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REPORTS OF ORIGINAL INVESTIGATIONS

Malignant hyperthermia susceptibility in patients with exertional rhabdomyolysis: a retrospective cohort study and updated systematic review

Susceptibilité à l'hyperthermie maligne chez les patients atteints de rhabdomyolyse d'effort: une étude de cohorte rétrospective et une revue systématique mise à jour

Natalia Kraeva, PhD · Alexander Sapa, BHSc · James J. Dowling, MD, PhD · Sheila Riazi, MSc, MD

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Abstract

Introduction *Two potentially fatal syndromes, malignant hyperthermia (MH), an adverse reaction to general anesthesia, and exertional rhabdomyolysis (ER) share some clinical features, including hyperthermia, muscle rigidity, tachycardia, and elevated serum creatine kinase. Some patients with ER have experienced an MH event and/or have been diagnosed as MH susceptible (MHS). In order to assess the relationship between ER and MH further, we conducted a retrospective cohort study summarizing clinical and genetic information on Canadian patients with ER who were diagnosed as MHS. In addition, a systematic literature review was performed to compile further evidence on MH susceptibility and RYR1 and CACNA1S variants associated with rhabdomyolysis.*

Methods *Demographic, clinical, and genetic information was collected on Canadian MHS patients who presented with rhabdomyolysis. In addition, we performed a systematic review of the literature published during 1995-2016 on genetic screening of the RYR1 and CACNA1S genes in patients with ER.*

Results *Retrospective data on Canadian MHS patients with ER showed that ten out of 17 patients carried RYR1 or CACNA1S variants that were either known MH-causative mutations or potentially pathogenic variants. The systematic review revealed 39 different rare RYR1 variants, including 13 MH-causative/associated mutations and five rare potentially deleterious CACNA1S variants in 78% of patients with ER.*

Conclusion *Findings from the Canadian patient cohort and the systematic review all signal a potential association between MH susceptibility and ER. The presence of MH-causative mutations and putative deleterious RYR1 variants in ER patients without a history of adverse anesthetic reactions suggests their possible increased risk for MH.*

Résumé

Introduction *Deux syndromes potentiellement fatals, soit l'hyperthermie maligne (HM), un effet indésirable de l'anesthésie générale, et la rhabdomyolyse d'effort (RE), partagent certaines caractéristiques cliniques, notamment l'hyperthermie, la rigidité musculaire, la tachycardie et des taux sériques élevés de créatine kinase. Certains patients atteints de RE ont souffert d'un épisode d'HM et/ou ont reçu un diagnostic de susceptibilité à l'HM (SHM). Afin d'approfondir nos connaissances quant à la relation entre RE et HM, nous avons réalisé une étude de cohorte rétrospective résumant les informations cliniques et génétiques des patients canadiens atteints de RE et ayant reçu un diagnostic de SHM. En outre, une revue systématique de la littérature a été réalisée afin de compiler d'autres données probantes concernant la*

N. Kraeva, PhD · A. Sapa, BHSc · S. Riazi, MSc, MD (✉)
Malignant Hyperthermia Investigation Unit, Toronto General Hospital, 200 Elizabeth Street, Toronto, ON M5G 2C4, Canada
e-mail: sheila.riazi@uhn.ca

J. J. Dowling, MD, PhD
Division of Neurology, Genetics and Genome Biology Program, Hospital for Sick Children, Departments of Paediatrics and Molecular Genetics, University of Toronto, Toronto, ON, Canada

susceptibilité à l'HM et les variantes RYR1 et CACNA1S associées à la rhabdomyolyse.

