ANESTHESIOLOGY

Referral Indications for Malignant Hyperthermia Susceptibility Diagnostics in Patients without Adverse Anesthetic Events in the Era of Next-generation Sequencing

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EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- Most cases of malignant hyperthermia susceptibility are associated with variants in the gene encoding the skeletal muscle ryanodine receptor 1, *RYR1*
- Next-generation sequencing has resulted in a rapid increase in the identification of both the number of patients with an *RYR1* variant and the number of newly identified *RYR1* variants

What This Article Tells Us That Is New

- The hypothesis that there is an increased referral to malignant hyperthermia units of patients without a personal or family history of adverse anesthetic events suspected to be malignant hyperthermia was tested in a retrospective multicenter cohort study
- The proportion of patients referred without a personal or family history of adverse anesthetic events increased from 28.4% (61 of 215) between 2010 and 2014 to 43.6% (133 of 305) between 2015 and 2019
- Patients with a personal or family history of adverse anesthetic events were more frequently diagnosed as malignant hyperthermia–susceptible (133 of 220; 60.5%) than those without (47 of 120; 39.2%)

ABSTRACT

Background: The introduction of next-generation sequencing into the diagnosis of neuromuscular disorders has resulted in an increased number of newly identified *RYR1* variants. The hypothesis was that there is an increased referral of patients to malignant hyperthermia units without a personal/family history of adverse anesthetic events suspected to be malignant hyperthermia. This retrospective multicenter cohort study evaluates patient referral indications and outcomes for those without a history of an adverse anesthetic event.

Methods: Patients referred between 2010 and 2019 to the malignant hyperthermia units in Antwerp, Belgium; Lund, Sweden; Nijmegen, The Netherlands; and Toronto, Ontario, Canada were included. Previously tested patients and relatives of previously tested patients were excluded. Data collection included demographics, referral details, muscle contracture, and genetic testing results including Rare Exome Variant Ensemble Learner scores. Referral indications were categorized into those with a personal/family history of adverse anesthetic event and other indications including exertional and/or recurrent rhabdomyolysis, *RYR1* variant(s) detected in diagnostic testing in the neuromuscular clinic without a specific diagnosis (in a family member), diagnosed *RYR1*-related myopathy (in a family member), idiopathically elevated resting creatine kinase values, exertional heat stroke, and other.

Results: A total of 520 medical records were included, with the three most frequent referral indications as follows: personal history of an adverse anesthetic event (211 of 520; 40.6%), family history of an adverse anesthetic event (115 of 520; 22.1%), and exertional and/or recurrent rhabdomyolysis (46 of 520; 8.8%). The proportion of patients referred without a personal/family history of an adverse anesthetic event increased to 43.6% (133 of 305) between 2015 and 2019 compared to 28.4% (61 of 215) in 2010 to 2014 ($P < 0.001$). Patients with a personal/family history of an adverse anesthetic event were more frequently diagnosed as malignant hyperthermia–susceptible (133 of 220; 60.5%) than those without (47 of 120; 39.2%; $P < 0.001$). Due to missing data, 180 medical records were excluded.

Conclusions: The proportion of patients referred to malignant hyperthermia units without a personal/family history of an adverse anesthetic event has increased, with 39.2% (47 of 120) diagnosed as malignant hyperthermia–susceptible.

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Malignant hyperthermia (MH) is a potentially life-threatening pharmacogenetic disorder triggered by volatile anesthetics and/or depolarizing muscle relaxants in MH-susceptible individuals. MH susceptibility diagnostics rely on the caffeine–halothane contracture test (CHCT)[1,2] or the *in vitro* contracture test[3] on freshly biopsied muscle tissue and on genotyping.[4] In the majority of cases, MH susceptibility is associated with variants in

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RYR1, the gene encoding skeletal muscle ryanodine receptor 1.[5] Other MH-associated genes are *CACNA1S*, which encodes the α1 subunit of the dihydropyridine receptor,[6] and *STAC3*, which encodes the SH3 and cysteine-rich domain 3 proteins.[7]

Next-generation sequencing, recently introduced in most institutions in the Western world, facilitates faster, cheaper, and more accurate genetic analysis and has caused a significant paradigm shift in MH susceptibility diagnostics. Furthermore, as *RYR1* variants may cause a wide spectrum of muscle diseases,[8] next-generation sequencing is frequently used in the neuromuscular clinic for *RYR1* analysis in patients with an unresolved neuromuscular phenotype. This has resulted in a considerable rise in the number of newly identified *RYR1* variants,[9] as well as an increased number of referred patients from neuromuscular clinics to MH units to assess the potential risk of MH in patients with an *RYR1* variant of unknown significance, even though they have no personal or family history of adverse anesthetic events suspected to be MH.

For genetic variants to be used in diagnosing MH susceptibility, they need to be classified according to the ClinGen Variant Curation Expert Panel recommendations for *RYR1* pathogenicity classification[10] and/or the European Malignant Hyperthermia Group scoring matrix for classification of genetic variants in MH susceptibility (https://www.emhg.org/genetic-scoring-matrix). If a patient carries a variant that does not meet the criteria of being benign or pathogenic, a CHCT/*in vitro* contracture test is the only option to confirm or rule out MH susceptibility.[4] As currently only a minority of the *RYR1* variants have been classified as benign or pathogenic, MH diagnostics still relies on the CHCT/*in vitro* contracture test. Hence, counseling for MH susceptibility in patients with one or more *RYR1* variants of unknown significance without a personal or family history of adverse anesthetic events suspected to be MH can be challenging, and performing a CHCT/*in vitro* contracture test in all these patients might result in unnecessary invasive muscle biopsies.

We hypothesize that there is an increased referral of patients to MH units without a personal or family history of adverse anesthetic events suspected to be MH. This retrospective multicenter cohort study aims to evaluate the overall referral indications and the results of MH susceptibility diagnostics in patients without a history of adverse anesthetic events suspected to be MH. The knowledge obtained from this study can be used to improve counseling of patients referred to MH centers without a personal or family history of adverse anesthetic events suspected to be MH and to reassess guidelines to test for MH susceptibility.[4]

Materials and Methods

This study was performed with approval of the Research Ethics Boards from the Canisius Wilhelmina Hospital, Nijmegen, The Netherlands (registration No. 067-2020, date of approval September 8, 2020); Skane University Hospital, Lund, Sweden (registration No. 2019-03960, date of approval October 9, 2019); Toronto General Hospital, Toronto, Ontario, Canada (registration No. 19-5365, date of approval May 12, 2019); and Antwerp University Hospital/University of Antwerp, Antwerp, Belgium (registration No. 1805016N, date of approval September 28, 2015). Informed consent was waived due to the retrospective nature of the study.

Study Design

This retrospective multicenter cohort study focusing on the referral indications for MH susceptibility diagnostics consists of two parts in line with the two aims of the study. The primary analysis is a retrospective evaluation of the referral indications and the use of next-generation sequencing during the study period. The secondary analysis is a detailed evaluation of the MH diagnostics performed and the test results of patients referred to the participating MH units without a personal or family history of an adverse anesthetic event suspected to be MH.

Study Population

The medical records of patients referred to one North American (Toronto, Ontario, Canada) and three European (Antwerp, Belgium; Lund, Sweden; and Nijmegen, The

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Netherlands) MH investigation units were evaluated. All medical records of patients referred to the participating centers between January 1, 2010, and December 31, 2019, were reviewed. Medical records of the following patients were excluded from the primary analysis to prevent ascertainment bias:

- Relatives of previously tested patients
- Patients who underwent MH susceptibility diagnostics before 2010 and were referred again for genetic testing to facilitate MH susceptibility diagnostics by genotyping in family members

Medical records of the following patients were excluded from the secondary analysis:

- Patients referred because of a personal or family history of adverse anesthetic event suspected to be MH
- Patients referred because of a referral indication categorized as other (see Referral Indication section)
- Patients genetically investigated using a targeted technique (*e.g.*, targeted screening for pathogenic *RYR1* variants or a hotspot technique), except when referred because of a family history of a diagnosed *RYR1*-related myopathy or a family history of an *RYR1* variant detected in diagnostic testing in the neuromuscular clinic without a specific clinical or histopathologic diagnosis; these patients were parents of neuromuscular patients who were too young to be investigated by a CHCT/*in vitro* contracture test and were genetically investigated utilizing a targeted technique to identify which family members are carriers of the *RYR1* variant(s) identified in the neuromuscular patient.

