Malignant Hyperthermia Testing in Probands without Adverse Anesthetic Reaction

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ABSTRACT

Background: Malignant hyperthermia (MH) is triggered by reactions to anesthetics. Reports link nonanesthetic-induced MH-like reactions to a variety of disorders. The objective of the authors was to retrospectively investigate the reasons for referrals for MH testing in nonanesthetic cases and assess their phenotype. In addition, the response to the administration of oral dantrolene in nonanesthetic probands with positive caffeine–halothane contracture test (CHCT) was investigated.

Methods: Following institutional research ethics board approval, probands without reaction to anesthesia, who underwent CHCT, were selected. Clinical details and response to dantrolene were analyzed.

Results: In total, 87 of 136 (64%) patients referred for nonanesthetic indications tested positive to the CHCT. Of these, 47 with a high creatine kinase (CK), 9 with exercise-induced rhabdomyolysis and/or exercise intolerance, 2 with high CK and exercise-induced rhabdomyolysis and/or exercise intolerance, 15 with postviral chronic fatigue, and 14 with muscle weakness of unknown etiology had a positive CHCT. These patients had a higher CK compared with those with negative CHCT. Oral dantrolene improved the musculoskeletal symptoms in 28 of 34 (82%) CHCT-positive patients. Response to treatment was associated with a significantly higher pretreatment CK and a greater posttreatment CK reduction.

Conclusions: A positive CHCT may represent more than simply an anesthetic-related disorder. Individuals with positive CHCTs may exhibit muscle symptoms without exposure to MH-triggering anesthetics. Oral dantrolene may be useful in alleviating these symptoms. **(Anesthesiology 2015; 123:548-56)**

MALIGNANT hyperthermia (MH; Mendelian Inheritance in Man 145600) is a syndrome characterized by the dysregulation of calcium homeostasis in skeletal muscle cells.[1] The condition is triggered by volatile anesthetics and/or succinylcholine leading to a potentially fatal hypermetabolic crisis in susceptible individuals.[2,3] In humans, MH is usually inherited as an autosomal dominant trait.[4]

To confirm MH susceptibility in patients with a suspected MH anesthetic event, the proband may undergo the caffeine–halothane contracture test (CHCT) and/or genetic testing. The CHCT or the European equivalent, the *in vitro* contracture test (IVCT), measures contracture response of muscle biopsy samples to caffeine and halothane, with a reported sensitivity and specificity of 97 and 78% for the CHCT and 99, and 94% for the IVCT, respectively.[5–7] The thresholds of response are designed not to miss susceptible individuals. These thresholds were determined by comparing the responses of patients with an "almost certain" anesthetic-induced MH reaction (as judged by the MH clinical grading scale and confirmed by MH experts) to subjects without personal or familial history of adverse anesthetic reactions.[8] Although the CHCT is the accepted standard diagnostic test to diagnose MH susceptibility in patients or family members

What We Already Know about This Topic

- Evaluation of malignant hyperthermia susceptibility involves testing with the use of a caffeine–halothane contracture test (CHCT)
- Some patients with persistently increased creatine kinase and exercise-induced rhabdomyolysis have a positive CHCT

What This Article Tells Us That Is New

- Approximately two thirds of patients referred for nonanesthetic reasons, including postviral chronic fatigue and muscle weakness of unknown etiology, tested positive to the caffeine–halothane contracture test
- Oral dantrolene therapy improved musculoskeletal symptoms in 82% of CHCT-positive patients

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with adverse anesthetic histories, the significance of a positive CHCT/IVCT in patients without such an anesthetic history is unclear. Thresholds for the diagnosis of susceptibility to other disorders have not been established in our laboratory or by others. Currently, two genes have been linked with MH susceptibility: the ryanodine receptor type 1 (*RYR1*) gene and the calcium channel, voltage-dependent, L type, α 1S subunit (*CACNA1S*) gene.[9,10] Variants in these genes are found to occur in 50 to 80% of MH-susceptible families.[11–17]