Méthode *Les renseignements démographiques, cliniques et génétiques ont été colligés concernant les patients canadiens ayant une SHM et atteints de rhabdomyolyse. En outre, nous avons réalisé une revue systématique de la littérature publiée entre 1995 et 2016 sur le dépistage génétique des gènes RYR1 et CACNA1S chez les patients atteints de RE.*

Résultats *Les données rétrospectives portant sur les patients canadiens atteints de SHM et de RE ont montré que 10 des 17 patients étaient porteurs de variantes RYR1 ou CACNA1S connues comme étant des mutations causatives d'HM ou de variantes potentiellement pathogènes. La revue systématique a révélé 39 différentes variantes rares du RYR1, y compris 13 mutations causatives/associées à l'HM et cinq variantes potentiellement délétères rares du CACNA1S chez 78 % des patients atteints de RE.*

Conclusion *Les résultats de la cohorte canadienne de patients et de notre revue systématique indiquent tous une association potentielle entre une susceptibilité à l'HM et la RE. La présence de mutations causatives d'HM et de variantes du RYR1 présumées comme étant délétères chez les patients souffrant de RE sans antécédent de réactions anesthésiques indésirables laisse penser qu'ils courent possiblement un risque accru d'HM.*

Malignant hyperthermia (MH) is a potentially fatal pharmacogenetic disorder triggered by volatile anesthetics and depolarizing muscle relaxants. Malignant hyperthermia often presents with hypercarbia, muscle rigidity, hyperthermia, tachycardia and, if not treated, can lead to hyperkalemia and elevated levels of serum myoglobin and creatine kinase (CK).[1,2] Malignant hyperthermia susceptibility is diagnosed *ex vivo* upon increased contractions of surgically cut muscle bundles following exposure to halothane and/or caffeine during the caffeine-halothane contracture test (CHCT).[3] The sensitivity and specificity of the CHCT is 97% and 78%, respectively.[4] Malignant hyperthermia susceptibility is associated with mutations in the genes whose products are involved in the excitation-contraction coupling in skeletal muscle, i.e., *RYR1*, *CACNA1S,* and *STAC3*.[2]

Exertional rhabdomyolysis (ER), a severe potentially life-threatening condition, may occur after strenuous exercise in apparently healthy young adults and children. Exertional rhabdomyolysis shares some clinical features with MH and can present with muscle cramps, elevated temperature, tachycardia, tachypnea, hyperkalemia, and elevated levels of serum myoglobin and CK.[5] There are many sporadic reports and two reviews[6,7] of heat and exercise-induced MH-like reactions in MH susceptible (MHS) patients, suggestive of the possible existence of non-anesthetic triggers for MH. Additionally, patients with ER with no family or personal history of MH have tested positive for MH, either with contracture testing or genetics.[8] These reports suggest a connection between MH and ER. In both conditions, the dysregulation of myoplasmic calcium homeostasis could ultimately lead to skeletal muscle breakdown and release of potentially toxic muscle components into the circulation.[9] Moreover, although a genetic basis for the majority of ER cases remains unknown, MH-causative *RYR1* mutations have been reported in association with ER as early as in 2001-2002.[10,11] Further studies have indicated that MH-associated *RYR1* mutations may account for up to 30% of ER episodes.[7]

This retrospective cohort study presents clinical and genetic information on Canadian patients with ER who were diagnosed with MH susceptibility. In addition, a systematic review was performed to compile further evidence on MH susceptibility and *RYR1* and *CACNA1S* variants associated with ER.

Methods

Analysis of patients from the MH Investigation Unit

Following Research Ethics Board approval (January 2016) patients referred to the MH Investigation Unit (MHIU) in Toronto from January 1, 2004 to December 30, 2015 were included in this retrospective study if they experienced more than two episodes of ER and were subsequently diagnosed as MHS by the CHCT (according to the North American MH Group protocol).[3] Due to the retrospective nature of the study, the Research Ethics Board waived written informed consent.

Neurologists referred these patients after extensive workup for more common causes of ER, such as endocrine and inflammatory disorders, drugs, toxins, as well as metabolic myopathies.[12]

The following data were extracted from the MHIU database: patient demographics (sex and race), personal and/or familial history of MH, rhabdomyolysis and its triggers, fitness level of patients, baseline CK and maximum CK values where available, as well as the *RYR1* and *CACNA1S* sequencing results.