The study design and the selection process of medical records for the primary and secondary analysis are summarized in figure 1. Some of the medical records included in this study cohort have been described before.[8,11–16] Details of the overlapping medical records are summarized in Supplemental Digital Content 1 (http://links.lww.com/ALN/C833).

Data Collection

The collected data included demographic characteristics (age at referral and sex), referral details (date of referral, indication for referral, and the indication for *RYR1*, *CACNA1S*, or *STAC3* sequencing), clinical grading scale[17] for the referred probands, resting creatine kinase levels, details on the performed genetic tests (genes analyzed, technique used, and test results), and CHCT/*in vitro* contracture test results.

Referral Indication

Referral indications were categorized into two main groups: those with a personal or family history of adverse anesthetic events suspected to be MH and those without. Since our objective was to improve counseling for patients without a history of adverse anesthetic events, referral indications from the latter category were subcategorized into different groups.

A personal or family history of adverse anesthetic events suspected to be MH was defined as follows:

1. A personal history of an adverse anesthetic event suspected to be MH (probands)
2. A family history of an adverse anesthetic event suspected to be MH (relatives who were investigated instead of the proband)

Other referral indications without a history of adverse anesthetic events suspected to be MH were defined as follows:

3. Personal history of exertional and/or recurrent rhabdomyolysis, defined as a creatine kinase value during the rhabdomyolysis event of more than 10,000 U/l, where recurrent is defined as at least two episodes of rhabdomyolysis
4. Personal history of a diagnosed *RYR1*-related myopathy (central core disease, multiminicore disease, centronuclear myopathy, congenital fiber type disproportion, King–Denborough syndrome, periodic paralysis, and axial myopathy)
5. Family history of a diagnosed *RYR1*-related myopathy
6. Personal history of an *RYR1* variant detected on diagnostic testing in the neuromuscular clinic for symptoms suggestive of a neuromuscular disorder but without a specific clinical or histopathologic diagnosis and/or fulfilling one of the other referral criteria; these are often coincidentally found *RYR1* variants; frequent reasons for *RYR1* sequencing in the neuromuscular clinic are myalgia, muscle cramps, and muscle weakness[18,19]
7. Family history of an *RYR1* variant detected during diagnostic testing in the neuromuscular clinic for symptoms suggestive of a neuromuscular disorder but without a specific clinical or histopathologic diagnosis and/or fulfilling one of the other referral criteria
8. Personal history of idiopathically elevated resting creatine kinase values
9. Personal history of exertional heat stroke defined as a temperature higher than 40°C/104°F with central nervous system dysfunction
10. Other

Final Diagnosis after Full MH Diagnostic Process

Based on the available information concerning genetic and CHCT/*in vitro* contracture test results, all medical records were classified as MH-susceptible, non–MH-susceptible, or unknown. Those who tested positive for both halothane and caffeine, positive for halothane only, or positive for caffeine only by CHCT/*in vitro* contracture test were

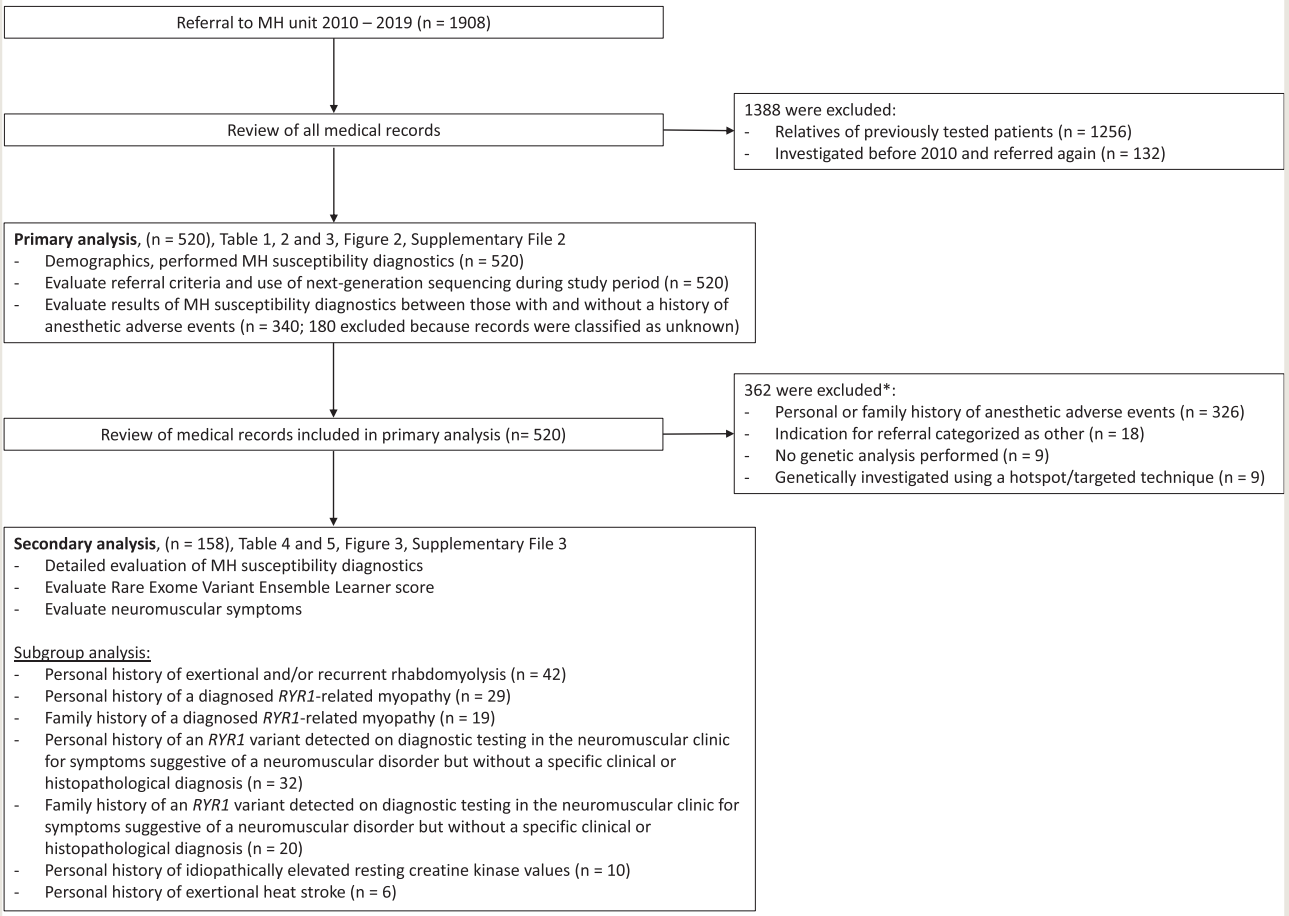

Fig. 1. A summary of the study design and study selection process. *Some medical records meet more than one exclusion criterion. MH, malignant hyperthermia.

classified as MH-susceptible. Those who were tested negative for both halothane and caffeine by CHCT/*in vitro* contracture test were classified as non–MH-susceptible. Patients with an *RYR1*/*CACNA1S* variant diagnostic for MH according to the European Malignant Hyperthermia Group list of diagnostic variants (www.emhg.org/diagnostic-mutations; Lund, Sweden) were classified as MH-susceptible.[20]

In cases in which the medical records did not support any of the above criteria, the records were classified as unknown.[4] Based on the available information concerning the diagnostic procedures performed, these records could not be classified as MH-susceptible or non–MH-susceptible because the relevant investigations (genetic testing and/or CHCT/*in vitro* contracture test) were not (yet) performed due to the waiting lists in the participating MH units, patient's refusal, missing data, or a combination of these reasons.

