihood of a patient having experienced an MH episode.[37] The CGS scores rigidity, muscle breakdown, respiratory acidosis, temperature increases, cardiac involvement, and family history to evaluate the likelihood of MH. The CGS requires careful documentation of the clinical events, temperature monitoring, and blood gas analysis in order to develop an accurate ranking.

The disadvantages of both contracture tests - their reliance on invasive muscle biopsies and the cost of maintaining testing centers - has led to the closure of several centers in the Western Hemisphere and to a decrease in number of CHCT tests performed annually.

Dr. S. Riazi (University of Toronto) discussed the disparities of phenotype and genotype concordance results from the Canadian database. These studies found that 22 patients (from 8 families, containing 2 probands) were identified as MHS by CHCT, yet they did not carry their familial RYR1-variants during workup. Of these, 17 patients (from 3 families) lacked familial mutations identified as causative in the EMHG database. The preponderance of discordance can be explained either as false positivity of CHCT or a lower than expected reliability of the current genetic testing. Thus caution should be taken in counseling family members based solely on genetic results, which emphasizes a) the complementary role of CHCT or IVCT analyses in making a definitive MH diagnosis, and b) the need for more definitive identification of genes and physiology that confer both MH susceptibility and define the hypermetabolic crisis that is MH.

Advances in MH genetic testing

A prominent focus of the conference centered on discussion of how novel genetic analytical techniques can be used to provide insights into the complex genetic basis of MH. Next-generation sequencing (NGS) platforms enable fast and cost-efficient sequencing of all protein coding regions - whole exome sequencing (WES) - in the human genome. WES based on NGS holds great promise for overcoming significant challenges in MH genetic research such as MH genetic heterogeneity, as well as the large and complex structures of the two known MH candidate genes - *RYR1* and *CACNA1S*.

[1] www.mhaus.org

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A key challenge of using WES for patient diagnosis is the ability to distinguish between disease-causing variants and benign polymorphisms. The American College of Medical Genetics and Genomics (ACMG) has developed recommendations for interpretation of sequence variations using standardized terminology.[38,39] Genetic variants are typically grouped into 4 categories: pathogenic, benign, variant of unknown significance (VUS), and incidental findings (IFs)[40] (Table 3).

Two studies presented at the conference revealed high levels of allelic heterogeneity in the *RYR1* and *CACNA1S* genes. Drs. Hopkins and Kim[41] analyzed *RYR1* and *CACNA1S* variants identified using WES in 5,379 of individuals as part of the National Heart, Lung, and Blood Institute (NHLBI) Grand Opportunity (GO) Exome Sequencing Project (ESP). Their analyses showed that *RYR1* is more genetically diverse than 74–79% of other genes in European-Americans and African Americans, while *CACNA1S* is more genetically diverse than 77–82% of other genes in these cohorts.[41] Additionally, the data analyses indicated that most detected rare *RYR1* variants - with frequency less than1% - are likely to be benign polymorphisms. The study also showed that current *in silico* bioinformatics prediction tools poorly discriminate between pathological and presumed non-pathological *RYR1* variants.

In the study by Gonsalves *et al*[42], the authors evaluated exome sequencing (ES) data from the ClinSeq® study on a cohort of 870 volunteers not selected for MHS. The study identified 70 *RYR1* variants and 53 *CACNA1S* variants in this cohort. Remarkably, a known MH-causal mutation was detected in one study participant without a medical or family history of MH; the finding was reported to the patient's family doctor as an incidental finding. As in the previously described study[41], most of the novel variants were predicted to be benign. The authors also reported that a significant proportion of *RYR1* sequence variants in human gene mutation database (HGMD) were erroneously classified as "disease-causing mutations" due to the absence of adequate genetic (*e.g.*, co-segregation) or functional data, and should be reclassified as being benign, probably benign, or a variant of unknown significance.

Further studies into creating useful algorithms to predict a given variant's pathogenicity are clearly warranted, and would require additional clinical and genetic data such as segregation patterns in large families and validation of altered RyR1 function in either cell systems or genetically engineered animals. As of today, only 31 out of more than 300 *RYR1* coding variants are officially classified as MH-causative and thus, are used for clinical genetic testing for MH[2]. Dr. N. Sambuughin (USUHS) reported the use of WES for genetic analysis of MHS patients negative for *RYR1* variants. Their study revealed that such patients represent a clinically and genetically heterogeneous group of muscle disorders that may carry variants in genes previously not implicated in MHS.

