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REPORTS OF ORIGINAL INVESTIGATIONS

Idiopathic hyperCKemia and malignant hyperthermia susceptibility

HyperCKémie idiopathique et susceptibilité à l'hyperthermie maligne

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Abstract

Purpose *HyperCKemia is a persistent rise in serum creatine kinase (CK) levels of at least 1.5 times the normal value, as evidenced by a minimum of two measurements at 30-day intervals. One of the neuromuscular diseases associated with hyperCKemia is malignant hyperthermia (MH). This study investigated the susceptibility to MH in patients with hyperCKemia via in vitro contracture testing (IVCT) and a search of mutations in the RYR1 gene.*

Methods *Patients in an MH centre were followed from 1997-2012, and their epidemiologic, clinical, and laboratory data were analyzed, including IVCT, muscle histochemical analysis, and next-generation sequencing molecular analysis.*

Results *There were nine patients (eight male) in our study with a mean (SD) age of 33 (12) yr. Four patients were Caucasian and five were African Brazilian. Most complained about myalgia or cramps, but all had a normal neurological examination. They persistently presented with hyperCKemia from three months to ten years, with a mean (SD) CK value of 788 (507) IU·L^{-1} ranging from 210-1,667 IU·L^{-1}. These values corresponded to a 1.5- to nine-fold increase in the normal value (mean increase, 3.7-fold). Six patients were MH susceptible (MHS) after a positive IVCT. Histopathological muscular analysis disclosed unspecified changes in four of the MHS patients. Mitochondrial proliferation was observed in the other two MHS patients and in three MH negative patients. No pathogenic mutations were identified in the RYR1 gene in the five patients evaluated.*

Conclusion *When investigating patients with idiopathic hyperCKemia, susceptibility to MH should be taken into account, and guidance should be offered to prevent anesthetic complications in the family.*

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Résumé

Objectif *L'hyperCKémie est définie comme étant l'élévation persistante des taux sériques de créatine kinase (CK) d'au moins 1,5 fois les valeurs normales, tel qu'attesté par un minimum de deux mesures prises à 30 jours d'intervalle. L'une des maladies neuromusculaires associées à l'hyperCKémie est l'hyperthermie maligne*

(HM). Cette étude a évalué la susceptibilité à l'HM de patients atteints d'hyperCKémie via un test de contracture in vitro (ou IVCT, pour in vitro contracture testing) et une recherche des mutations du gène RYR1.

Méthode *Des patients d'un centre d'HM ont été suivis entre 1997 et 2012 et leurs données épidémiologiques, cliniques et de laboratoire ont été analysées, notamment par IVCT, par analyse histochimique musculaire et par analyse moléculaire séquentielle de nouvelle génération.*

Résultats *Neuf patients (huit hommes) ont été inclus dans notre étude, d'un âge moyen (ÉT) de 33 (12) ans. Quatre patients étaient d'origine caucasienne et cinq d'origine afro-brésilienne. La plupart se plaignaient de myalgie ou de crampes, mais l'examen neurologique était normal chez tous les patients. Ils ont présenté de façon persistante une hyperCKémie allant de trois mois à dix ans, avec une valeur moyenne de CK (ÉT) de 788 (507) IU·L^{-1}, allant de 210 à 1667 IU·L^{-1}. Ces valeurs correspondent à une augmentation de 1,5 à 9 fois les valeurs normales (augmentation moyenne, 3,7 fois). Six patients étaient susceptibles à l'HM (SHM) après un IVCT positif. L'analyse musculaire histopathologique a révélé des changements non spécifiés chez quatre des patients SHM. Une prolifération mitochondriale a été observée chez les deux autres patients SHM et chez trois patients négatifs à l'HM. Aucune mutation pathogénique n'a été identifiée sur le gène RYR1 chez les cinq patients évalués.*