Individuals referred to the MH unit in Toronto were investigated by CHCT according to the North American MH protocol. The CHCT has a sensitivity of 97% and specificity of 78%.[1,2] Individuals referred to Nijmegen, Lund, and Antwerp were investigated by *in vitro* contracture test according to the European Malignant Hyperthermia Group protocol. The *in vitro* contracture test has a sensitivity of 100% and specificity of 94%.[3,4]

RYR1 Pathogenicity Classification Using Computational Evidence

To study whether computational evidence is useful when counseling patients without a history of an adverse anesthetic event suspected to be MH, the Rare Exome Variant Ensemble Learner score[21] was used. During the secondary analysis, the Rare Exome Variant Ensemble Learner score was calculated for the *RYR1* missense variants that were not on the European Malignant Hyperthermia Group

list of diagnostic variants.[20] As the Rare Exome Variant Ensemble Learner score was developed for missense variants, other types of *RYR1* variants (*e.g.*, duplication or deletion variants) were excluded from the analysis. When two or more *RYR1* missense variants were identified, the variant with the highest Rare Exome Variant Ensemble Learner score was included in the analysis. According to the ClinGen Variant Curation Expert Panel for *RYR1* pathogenicity classification recommendations for *RYR1* pathogenicity assertions in MH susceptibility,[10] a Rare Exome Variant Ensemble Learner score of 0.5 or lower is evidence against pathogenicity, a Rare Exome Variant Ensemble Learner score of 0.5 to 0.85 neither is evidence against nor supports pathogenicity, and a Rare Exome Variant Ensemble Learner score of 0.85 or higher supports pathogenicity.

Statistical Analysis

The sample size was based on the available data. Therefore, no statistical power analysis was performed. Normality of continuous variables was assessed with the use of histograms. Continuous variables were reported as mean ± SD for normally distributed data and as median and interquartile range for nonnormally distributed data. Categorical variables were reported using frequencies and percentages. The chi-square test was used to compare categorical variables. Records classified as unknown were included in the descriptive statistics regarding MH diagnostic tests used and also in the analysis regarding the referral criteria during the study period. These records were excluded from the statistical analysis comparing the proportion of records classified as MH-susceptible between those with or without personal or family history of adverse anesthetic events suspected to be MH. All statistical analyses were performed using IBM SPSS Statistics for Windows version 24.0 (USA). A two-tailed P value < 0.05 was used as a cutoff for significance. The statistical analysis plan, definition of the subgroups, and outcomes were documented before accessing the data.

Results

Primary Analysis

Demographic Characteristics. In the primary analysis, 520 medical records were included (fig. 1). Median age at referral was 37 yr (interquartile range, 28 to 50), and 265 (51.0%) were male. Based on the information in their MH unit medical records, 180 (34.6%) patients were categorized as MH-susceptible, 160 (30.8%) were categorized as non–MH-susceptible, and 180 (34.6%) were categorized as unknown. In seven (1.8%) medical records, the result of the genetic analysis could not be identified. There were no missing CHCT/*in vitro* contracture test results. The clinical grading scale[17] was available for 170 probands, of which 109 completed the full diagnostic process of MH susceptibility. Resting creatine kinase values were available in 134 (25.8%) medical records, of which 115 completed the full diagnostic process for MH susceptibility. The demographic characteristics are summarized in table 1.

Referral Indications and Use of Next-generation Sequencing during the Study Period. The most frequent referral indication was a personal history of an anesthetic adverse event suspected to be MH (n = 211; 40.6%). A total of 194 (37.3%) patients referred to the participating MH units did not have a personal or family history of adverse anesthetic events suspected to be MH. Referral indications changed during the study period; 28.4% (61 of 215) of the patients referred between 2010 to 2014 did not have a personal or family history of an adverse anesthetic event suspected to be MH, while this increased to 43.6% (133 of 305) between 2015 to 2019 (P < 0.001). Distribution of the referral criteria for each MH unit is summarized in table 2.

A CHCT/*in vitro* contracture test was performed in 288 (55.4%) patients, genetic investigation was performed in 399 (76.7%) patients, and 192 (36.9%) were investigated both genetically and by CHCT/*in vitro* contracture test. Of the 399 genetically investigated patients, 168 (42.1%) were investigated by sequencing of both the *RYR1* and *CACNA1S* genes. The use of next-generation sequencing increased during the study period; 49.3% (106 of 215) of the patients referred between 2010 to 2014 were investigated by next-generation sequencing, while this increased to 68.2% (208 of 305) between 2015 and 2019 (P < 0.001). Referral indication and the use of next-generation sequencing during the study period are summarized in figure 2.

In the subgroup of those without a personal or family history of an adverse anesthetic event suspected to be MH (n = 194), 47 were diagnosed as MH-susceptible, 73 were diagnosed as non–MH-susceptible, and 74 were classified as unknown. Patients with a personal or family history of an anesthetic adverse event suspected to be MH were more frequently diagnosed as MH-susceptible (133 of 220; 60.5%) than those without (47 of 120; 39.2%; P < 0.001). A total of 180 records were excluded from the analysis because of insufficient information, resulting in classification as unknown. Referral indications and details of the diagnostic tests performed are summarized in table 1.

The results of the genetic analysis, overall and for the subgroups (MH-susceptible, non–MH-susceptible, and unknown), are summarized in table 3. We did not identify any *STAC3* variants; however, this gene was specifically investigated in only one patient. All identified variants in the *RYR1* and *CACNA1S* genes and in other genes relevant for the neuromuscular clinic are given in Supplemental Digital Content 2 (http://links.lww.com/ALN/C834).

Table 1. Patient Characteristics, Referral Indications, and Diagnostic Tests Performed

Characteristics and Analyses	MH-susceptible, n (%)	Non–MH-susceptible, n (%)	Unknown, n (%)	Total, n
MH investigation unit				
Antwerp (Belgium)	36 (43.9)	45 (54.9)	1 (1.2)	82
Lund (Sweden)	55 (50.9)	37 (34.3)	16 (14.8)	108
Nijmegen (The Netherlands)	31 (21.1)	64 (43.5)	52 (35.4)	147
Toronto (Canada)	58 (31.7)	14 (7.7)	111 (60.7)	183
Total	180 (34.6)	160 (30.8)	180 (34.6)	520
Median age at referral, yr [interquartile range]	36 [26 to 48]	38 [31 to 50]	39 [27 to 53]	37 [28 to 50]
Sex				
Male	118 (44.5)	56 (21.1)	91 (34.3)	265
Female	61 (24.0)	104 (40.9)	89 (35.0)	254
Unknown	1 (100.0)	0 (0.0)	0 (0.0)	1
Referral indication				
Personal history of adverse anesthetic events suspected to be MH	93 (44.1)	42 (19.9)	76 (36.0)	211
Family history of adverse anesthetic events suspected to be MH	40 (34.8)	45 (39.1)	30 (26.1)	115
Exertional and recurrent rhabdomyolysis	19 (41.3)	10 (21.7)	17 (37.0)	46
Personal *RYR1* variant detected in neuromuscular clinic testing without specific diagnosis	13 (35.1)	13 (35.1)	11 (29.7)	37
A diagnosed *RYR1*-related myopathy	6 (18.8)	13 (40.6)	13 (40.6)	32
Family history of an *RYR1* variant detected in diagnostic testing without specific diagnosis	2 (10.0)	11 (55.0)	7 (35.0)	20
Family history of diagnosed *RYR1*-related myopathy	1 (5.3)	9 (47.4)	9 (47.4)	19
Idiopathically elevated resting creatine kinase values	1 (7.1)	6 (42.9)	7 (50.0)	14
Exertional heat stroke	2 (25.0)	2 (25.0)	4 (50.0)	8
Other	3 (16.7)	9 (50.0)	6 (33.3)	18
Clinical grading scale in points [interquartile range] (n = 170)	35 [23 to 44]	17 [15 to 20]	35 [30 to 43]	33 [18 to 40]
Resting creatine kinase values in U/l [interquartile range] (n = 134)	301 [156 to 708]	102 [72 to 187]	567 [217 to 854]	153 [83 to 476]
Type of diagnostic tests performed				
CHCT/*in vitro* contracture test performed	128 (44.4)	160 (55.6)	0 (0.0)	288
Genetic testing performed	155 (38.8)	88 (22.1)	156 (39.1)	399
CHCT/*in vitro* contracture test and genetic testing were both performed	104 (54.2)	88 (45.8)	0 (0.0)	192
Type of genetic tests performed				
RYR1 hot spots or familial *RYR1*/*CACNA1S*-variant	39 (60.0)	13 (20.0)	13 (20.0)	65
RYR1 + *CACNA1S* sequencing (entire genes)	53 (31.5)	13 (7.7)	102 (60.7)	168
RYR1 sequencing (entire gene)	45 (45.0)	26 (26.0)	29 (29.0)	100
Whole-exome sequencing	7 (26.9)	14 (53.8)	5 (19.2)	26
RYR1 + *CACNA1S* + *STAC3* sequencing (entire genes)	0 (0.0)	0 (0.0)	1 (100.0)	1
Other (*e.g.*, *RYR1* + other relevant genes for neuromuscular diagnostics)	4 (19.0)	13 (61.9)	4 (19.0)	21
Unknown	8 (44.4)	9 (50.0)	1 (5.6)	18

The table shows the patient characteristics, referral indications, and diagnostic tests performed for all patients referred from 2010 through 2019 (n = 520).
CHCT, caffeine–halothane contracture test; MH, malignant hyperthermia.