Although the triggering of MH reactions by the administration of potent inhalational anesthetics or succinylcholine has long been reported,[1,18] there is an expanding collection of reports detailing nonanesthetic-induced MH-like reactions or positive CHCT/IVCT in some individuals suffering from a variety of disorders. These include heat stroke,[19–23] exercise-induced rhabdomyolysis,[24–28] postexertional muscle cramping,[29] statin-induced myopathy and myopathies of unknown etiology,[30] idiopathic-elevated creatine kinase (CK),[31,32] and infection-induced muscle dysfuntion.[20] *RYR1*-related myopathies, particularly central core disease, also carry a risk of MH susceptibility.[31] In addition, occurrence of nonanesthetic-induced MH-like reactions was recently demonstrated by the case of a fatal awake MH-like reaction in a 6-yr-old boy.[31,32] Overall, these reports have contributed to a range of referrals of patients with no personal or family history of anesthesia-related MH reaction to testing facilities.

Currently, except for anecdotal or case series reports, there is no large single study analyzing the CHCT outcomes of patients referred for nonanesthetic indications. Therefore, we present data from the Malignant Hyperthermia Investigation Unit (MHIU) of Toronto, with the objective to investigate reasons for referrals in probands undergoing CHCT testing who have not suffered any adverse anesthetic reaction and to assess their phenotype. We also summarized data on effects of oral dantrolene in CHCT-positive nonanesthetic probands with musculoskeletal symptoms.

Materials and Methods

Patient Population

Research Ethics Board at University Health Network, Toronto, Ontario, Canada, approved this retrospective study to gather reported data and waived the informed consent because of the retrospective nature of the study. All probands who underwent the CHCT at MHIU at Toronto General Hospital between January 1, 1992, and August 30, 2014, were identified. Probands with personal or familial history of adverse anesthetic reactions were excluded. Remaining probands were divided based on the results of the standardized North American CHCT test protocol.[5] Contracture of more than 0.7 g to 3% halothane or contracture of more than 0.3 g to 2.0 mmol/l caffeine in at least one muscle fascicle was considered to be a positive CHCT. None of the probands were related to one another. There were no CHCT values from normal volunteers, who were not members of pedigrees in which MH is segregating and who had not been referred for evaluation of a skeletal muscle complaint, for comparison as controls.

Clinical Information

Collected data included referral indication including neurologist-referred unexplained high CK (CK > 250 IU/l), postviral chronic fatigue (persisting fatigue, muscle pain, weakness, and/or cramps 6 months after viral illness including influenza, Epstein–Barr, and cytomegalovirus, and interfering with functional ability), postexercise rhabdomyolysis, heat stroke, and weakness of unknown etiology, referred by neurologists. Also collected were demographics (gender and age), previous anesthetic history, musculoskeletal symptoms including fatigue and cramps, complete neurological examination, baseline CK, nerve conduction velocity studies in 32 patients only, sequence data of entire coding regions of *RYR1* gene and *CACNA1S* gene in all consenting individuals (between 2003 and 2014), according to the previously published methods,[16,33] and histomorphology. These nonanesthetic probands were compared with anesthetic probands (tested in our unit during the same time period) as regards to response to caffeine and halothane contracture response.

Dantrolene Treatment

A subgroup of CHCT-positive probands was identified, who were treated with oral dantrolene in an effort to alleviate musculoskeletal symptoms such as myalgia, fatigue, muscle weakness, and muscle cramps. There was no specific criterion for patient selection for oral dantrolene treatment. Patients were started on oral dantrolene based on the severity of symptoms and desire for treatment. Initial starting dose was 25 mg once daily. Dantrolene dose frequency was increased up to four times a day, followed by increase in each dose until the resolution of reported musculoskeletal symptoms. Patients were maintained on the minimum dose with the best subjective response. Data gathered included (1) dantrolene dose (starting and final dose), (2) pretreatment CK and CK 3 months after the start of treatment, (3) side effects while on dantrolene therapy, and (4) liver function tests (LFTs) including aspartate aminotransferase, alanine transaminase, alkaline phosphatase, and bilirubin were recorded quarterly. Patients were followed up by MHIU for 3 months to 1 yr and thereafter followed up by their family doctors. Except for the initial dropouts, patients remained on the final dose. Results were retrieved from family doctors with patients' permission. Response to dantrolene was defined as patient-reported improvement of musculoskeletal symptoms, including fatigue.