Conclusion *Lorsqu'on étudie des patients atteints d'hyperCKémie idiopathique, la susceptibilité à l'HM devrait être prise en compte, et il convient de conseiller ces patients afin de prévenir les complications anesthésiques dans la famille.*

The serum creatine kinase enzyme (CK) breaks up creatine phosphate to generate energy for cell use in very short-lived activities. The CK is present in various tissues, particularly in the muscle, and it can be separated into three fractions: CK-MM (prevalent in the skeletal muscle), CK-BB (brain), and CK-MB (cardiac muscle).[1] A steep rise in CK levels, more than ten times the reference value, with a quick return to the normal range is called rhabdomyolysis. It is associated with extensive destruction of muscle fibre accompanied by severe muscular symptoms and myoglobinuria.[2] A persistent rise in serum CK levels, defined by at least a 1.5-fold increase in the normal value, as evidenced by a minimum of two measurements at 30-day intervals, is known as hyperCKemia.[3]

HyperCKemia may be found in apparently healthy individuals, but it is also related to intense physical exercise, muscle lesions, pregnancy, use of alcohol and/or drugs (legal or illegal), neoplasms, infections, endocrinopathies, hemopathies, and neuromuscular disorders (clinically evident, subclinical, or even preclinical).[4] A diagnosis of idiopathic hyperCKemia is made when the hyperCKemia patient is asymptomatic (no muscular weakness or other significant neuromuscular symptoms), has no family history of a neuromuscular disease, and the complementary probes yield normal results (e.g., neurological examination, electroneuromyography, muscular anatomopathological analysis). In this context, idiopathic hyperCKemia may be a benign autosomal dominant condition with a greater prevalence in males and possibly resulting from combined changes in several genes.[3]

Nevertheless, patients with a previous diagnosis of idiopathic hyperCKemia could have their disease ascribed to other etiologies as research widens the range of complementary examinations in the field of neuromuscular disorders through such methods as biochemical assays and genetic studies. One of the neuromuscular diseases associated with hyperCKemia is malignant hyperthermia (MH).[5] This disorder is associated with mutations in the ryanodine receptor 1 (*RYR1*) gene, the calcium release channel in the sarcoplasmic reticulum, and it is expressed as a severe hypermetabolic crisis during anesthesia with halogenated anesthetics and/or succinylcholine. Malignant hyperthermia may also be expressed as a chronic myopathy, such as central core disease.[6] Susceptibility to MH is hereditary and autosomal dominant, and an *in vitro* muscular contracture test (IVCT) in response to exposure to halothane and caffeine is the gold standard for the diagnosis.[7]

This study investigated susceptibility to MH in patients with hyperCKemia via the IVCT and a search of mutations in the *RYR1* gene.

Methods

Design

This study was performed by collecting and analyzing the clinical and laboratorial data of patients (of all ages and both sexes) followed from 1997–2012 at the Center for Study, Diagnosis, and Research of Malignant Hyperthermia (CEDHIMA)—Discipline of Anesthesiology, Pain, and Intensive Care, Federal University of São Paulo, Brazil. This study was approved by the Ethics Committee of Federal University of São Paulo, and it was performed in accordance with the ethical standards presented in the 1964 Declaration of Helsinki and its later amendments (CEP-UNIFESP 0970/08). All patients provided written informed consent prior to participating in the study. Ten (5.8%) of a sample of 172

patients investigated for MH susceptibility with the IVCT were selected to participate in the study due to referral for a previous diagnosis of idiopathic hyperCKemia. One MH-susceptible (MHS) patient was excluded, however, due to unavailability of anatomopathological material. The diagnosis of idiopathic hyperCKemia was based on a serum CK at least 1.5-fold higher than the upper limit of normal (measured in at least two specimens at 30-day intervals), previous exclusion of non-muscular aetiologies, and investigation of underlying myopathies by anatomopathological analysis (at least histologic and histochemical assays). The CK values < 190 IU·L^{-1} in males and < 170 IU·L^{-1} in females were deemed normal.[8]