Table 2. Distribution of Referral Criteria for Each MH Unit

Referral Indication	Antwerp, n (%)	Lund, n (%)	Nijmegen, n (%)	Toronto, n (%)
Personal history of anesthetic adverse event suspected to be MH	19 (23.2)	77 (71.3)	42 (28.6)	73 (39.9)
Family history of anesthetic adverse event suspected to be MH	33 (40.2)	22 (20.4)	19 (12.9)	41 (22.4)
Exertional and recurrent rhabdomyolysis	7 (8.5)	2 (1.9)	12 (8.2)	25 (13.7)
RYR1 variant detected in diagnostic testing without a specific diagnosis	4 (4.9)	1 (0.9)	27 (18.4)	5 (2.7)
A diagnosed *RYR1*-related myopathy	4 (4.9)	2 (1.9)	16 (10.9)	10 (5.5)
Family history of an *RYR1* variant detected in diagnostic testing without a specific diagnosis	2 (2.4)	0 (0.0)	16 (10.9)	2 (1.1)
Family history of a diagnosed *RYR1*-related myopathy	3 (3.7)	1 (0.9)	7 (4.8)	8 (4.4)
Idiopathically elevated resting creatine kinase values	4 (4.9)	0 (0.0)	1 (0.7)	9 (4.9)
Exertional heat stroke	0 (0.0)	1 (0.9)	1 (0.7)	6 (3.3)
Other	6 (7.3)	2 (1.9)	6 (4.1)	4 (2.2)
Total	82	108	147	183

MH, malignant hyperthermia.

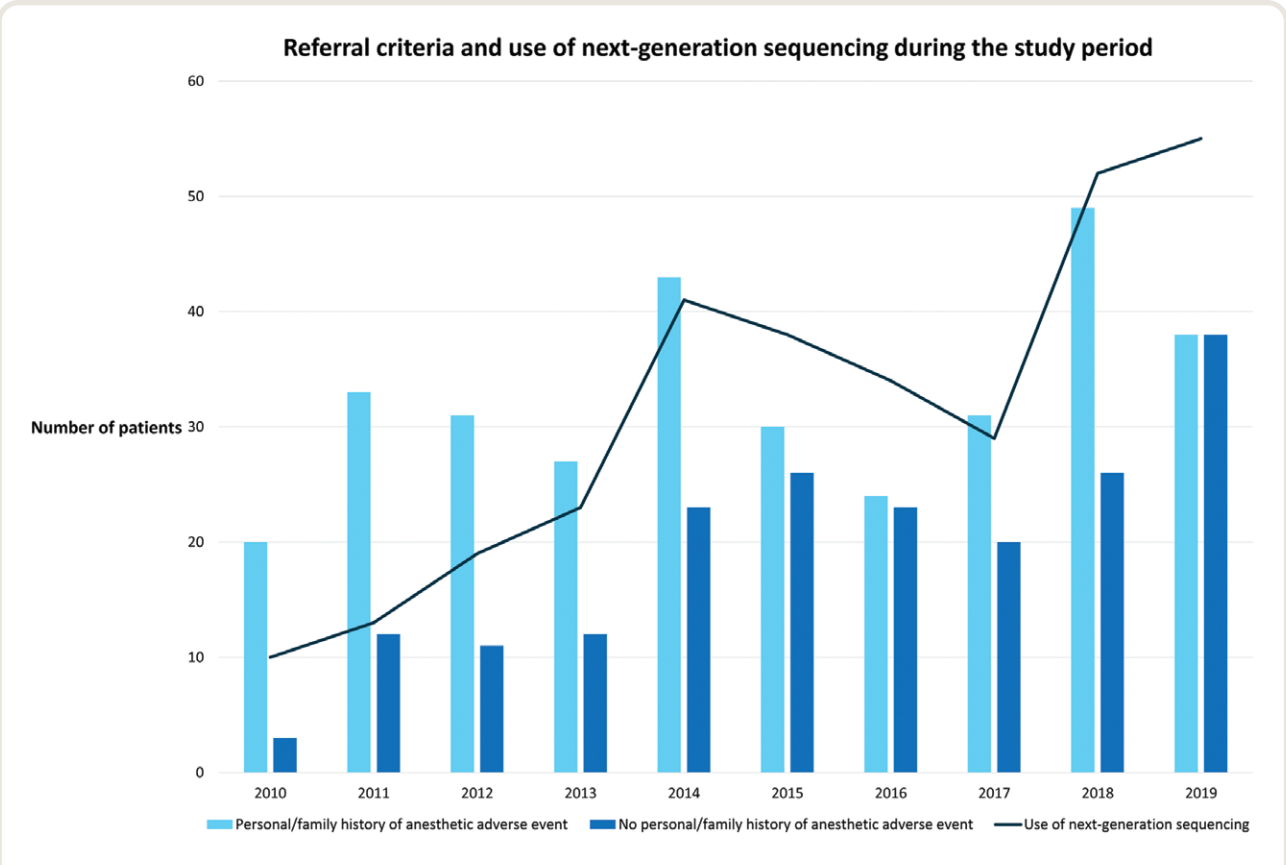

Fig. 2. Referral criteria and use of next-generation sequencing during the study period.

Secondary Analysis

Results of MH Diagnostics of Patients without a Personal or Family History of an Adverse Anesthetic Event Suspected to Be MH. The medical records of 158 patients without a personal or family history of an anesthetic adverse event suspected to be MH were included in the secondary analysis (fig. 1). A total of 42 referred because of a personal history of exertional and/or recurrent rhabdomyolysis were included in the secondary analysis. Based on the information contained in these records, 16 of 42 were diagnosed as MH-susceptible. Only one of six patients referred because of exertional heat stroke was diagnosed as MH-susceptible.

Table 3. Results of Genetic Testing of Patients Investigated by Genotyping

Test Result	MH-susceptible (n = 155), %	Non–MH-susceptible (n = 88), %	Unknown (n = 156), %	Total (n = 399), %
No variant in *RYR1* or *CACNA1S*	43 (27.7)	32 (36.4)	74 (47.4)	149 (37.3)
Variant(s) of unknown significance in *RYR1*	27 (17.4)	49 (55.7)	65 (41.7)	141 (35.3)
Diagnostic *RYR1* variant for MH susceptibility	67 (43.2)	0 (0.0)	0 (0.0)	67 (16.8)
Variant(s) of unknown significance in *CACNA1S*	5 (3.2)	1 (1.1)	9 (5.8)	15 (3.8)
RYR1 variant(s) + variant in other relevant gene(s) for neuromuscular diagnostics	2 (1.3)	5 (5.7)	3 (1.9)	10 (2.5)
Diagnostic *CACNA1S* variant for MH susceptibility	2 (1.3)	0 (0.0)	0 (0.0)	2 (0.5)
Diagnostic *RYR1/CACNA1S* variant for MH susceptibility + variant of unknown significance in *RYR1/CACNA1S*	4 (2.6)	0 (0.0)	0 (0.0)	4 (1.0)
Variant of unknown significance in *RYR1* + *CACNA1S*	1 (0.6)	0 (0.0)	3 (1.9)	4 (1.0)
Unknown	4 (2.6)	1 (1.1)	2 (1.3)	7 (1.8)

Percentages of the performed tests are given for all patients included in the study and per subgroup (MH-susceptible, non–MH-susceptible, and unknown). In the medical records of seven patients, the results of the genetic testing could not be identified.

