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Risk of malignant hyperthermia in patients carrying a variant in the skeletal muscle ryanodine receptor 1 gene

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ABSTRACT

Malignant hyperthermia is a life-threatening disorder, which can be prevented by avoiding certain anesthetic agents. Pathogenic variants in the skeletal muscle ryanodine receptor 1-gene are linked to malignant hyperthermia. We retrospectively studied 15 patients who presented to our clinic with symptoms of muscle dysfunction (weakness, myalgia or cramps) and were later found to have a variant in the skeletal muscle ryanodine receptor 1-gene. Symptoms, creatine kinase levels, electromyography, muscle biopsy and *in vitro* contracture test results were reviewed. Six out of the eleven patients, with a variant of unknown significance in the skeletal muscle ryanodine receptor 1-gene, had a positive *in vitro* contracture test, indicating malignant hyperthermia susceptibility. In one patient, with two variants of unknown significance, both variants were required to express the malignant hyperthermia-susceptibility trait. Neurologists should consider screening the skeletal muscle ryanodine receptor 1-gene in patients with myalgia or cramps, even when few to no abnormalities on ancillary testing.

1. Introduction

Malignant hyperthermia (MH) is a rare, but life-threatening condition [1]. This pharmacogenetic disorder of skeletal muscle presents as a hypermetabolic response to potent volatile anesthetic gasses such as halothane and isoflurane and the depolarizing muscle relaxant succinylcholine [2, 3]. The clinical signs of MH include hyperthermia, tachycardia, tachypnea, increased carbon dioxide production, increased oxygen consumption, acidosis, muscle rigidity and rhabdomyolysis [4–6]. The pathophysiologic changes of MH are due to an uncontrolled increase in myoplasmic calcium, leading to increased muscle activation [7]. As a result, the muscle membrane integrity becomes compromised due to ATP depletion, which eventually leads to hyperkalemia and rhabdomyolysis [1, 8, 9]. By identifying those at risk of this potentially fatal condition, it can be avoided. Avoidance of anesthetic triggers and monitoring of patients at risk reduces the risk of a fatal outcome [10]. Treatment of a MH-crisis should include immediate cessation of triggering agents and administration of the muscle relaxant dantrolene, which will counteract the uncontrolled rise in calcium and its potentially fatal consequences [7, 11].

Central in the pathogenesis of MH is the ryanodine receptor of skeletal muscle, further referred to as RyR1 [12–14]. The ryanodine receptors (RyR) form a group of intracellular calcium release channels on the sarcoplasmic reticulum (SR) or endoplasmic reticulum (ER) in muscles and nerves [15, 16]. Alongside MH, pathogenic variants in the *RYR1*-gene are linked to exertional rhabdomyolysis, King-Denborough syndrome, late-onset axial myopathy and congenital myopathies such as central core disease and minicore myopathy with external ophtalmoplegia [17, 18]. Malignant hyperthermia has an autosomal dominant inheritance, congenital myopathies an autosomal recessive or dominant inheritance. There is growing evidence for a clinical and histopathological continuum between patients with MH-susceptibility (MHS) and congenital myopathies [19]. The phenotype is dependent on the pathogenic variant, but some patients with central core or multiminicore disease will also suffer from MH [7]. Currently more than 400 variants of unknown significance in the *RYR1*-gene have been identified, but the specific MH risk for each of these variants is uncertain [20]. Until

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now, 48 variants have been identified as pathogenic for MHS according to the European Malignant Hyperthermia Group (EMHG) (www.emhg.org). The criteria of the EMHG recommend that, when a variant of unknown significance is detected, a functional assay with a muscle biopsy and *in vitro* contracture test (IVCT) is required [21]. Thus, a variant must be both genetically and functionally characterized before being reclassified [22].