Methodology

As with all patients referred for IVCT, the nine patients were evaluated by a neurologist (medical history, physical and neurological examinations) and an anesthesiologist (preanesthesia assessment) and underwent laboratory tests (CK, hemogram, renal function, glucose, coagulogram, electrocardiogram, and a chest *x-ray*). Assessment proceeded with a vastus lateralis muscle biopsy to obtain, at the same moment, material for anatomopathological analysis (histologic, histochemical, and enzymatic assays) and IVCT. We did not perform metabolic studies of fatty acid transport/beta oxidation or glycolytic enzymes. Every histological and histochemical technique was carried out in accordance with reported methodology.[9] For the histological assays, the specimens were stained with hematoxylin and eosin, Gomori trichrome, Sudan, and periodic acid Schiff. The histochemical assays consisted of the following techniques: nicotinamide adenine dinucleotide dehydrogenase (NADH), succinate dehydrogenase, cytochrome *c* oxidase, and myofibrillar adenosine triphosphatase (ATPase) with alkaline (pH 9.4) and acid (pH 4.3) preincubation. Every pathological change was annotated (present/absent). Furthermore, we evaluated the percentage and cross-sectional area of types I and II fibres, the variability coefficient, and the percentage of fibres with nuclear centralization. To determine the cross-sectional area, approximately 150 viable skeletal muscle fibres of each type and from each patient were analyzed by optical microscopy in ATPase pH 9.4 in transverse sections. To obtain the variability coefficient, the standard deviation (SD) of the mean of the values obtained when measuring the cross-sectional area of muscle fibres was multiplied by 1,000 and divided by the mean of the values of the cross-sectional area. Values ≤ 250 were considered normal.[9] For the enzymatic assays of the respiratory chain, first protein concentration was measured by the mini method.[10] Enzyme activities were then quantified in a spectrophotometer at 30°C, as previously described for citrate synthase in the respiratory chain complexes (I—NADH ubiquinone oxidoreductase; II—succinate-ubiquinone oxidoreductase; II+III—succinate-cytochrome *c* reductase; III—ubiquinol-cytochrome *c* oxidoreductase; IV—cytochrome *c* oxidase).[11,12] The IVCT was performed following the protocol of the European Malignant Hyperthermia Group. The result was regarded as positive when there was muscle contracture in the presence of either halothane or caffeine, defined as an abnormal rise of at least 0.2 g from baseline in the presence of 2% halothane or 2 mM of caffeine.[7]

Molecular analysis

Molecular analysis was performed using genomic DNA extracted from peripheral blood lymphocytes in five patients with available DNA for the analysis. Next-generation sequencing was done using a customized NGS panel with 95 genes involved in neuromuscular disorders (NMD) (http://laboratorio.genoma.ib.usp.br/search?painel[]=Neuro-NGS), with Illumina's Nextera® kits for library preparation and custom capture of these genes. Sequencing was performed at the Illumina MiSeq sequencer, and variants were filtered and compared with the control populations of 1,000 Genomes, National Institutes of Health (NIH), 6500 Exome Sequencing Project from Washington University and the recently created Online Archive of Brazilian Mutations (ABraOM) (http://www.abraom.ib.usp.br/).[13] Rare variants were checked in the *RYR1* (OMIM#180901), *DNM2* (OMIM#602378), *CACNA1S* (OMIM#114208), and *PYGM* (OMIM#608455) genes and analyzed using bioinformatics tools. The pathogenicity of *de novo* variants was analyzed in prediction sites, including Mutation Taster, Predict SNP1, CADD, DANN, FATHMM, FunSeq2, GWAVA, VEP, SIFT, Polyphen1, Polyphen2, and Human Splicing Finder3.0.

Results

Epidemiological data

The mean (SD) age of the nine patients was 33 (12) yr, with an age range of 13–56. There were eight male patients, and five patients were of African descent.