Statistical Analysis

Sample sizes were based on the available data and not determined by a statistical power analysis. Continuous variables were

represented by means, SD, medians, and ranges. Categorical variables were represented by frequencies and percentages. Wilcoxon rank sum test and Fisher exact test were used for comparing groups on numerical and categorical parameters, respectively.

Kruskal-Wallis H test was used for comparing more than two groups on continuous variables. Statistical analyses were performed using IBM SPSS Statistics Version 21.0.[34] A two-tailed $P < 0.05$ was used to define statistical significance of all hypotheses tested.

Results

Referral Indications

A total of 136 probands with no anesthetic reaction were tested with CHCT. Of these, 87 were CHCT positive (64%). A similar rate of positive CHCT results was observed in probands with anesthetic reaction (221 of 346 [64%]), who were tested at our unit during the same time period. Referral indications included high CK (n = 71, 52.2%), postexercise rhabdomyolysis and/or exercise intolerance (n = 14, 10.3%), postviral chronic fatigue (n = 25, 18.4%), muscle weakness of unknown etiology (n = 22, 16.2%), heat stroke (n = 1, 0.7%), and high CK and postexercise rhabdomyolysis (n = 3, 2.2%). Forty-seven patients with high CK (54.0%), 9 patients with postexercise rhabdomyolysis (10.3%), 2 patients with high CK and postexercise rhabdomyolysis (2.3%), 15 patients with postviral chronic fatigue (17.2%), and 14 patients with muscle weakness of unknown etiology (16.1%) tested positive to the CHCT.

Characteristics of Referred Probands

Clinical characteristics of CHCT-positive and CHCT-negative patients are compiled in table 1. CHCT-positive patients were predominantly male compared with CHCT-negative patients (63.2 *vs.* 34.7%, $P = 0.002$). A difference in overall mean baseline CK was observed between CHCT-positive and CHCT-negative patients (777.9 *vs.* 333.9 IU/l, $P = 0.002$). There were also significant differences detected when data were stratified by gender (table 1). There was no difference in age, cramps, weakness, and heat intolerance between CHCT-positive and CHCT-negative patients. More than a quarter of CHCT-positive patients (27.6%) reported having at least one anesthetic with one or more drugs known to trigger MH in susceptible individuals.

Table 1. Characteristics of Referred Probands without Adverse Anesthetic Reactions

	CHCT + (n = 87)	CHCT − (n = 49)	*P* Value
Demographics			
Age (yr)			
Mean (SD)	40 (13.5)	44 (14.7)	0.195*
Median (range)	41 (14–69)	45 (15–76)	
Male gender	55/87 (63.2%)	17/49 (34.7%)	0.002†
Symptoms			
Cramps	60/87 (69%)	34/49 (69.4%)	1†
Weakness	44/87 (50.6%)	20/49 (40.8%)	0.289†
Heat intolerance	13/87 (14.9%)	6/49 (12.2%)	0.799†
Two of the above symptoms	38/87 (43.7%)	19/49 (38.8%)	0.593†
Three of the above symptoms	4/87(4.6%)	1/49 (2%)	0.654†
No symptoms	16/87 (18.4%)	10/49 (20.4%)	0.822†
Previous unremarkable anesthetics			
n (%)	24/87 (27.6)	13/49 (26.5)	1†
Range	0–6	0–6	
Referral CK			
Overall (IU/l)			
Median (IQR)	397 (763)	320 (455.5)	0.002*
Range	23–7000	23–1210	
Male (IU/l)			
Median (IQR)	478 (548)	401 (265.5)	0.016*
Range	23–7000	23–1210	
Female (IU/l)			
Median (IQR)	224 (978.8)	230 (465.8)	0.028*
Range	32–4782	23–741	
Abnormal histomorphology	29/87 (33.3%)	5/49 (10.2%)	0.003†
Genetic testing			
All	15/48 (31.3%)	0/3 (0%)	0.546†
Causative	3/48 (6.3%)	0/3 (0%)	1†
VUS	12/48 (25%)	0/3 (0%)	1†

P values calculated using * Wilcoxon rank sum test and † Fisher exact test.
CHCT = caffeine–halothane contracture test; CK = creatine kinase; IQR = interquartile range; VUS = variant of uncertain significance.