Systematic review of literature

The authors used the guidelines set out by the Preferred Reporting Items for Systematic Reviews and Meta-

Analyses (PRISMA) for this review.[13] Prior to starting, a research protocol was established outlining the research question, search strategies, and databases to be used. We selected studies based on the criteria that they were related to exertional heat illness or ER and involvement of *RYR1* and /or *CACNA1S* genes. We aimed to answer the question, "What *RYR1* and *CACNA1S* variants have been reported in patients with exertional rhabdomyolysis?"

Literature search

We searched the following sources for the period January 1, 1995 to July 31, 2016: the U.S. National Library of Medicine database (MEDLINE®), electronic publication (ePub) ahead of Print/MEDLINE In-Process & Other Non-Indexed Citations database, the Excerpta Medica database (EMBASE™), the Cochrane Central Register of Controlled Trials database, the Cochrane Database of Systematic Reviews, the Web of Science Core Collection database, the ClinicalTrials.gov database, and the World Health Organization International Clinical Trials Registry Platform. For our search, we used the following medical subject headings, text terms, and controlled vocabulary terms specific to our research question: 1) malignant hyperthermia, 2) heat stress disorders, 3) exertional rhabdomyolysis, 4) *RYR1*, and 5) *CACNA1S*. These terms, along with a number of related terms, were used individually and in combination in the search. Bibliographies of relevant articles were also manually searched for additional papers meeting the inclusion criteria.

Eligibility criteria

Articles and case reports were included if they contained data on patients with rhabdomyolysis who were screened genetically for *RYR1* and/or *CACNA1S* (our research question). Exclusion criteria were articles not written in English, articles on animal models or on statin-induced myopathies, articles with no genetic screening results, or articles describing MH episodes attributed solely to volatile anesthetics and depolarizing muscle relaxants.

Selection of included studies

Two authors (A.S. and S.R.) independently evaluated the de-duplicated results of the literature search, first by title and abstract, followed by full-text articles. The decision to include qualified studies in the review required consensus between the two authors. The authors resolved discrepancies by re-examining the source date and considering the opinion of a third author (N.K.).

Data extraction

Extracted data from chosen articles included the primary author, number of subjects with exercise-induced rhabdomyolysis, and number carrying *RYR1* or *CACNA1S* variants, including their respective CHCT results where available. In studies with multiple groups, only subjects from the exercise- or heat-induced group were included. Exercise-induced manifestations were evaluated by the authors according to the description of the studies.

Results

Canadian retrospective cohort

A summary of the clinical and genetic findings for the Canadian cohort is presented in Table 1. The Canadian cohort of patients with ER included 17 patients from 16 unrelated families—12 males and five females, most of them young, physically fit individuals. Every patient of this cohort had positive CHCT results according to the North American MH criteria.[3] *RYR1* variants were detected in nine/17 patients. Two of the identified variants were known MH-causative mutations, p.Val2168Met and p.Gly2434Arg, and one variant, p.Val4849Ile, has been associated with MH in several MH families worldwide. Noteworthy, there was no history of MH in families of the carriers of p.Gly2434Arg (Family 13) and p.Val4849Ile (Family 2). One patient in this cohort carried a rare *CACNA1S* variant, Thr852Met, of unknown significance and with a frequency of 0.02% in the general population.

Two patients (Families 15 and 16) (Table 1) developed rhabdomyolysis 24 hr after termination of a volatile anesthetic. One patient (Family 15) also received succinylcholine. A review of anesthetic records in these two patients did not reveal any signs of MH reaction (i.e., hypercarbia, hyperthermia, tachycardia, or hypertension) intraoperatively or up to one hour postoperatively. The procedures were breast augmentation and tonsillectomy, respectively, and were completed as day surgery procedures.