Laboratory and clinical data

Five patients complained about effort intolerance and five complained about cramps and/or myalgia. In terms of family history, there were relatives with cleft palate/strabismus (patient 9), muscle hypertrophy (patient 7) or relatives who died during physical effort (patient 5) or surgery (patient 3). Four patients exhibited muscle hypertrophy, which was also reported in their relatives. A

Table 1 Clinical and laboratory data of patients with hyperCKemia

Patient	*n*/interval CK analysis	CK rise (x normal)	Personal history	Physical examination	%Type I fibre (normal≤55%)	%Type II fibre (normal≤80%)	Cross-sectional area type I fibre Mean (SD) (μm^2)	Cross-sectional area type II fibre Mean(SD) (μm^2)	VC	NC (%)	Other Changes
MHSh											
1	3 in 3 months (2010)	1.5 x	-	Everted ears Strabismus	43	57	5,263 (923)	6,028 (1,220)	203	↑4	-
2	3 in 3 months (2006/2007)	4 x	Effort intolerance	-	41	59	↓3,056 (1,351)*	5,203 (1,634)	↑445	↑5	-
3	12 in 3 years (2006-2008)	9 x	Effort intolerance	High arched palate Calf hypertrophy	45	55	↑8,523 (2,251)†	↑9,441 (3,299)†	↑321	0	Myofibrillar disorganization (*moth-eaten*)
4	3 in 3 months (2010)	2 x	Effort intolerance	Everted ears Strabismus Ptosis,Muscular hypertrophy	39	61	6,285 (1,356)	7,129 (1,558)	226	1	MP
5	8 in 10 years (2000-2009)	4 x	Cramps/myalgia LL	Everted ears Ptosis	40	60	3,512 (790)	4,039 (753)	214	0	MP
6	2 in 30 days (1997)	3.5 x	-	-	39	61	6,712 (3,246)	↑8,169 (2,587)†	↑483	0	Necrosis ↑conjunctive
MHN											
7	4 in 2 years (2009-2010)	4 x	Effort intolerance	Global muscular hypertrophy	↑63	37	7,427 (2,395)	↑8,166 (1,574)†	↑268	1	MP, COX-fibres Lipid accumulation
8	3 in 3 months (2010)	4.5 x	Effort intolerance	Everted ears Global muscular hypertrophy	52	48	↑8,678 (2,087)†	↑8,513 (2,212)†	↑254	1	MP, COX-fibres Lipid accumulation
9	4 in 7 months (2001)	1.5 x	-	-	↑61	39	↑5,261 (1,205†	3,942 (1,116)	289↑	↑5	MP, Necrosis Atrophy, Lipid accumulation Inflammation

CK = creatine kinase; COX = cytochrome *c* oxidase; LL = lower limbs; MHN = malignant hyperthermia negative; MHSh = susceptibility to malignant hyperthermia with contracture only in the presence of halothane; MP = mitochondrial proliferation; NC = nuclear centralization; VC = variability coefficient; ↓Decrease; ↑Increase; μm^2 = squared micrometer; *Atrophy; †Hypertrophy of muscle fibres