Table 2. Referral Indications in CHCT-positive Probands without Adverse Anesthetic Reaction (n = 15) Carrying *RYR1* and *CACNA1S* Variants

High creatine kinase	Nucleotide Change	Amino Acid Change	Causal Potential
	c.1840C>T	p.Arg614Cys	Causative
	c.7007G>A	p.Arg2336His	Causative
	c.7522C>T	p.Arg2508Cys	Causative
	c.4999C>T	p.Arg1667Cys	VUS, MAF T = 0.0022; MH associated[15]
	c.6599C>T	p.Ala2200Val	VUS, absent from dbSNP; MH associated[15]
	c.10616G>A	p.Arg3539His	VUS, MAF A = 0.0006; well conserved
	c.12310G>C	p.Gly4104Arg	VUS, absent from dbSNP; not conserved[15]
	c.14524G>A	p.Val4842Met	VUS, highly conserved; absent from dbSNP; MH associated[15]
	c.14545G>A	p.Val4849Ile	VUS, highly conserved; absent from dbSNP; MH associated[15]
Postexercise rhabdomyolysis and/or exercise intolerance	c.14968A>G	p.Met4990Val	VUS, not in dbSNP; moderately conserved[15]
	c.418G>A	p.Ala140Thr	VUS, MAF* A = 0.000; well conserved; MH associated[15]
Postviral chronic fatigue	c.9850T>A	p.Trp3284Arg	VUS, highly conserved; absent from dbSNP; MH associated[15]
	c.6377G>A	p.Arg2126Gln	VUS, highly conserved; absent from dbSNP; MH associated[15]
	c.14968A>G	p.Met4990Val	VUS, not in dbSNP; moderately conserved[15]
	c.4615C>T†	p.Arg1539Cys†	Likely benign[37]; MAF A = 0.0841

* NHLBI exome sequencing project.[36] †CACNA1S variant: dbSNP, VUS. MAF in the 1000 genome phase 1 genotype data.[35]

CACNA1S = calcium channel, voltage dependent, L type, α 1S subunit; CHCT = caffeine–halothane contracture test; dbSNP = the single-nucleotide polymorphism database; MAF = minor allele frequency; MH = malignant hyperthermia; *RYR1* = ryanodine receptor type 1 gene; VUS = variant of uncertain significance.

Abnormalities in Histomorphology

A difference in the incidence of abnormal histomorphology was observed between CHCT-positive (n = 28, 33.3%) and CHCT-negative patients (n = 5, 10.2%; 33.3 *vs.* 10.2%, P = 0.003). The most common reported abnormalities included type II atrophy, increased internal nuclei, and inflammatory changes. Histomorphologic abnormalities across CHCT-positive and CHCT-negative patients were similar. Three CHCT-positive patients had evidence of cores on histomorphology. These patients were referred for idiopathic hyperCKemia. Congenital myopathy and atypical fiber disproportionate syndrome were also reported in two other CHCT-positive patients. In addition, phosphofructokinase deficiency was identified in one CHCT-positive patient.

Genetic Testing

Forty-eight CHCT-positive and three CHCT-negative patients underwent genetic testing. Although there were no variants in the *RYR1* and *CACNA1S* genes in the CHCT-negative patients, 15 CHCT-positive patients (31.3%) carried 14 nonsynonymous *RYR1* variants and 1 *CACNA1S* variant (table 2); no variants were present in a compound heterozygous state. Among the identified *RYR1* variants were three MH causative mutations (as defined by the European Malignant Hyperthermia Group[38]); and the remaining were variants of uncertain significance (VUS) with four variants previously found in association with MH.[39] One variant (c.14968A>G, p.Met4990Val) was identified in two patients: one complaining of postexercise rhabdomyolysis and exercise intolerance and the other with postviral chronic fatigue. Details of the identified variants and respective referral indications are outlined in table 2.