MH, malignant hyperthermia.

However, only two of six were investigated by CHCT/*in vitro* contracture test. The details of the CHCT/*in vitro* contracture test and genetic analysis results from patients referred because of a personal history of exertional and/or recurrent rhabdomyolysis and exertional heat stroke are summarized in table 4.

Of 29 patients referred for a *RYR1*-related myopathy, 5 were diagnosed MH-susceptible. Of 19 patients for whom referral was for a family history of *RYR1*-related myopathy, 1 was diagnosed as MH-susceptible. A total of 11 of 32 patients were diagnosed as MH-susceptible when the referral indication was an *RYR1* variant detected in patients seen in the neuromuscular clinic and tested for symptoms suggestive of a neuromuscular disorder but without a specific clinical or histopathologic diagnosis. Of 20 patients for whom a family history of an *RYR1* variant detected in diagnostic testing in the neuromuscular clinic without a specific clinical or histopathologic diagnosis was the referral indication in the medical records, 2 were diagnosed as MH-susceptible. Idiopathically elevated creatine kinase level was the referral indication in the medical records of 10 patients, where 1 was diagnosed as MH-susceptible.

The results of the performed CHCT/*in vitro* contracture test and genetic analysis of patients referred because of a personal or family history of *RYR1*-related myopathies, idiopathically elevated creatine kinase values, and a personal or family history of an *RYR1* variant detected on diagnostic testing in the neuromuscular clinic are summarized in table 5. All neuromuscular symptoms reported in the medical records of patients with an *RYR1* variant detected in the neuromuscular clinic without a specific clinical or histopathologic diagnosis are summarized in Supplemental Digital Content 3 (http://links.lww.com/ALN/C835).

RYR1 *Pathogenicity Classification Using Computational Evidence.* A total of 71 patients without a personal or family of an adverse anesthetic event suspected to be MH had at

Table 4. Results of Genetic Analysis and CHCT/*In Vitro* Contracture Test of Patients with Episodic *RYR1*-related Phenotypes

Characteristics, Analyses, and Test Results	Exertional and/or Recurrent Rhabdomyolysis (n = 42)	Exertional Heat Stroke (n = 6)
Sex		
Male	32	4
Female	10	2
Genes analyzed		
RYR1 + *CACNA1S*	25	5
RYR1	8	1
RYR1 + other relevant genes for neuromuscular diagnostics	8	0
Whole-exome sequencing	1	0
Results of genetic analysis and CHCT/*in vitro* contracture test categorized according to the result of the genetic analysis		
Diagnostic *RYR1* variant for MH (total)	6	0
Tested positive for both halothane and caffeine by CHCT/*in vitro* contracture test	1	0
No CHCT/*in vitro* contracture test performed	5	0
Variant of unknown significance in *RYR1* (total)	16	1
Tested positive for both halothane and caffeine by CHCT/*in vitro* contracture test	2	0
Tested positive for halothane only by CHCT/*in vitro* contracture test	1	0
Non–MH-susceptible	6	1
No CHCT/*in vitro* contracture test performed	7	0
Diagnostic *RYR1* variant for MH susceptibility + variant of unknown significance in *RYR1* (total)	1	0
Tested positive for both halothane and caffeine by CHCT/*in vitro* contracture test	1	0
Diagnostic *CACNA1S* variant for MH (total)	1	1
Tested positive for both halothane and caffeine by CHCT/*in vitro* contracture test	1	1
Variant of unknown significance in *CACNA1S* (total)	5	1
Tested positive for halothane only by CHCT/*in vitro* contracture test	2	0
No CHCT/*in vitro* contracture test performed	3	1
No variant in *RYR1*/*CACNA1S* (total)	12	2
Tested positive for both halothane and caffeine by CHCT/*in vitro* contracture test	1	0
Tested positive for halothane only by CHCT/*in vitro* contracture test	1	0
Tested positive for caffeine only by CHCT/*in vitro* contracture test	1	0
Non–MH-susceptible	3	0
No CHCT/*in vitro* contracture test performed	6	2
RYR1 variant + variant(s) in other relevant gene(s) (total)*	1	1
Non–MH-susceptible	1	0
No CHCT/*in vitro* contracture test performed	0	1

Shown are the results of the genetic analysis and the CHCT/*in vitro* contracture test of 48 patients referred because of the episodic *RYR1*-related phenotypes (exertional and/or recurrent rhabdomyolysis and exertional heat stroke).

*Genes relevant for the neuromuscular clinic.

CHCT, caffeine–halothane contracture test; MH, malignant hyperthermia.

least one missense *RYR1* variant. Most of the Rare Exome Variant Ensemble Learner scores (37 of 71) were between 0.5 and 0.85 and therefore not helpful in *RYR1* pathogenicity classification; 9 of 71 *RYR1* variants had Rare Exome Variant Ensemble Learner scores of 0.5 or lower, and 25 of 71 *RYR1* variants had Rare Exome Variant Ensemble Learner scores of 0.85 or higher.

A total of 34 of 71 *RYR1* variants had a Rare Exome Variant Ensemble Learner score of 0.5 or lower or 0.85 or higher, indicating a benign (0.5 or lower) or pathogenic

Table 5. Results of Genetic Analysis and CHCT/*In Vitro* Contracture Test of Patients with History of *RYR1*-related Myopathies, Idiopathically Elevated Creatine Kinase Values, and History of *RYR1* Variant

Characteristics and Analyses	A Diagnosed *RYR1*-related Myopathy (n = 29)	Family History of Diagnosed *RYR1*-related Myopathy (n = 19)	*RYR1* Variant Detected in Neuromuscular Diagnostics (n = 32)	Family History of *RYR1* Variant Detected in Neuromuscular Diagnostics (n = 20)	Idiopathically Elevated Resting Creatine Kinase Values (n = 10)
Sex					
Male	12	8	21	6	4
Female	17	11	11	14	6
Genes analyzed					
RYR1 + *CACNA1S*	8	8	5	2	8
RYR1	12	3	10	5	0
RYR1 targeted/hotspot technique	Not applicable	5	Not applicable	12	Not applicable
RYR1 + other relevant genes	2	0	8	1	1
Whole-exome sequencing	7	3	9	0	0
RYR1 + *CACNA1S* + *STAC3*	0	0	0	0	1
Results genetic analysis and CHCT/*in vitro* contracture test categorized according to results of the genetic analysis					
Diagnostic *RYR1* variant for MH (total)	2	1	5	0	1
Tested positive for both halothane and caffeine by CHCT/*in vitro* contracture test	0	0	2	0	1
Tested positive for halothane only by CHCT/*in vitro* contracture test	0	0	0	0	0
Tested positive for caffeine only by CHCT/*in vitro* contracture test	0	0	1	0	0
No CHCT/*in vitro* contracture test performed	2	1	2	0	0
Variant of unknown significance in *RYR1* (total)	19	13	20	18	2
Tested positive for both halothane and caffeine by CHCT/*in vitro* contracture test	1	0	3	1	0
Tested positive for halothane only by CHCT/*in vitro* contracture test	0	0	0	1	0
Tested positive for caffeine only by CHCT/*in vitro* contracture test	1	0	1	0	0
Non–MH-susceptible	9	4	9	3	2
No CHCT/*in vitro* contracture test performed	8	9	7	13	0
Diagnostic *RYR1* variant for MH + variant of unknown significance in *RYR1* (total)	0	0	1	0	0
No CHCT/*in vitro* contracture test performed	0	0	1	0	0
Variant of unknown significance in *CACNA1S* (total)	0	0	0	0	1
No CHCT/*in vitro* contracture test performed	0	0	0	0	1
No variant in *RYR1*/*CACNA1S*/STAC3 (total)	6	3	0	1	6
Non–MH-susceptible	1	2	0	0	1
No CHCT/*in vitro* contracture test performed	5	1	0	1	5
RYR1 variant(s) + variant in other relevant gene(s)* (total)	2	1	6	1	0
Tested positive for both halothane and caffeine by CHCT/*in vitro* contracture test	1	0	1	0	0
Non–MH-susceptible	1	0	2	1	0
No CHCT/*in vitro* contracture test performed	0	1	3	0	0
Results of genetic analysis unknown	0	1	0	0	0

Shown are the results of the genetic analysis and the CHCT/*in vitro* contracture test of 110 patients referred because of a personal or family history of *RYR1*-related myopathies, idiopathically elevated creatine kinase values, and a personal or family history of an *RYR1* variant detected on diagnostic testing in the neuromuscular clinic. The patients with the following myopathies were classified as *RYR1*-related myopathies: axial myopathy, central core disease, King–Denborough syndrome, fiber disproportion disorder, periodic paralysis, centronuclear myopathy, and multiminicore disease.