Systematic review

The original search yielded 1,212 results. Following removal of duplicates, 842 records remained. After screening of abstracts and titles based on our eligibility criteria, 815 records were excluded, leaving 27 articles. Seven of these 27 full-text articles were excluded as 1) one did not contain detailed genetic information; 2) three were editorials commenting on existing articles; 3) one described an episode specific to general anesthesia; and

Table 1 Genetic and clinical findings in Canadian MHS patients with non-anesthetic rhabdomyolysis

Family No.	Genetics	Sex	Fitness	Trigger/symptoms	MH family	MH event	CK ($IU{\cdot}L^{-1}$)	Max CK ($IU{\cdot}L^{-1}$)
1	p.Val2168Met*	F	Athletic	Exercise	Yes	No	619	20,000
2	p.Val4849Ile	M	Athletic	Exercise, heat	No	No	316	N/A
3	p.Glu176Lys	M	Fit	Exercise, heat	No	No	234	170,000
4	CACNA1S: p.Thr852Met	M	Athletic	Strenuous exercise	No	No	412	13,000
5	Negative†	M	Athletic	Strenuous exercise	No	No	278	400
6	Negative	M	Athletic	Strenuous exercise	No	No	270	35,000
7	Negative	M	Athletic	Exercise	No	No	586	N/A
8	Negative	F	Athletic	Exercise, heat	Yes	No	343	N/A
9	p.Ala933Thr; p.Ala1352Gly	M	Athletic	Exercise, viral infection	No	No	994	N/A
10	Thr4288Ala4290dup	M	Child	Viral illness	Yes	No	266	N/A
11	Negative	M	Obese	Exercise, viral infection	No	No	1105	80,000
12	Negative	F	Fit	Exercise, heat	No	No	769	N/A
13‡	p.Leu2695Arg	M	Athletic	Strenuous exercise	No	No	150	2,500
13	p.Gly2434Arg; p.Leu2695Arg	M	Fit	Strenuous exercise	No	No	471	569
14	Negative	F	Fit	Exercise, heat	Yes	No	212	N/A
15	p.Val4849Ile	F	Fit	Delayed rhabdo§	No	Yes	290	7,000
16	p.Gly2434Arg	M	Fit	Delayed rhabdo§	No	Yes	327	7,500

CK = creatine kinase; F = female; $IU{\cdot}L^{-1}$ = international unit per litre; M = male; MH = malignant hyperthermia; MHS = malignant hyperthermia susceptible; N/A = not available

*The variants are *RYR1* variants unless mentioned otherwise. The amino acid numbering corresponds to the GenBank® accession number NP_000531.2 for *RYR1* and NP_000060.2 for *CACNA1S*. †Negative sequencing results for *RYR1* and *CACNA1S*. ‡Family 13 includes two patients with exertional rhabdomyolysis. §Delayed rhabdomyolysis (>24 hr) after anesthesia

4) two were reviews with no new patients. Therefore, 20 articles were included in the final results (Figure).

The strength of the articles based on Grading of Recommendations Assessment, Development and Evaluation (GRADE)[14] was not performed, as they were all observational case reports or case series studies.

Fifty-nine subjects presenting with exercise- or heat and exercise-induced symptoms were included in the analysis. Table 1 summarizes the extracted data from relevant articles. There were 34 missense *RYR1* variants and five insertion/deletions in 46/59 (78%) patients with ER.

There were 12 ER patients carrying six different MH-causative mutations: p.Arg163Cys,[10,15] p.Gly341Arg,[10] p.Arg614Cys,[16] p.Thr2206Met,[17] p.Gly2434Arg,[10,17-19] p.Arg2454Cys,[20] with six unrelated patients carrying the same MH-causative mutation p.Gly2434Arg. The most common among insertion/deletion variants was p.Thr4288_Ala4290dup found in four unrelated ER patients of Afro-Caribbean ancestry, while four of their relatives—carriers of this variant—were asymptomatic.[18,21] The remaining *RYR1* variants were rare variants of unknown significance (VUS) and several neutral polymorphisms (Table 2).[17-29]

There were five *CACNA1S* variants, p.Arg498His, p.Ser516Leu, p.Ser606Asn, p.Arg683Cys, p.Thr852Met, identified in this cohort. These rare variants (minor allele frequency $< 1\%$) are of unknown significance and have not been reported in association with MH.