Contracture Testing

Mean contracture responses of CHCT-positive probands are reported in table 3. Forty-one probands tested positive to both caffeine and halothane. Forty-three probands tested positive to only halothane and three probands tested positive to only caffeine. The mean contracture of all CHCT-positive probands was 0.5 g to caffeine and 2.3 g to halothane. High CK and postviral chronic fatigue were the referral indications with highest mean contracture responses (0.6 g to caffeine and 2.4 g to halothane, and 0.5 g to caffeine and 2.6 g to halothane, respectively). However, there was no statistically significant difference in caffeine or halothane contracture responses among referral indications (table 3).

We also compared contracture responses of probands without anesthetic reaction with contracture responses from 221 probands with anesthetic reaction and a positive CHCT from our unit during the same time period. A significantly higher contracture response to both caffeine and halothane was observed in probands with anesthetic reaction (table 3).

Probands without anesthetic reaction, who carried an MH causative mutation or VUS, had a greater contracture response when compared with those who had negative genetic results. A significant difference was found in both caffeine and halothane contracture responses between probands harboring genetic variants (VUS and causative) and probands with negative genetic test results (P = 0.032 and 0.009, respectively).

Table 3. Comparison of Contracture Responses of CHCT-positive Probands

	Caffeine (2.0 mmol/l)	Halothane (3%)
All CHCT + probands without adverse anesthetic reaction		
Mean (SD)	0.5 (0.8)	2.3 (1.7)
Median (range)	0.3 (0.0–5.6)	1.8 (0.1–8.7)
Referral indications		
High CK		
Mean (SD)	0.6 (1.0)	2.4 (1.8)
Median (range)	0.3 (0.0–5.6)	1.8 (0.1–8.7)
Postexercise rhabdomyolysis and/or exercise intolerance		
Mean (SD)	0.3 (0.3)	2.3 (1.9)
Median (range)	0.2 (0.0–1.0)	1.3 (0.7–6.4)
Postviral chronic fatigue		
Mean (SD)	0.5 (0.4)	2.6 (1.7)
Median (range)	0.2 (0.0–2.1)	2.2 (0.7–7.2)
High CK and postexercise rhabdomyolysis		
Mean (SD)	0.2 (0.0)	2.1 (0.1)
Median (range)	0.2 (0.2–0.2)	2.1 (2.0–2.2)
Muscle weakness of unknown etiology		
Mean (SD)	0.4 (0.6)	1.7 (1.5)
Median (range)	0.2 (0.0–2.1)	1.2 (0.2–6.0)
P value*	0.761	0.328
Probands with anesthetic reaction (n = 221)		
Mean (SD)	2.2 (2.0)	4.0 (1.2)
Range	0–8.6	0.3–12
P value†	0.005	0.003
Genetic testing		
Causative		
Mean (SD)	3.1 (2.2)	5.8 (3.1)
Median (range)	2.2 (1.6–5.6)	6.0 (2.7–8.8)
VUS		
Mean (SD)	0.6 (0.4)	3.7 (1.9)
Median (range)	0.5 (0.1–1.2)	3.1 (1.2–7.2)
Negative		
Mean (SD)	0.5 (0.5)	2.4 (1.5)
Median (range)	0.4 (0.0–2.2)	2.1 (0.2–7.2)
P value‡	0.032	0.009

* Kruskal-Wallis H test is used to compare contracture values among five referral reasons. † Wilcoxon rank sum test is used to compare contracture values between probands with and without anesthetic reaction. ‡ Wilcoxon rank sum test is used to compare contracture values between patients with positive (causative and VUS) and negative genetic results.
CHCT = caffeine-halothane contracture test; CK = creatine kinase; VUS = variant of uncertain significance.