*Genes relevant for the neuromuscular clinic.

CHCT, caffeine–halothane contracture test; MH, malignant hyperthermia.

(0.85 or higher) variant. The full diagnostic process of MH susceptibility was completed by 19 of 34. In 10 of 19, the Rare Exome Variant Ensemble Learner scores were discordant with the results of MH susceptibility diagnostics. Rare Exome Variant Ensemble Learner scores and the results of MH susceptibility diagnostics are summarized in figure 3.

Discussion

This retrospective multicenter cohort study shows that the indications for referral to MH units have changed. An increasing number of patients referred to MH units do not have a personal or family history of an adverse anesthetic event suspected to be MH. This trend coincides with the publication of the European Malignant Hyperthermia Group guideline for investigation of MH susceptibility in 2015.[4] This guideline recommends referral to an MH unit for patients with exertional and/or recurrent rhabdomyolysis, *RYR1*-related myopathies, and other *RYR1*-related phenotypes. This might, at least partly, explain the increasing number of referrals concerning patients without a personal or family history of an adverse anesthetic event. These patients carry *RYR1* variants identified during the diagnostic workup for exertional and/or recurrent rhabdomyolysis, exertional heat stroke, *RYR1*-related myopathies, or an unresolved nonspecific neuromuscular phenotype reflecting the wide spectrum of *RYR1*-related phenotypes.[8] Since 39.2% of the patients referred to an MH unit without a personal or family history of an anesthetic adverse event suspected to be MH were diagnosed as MH-susceptible, these patients can be at risk of MH when exposed to triggering anesthetic agents. On the other hand, 60.8% of the patients without a personal or family history of an adverse anesthetic event were diagnosed as non–MH-susceptible, indicating the importance of MH susceptibility diagnostics; a non–MH-susceptible test result enables anesthesiologists to treat carriers of *RYR1* variants and their family members without MH precaution measures.[4,22]

In our study, 16 of 42 of the patients with exertional and/or recurrent rhabdomyolysis were diagnosed as MH-susceptible, which compared to previous case studies is less frequent than 11 of 12[23] and 5 of 6[24] but more frequent than 2 of 14.[18] This variability can, at least partly, be explained by selection bias concerning some of these study cohorts. Another cohort study reporting 17 MH-susceptible patients who suffered more than two episodes of exertional rhabdomyolysis identified 9 patients with *RYR1* variants, including two pathogenic variants for MH.[11] Previous studies on MH susceptibility in exertional heat stroke patients reported a positive *in vitro* contracture test in 12 of 28,[25] which is higher than in our study, probably due to the low sample size of the exertional heat

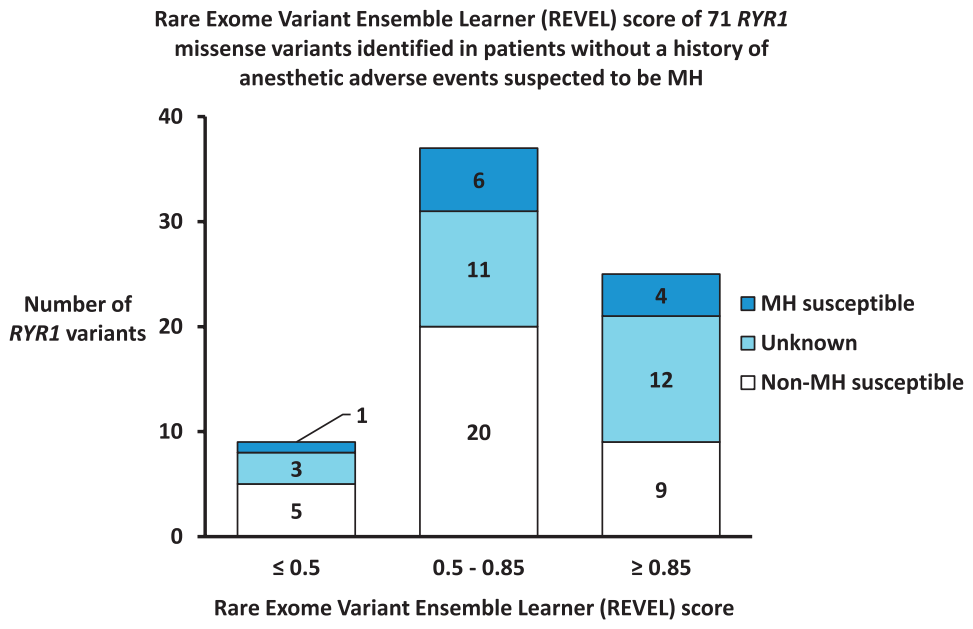

Fig. 3. Rare Exome Variant Ensemble Learner cores of 71 *RYR1* missense variants identified in patients without a history of anesthetic adverse events had a missense variant of unknown significance in *RYR1*. A total of 37 of 71 *RYR1* variants had Rare Exome Variant Ensemble Learner scores between 0.5 and 0.85 and therefore were not helpful in *RYR1* pathogenicity classification. A total of 9 of 71 *RYR1* variants had Rare Exome Variant Ensemble Learner scores of 0.5 or lower, of which 1 record was classified MH-susceptible. A total of 25 of 71 *RYR1* variants had Rare Exome Variant Ensemble Learner scores of 0.85 of higher, of which 9 records were classified as non–MH-susceptible. MH, malignant hyperthermia.

stroke cohort and the high number of patients classified as unknown in our study.

There are several case reports reporting MH reactions and studies reporting MH susceptibility in patients with *RYR1*-related myopathies,[26–29] but we did not identify any cohort studies or large case series on MH susceptibility in patients with *RYR1*-related myopathies. The same applies to patients with an *RYR1* variant detected in diagnostic testing in the neuromuscular clinic without a specific clinical or histopathologic diagnosis.[18]

As next-generation sequencing has become faster and more cost-effective, it is gradually becoming the first-line diagnostic test for genetically heterogenous disorders (such as congenital myopathies). This has resulted in a rapid increase in the identification of both the number of patients with an *RYR1* variant and the number of newly identified *RYR1* variants. Only a limited number of these *RYR1* variants are classified as pathogenic or benign according to the Variant Curation Expert Panel recommendations for *RYR1* pathogenicity classification in MH susceptibility[10] and/or the European Malignant Hyperthermia Group scoring matrix for classification of genetic variants in MH susceptibility. However, only variants classified as (likely) pathogenic or benign can be used for genetic MH susceptibility diagnostics.[10] The increasing number of patients without a personal or family history of an adverse anesthetic event with a variant of unknown significance in *RYR1* will be a major challenge for MH units in future.

As our study shows, bioinformatic prediction tools are currently insufficient to classify *RYR1* missense variants of unknown significance. In 37 of 71 of the patients with a missense variant of unknown significance in *RYR1* who did not have a history of an adverse anesthetic event, the Rare Exome Variant Ensemble Learner scores were between 0.5 and 0.85 and were therefore not helpful.[10] Furthermore, the Rare Exome Variant Ensemble Learner score does not take into consideration the possibility of two or more *RYR1* variants interacting in a synergistic manner with regards to their pathogenicity. Rare Exome Variant Ensemble Learner scores of 0.5 or lower and 0.85 or higher may be useful as preliminary guidance but are currently not validated to confirm or rule out MH susceptibility. We identified 10 of 19 cases of discordance between the CHCT/*in vitro* contracture test result and the Rare Exome Variant Ensemble Learner score (fig. 3). These findings are in line with other *in silico* predictors of pathogenicity in MH.[30,31]

Our results can be used to improve counseling of patients referred to MH units without a personal or family history of an adverse anesthetic event suspected to be MH. Furthermore, these results can also be useful for geneticists, neurologists, and other specialists investigating patients by *RYR1* sequencing or whole-exome sequencing. They need to be aware of and inform patients about the possibility of identifying a variant of unknown significance in *RYR1* and the potential subsequent need for a muscle biopsy for CHCT/*in vitro* contracture test and, if relevant, cascade family testing in all first-degree family members before they perform *RYR1* sequencing (either targeted or by next-generation sequencing).