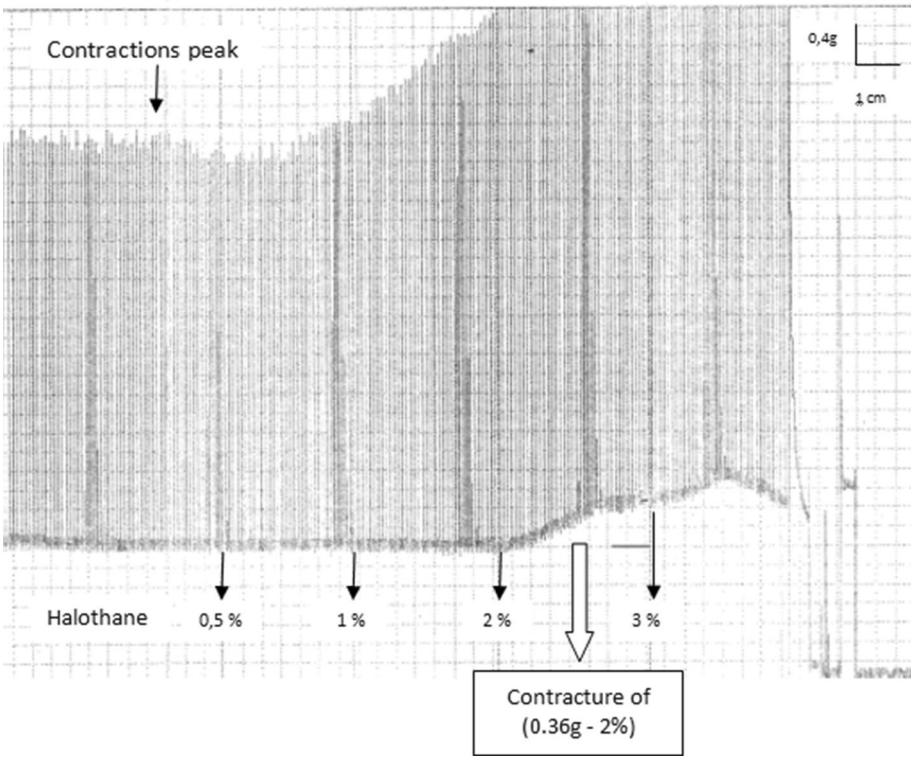

Figure Positive *in vitro* muscle contracture test in response to halothane (patient 5). Black arrows indicate the moment at which the halothane was added. White arrow shows contracture of 0.36 g at a concentration of 2% halothane, a finding consistent with a positive result, thus indicating susceptibility to malignant hyperthermia (MHSh)

variety of dysmorphisms were present in the group, such as everted ears, ptosis of the eyelids, strabismus, high palate, and short fingers. None of the patients exhibited changes in gait, muscle weakness, hypotonia, hypotrophy, myotonia, or sensitivity changes. None of the patients used any medications that could increase CK. In particular, none of the patients with dyslipidemia had used cholesterol-reducing agents, such as statins.

All patients showed high CK levels persistently from three months to ten years, with a mean (SD) CK value of 788 (507) IU·L^{-1} ranging from 210-1,667 IU·L^{-1}, which corresponds to a 1.5- to nine-fold increase in normal value (mean increase, 3.7-fold) (Table 1). Two patients (3 and 7) had normal electroneuromyography.

In vitro contracture test

The *IVCT* was positive in six of the patients with idiopathic hyperCKemia. All positive tests revealed MHSh—i.e., susceptibility to malignant hyperthermia with contracture only in the presence of halothane (Figure) (Table 1). There was no correlation between an increase in CK and contracture on IVCT at 2% halothane (r = 0.25; P = 0.63).

Histomorphometric analysis and enzymatic assays of the respiratory chain

The data related to qualitative and morphometric histopathological findings are listed in Table 1. A predominance of type I fibres was found in two nonsusceptible patients; there was no predominance of type II fibres. One patient susceptible to MH exhibited atrophy of type I fibres. Hypertrophy of fibres I or II was present in two MHS patients and in all three nonsusceptible patients. The coefficient of variability was higher in three of the MHS patients as well as in the three nonsusceptible patients. Increased nuclear centralization was found in two MHS patients and in one of the nonsusceptible patients. Subsarcolemmal mitochondrial proliferation was the most common qualitative change found in two of the MHS patients and in the three nonsusceptible patients. Lipid accumulation was the second most common qualitative change, which was exhibited by three nonsusceptible patients. The final diagnosis in the MHS patients was mainly minimal and unspecified changes (4), followed by mitochondrial proliferation (2); and the final diagnosis in all nonsusceptible patients was mitochondrial proliferation. Enzymatic assays of the respiratory chain were performed in patient 9 and were normal.