Dantrolene Treatment

Thirty-four CHCT-positive patients without a history of adverse anesthetic reaction but with complaints of myalgia, muscle weakness, muscle cramps, rigidity, and/or fatigue received oral dantrolene. Twenty-eight (82.4%) dantrolene-treated patients responded with diminution of symptoms. CHCT referral indications for these patients are listed in table 4. The mean end dose received by responders was 111 mg/day (SD, 84.2; range, 25–400 mg/day). There were no differences observed in age or gender between responders and nonresponders (table 3). Nerve conduction velocity findings were normal. Three responders had mild proximal muscle weakness before initiating dantrolene. Responders had an overall higher pretreatment CK (774.9 *vs.* 127, $P = 0.028$) and a larger reduction in CK (–671 *vs.* –60, $P = 0.028$). These CK differences remained when the responder *versus* nonresponders were analyzed in a subgroup divided by gender. Seventeen responders underwent genetic testing identifying one causative mutation and five VUS (specific variants outlined in table 2). Histomorphologic abnormalities were reported in six responsive patients with none found in nonresponsive patients ($P = 1$). Histomorphologic features were not consistent but most commonly included type II atrophy and inflammatory changes.

In total, 11 responders reported dantrolene side effects including weakness (n = 4), abnormal LFTs (n = 3), headache (n = 2), pruritus (n = 1), and fatigue (n = 1; table 3). The mean maximum dose received by individuals reporting side effects was 172.7 mg/day (SD, 112.6; range, 25–300 mg/day). Responders who did not report side effects received a mean maximum dose of 117.7 mg/day (SD, 96.3; range, 25–400 mg/day). Six patients had their dantrolene dose reduced. A reduced dantrolene dose eliminated side effects in four of these patients (fig. 1). Five responders withdrew from treatment because of weakness (n = 2), headache (n = 1), abnormal LFTs (n = 1), and pruritus (n = 1). No patient developed fulminant hepatitis.

Discussion

This is the first large study analyzing the characteristics in CHCT-positive patients and no personal or familial history of adverse anesthetic reaction. These patients were predominantly male and had a higher CK than CHCT-negative patients. Our results support the evidence that patients with a high CK[40–42] and exercise-induced rhabdomyolysis and/or exercise intolerance may have positive CHCT results.[24,25,28,43] In addition, we identified two new phenotypes associated with a positive CHCT namely postviral chronic fatigue and weakness of unknown etiology.

Individuals with positive CHCT in this study carried fewer genetic variants (31.3%) compared with individuals with a personal or familial history of anesthetic-induced MH (52 to 86%).[16,17] However, they had a similar incidence of abnormal histomorphology compared with probands with a personal or familial history of MH reaction (33.3 *vs.* 29.9%, respectively).[43] The histomorphologic features were also similar to probands with a history of MH reaction.[43] Mean contracture responses were highest in probands without anesthetic reaction, who harbored MH causative *RYR1* mutations consistent with published reports of CHCT testing in individuals with a personal or familial history of an adverse anesthetic reaction.[17]

Fifteen of 20 (75%) patients presenting with postviral chronic fatigue had a positive CHCT and had one of the highest mean contracture responses of all referral indications (0.5 g to caffeine and 2.6 g to halothane). This suggests that patients with persistent musculoskeletal symptoms after certain viral illnesses may test positive to the CHCT, supporting further

Table 4. Characteristics of Dantrolene-treated CHCT-positive Probands without Adverse Anesthetic Reaction (n = 34)