Our study has some limitations. Some referral indications were disproportionally distributed between the participating MH units, probably caused by the close collaboration between the MH units in Toronto and Nijmegen and the university hospital neuromuscular clinic, in contrast to the MH unit in Lund, which does not have any collaborations with the local neurology department. In addition, only a limited number of patients were tested for *CACNA1S* and *STAC3* variants, and several patients referred because of a family history of an *RYR1*-related myopathy (5 of 19) or a family history of an *RYR1* variant detected in diagnostic testing in the neuromuscular clinic without a specific clinical or histopathologic diagnosis (12 of 20) were genetically investigated using a targeted technique. Therefore, we are not sure whether these unaffected family members carried *RYR1* variant(s) other than those identified in their relatives with neuromuscular symptoms. Furthermore, due to the low penetrance of MH susceptibility,[10,12,32] currently unresolved modifying factors in the occurrence of MH, and ethical limitations, it is not possible to study which MH-susceptible patients suffer an MH reaction when exposed to triggering agents. Since the CHCT/*in vitro* contracture test and screening for diagnostic variants are the accepted standard in MH susceptibility diagnostics[4] and an MH reaction can be life-threatening,[13,33] all patients diagnosed as MH-susceptible should be considered at risk for MH when in need of anesthesia.[22]

It is important to mention that not all patients who suffer a rhabdomyolysis and/or exertional heat stroke episode will be referred to MH units as only a limited number of patients have a genetic background associated with an increased susceptibility to rhabdomyolysis[34] or exertional heat stroke.[25,35,36] Neurologists and sport physicians only refer patients to an MH unit with signs of an increased genetic susceptibility to rhabdomyolysis and/or exertional heat stroke, resulting in a selection bias. The same selection bias arises for the *RYR1* variants within the study cohort. Patients with *RYR1* variants resulting in a loss of function in ryanodine receptor 1 or a high prevalence in control populations are unlikely to cause MH.[10] Carriers of these variants are therefore less likely to be referred to an MH unit.

Last, the number of patients in our cohort who did not complete the full process of MH susceptibility diagnostics might have affected our results but probably also reflect the problem our study addresses. The CHCT/*in vitro* contracture test is an invasive procedure, and some patients referred for MH susceptibility diagnostics refuse muscle biopsy or are unable to undergo muscle biopsy and consider themselves MH-susceptible without confirmation of the diagnosis. Furthermore, worldwide knowledge and expertise needed to perform a reliable CHCT/*in vitro* contracture test are limited.

In some countries, there are no CHCT/*in vitro* contracture test laboratories, and the established MH units in other countries have long waiting lists, resulting in a very large number of patients carrying *RYR1* variants of unknown significance with limited possibilities for MH susceptibility diagnostics. This is also the case for the MH unit in Toronto; most patients who did not complete the full process of MH susceptibility are on the waiting list to be investigated.

Future strategies for MH susceptibility diagnostics should focus on classification of *RYR1*, *CACNA1S*, and *STAC3* variants utilizing common databases and functional studies. This should not be limited to suspected pathogenic variants because classification of a variant as benign could prevent unnecessary invasive diagnostic procedures. Other potential fields of interest for future research are identification of new genes of interest as 27.7% of the MH-susceptible–diagnosed patients did not have a variant in *RYR1*, *CACNA1S*, or *STAC3*. As our study demonstrates, currently available bioinformatic models such as Rare Exome Variant Ensemble Learner[21] are insufficient for MH susceptibility diagnostics, but more useful alternatives may emerge in the future.

Conclusions

The proportion of patients referred to MH units without a personal or family history of adverse anesthetic events suspected to be MH has increased. These patients carry *RYR1* variants identified during the diagnostics workup for exertional or recurrent rhabdomyolysis, exertional heat stroke, *RYR1*-related myopathies, or an unresolved neuromuscular phenotype. Since 39.2% of the patients referred to an MH unit without a personal or family history of an anesthetic adverse event suspected to be MH were diagnosed as MH-susceptible, these patients can be at risk for MH when exposed to MH triggering agents, and the referral of such patients to MH units is therefore indicated.

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Competing Interests

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References

1. Larach MG: Standardization of the caffeine halothane muscle contracture test. North American Malignant Hyperthermia Group. Anesth Analg 1989; 69:511–5
2. Allen GC, Larach MG, Kunselman AR: The sensitivity and specificity of the caffeine–halothane contracture test: A report from the North American Malignant Hyperthermia Registry. The North American Malignant Hyperthermia Registry of MHAUS. Anesthesiology 1998; 88:579–88
3. European Malignant Hyperpyrexia Group: A protocol for the investigation of malignant hyperpyrexia (MH) susceptibility. Br J Anaesth 1984; 56:1267–9
4. Hopkins PM, Rüffert H, Snoeck MM, Girard T, Glahn KP, Ellis FR, Müller CR, Urwyler A; European Malignant Hyperthermia Group: European Malignant Hyperthermia Group guidelines for investigation of malignant hyperthermia susceptibility. Br J Anaesth 2015; 115:531–9
5. Sambuughin N, Holley H, Muldoon S, Brandom BW, de Bantel AM, Tobin JR, Nelson TE, Goldfarb LG: Screening of the entire ryanodine receptor type 1 coding region for sequence variants associated with malignant hyperthermia susceptibility in the North American population. Anesthesiology 2005; 102:515–21
6. Monnier N, Krivosic-Horber R, Payen JF, Kozak-Ribbens G, Nivoche Y, Adnet P, Reyford H, Lunardi J: Presence of two different genetic traits in malignant hyperthermia families: Implication for genetic analysis, diagnosis, and incidence of malignant hyperthermia susceptibility. Anesthesiology 2002; 97:1067–74
7. Horstick EJ, Linsley JW, Dowling JJ, Hauser MA, McDonald KK, Ashley-Koch A, Saint-Amant L, Satish A, Cui WW, Zhou W, Sprague SM, Stamm DS, Powell CM, Speer MC, Franzini-Armstrong C, Hirata H, Kuwada JY: Stac3 is a component of the excitation–contraction coupling machinery and mutated in Native American myopathy. Nat Commun 2013; 4:1952
8. Snoeck M, van Engelen BG, Küsters B, Lammens M, Meijer R, Molenaar JP, Raaphorst J, Verschuuren-Bemelmans CC, Straathof CS, Sie LT, de Coo IF, van der Pol WL, de Visser M, Scheffer H, Treves S, Jungbluth H, Voermans NC, Kamsteeg EJ: RYR1-related myopathies: A wide spectrum of phenotypes throughout life. Eur J Neurol 2015; 22:1094–112