Discussion

This study compiles the genetic findings in a Canadian cohort of MHS patients with exertional rhabdomyolysis. It is complemented by a literature review listing *RYR1* and *CACNA1S* variants identified in patients with ER to date.

Canadian patients with ER were either asymptomatic or had mild exercise-induced myalgia (which developed after the first episode of ER). The majority of the patients were physically fit male athletes who experienced the rhabdomyolysis event unexpectedly after a regular intensity exercise. In several cases, ER episodes were triggered by a combination of either exercise and hot ambient temperature or exercise and viral illness.

These findings were in line with the characteristic features of *RYR1*-related ER reported in the systematic articles.[6,7]

The presence of MH-causative or -associated mutations in ER patients without a personal or familial history of MH as well as reports of positive results in many ER patients on the CHCT and *in vitro* contracture test (IVCT) strengthen the argument in favour of a connection between MH

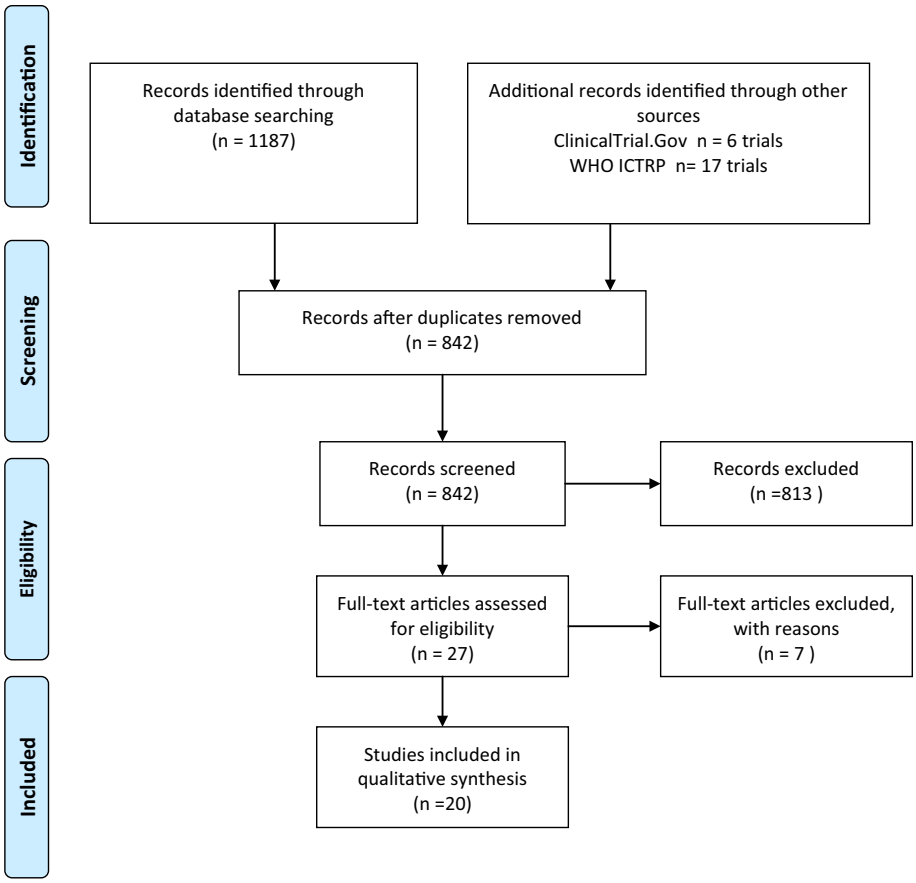

Figure Flowchart for selection of the studies included in the analysis using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines

susceptibility and rhabdomyolysis triggered by exertion, heat, and even viral illness. Although the contracture tests (CHCT/IVCT) were originally designed for testing patients with suspected anesthetic-induced MH reaction, a positive CHCT in patients with rhabdomyolysis caused by non-anesthetic triggers may imply existence of a common muscle defect.