All the presenters in this session emphasized that at present, targeted sequencing (TS) through a gene panel that uses the NGS platform seems to be the most practical approach for screening MH candidate genes, including *RYR1* and *CACNA1S*. TS has a higher coverage (up to 99%) and accuracy, and therefore, higher sensitivity than WES. Depending on the

[2]https://emhg.org/genetics/

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laboratory, TS panels are also supplemented with traditional Sanger sequencing to "backfill" exons that are poorly or incompletely covered by NGS. This gives an added level of coverage and reduces the potential for false negative results. Thus, accurate MH diagnosis could be improved through the use of TS of *RYR1* and *CACNA1S* as part of an integrated approach that also includes careful consideration the patient's anesthetic and family history, CHCT/IVCT results, genotype-phenotype correlations, histopathology and clinical evaluation for neuromuscular disorders.

Update on treatment of MH

Dantrolene remains the only specific drug treatment for acute MH. Use of dantrolene along with greater awareness and education by such groups as MHAUS has led to a reduction of MH mortality from 80% in the 1960s/1970s to less than 10% today. A multicenter study, presented by Drs. Pawar and Rosenberg, showed that dantrolene is also effective in treating related MH-like syndromes, such as neuroleptic malignant syndrome (NMS), sepsis, and cocaine intoxication.[43,44] Dantrolene is a relatively safe medication as indicated by an analysis of 368 AMRA reports submitted to the NAMHR.

Other potential drugs were also discussed at the conference. Dr. Hamilton reported that 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR), an AMPK (AMP- activated protein kinase) activator, prevented heat-induced sudden death and decreased cytoplasmic Ca^{2+} levels in a mutant RyR1 knock-in mouse model of MH.[45]

Additionally, given similar roles of RyR1 and RyR2 in skeletal and cardiac muscle, respectively, it is possible that carvedilol, which suppresses the store-overload-induced Ca^{2+} release in cardiac myocytes and prevents ventricular tachy-arrhythmias in heart failure, may also be useful as a potential MH pharmacotherapy.[10]

Dr. J. Philips (West Virginia University) presented a poster emphasizing the importance of intraoperative cognitive aids to successfully treat MH. This field will likely be facilitated by the recent introduction of the MHApp. This iPhone application not only guides users through the MH management steps, but also generates a report of the event that can be filed with the NAMHR.

Integration of MH data with global databases of rare diseases

MH is one of ~ 6000 rare diseases that together affect 6–8% of the global population.[46] Single medical research centers do not typically have access to a sufficient patient population on their own to conduct comprehensive clinical or translational research studies for a particular rare disease, hampering the development and validation of effective treatments for these conditions. In 2010, the Office of Rare Diseases Research (ORDR) at the NIH initiated the creation of a global patient registry of rare diseases (GRDR) and the development of a repository of rare disease biospecimens.[47] Drs. B. Brandom (NAMHR) and D. Maglott (National Center of Biological Information) discussed the role of the ORDR and genetic databases in MH risk assessment, and as a resource for clinicians and scientists. Dr. Brandom reported that the NAMHR of MHAUS was selected to be one of 12 existing organizations to contribute data to the Global Rare Diseases Patient Registry and Data

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Repository (GRDR), a 2-year pilot project with the aim of facilitating data analyses across different rare diseases and promoting the development of clinical trials and translational research for rare diseases.

Dr. Maglott emphasized the importance of genetic variation databases for clinical genetics. Next-generation sequencing has resulted in an exponential growth in the identification of genetic variants, making it difficult to keep up with new information added to genetic variation databases such as LOVD, OMIM, HGMD, and UniProt/Swiss-Prot. The newly released ClinVar database provides a comprehensive resource for disease variants (https://www.ncbi.nlm.nih.gov/pubmed/24234437).

The combination of the complex genetic nature of MH, and the genetic diversity of *RYR1* and *CACNA1S* genes, make these databases and bioinformatics tools particularly valuable in the assessment of the pathogenic potential of newly identified genetic variants.

In his presentation, Dr. H. Rosenberg, President of MHAUS, emphasized that the mission of MHAUS is to promote education, training, preparedness, and research related to MH and MH-like syndromes. The MH Hotline provides invaluable direct, real-time assistance to clinicians dealing with potential MH cases. About 600 calls per year are fielded by the 32 volunteer hotline consultants. Although support for clinical and laboratory research through MHAUS funding is limited, the organization serves a critical role in sharing new information and insights among the clinical and patient communities and promoting initiatives to enhance MH preparedness, patient care, and to reduce morbidity from MH. As a result of conferences such as this one, supported by both MHAUS and the University of Toronto, Department of Anesthesia, the significance of *RYR1* variants in medicine beyond those related to anesthesia is better appreciated, and thus, will stimulate further advances that will benefit patients and clinicians alike. Malignant Hyperthermia is probably the most dramatic example of how *RYR1* variants may lead to alteration of muscle physiology and biochemistry, but is certainly not the only syndrome or disease state affected by this channel. The conference demonstrated the wide-spread interest in better understanding the role and significance of RyR1 and related proteins in muscle physiology and a wide range of clinical syndromes.