9. Riazi S, Kraeva N, Hopkins PM: Malignant hyperthermia in the post-genomics era: New perspectives on an old concept. Anesthesiology 2018; 128:168–80
10. Johnston JJ, Dirksen RT, Girard T, Gonsalves SG, Hopkins PM, Riazi S, Saddic LA, Sambuughin N, Saxena R, Stowell K, Weber J, Rosenberg H, Biesecker LG: Variant curation expert panel recommendations for RYR1 pathogenicity classifications in malignant hyperthermia susceptibility. Genet Med 2021; 23:1288–95
11. Kraeva N, Sapa A, Dowling JJ, Riazi S: Malignant hyperthermia susceptibility in patients with exertional rhabdomyolysis: A retrospective cohort study and updated systematic review. Can J Anaesth 2017; 64:736–43
12. Ibarra Moreno CA, Hu S, Kraeva N, Schuster F, Johannsen S, Rueffert H, Klingler W, Heytens L, Riazi S: An assessment of penetrance and clinical expression of malignant hyperthermia in individuals carrying diagnostic ryanodine receptor 1 gene mutations. Anesthesiology 2019; 131:983–91
13. Riazi S, Larach MG, Hu C, Wijeysundera D, Massey C, Kraeva N: Malignant hyperthermia in Canada: Characteristics of index anesthetics in 129 malignant hyperthermia susceptible probands. Anesth Analg 2014; 118:381–7
14. van den Bersselaar LR, Greven T, Bulger T, Voermans NC, van Petegem F, Schiemann AH, Parker R, Burling SM, Jungbluth H, Stowell KM, Kamsteeg EJ, Snoeck MMJ: RYR1 variant c.38T>G, p.Leu13Arg causes hypersensitivity of the ryanodine receptor-1 and is pathogenic for malignant hyperthermia. Br J Anaesth 2021; 127:e63–5
15. van den Bersselaar LR, Hollmann MW, van den Goor JMMH, Winkelman JA, Snoeck MMJ, Corsmit OT: Malignant hyperthermia during cardiac surgery successfully treated by deep hypothermia using cardiopulmonary bypass: A case report. A A Pract 2021; 15:e01546
16. Kruijt N, den Bersselaar LV, Snoeck M, Kramers K, Riazi S, Bongers C, Treves S, Jungbluth H, Voermans N: RYR1-related rhabdomyolysis: A spectrum of hypermetabolic states due to ryanodine receptor dysfunction. Curr Pharm Des 2022; 28:2–14
17. Larach MG, Localio AR, Allen GC, Denborough MA, Ellis FR, Gronert GA, Kaplan RF, Muldoon SM, Nelson TE, Ording H: A clinical grading scale to predict malignant hyperthermia susceptibility. Anesthesiology 1994; 80:771–9
18. Dlamini N, Voermans NC, Lillis S, Stewart K, Kamsteeg EJ, Drost G, Quinlivan R, Snoeck M, Norwood F, Radunovic A, Straub V, Roberts M, Vrancken AF, van der Pol WL, de Coo RI, Manzur AY, Yau S, Abbs S, King A, Lammens M, Hopkins PM, Mohammed S, Treves S, Muntoni F, Wraige E, Davis MR, van Engelen B, Jungbluth H: Mutations in RYR1 are a common cause of exertional myalgia and rhabdomyolysis. Neuromuscul Disord 2013; 23:540–8
19. van den Bersselaar LR, Kruijt N, Scheffer GJ, van Eijk L, Malagon I, Buckens S, Custers JA, Helder L, Greco A, Joosten LA, van Engelen BG, van Alfen N, Riazi S, Treves S, Jungbluth H, Snoeck MM, Voermans NC: The neuromuscular and multisystem features of RYR1-related malignant hyperthermia and rhabdomyolysis: A study protocol. Medicine (Baltimore) 2021; 100:e26999
20. European Malignant Hyperthermia Group: Diagnostic MH mutations. Available at: https://www.emhg.org/diagnostic-mutations. Accessed October 1, 2021.
21. Ioannidis NM, Rothstein JH, Pejaver V, Middha S, McDonnell SK, Baheti S, Musolf A, Li Q, Holzinger E, Karyadi D, Cannon-Albright LA, Teerlink CC, Stanford JL, Isaacs WB, Xu J, Cooney KA, Lange EM, Schleutker J, Carpten JD, Powell IJ, Cussenot O, Cancel-Tassin G, Giles GG, MacInnis RJ, Maier C, Hsieh CL, Wiklund F, Catalona WJ, Foulkes WD, Mandal D, Eeles RA, Kote-Jarai Z, Bustamante CD, Schaid DJ, Hastie T, Ostrander EA, Bailey-Wilson JE, Radivojac P, Thibodeau SN, Whittemore AS, Sieh W: REVEL: An ensemble method for predicting the pathogenicity of rare missense variants. Am J Hum Genet 2016; 99:877–85
22. Rüffert H, Bastian B, Bendixen D, Girard T, Heiderich S, Hellblom A, Hopkins PM, Johannsen S, Snoeck MM, Urwyler A, Glahn KPE; European Malignant Hyperthermia Group: Consensus guidelines on perioperative management of malignant hyperthermia suspected or susceptible patients from the European Malignant Hyperthermia Group. Br J Anaesth 2021; 126:120–30
23. Wappler F, Fiege M, Steinfath M, Agarwal K, Scholz J, Singh S, Matschke J, Schulte Am Esch J: Evidence for susceptibility to malignant hyperthermia in patients with exercise-induced rhabdomyolysis. Anesthesiology 2001; 94:95–100
24. Poels PJ, Joosten EM, Sengers RC, Stadhouders AM, Veerkamp JH, Benders AA: *In vitro* contraction test for malignant hyperthermia in patients with unexplained recurrent rhabdomyolysis. J Neurol Sci 1991; 105:67–72
25. Fiszer D, Shaw MA, Fisher NA, Carr IM, Gupta PK, Watkins EJ, Roiz de Sa D, Kim JH, Hopkins PM: Next-generation sequencing of RYR1 and CACNA1S in malignant hyperthermia and exertional heat illness. Anesthesiology 2015; 122:1033–46
26. Robinson R, Carpenter D, Shaw MA, Halsall J, Hopkins P: Mutations in RYR1 in malignant hyperthermia and central core disease. Hum Mutat 2006; 27:977–89
27. McCarthy TV, Quane KA, Lynch PJ: Ryanodine receptor mutations in malignant hyperthermia and central core disease. Hum Mutat 2000; 15:410–7

28. Murayama T, Kurebayashi N, Ogawa H, Yamazawa T, Oyamada H, Suzuki J, Kanemaru K, Oguchi K, Iino M, Sakurai T: Genotype–phenotype correlations of malignant hyperthermia and central core disease mutations in the central region of the RYR1 channel. Hum Mutat 2016; 37:1231–41
29. Taylor A, Lachlan K, Manners RM, Lotery AJ: A study of a family with the skeletal muscle RYR1 mutation (c.7354C>T) associated with central core myopathy and malignant hyperthermia susceptibility. J Clin Neurosci 2012; 19:65–70
30. Kim JH, Jarvik GP, Browning BL, Rajagopalan R, Gordon AS, Rieder MJ, Robertson PD, Nickerson DA, Fisher NA, Hopkins PM: Exome sequencing reveals novel rare variants in the ryanodine receptor and calcium channel genes in malignant hyperthermia families. Anesthesiology 2013; 119:1054–65
31. Schiemann AH, Stowell KM: Comparison of pathogenicity prediction tools on missense variants in RYR1 and CACNA1S associated with malignant hyperthermia. Br J Anaesth 2016; 117:124–8
32. Shaw MA, Hopkins PM: Mission impossible or mission futile?: Estimating penetrance for malignant hyperthermia. Anesthesiology 2019; 131:957–9
33. Larach MG, Brandom BW, Allen GC, Gronert GA, Lehman EB: Malignant hyperthermia deaths related to inadequate temperature monitoring, 2007–2012: A report from the North American malignant hyperthermia registry of the malignant hyperthermia association of the United States. Anesth Analg 2014; 119:1359–66
34. Kruijt N, van den Bersselaar LR, Kamsteeg EJ, Verbeeck W, Snoeck MMJ, Everaerd DS, Abdo WF, Jansen DRM, Erasmus CE, Jungbluth H, Voermans NC: The etiology of rhabdomyolysis: An interaction between genetic susceptibility and external triggers. Eur J Neurol 2021; 28:647–59
35. Gardner L, Miller DM, Daly C, Gupta PK, House C, Roiz de Sa D, Shaw MA, Hopkins PM: Investigating the genetic susceptibility to exertional heat illness. J Med Genet 2020; 57:531–41
36. Roux-Buisson N, Monnier N, Sagui E, Abriat A, Brosset C, Bendahan D, Kozak-Ribbens G, Gazzola S, Quesada JL, Foutrier-Morello C, Rendu J, Figarella-Branger D, Cozonne P, Aubert M, Bourdon L, Lunardi J, Fauré J: Identification of variants of the ryanodine receptor type 1 in patients with exertional heat stroke and positive response to the malignant hyperthermia *in vitro* contracture test. Br J Anaesth 2016; 116:566–8