Molecular analysis

No known pathogenic mutations in the 95 genes involved in NMD were found in the five studied patients. On the other hand, a detailed analysis of the *RYR1*, *CACNA1S, DNM2,* and *PYGM* genes identified some polymorphisms in these five patients (Table 2). All the identified variants were described in the Single Nucleotide Polymorphism database (dbSNP), and according to several prediction software programs, all were considered neutral with low prediction of pathogenicity.

Table 2 Genetic variants identified in the five MHSh patients molecularly studied

Patient	Gene	Region	Variant - heterozygous	Predicted pathogenicity	References
1	*CACNA1S*	Exonic	*CACNA1S*:NM_000069:exon26:c.A3261G:p.Q1087Q	Low	
	RYR1	Intronic	pos.38948111 C>G	Low	
2	*CACNA1S*	Exonic	*CACNA1S*:NM_000069:exon40:c.T5008A:p.Y1670N	Low	
3	*DNM2*	Exonic	*DNM2*:NM_001190716:exon20:c.C2514T:p.I838I	Low	
4	*DNM2*	Intronic	rs113192269 pos.10870493 A>G (promoter)	Low	
	DNM2	Intronic	rs200354263 pos.10904363 G>A	Low	
5	*CACNA1S*	Exonic	*CACNA1S*:NM_000069:exon11:c.G1548A:p.S516S	Low	(38)
	RYR1	Exonic	*RYR1*:NM_001042723:exon7:c.C573T:p.D191D	Low	(18,20,39)
	RYR1	Exonic	*RYR1*:NM_001042723:exon10:c.G897C:p.V299V	Low	
	RYR1	Exonic	*RYR1*:NM_001042723:exon28:c.C3876A:p.L1292L	Low	
	RYR1	Exonic	*RYR1*:NM_001042723:exon28:c.C4055G:p.A1352G	Low	
	RYR1	Exonic	*RYR1*:NM_001042723:exon40:c.C6612T:p.H2204H	Low	
	RYR1	Exonic	*RYR1*:NM_001042723:exon48:c.G7737A:p.V2579V	Low	

MHSh = susceptibility to malignant hyperthermia with contracture only in the presence of halothane

Discussion

Patients with hyperCKemia may be identified incidentally during a routine examination, a preoperative evaluation, or a laboratory evaluation prior to the introduction of statins. Another possibility is the investigation of effort intolerance or muscle pain or cramps. These complaints were present in over half of our sample and in other similar series.[5,14-16]

Initial screening of idiopathic hyperCKemia must be oriented towards exclusion of obvious situations, such as systemic diseases, drug side effects, and muscle trauma. If the initial evaluation is normal, the patient must undergo neurological assessment and be evaluated for neuromuscular diseases.[16] Among these, ryanodinopathies, muscle diseases related to *RYR1,* have been detected, especially after the introduction of the next-generation sequencing methods.[17] Nevertheless, the results of MH-susceptibility research in patients with idiopathic hyperCKemia are greatly diversified and dependent on the patients' background and the selection criteria. Moreover, diagnostic criteria for MH susceptibility by IVCT were specifically determined for individuals with anesthetic-induced MH. In myopathies not related to anesthetic-induced MH, an MH-susceptible IVCT has unclear significance, though there is general agreement that these patients be treated as MHS. On the other hand, an increasing number of myopathies have been linked with causal *RYR1* mutations.[5,7,14,15,18] After a positive IVCT in 49 patients, Weglinski *et al*.,[5] in the United States, reported that 49% of the patients were MHS with no neurological alterations but with a persistent increase in CK levels. On the other hand, two Italian studies showed divergent findings. Malandrini *et al.*[15] found that 5% of 37 patients with hyperCKemia were MHS, while Prelle *et al.*[4] found that 23% of 13 patients with idiopathic hyperCKemia (investigated through IVCT) were MHS. A recent Canadian study of IVCT testing in probands without adverse reaction to anesthetics found MH susceptibility in 66% of 71 patients with hyperCKemia.[19] The higher frequency of MH susceptibility in our study, namely two-thirds, may be related to different ethnic characteristics (i.e., predominance of African descendants—which may also occur in the USA) and the fact that almost half of the patients in our sample exhibited muscular hypertrophy. This condition in families suspected of having malignant hyperthermia might be an indicator of both MH susceptibility and more severe MH crises.[20-22] In MH, both muscular hypertrophy and atrophy could be dependent on changes in intracellular calcium metabolism, so that minimal calcium leakage would lead to hypertrophy and excessive leakage or decoupling of the excitation-contraction mechanism would be associated with atrophy and weakness.[23]