The IVCT is currently considered the gold standard for diagnosis of an individual's MHS [21]. The IVCT is based on the contractile response of the individual's fresh muscle tissue in the presence of caffeine or halothane. The sensitivity of the IVCT conducted according to the EMHG protocol has been evaluated as 99% with a specificity of 94% [7, 21, 23]. Therefore, in patients with a negative IVCT, MHS is reasonably ruled out [21]. Several in silico prediction methods have been developed to reveal the pathogenicity of a detected variant, but none can replace the IVCT so far [24, 25].

The aim of this study is to describe the findings on clinical assessment and ancillary testing (creatine kinase (CK) levels, EMG, muscle biopsy, IVCT) in patients with neuromuscular complaints that turned out to be secondary to MHS, as determined by IVCT. Additionally, we want to stress the importance of considering a *RYR1*-variant in patients with muscle complaints, given the potential medico-legal issues when MHS is missed.

2. Methods

This study is a retrospective analysis of data from 15 individuals referred to the adult neuromuscular outpatient clinic of Ghent University Hospital and Sint Lucas General Hospital Ghent. The patients presented to one of these hospitals between 1/1/2016 and 30/06/2021 and were included if a variant of unknown significance or (likely) pathogenic variant in *RYR1*-gene was found in the work-up for their neuromuscular complaints (or when performing segregation analysis). Information for this study was gathered from electronic medical records of the included individuals. We retrospectively reviewed the initial symptoms at presentation, serum CK, family history, results of the muscle biopsy, EMG, genetic testing, IVCT and the pathogenicity prediction. When patients presented to us with unexplained muscle weakness and/or myalgia/cramps, which were considered to be neuromuscular in origin by the clinician, CK levels and EMG were usually performed first. If no cause was identified, we proceeded to muscle biopsy +/- genetic testing, depending on the urgency (for example, clinical deterioration, wish for children, impeding anesthesia and other factors). The indications for performing a 216-gene panel, including well-known neuromuscular diseases were: (1) various unexplained complaints, such as muscle weakness, cramps, non-specific myalgia, with or without increased serum CK or (2) an adverse anesthetic event of the individual.

Thirteen of the 15 individuals underwent this panel. Some underwent additional testing for other genes (*e.g.* facioscapulohumeral dystrophy or myotonic dystrophies) depending on their clinical presentation. Two of the 15 individuals (patient 7 and 8) underwent targeted mutation analysis because of a MH-susceptible child (patient 6) who carried two variants in the *RYR1*-gene. In addition to genetic screening, an IVCT was performed at the University of Antwerp (J. De Puydt). Genetic testing for MHS and IVCT were carried out in accordance with the guidelines of the EMHG (https://www.emhg.org/testing-for-mh) [21]. Written informed consent was obtained from individuals prior to revision of their electronic records and the study design was approved by the Ethics Committee of the University Hospital of Ghent, Belgium.

Genetic screening – Gene panel analysis for neuromuscular diseases (including the *RYR1*-gene) was performed by applying whole-exome sequencing (WES) on genomic DNA extracted from blood samples of the 13 individuals using the SureSelect XT Human All Exon V6 enrichment kit (Agilent Technologies), followed by sequencing on the HiSeq3000 sequencer (Illumina). Variants with a low-quality score were confirmed with an independent test (polymerase chain reaction (PCR) amplification followed by Sanger sequencing). Nucleotide numbering is according to the Human Genome Variation Society guidelines (HGVS). Variant classification for MHS is based on the recently published guidelines by the ClinGen expert panel [26]. Since MHS primarily results from missense alterations in *RYR1*, this classification system is developed for missense variants. Other alterations (*e.g.* nonsense, splice site) rather result in a loss of function and are thus more likely to potentially cause a *RYR1*-related myopathy. Variant classification for *RYR1*-related myopathies is performed using an in house developed tool based on the ACMG [27] and ACGS (www.acgs.uk.com/quality/best-practice-guidelines/) guidelines in the following classes: (1) benign, (2) likely benign (>95% certainty that the variant is benign), (3) variant of unknown significance, (4) likely pathogenic (>95% certainty that the variant is pathogenic), (5) pathogenic. The classification system cannot distinguish between *RYR1*-variants causing MHS and variants causing a *RYR1*-relateded myopathy [7].