The earliest implication of a link between MH and ER goes back to porcine stress syndrome. An *RYR1* variant in pigs causes a condition similar to MH in humans, but triggered mainly with intense activity and heat.[30] Campbell *et al.*[31] suggested a possible link between MH susceptibility and ER when comparing MHS subjects with controls during exercise. Malignant hyperthermia susceptible subjects not only developed higher core temperatures during exercise but they also dissipated peripheral heat poorly. This could be seen through slower rising thumb and chest wall temperatures following an increase in core temperature.[31] Nevertheless, MHS subjects did not develop serum lactate levels higher than controls or other rhabdomyolysis indicators, such as CK or myoglobin (that were not measured in this study).

Bendahan *et al.*[32] used ^{31}P magnetic resonance spectroscopy to compare patients with a previous MH reaction with those who had exertional heat stroke and abnormal CHCT results. The authors detected a similarly abnormal glycolytic activity in both groups, with a preference for glycogenolysis caused by high cytosol Ca^{2+} concentrations.[32]

Studies on a mouse model of MH demonstrated that certain *RYR1* mutations could cause a Ca^{2+} leak into the cytoplasm, resulting in increased production of reactive nitrogen species. This nitrosative stress could potentially result in S-nitrosylation of the RyR1 receptor, leading to increased temperature sensitivity and a higher risk of heat-induced sudden death in mice.[33] Another study by the same group showed that MHS mice did not develop exercise-induced rhabdomyolysis if their core body temperature was maintained at $< 36°C$, suggesting the importance of a combination of mechanical and thermal stress for triggering the MH-related rhabdomyolysis.[34] These studies raised awareness of a possible MH susceptibility in patients with ER, especially in those carrying *RYR1* variants.

Nevertheless, the extent of the overlap or connection between ER and MH remains unknown as both disorders are multifactorial with complex etiology. First, the majority of the *RYR1* variants found in ER patients are rare VUS. Although their disease-causing potential can be predicted using bioinformatics software tools, including commonly used Sorting Intolerant From Tolerant (SIFT),[35] PolyPhen-2,[36] and Combined Annotation-Dependent Depletion

Table 2 A systematic review of *RYR1* variants reported in patients with non-anesthetic rhabdomyolysis