With respect to ethnicity, study of a Dutch population showed higher CK levels in African descendants than in Caucasians, and values in both groups were higher on average than the normal reference values. This study also drew attention to the need for three days without intense physical activity before taking CK measurements in order to avoid increases due to the effort.[24] Despite the presence of African descendants in our sample, we have chosen a unique CK upper limit due to the high degree of miscegenation of the Brazilian population. This makes it impractical to propose a clear separation of African, European, and Amerindian ethnic contribution. Moreover, an assessment of Brazilian healthy individuals agrees with the CK limit used.[25,26]

As with other studies, male patients and those with complaints such as myalgia and cramps predominated in our study.[5,15] The CK level is lower in females, a fact

possibly related to higher estrogenic levels.[27] Greater CK levels in males is attributed to greater muscle mass and to potential differentiated expression of proteins connected with calcium metabolism in males.[28,29] Muscle pain is a common complaint in *RYR1*-related myopathies. There is increasing awareness of the relationship of *RYR1* mutations with rhabdomyolysis and/or effort myalgia.[14,19,30]

In our study, the death of relatives during surgery or physical activity was an indicator of subsequent MH susceptibility, and it is clinical information that could guide research early on towards MH. In this regard, Sunohara *et al.*[31] reported a patient with idiopathic hyperCKemia whose sister presented with MH, and Kasi[32] emphasizes that families with idiopathic hyperCKemia should receive guidance and be investigated for MH, even if family members were asymptomatic. Kasi reported a family with idiopathic hyperCKemia where a member developed anesthetic-induced MH and was found to have a causative *RYR*1 mutation. This suggests that patients with idiopathic hyperCKemia may have an *RYR1*-associated myopathy, and it emphasizes the need for extensive investigation before establishing the diagnosis of idiopathic hyperCKemia.[32]

The presence of dysmorphisms detected during a physical examination in most of the MHS patients in this study could be another factor raising the possibility of MH in the investigation of idiopathic hyperCKemia. This is further supported by the presence of dysmorphisms in some diseases associated with MH, such as the King-Denborough syndrome and Native American myopathy.[6,33]

Study of muscular anatomopathology in the MHS patients resulted mostly in a final diagnosis of unspecified changes; whereas, in the case of patients nonsusceptible to MH, the resulting diagnosis was mitochondrial proliferation. Anatomopathological study does not allow for a diagnosis of MH susceptibility as up to 78% of the MHS patients can have normal features.[34] Histomorphometric analysis does not reveal any distinctive features in the MHS patients, with the exception of occasional cores (regions without oxidative activity in the muscle fibre). This technique, however, makes it possible to suspect or exclude other muscle diseases that may express as idiopathic hyperCKemia, such as muscular dystrophies (dystrophinopathy, dyspherlinopathy, caveolinopathy, and calpainopathy), myofibrillar myopathies, inflammatory myopathies, metabolic myopathies (glycogenosis or mitochondrial myopathies), adenylate deaminase deficiency, myopathy with tubular aggregates, carnitine palmitoyltransferase II deficiency, and myotonia.[4,15,16] According to the population and the selection criteria, a normal muscular anatomopathological study can be found in 8–71% (3/37 to 10/14) of patients with idiopathic hyperCKemia.[16]