	Responders (n = 28)	Nonresponders (n = 6)	*P* Value
Referral indications, n (%)			
High creatine kinase	12/28 (42.9)	1/6 (16.7)	0.37*
Postexercise rhabdomyolysis and/or exercise intolerance	2/28 (7.1)	0/6 (0)	1*
Postviral chronic fatigue	4/28 (14.3)	4/6 (66.7)	0.018*
Muscle weakness of unknown etiology	10/28 (35.7)	1/6 (16.7)	0.638*
Demographics			
Age (yr)			
Mean (SD)	42 (12)	45 (15)	1†
Median (range)	43 (21–42)	41 (28–63)	
Male gender	18/28 (64.3%)	3/6 (50%)	0.653*
Abnormal histomorphology	6/28 (21.4%)	0/6 (0%)	0.562*
Creatine kinase (IU/L)			
Overall pretreatment CK			
Mean (SD)	774.9 (1311.8)	127.7 (160)	
Median (range)	368 (32–6232)	53.5 (45–449)	
Overall change in CK with treatment			
Mean	–671	–60	0.028†
Male pretreatment CK			
Mean (SD)	964.9 (1524.0)	75.7 (38.4)	
Median (range)	430.5 (63–6232)	54 (53–120)	
Male change in CK with treatment			
Mean	–839.3	–11	0.007†
Female pretreatment CK			
Mean (SD)	433 (757.2)	179.7 (233.2)	
Median (range)	134 (32–2532)	45 (45–449)	
Female change in CK with treatment			
Mean	–368	–109	0.016†
Genetic testing			
All	6/17	0/3	0.521*
Causative	1/17	0/3	1*
VUS	5/17	0/3	0.539*
Abnormal EMG	0/27	0/5	1*
Proximal muscle weakness	3/28 (10.7%)	0/6 (0%)	1*
Dose range	25–400	25–300	
Reported side effects			
Total	11/28 (39.3%)	0/6 (0%)	0.145*
Weakness	4	0	1*
Abnormal LFTs	3	0	1*
Headache	2	0	1*
Pruritus	1	0	1*
Fatigue	1	0	1*

P values calculated using * Fisher exact test and † Wilcoxon rank sum test.

CHCT = caffeine–halothane contracture test; CK = creatine kinase; EMG = electromyography; LFT = liver function test; VUS = variant of uncertain significance.

study of disordered calcium homeostasis in the skeletal muscle of patients with chronic fatigue.[44] Five CHCT-positive individuals suffering from postviral chronic fatigue carried *RYR1* or *CACNA1S* variants. Seventy percent of patients (n = 14) referred with weakness of unknown etiology tested positive to the CHCT; however, no genetic variants were identified. However, recent screening for *RYR1* mutations in patients with myopathies of unknown etiology presenting with features including weakness have reported MH causative *RYR1* mutations in a small proportion of patients, thus supporting an association of muscle weakness with MH susceptible phenotype.[30]

It is important to consider the limitations of CHCT. Using consensual thresholds, the CHCT has a specificity of 78% and consequently will overdiagnose MH susceptibility.[6] The CHCT has been validated only for classical reactions in association with anesthetic agents and may lack specificity in the interpretation of contractures of nonanesthetic probands with underlying muscle dysfunction.[45] Therefore, classification of patients with conditions such as postexercise rhabdomyolysis as MH susceptible on the basis of contracture testing has been questioned.[46] The possibility of false-positive CHCT in novel phenotypes

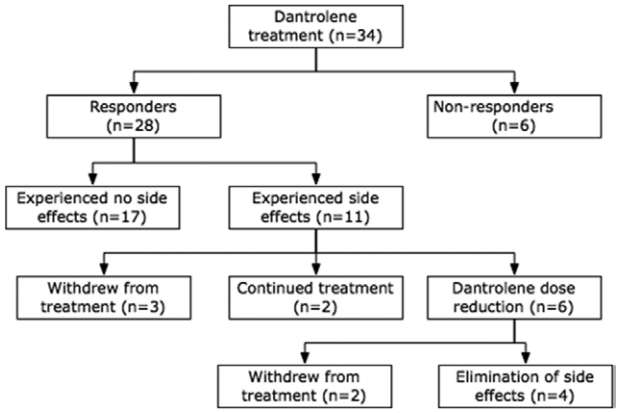

Fig. 1. Flow chart of dantrolene treatment in probands with positive caffeine–halothane contracture test and persistent musculoskeletal symptoms.