RYR1 variant	#cases*	Triggers	CHCT	dbSNP† reference number	MAF ExAC‡, %	ClinVar§ public archive	MH-causal potential	References
p. Arg163Cys	*n* = 2	E, H	MHS	rs118192161	ND	pathogenic	Causative	10, 15
p.Gly341Arg	*n* =1	E	MHS	rs121918592	ND	pathogenic	Causative	10
p.Arg614Cys	*n* = 1	E	ND	rs193922772	0.002	pathogenic	Causative	16
p.Thr2206Met	*n* = 1	VIR	ND	rs118192177	0.003	pathogenic	Causative	17
p.Gly2434Arg	*n* = 6	E, VIR	MHS	rs121918593	0.002	pathogenic	Causative	10, 17, 18, 19
p.Arg2454Cys	*n* = 1	E, H	MHS	rs193922816	0.0008	pathogenic	Causative	20
p. Arg401Cys	*n* = 2	E	MHS	rs193922764	ND	untested allele	Associated	11
p. Arg492His	*n* = 1	E,H	MHS	ND	ND	ND	ND	22
p. Arg830Trp	*n* = 1	E, H	MHN	rs142548565	0.010	ND	ND	17
p.Ala933Thr (p.Ser1342Gly, p.Ala1352Gly)	*n* = 1	E	MHS	rs148623597	0.10	likely benign	ND	21
Arg1109Lys	*n* = 1	E, H	MHS	rs35719391	0.17	ND	ND	19
p.Ser1342Gly	*n* = 3	E	MHS	rs34694816	2.21	benign	ND	16, 21, 22
p.Ala1352Gly (p.Ala933Thr, p.Ser1342Gly)	*n* = 1	E	MHS	rs112105381	0.28	likely benign	ND	21
p.Ala1352Gly (p.Ser1342Gly, Thr4288_Ala4290dup)	*n* = 1	E, H	MHS	rs112105381	0.28	likely benign	ND	21
p. Lys1393Arg;	*n* = 1	E, H	ND	rs137933390	0.51	likely benign	ND	18, 23
p.Lys1393Arg (Arg4737Gln)	*n* = 2	E	ND	ND	ND	ND	ND	18
p. Asp2129Asn	*n* = 1	E, H	ND	rs772695891	0.006	untested allele	Associated	18
p. Gly2132Ser	*n* = 3	E	ND	ND	ND	ND	ASY	17, 18
p.Ile2321Val (p.Val4849Ile)	*n* = 1	E, H, D‖	MHS	rs34390345	0.04	likely benign	Associated	24
p. Tyr2426Cys	*n* = 1	E, D**	ND; MHN	ND	ND	ND	ND	17, 18
Val3088Met	*n* = 1	E,H	MHS	rs145044872	0.06	VUS	ND	19
p. Ile3253Thr	*n* = 2	E,H	MHS;MHS	rs375626634	0.004	VUS	ND	19, 22
p. Ala3407Ser	*n* = 1	VIR	ND	ND	ND	ND	ND	25
p. His3981Tyr (p.Ser1342Gly)	*n* = 1	E,H	MHS	rs148772854	0.13	VUS	ND	22
p. Arg3983Cys	*n* = 2	E, VIR	ND; MHS	ND	ND	ND	Associated	26, 27
p. Arg3983Cys (p.Asp4505His)	*n* = 1	E, H, VIR	MHS	ND	ND	ND	ND	26
Leu4282Val	*n* = 1	E, H	MHN	ND	ND	ND	ND	22
p.Thr4294Met (p.S1342G, A1352G, T4288_A4290dup)	*n* = 1	E	MHS	rs587784372	ND	VUS	ND	21
p. Ala4295Val	*n* = 2	E, H	ND; MHN	rs193922855	ND	ND	ASY	18, 22
p. Ala4331Thr	*n* = 1	E, H	MHN	ND	ND	ND	ND	22
Val4438Gly	*n* = 1	E, H	MHS	ND	ND	ND	ND	19
p. Pro4501Leu	*n* = 1	E, H	ND	rs73933023	0.48	likely benign	ND	16
p. Asp4505His (p.Arg3983Cys)	*n* = 1	E, H	MHS	rs150396398	0.61	VUS	ND	26
p. Arg4645Gln (p.L4320_R4322dup)	*n* = 1	E, H	ND	rs193922860	0.004	ND	Associated	28
p. Arg4737Gln (p. Lys1393Arg)	*n* = 2	E	MHS	rs193922868	0.0008	ND	Associated	18
p. Gly4820Arg	*n* = 1	E, H, D‡‡	ND	ND	ND	ND	ND	29
p.Val4849Ile (p.Ile2321Val)	*n* = 1	E, H, D‖	MHS	rs118192168	0.0008	Pathogenic	ND	24
c.957+5_957+29del	*n* = 1	E	ND	ND	ND	ND	ASY	18
c.12282+57_12282+60delins238	*n* = 1	VIR	ND	ND	ND	ND	ASY	17
Thr4288_Ala4290dup	*n* = 4	E, H	MHS	ND	ND	ND	ASY	18, 21
Leu4320_Arg4322dup (p.Arg4645Gln)	*n* = 1	H	ND	ND	ND	ND	Associated	28

Table 2 continued

RYR1 variant	#cases*	Triggers	CHCT	dbSNP† reference number	MAF ExAC‡, %	ClinVar§ public archive	MH-causal potential	References
Ala4415_4421del	*n* = 1	E, H	MHS	ND	ND	ND	ND	19