IVCT – MHS was diagnosed using the IVCT in accordance with the recommendations of the EMHG [21]. The IVCT determines the contracture susceptibility of surgically excised muscle specimens to halothane and caffeine. Muscle samples from all 15 individuals were tested, irrespective of whether a pathogenic mutation or a variant of unknown significance (VUS) was detected. Biopsies were taken from the vastus lateralis muscle under locoregional anesthesia. Muscle bundles from individuals who are MHS have lower contracture thresholds to halothane and caffeine relative to MH-negative (MHN) individuals. A contracture force of $\geq$0,2 gr at a halothane concentration $\leq$2% and a caffeine concentration $\leq$2 mM defines a positive result for MHS [21].

3. Results

Results on individual patients are summarized in Table A.

3.1. Genetic data

Based on the ClinGen criteria for MHS, the variants were classified as follows: of the 15 individuals with a variant in the *RYR1*-gene, 9 individuals (patient 1, 2, 3, 4, 5, 7, 8, 9, 11) harbored one variant of unknown significance (VUS, class 3) and one individual (patient 6) was compound heterozygous for two VUS in trans. Patient 11 also carried an intronic variant in cis, that was classified as a pathogenic variant (class 5) using the ACMG/ACGS-criteria for *RYR1*-related myopathies. Patient 14 and 15 had a likely pathogenic variant according to the ClinGen classification, but since these variants were known to be an EMHG-mutation, they were upgraded to pathogenic. Also patient 13 had a variant that was known to be an EMHG-mutation. The variant classification was mostly the same when the variant was classified using the ClinGen classification (for MHS) or the ACMG/ACGS-criteria for *RYR1*-related myopathies. An exception were the nonsense (patient 12) and splice site variants (patient 10, 11), for which the ClinGen classification is not useful, since these type of genetic defects are rather known to result in a loss of function and thus a possible *RYR1*-related myopathy (instead of MHS). In total, forteen different variants in the *RYR1*-gene were detected. Ten of these variants were absent in the gnomAD v2.1.1 population databases: p.(Arg4825His) (c.14474G>*A* in exon 100), p.(Met3266Leu) (c.9796A>C in exon 66), p.(Ile2162Met) (c.6486C>G in exon 39), p.(Pro4587Leu) (c.13760C>T in exon 95), p.(Met4640Val) (c.13918A>*G* in exon 95), (c.8401–8C>G in

Table A
Schematic overview of the key patient data.