such as muscle weakness of unknown etiology in which no genetic variants were identified requires careful consideration. Previous reports have highlighted that individuals with diagnosed neuromuscular disease[46–48] and high CK[40] can have abnormal contracture responses; however, the mean contractures reported in our study, particularly to halothane, show a larger mean contracture response than previous studies albeit not as great as patients with documented anesthetic reaction. Although more than 25% of CHCT-positive probands had undergone uneventful anesthetics, many individuals who experience MH events also have a history of normal anesthetics. Indeed, study from North American MH Registry has highlighted that 50.7% of patients with a "very likely" or "almost certain" MH event had two or more previous unremarkable anesthetics.[49] Also in our unit, 13.2% of index cases of anesthetic MH reaction had previous unremarkable anesthetics.[50] However, it should be considered that a positive CHCT in probands with no adverse anesthetic reactions may not be necessarily associated with MH and that ultimately further study is required to elucidate underlying genetic and environmental factors involved in the pathophysiology of these complex phenotypes. The probands in our study had an overall lower contracture response when compared with probands who have had an MH reaction. Furthermore, in this cohort of patients, those with *RYR1* variants had a greater contracture response. It may be that lower or borderline contracture responses are associated with a false-positive CHCT. However, although the significance of a positive CHCT (and its sensitivity and specificity) in patients with no personal or familial history of MH needs to be investigated further, we advise these patients to avoid known MH triggers. The data presented here may help future interpretation of CHCT testing in patients without adverse anesthetic reaction.

An interesting observation in our patient population was the reported improvement in musculoskeletal symptoms including cramps, rigidity, myalgia, and muscle weakness with oral dantrolene treatment. This was an off-label use of dantrolene, based on the rationale that reported symptoms in CHCT-positive patients may be due to mini-focal MH crisis in the skeletal muscle. Twenty-eight of 34 (82%) CHCT-positive patients treated with dantrolene reported improved musculoskeletal symptoms. Response to treatment was associated with a higher pretreatment CK (774.9 *vs.* 127.7, $P = 0.028$) and a corresponding greater reduction in CK (−671 *vs.* −60, $P = 0.028$). It would have been interesting to see whether patients with musculoskeletal symptoms in this study who tested negative with CHCT also respond to dantrolene. However, because of the retrospective nature of this study, these data were not available. Currently, there are only two case reports of successful use of oral dantrolene in treatment of musculoskeletal symptoms in MH-susceptible patients.[51,52] Although the precise mechanism of action of dantrolene is unknown, it has been established that dantrolene depresses the intrinsic mechanisms of excitation–contraction in the skeletal muscle.[52] The reported response to treatment presented here supports the assertion of some degree of muscle dysfunction in our CHCT-positive cohort. Intriguingly there is no clear explanation for symptom improvement with dantrolene in patients suffering from weakness of unknown etiology.[53]

There are limitations associated with the study. As a retrospective study, our findings are limited to recorded data and data availability. Ethnicity data were not available for all patients, and therefore, CK values could not be corrected for this parameter.[54] Genetic testing was only carried out in a small proportion of probands, and the genetic methods used were not exhaustive. Patients have been followed up in our unit, which minimizes the extent of missing data; however, careful consideration is required when interpreting retrospective data. Dantrolene treatment was assessed retrospectively in a small highly selected sample with no controls. Ideally, oral dantrolene therapy requires assessment in a larger sample adopting a prospective, randomized, placebo-controlled trial approach.

In conclusion, this is the first study summarizing the characteristics of CHCT-positive probands with no history of adverse anesthetic reaction. Overall our findings show that patients referred for CHCT testing with a history of nonanesthetic neuromuscular disorders may have abnormal CHCT results similar to but less aberrant than those observed in patients referred for CHCT testing with a history of anesthetic-triggered MH. In addition, our report highlights the potential value of oral dantrolene for the treatment of musculoskeletal symptoms in patients with positive CHCT.

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Competing Interests

Dr. Rosenberg received a one-time speaking fee from Eagle Pharmaceuticals (Woodcliff Lakes, New Jersey), which manufactures Ryanodex, a concentrated formulation of dantrolene approved for the treatment of malignant hyperthermia. The other authors declare no competing interests.

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