*Number of patients with rhabdomyolysis; †dbSNP = Single Nucleotide Polymorphism database: the National Center for Biotechnology Information (NCBI) database of nucleotide sequence variation (https://www.ncbi.nlm.nih.gov/projects/SNP); ‡MAF ExAC = minor allele frequency from the Exome Aggregation Consortium (http://exac.broadinstitute.org); §ClinVar = ClinVar is a public archive of interpretations of clinically relevant variants (https://www.ncbi.nlm.nih.gov/clinvar/)

D = drug (‖caffeine gel, **Treated with olanzapine, ‡‡succinylcholine); ASY = asymptomatic carrier; CHCT = caffeine-halothane contracture test; E = exertion; H = heat; MH = malignant hyperthermia; MHS = MH susceptible; MHN = MH negative; ND = not determined; VIR = viral infection - triggered rhabdomyolysis; VUS = variants of unknown significance

Malignant hyperthermia (MH)-causative *RYR1* mutations are in bold

(CADD),[37] such predictions should be treated with caution. The sensitivity of predictions for *RYR1* and *CACNA1S* has been estimated[38] to range from 84-100%, with specificity ranging from 25-83%. Therefore, other approaches, such as segregation analysis and functional studies, are still required for accurate validation of the role of each VUS in rhabdomyolysis as well as in the MH phenotype. Secondly, variable penetrance of *RYR1* variants hampers validation of their pathogenic potential as well as their involvement in the MH or ER phenotype. In our cohort, the index patient with ER in Family 1, a family with a history of MH reaction, carried an MH-causative mutation, p.Val2168Met. Nonetheless, neither the patient's father, who survived an MH event and carried the same mutation, nor several MHS family members were reported to have experienced episodes of rhabdomyolysis or excessive exertional myalgia. In Family 13, the physically fit father and athletic son had at least one rhabdomyolysis event each. There was no family history of MH in this family. The father was found to be compound heterozygous for p.Gly2434Arg, a MH-causative mutation, and a novel variant, p.Leu2695Arg, while the son carried only p.Leu2695Arg. This variant is not present in the ClinVar public archive, the Human Gene Mutation Database (HGMD®), or the Leiden Open (source) Variation Database (LOVD) and is predicted to be deleterious based on programs such as PolyPhen-2 and SIFT. Another example of an ER-associated variant with variable penetrance is p.Thr4288Ala4290dup, which has been found in five patients of African-Caribbean ancestry with rhabdomyolysis, including one from the Canadian cohort, as well as in several asymptomatic carriers. Moreover, some of the asymptomatic subjects with ER-associated *RYR1* variants have actually been tested as MH negative (Table 2). Reduced penetrance of the putative deleterious *RYR1* variants complicates validation of their impact and suggests presence of additional yet not identified genetic or non-genetic factors that might influence the phenotype.

Therefore, because of the complex nature of both syndromes, patients with ER should be carefully evaluated by neuromuscular specialists to rule out non-MH-related etiologies, such as endocrine, inflammatory, or drugs. Screening should be performed for metabolic disorders causing exercise intolerance, including, phosphofructokinase deficiency, McArdle's disease, Tarui's disease, phosphoglycerate kinase deficiency, phosphoglycerate mutase deficiency, lactate dehydrogenase deficiency, glycogen, phosphorylase A total deficiency, phosphorylase B kinase deficiency, carnitine palmitoyltransferase-2 deficiency, and myoadenylate deaminase deficiency.[5,39] Nevertheless, if no clear etiology is found in patients with repeated ER events, MH susceptibility should be assessed.

In conclusion, our findings from the Canadian patient cohort and the systematic review all signal toward a potential association between MH susceptibility and rhabdomyolysis. Presence of MH-causative mutations and putative deleterious *RYR1* variants in patients with unanticipated or recurrent exertional rhabdomyolysis and without a history of adverse anesthetic reactions suggests their increased risk for MH.

Conflict of interest We confirm that we have read the *Journal's* position on issues involved in ethical publication and affirm that this report is consistent with those guidelines. None of the authors have any conflict of interest to disclose.

Editorial responsibility This submission was handled by Dr. Hilary P. Grocott, Editor-in-Chief, *Canadian Journal of Anesthesia.*

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