Patient	Classification (for MHS, prior to IVCT)	Region	IVCT	Symptoms at presentation	Serum CK (U/L)	EMG	Muscle biopsy	General anesthesia in medical history
1	Variant of unknown significance	c.14474G>*A*, p.(Arg4825His), exon 100	+	Myalgia mainly post exercise	286 - 81 000	Non-irritable myopathy	Mild myopathic changes	–
2	Variant of unknown significance	c.3379C>T, p.(Arg1127Cys), exon 25	+	Myalgia and spasms in proximal muscles	70	Normal	Normal	+
3	Variant of unknown significance	c.3877C>A, p.(Pro1293Thr), exon 28	+	Myalgia, proximal and facial weakness and contractures	118	Minimal myopathic changes in biceps muscle	Normal	+
4	Variant of unknown significance	c.9796A>C, p.(Met3266Leu), exon 66	+	Proximal muscle weakness, mild distal weakness	221 - 450	Non-irritable myopathy	Myopathy with central cores	–
5	Variant of unknown significance	c.6486C>G, p.(Ile2162Met), exon 39	+	Muscle atrophy, proximal muscle weakness, cramps	990	Normal	Mild muscular dystrophy	–
6	Two variants of unknown significance	c.13760C>T, p.(Pro4587Leu), exon 95 and c.13918A>*G*, p.(Met4640Val), exon 95	+	Proximal muscle weakness and fatigue	159	Mild non-irritable myopathy	Myopathy with core formations	–
7	Variant of unknown significance	c.13918A>*G*, p.(Met 4640Val), exon 95 (father of patient 6)	–	No symptoms	155	ND	Myopathy with core formations	+
8	Variant of unknown significance	c.13760C>T, p.(Pro4587Leu), exon 95 (mother of patient 6)	–	No symptoms	71	ND	Normal	+
9	Variant of unknown significance	c.3953C>T, p.(Ala1318Val), exon 28	–	Myalgia, exercise intolerance	51 - 32,000	Normal	Normal	+
10	NA, (variant of unknown significance)	c.8401–8C>G, p. (?), intron 53	–	Diffuse muscle weakness and myalgia	92 - 596	Normal	Normal	+
11	Variant of unknown significance and NA, (pathogenic variant)	(3) c.13664A>*G*, p.(Tyr4555Cys), exon 49 (5) c.10347+1G>*A*, p.(?), intron 68	–	Proximal muscle weakness and myalgia	572 - 3400	ND	Unspecific myopathy, mild increased variation in muscle fiber size	–
12	NA, (pathogenic variant)	c.10859G>*A*, p.(Trp3620Ter), exon 74	–	Myalgia after exercise	743 - 1200	Normal	Multiple fibers with central attenuation of the intermyofibrillar network, sometimes with the morphology of classic cores	–
13	Pathogenic variant)	c.14545G>*A*, p.(Val4849Ile), exon 101	+	Muscle cramps and myalgia	306 - 700	ND	Multiple fibers with central attenuation of the intermyofibrillar network, without the morphology of classic cores	+
14	Pathogenic variant	c.7042_7044del, p.(Glu2348de), exon 44	+	Myalgia in rest and during exercise	406	Normal	Myopathic changes with mild changes of the intermyofibrillar network without cores	–
15	Pathogenic variant	c. 7523G>*A*, p.(Arg2508His), exon 47	+	Muscle cramps, mild proximal muscle weakness	252	ND	Myopathy with central cores	+, resulting in MH

Schematic overview of the relevant patient data *i.e.* classification of the variant based on the criteria for MHS by the ClinGen expert panel [26], location of the variant in the *RYR1*-gene, result of the IVCT, symptoms, CK levels (normal values are 10–195 U/L for men and 10–170 U/L for women), EMG and muscle biopsy results, previous exposure to general anesthesia. The ClinGen classification in not useful for splice variants (patients 10 and 11) and nonsense variants (patient 12) since these are considered to possibly cause *RYR1*-related myopathies instead of MHS. The genetic classification following the ACMG/ACGS-criteria for these 3 patients is noted between brackets. The variants in patient 13, 14 and 15 are EMHG diagnostic mutations. Therefore, the variants in patient 14 and 15, who were identified as likely pathogenic by ClinGen were changed to pathogenic. ND =not done. NA = no information available.

intron 53), p.(Trp3620Ter) (c.10859G>*A* in exon 74), p.(Tyr4555Cys) (c.13664A>*G* in exon 49), splice site variant c.10347+1G>*A* in intron 68 and p.(Ala1318Val) (c.3953C>T in exon 28).

3.2. IVCT data

In vitro contracture testing classified 9 of all 15 individuals with a variant in the *RYR1*-gene as MHS, whilst six were classified as MHN. Of the 9 individuals identified with one VUS, 5 were classified as MHS (patient 1, 2, 3, 4, 5). The one individual identified with two variants of unknown significance (patient 6) was classified as MHS. The individual identified with both a VUS (for MHS) and a pathogenic variant (for *RYR1*-myopathies) (patient 11) was classified as MHN. The three individuals with a pathogenic variant (patient 13, 14, 15) were classified as MHS. One positive IVCT result does not change the ClinGen result, since one IVCT result is not considered to be a strong enough argument to change the classification. However, one negative IVCT result in combination with other benign criteria or arguments allows downgrading a class 3 into a class 2 variant for MHS, as could be applied to patient 9.