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Implication Statement

This article reports on new insights into the molecular genetics, pathophysiology and clinical implications of malignant hyperthermia (MH) and related *RYR1*-linked diseases, as well as the relationship between MH and exertional rhabdomyolysis/exertional heat illness, which were presented at the 2013 MHAUS Scientific Conference in Toronto.

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Table 1

Key concepts and theories presented at the scientific conference

- Combining cryoEM and x-ray crystal structure analyses can model effects of N-terminal disease mutations on RyR1 function and mechanisms of *RYR1*-related diseases, thereby clarifying mechanisms by which disease-causing mutations act to alter RyR1 function.
- Parallels can be drawn between mechanisms by which *RYR1* and *RYR2* disease mutations alter excitation-contraction coupling that lead to diseases in skeletal muscle (MH) and cardiac muscle (CPVT).
- Increasingly, the *RYR1* gene mutations are implicated in various inherited myopathies, statin-induced myopathy, and heat/exercise-induced rhabdomyolysis.
- Clarification of which *RYR1*-related myopathies predispose to MH is necessary.
- Identification of genes other than *RYR1* and *CACNA1S* that are involved in MH is required before comprehensive genetic testing for MH is possible.
- The metabolic basis of MH needs to be understood.
- More work needs to be done to clarify which *RYR1*-related myopathies predispose to MH
- The diagnostic algorithm for MH was reviewed, with a focus on the characteristics of the clinical presentation, IVCT/CHCT, and genetic testing.
- MH therapeutic options are expanding including a new formulation of dantrolene and the possible utility of newer drugs such as AICAR and carvedilol derivatives.
- A diagnostic algorithm for myopathic patients was presented.
- The continued evolution of genetic databases will be instrumental in advancing improved understanding of the genotype/phenotype relation in MH.
- Next-generation sequencing has the potential to identify new MH-causative genes, improve the sensitivity and efficiency of MH genetic testing, and will be instrumental in a more accurate assessment of the MH susceptibility prevalence within the general population.

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Table 2

Research directions and patient care issues

- Further characterization of RyR1 structure will likely elucidate pathophysiological mechanisms for MH and other *RYR1*-related disorders.
- New alternative treatments for acute MH and related crises are under investigation (AICAR, carvedilol derivatives, altered formulations for dantrolene).
- Improved understanding of the pathologic potential of *RYR1* variants will be facilitated by next-generation sequencing and new genetic databases such as ClinVar.
- The need to identify the metabolic underpinnings of MH should be facilitated by a metabolomics approach.
- Access to rare diseases databases promoted by collaborations with the Office of Rare Diseases Research and their Global Rare Diseases Patient Registry and Data Repository may reveal new links between MH and other rare disease entities.
- The need to further elucidate the relationship between exercise-induced rhabdomyolysis, heat stroke, MH and *RYR1* variants.
- The role of dantrolene and other MH therapies in ER and EHI should be explored.
- The importance of preoperative identification of MH risk among patients suffering from congenital myopathies.
- Epidemiology of MH across the globe and increased preparedness for treatment of MH in other countries and non-hospital sites where anesthesia is administered.

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Table 3

Classification of Variants Obtained From NGS Data Using RYR1 as an Example*

Variant	Definition	Example	Significance	Report
Pathogenic	Confirmed as a disease-causing mutation	R614C	MHS Diagnosis	Yes
Benign polymorphism	Natural variation in DNA with no known adverse effect	Variant observed in healthy populations with high frequency	Could be a genetic modifier in future studies	No
VUS	Low frequency variant of unknown significance	Missense variant in a less evolutionary conserved RYR1 region	May change status to pathogenic with additional data	Yes
Incidental finding	Variant that carries a potential risk of a disease state but is not relevant to the patient's primary diagnosis	Carrier state for mutations in CFTR gene	Not diagnostic for MH. Assessment of clinically actionable defined by ACMG guidelines	Yes, if clinically actionable

*Modified from[40]

NGS – Next Generation Sequencing; VUS – variant of unknown significance; CFTR - cystic fibrosis transmembrane conductance regulator gene; ACMG - the American College of Medical Genetics and Genomics.