3.3. Clinical features

Eleven patients, that presented to our neuromuscular clinic, suffered from non-specific myalgia, muscle fatigue and/or cramps. Seven had muscle weakness, especially proximal. Of the 9 patients that turned out to be MHS, 4 only had myalgia or cramps, 1 only weakness and 4 both.

3.4. Ancillary investigations

Blood tests were performed on all individuals following symptom presentation looking primarily at the **CK levels**. In the table, CK levels at rest are included for all patients (in U/L). Two patients were referred to us after an episode of rhabdomyolysis (patient 1 and patient 9). Patient 1 had several episodes of exertional myalgia, sometimes with myoglobinuria and had elevated CK values at rest. Patient 9 only had rhabdomyolysis once, without a clear trigger, but had normal CK at rest. In 9 out of 15 individuals with a variant in the *RYR1*-gene, CK levels at rest were elevated. Out of the 9 patients who turned out to have a positive IVCT, 6 had elevated CK levels at baseline.

An **EMG** was performed on 10 of the 15 individuals. Four individuals out of the 10 showed an abnormal EMG with chronic myopathic findings: these patients were diagnosed with a VUS and were MHS.

Light microscopy studies of **muscle biopsy** were, in addition to the IVCT, performed on all individuals included in this study. Several abnormal muscle biopsies were found in patients diagnosed with a variant in the *RYR1*-gene. Individuals with a pathogenic variant all showed changes in the intermyofibrillar pattern with or without central cores. In addition, five patients with a VUS showed abnormal muscle biopsies and four of them were proven MHS.

3.5. Special findings

One individual (patient 6) was diagnosed with two variants of unknown significance in the *RYR1*-gene: p.(Met4640Val) (c.13918A>*G*) and p.(Pro4587Leu) (c.13760C>T). Since *in vitro* contracture testing showed this individual to be MHS, further investigations were conducted in both parents (patient 7 and 8). Genetic testing identified the p.(Met4640Val) (c.13918A>*G*) variant in one parent (patient 7) and the p.(Pro4587Leu) (c.13760C>T) variant in the other parent (patient 8). Remarkably, IVCT showed both these variants to be MHN, which indicates that probably only the combination of the two variants will pose a risk for MH. The individual with two variants initially presented with motor development delay in childhood and complaints of proximal muscle weakness and fatigue. CK levels were normal, but an EMG showed a mild non-irritable myopathy. The muscle biopsy showed a myopathy with core formations. Both parents, however, did not have muscle complaints and had normal CK levels.

4. Discussion

In this study, eleven individuals were characterized with one or two VUS. The patients included were seen and referred for IVCT, prior to the publication of the new classification system for RYR1/MHS. In retrospect, patient 10 and 12 should not have been referred for IVCT to rule out MHS, since these splice site and nonsense variants are unlikely to result in a gain of function. Out of these 11 patients, six individuals were eventually proven MHS by IVCT. This illustrates that it is important to further investigate individuals with a VUS in the *RYR1*-gene [21, 22]. Furthermore, there seems to be an overlap with some mutations that are associated with CCD, also increasing the risk of MH [7, 28]. Thus, functional testing for MHS in patients with a congenital myopathy and a (likely) pathogenic variant in the *RYR1*-gene can also be informative. According to the EMHG, the IVCT remains the gold standard test for (re)classification of variants in the *RYR1*-gene as pathogenic for MHS [20]. Patients with a positive IVCT, should be considered at risk for MH and given a warning card. Family members also need gene testing and should avoid undergoing anesthesia with MH-triggering agents while awaiting results [20, 34]. Relatives identified with the same variant as their affected family member are assumed to be MHS. Some of the patients in this study had a positive family history of muscle complaints in first degree relatives, often previously diagnosed as fibromyalgia, but none had a family member with MH. Recently, a large multi-center study among four MH-units also reported positive IVCT's in patients without a personal/family history of an adverse anesthetic event [29].

In this study, a particularly interesting family was observed: one patient had two VUS in the *RYR1*-gene and a positive IVCT. After segregation analysis both parents were identified with one VUS, but had a negative IVCT, implicating that only when both of these variants are present, the MHS-trait is expressed.

Of the patients with MHS, four had already undergone general anesthesia, only causing clinically obvious MH in patient 15. This patient, who has a pathogenic mutation in the *RYR1*-gene, presented with MH during general anesthesia for reconstruction of the middle ear. This discrepancy can be explained by the existence of incomplete penetrance, as well as the fact that some MH-crises are missed based on the variable manifestations [12, 30]. Incomplete penetrance of the MHS-trait implies that the genetic defect either requires additional factors for MH to occur, or that other factors can prevent the occurrence [31, 32]. This explains why up to 50% of the individuals with MHS described in literature have undergone anesthesia uneventfully despite the use of volatile anesthetics [4, 31, 33]. An additional explanation for the variable occurrence of MH-crises is that newer inhalational anesthetics are a less potent trigger for MH. Lastly, variable occurrence of MH-crises may also reflect the variable presence of additional modifying factors, such as intense exercise and/or pyrexia in genetically susceptible individuals [34].

Patients with muscular dystrophies (Becker, Duchenne), that are not MHS, can develop a MH-like reactions when exposed to succinylcholine [35, 36]. The management of this condition with rhabdomyolysis and hyperkalemia is different from that of MH and consists of cardiac support and reduction of potassium levels.

Such a MH-like reaction has also been described in patients with myotonic dystrophy type 1, certain metabolic and mitochondrial myopathies [37]. The sensitivity and specificity of the IVCT has been validated in patients with suspected MH, but might be different in a less specific population.

Of all 9 patients with MHS, 2 had a normal biopsy and 7 an abnormal one with findings ranging from mild changes to central cores. Previous studies already reported a broad spectrum of histopathologic abnormalities in patients with *RYR1*-related MH, with some also having normal biopsies [38].

Other aspects of *RYR1*-related MH described in our study have been confirmed previously. For instance, (non-specific) myalgia, muscle fatigue and/or cramps, present in all of our MHS-patients, have already been associated with *RYR1*-variants in several studies [39–41]. These symptoms have a significant effect on quality of life [42]. Five had muscle weakness, especially proximal, which has also been described in MH-related *RYR1*-variants [41, 43]. This underscores the wide spectrum of *RYR1*-related neuromuscular disease.

One of the six patients with a VUS didn't have any objective clinical finding or abnormality on ancillary testing (CK levels, EMG, biopsy). Although we are aware of the small numbers in this case series, requiring caution in drawing major conclusions, this is important to mention, especially given the prevalence of unexplained muscle complaints in a neuromuscular outpatient clinic.

The most important limitation of our study is its low number of patients and its retrospective nature. Nevertheless, given there is a preventative treatment for this life-threatening condition and given the potential medicolegal issues when missed, we consider it important to raise awareness amongst neuromuscular neurologists about testing for a variant in the *RYR1*-gene in patients with non-specific muscle complaints, even in the absence of muscle weakness or CK elevation. We would recommend considering this diagnosis in (young) patients with invalidating muscle aches/cramps, especially if there are abnormalities on clinical exam or (at least) one ancillary test (CK's, EMG, biopsy) and/or if there is a positive family history of similar complaints. From a therapeutic standpoint, if MHS is proven, dantrolene orally might provide a relief from their symptoms of myalgia and cramps [44]. Neurologists should keep in mind that, if a variant is found, this will likely lead to an IVCT, requiring a muscle biopsy to determine the relevance of the variant.

Study roles

L.J. was responsible for collection and analysis of the data. J.D.B. and S.H were responsible for intellectual content and study design. S.S and M.M performed the molecular genetic testing and interpretation. L.J and S.H were involved in drafting the manuscript. J.D.B., J.D., S.S were responsible for evaluation of the manuscript.

Declaration of Competing Interest

J.D.B. and S.H. are a member of the European Reference Network for Neuromuscular Diseases. There is no conflict of interest. No targeted funding was received for this study.

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