Mitochondrial alterations comprised the second most frequent anatomopathological diagnosis in the MHS group, but it can be a nonspecific alteration also present in the nonsusceptible MH group. Such changes, along with carnitine palmitoyltransferase deficiency, were previously reported in MH.[35,36] The transgenic *RYR1* mice, mostly male, also exhibit mitochondrial alterations, such as an increased number and abnormal distribution of mitochondria.[37] These mitochondrial alterations may be related to MH as a change secondary to a chronic regimen of increased intracellular calcium levels.[18] In idiopathic hyperCKemia, up to 5% of patients from other studies presented mitochondrial abnormalities, and a multiple deletion of mitochondrial deoxyribonucleic acid was reported in one patient.[4,15]

Molecular analysis failed to identify a concordance between a specific gene mutation and the phenotype in this study. On the other hand, several polymorphisms were found in the genes *RYR1*, *DNM2*, and *CACNA1S*, but with no particular pattern that could suggest an association with the hyperCKemia. Two *RYR*1 variants (D191D and A1352G) have been reported previously in association with MH, but A1352G was later considered a polymorphism of African American population with a frequency of 2.7%.[18,20,38,39] Interestingly, *RYR*1 variant A1352G was reported in homozygosis in two independent African American male patients, one with statin myopathy and the other with complaints of myalgia, cramps, weakness, rhabdomyolysis, and myoglobinuria.[18] While none of these variants is probably the primary cause of muscle changes, we cannot dismiss the possibility that these polymorphisms could act as a modifier or potentialize the action of any other yet unidentified gene mutations.

In conclusion, when investigating patients with idiopathic hyperCKemia, it is crucial to take into account potential susceptibility to MH and to offer specific guidance to prevent anesthetic complications in the family, such as avoiding the use of anesthetics that may have a triggering effect (halogenated anesthetics and succinylcholine) and warning surgical and anesthesia teams in advance.

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References

1. *Roman BB*, *Wieringa B*, *Koretsky AP*. Functional equivalence of creatine kinase isoforms in mouse skeletal muscle. J Biol Chem 1997; 272: 17790-4.
2. *Zutt R*, *van der Kooi AJ*, *Linthorst GE*, *Wanders RJ*, *deVisser M*. Rhabdomyolysis: review of the literature. Neuromuscul Disord 2014; 24: 651-9.
3. *Kyriakides T*, *Angelini C*, *Schaefer J*, *European Federation of Neurological Societies*, *et al*. EFNS guidelines on the diagnostic approach to pauci- or asymptomatic hyperCKemia. Eur J Neurol 2010; 17: 767-73.
4. *Prelle A*, *Tancredi L*, *Sciacco M*, *et al*. Retrospective study of a large population of patients with asymptomatic or minimally symptomatic raised serum creatine kinase levels. J Neurol 2002; 249: 305-11.
5. *Weglinski MR*, *Wedel DJ*, *Engel AG*. Malignant hyperthermia testing in patients with persistently increased serum creatine kinase levels. Anesth Analg 1997; 84: 1038-41.
6. *Almeida da Silva HC, dos Santos Almeida C, Mendes Brandão JC*, *et al*. Malignant hyperthermia in Brazil: analysis of hotline activity in 2009. Braz J Anesthesiol 2013; 63: 13-9.
7. *Hopkins PM*, *Rüffert H*, *Snoeck MM*, *European Malignant Hyperthermia Group*, *et al*. European Malignant Hyperthermia Group guidelines for investigation of malignant hyperthermia susceptibility. Br J Anaesth 2015; 115: 531-9.
8. *Thomas L*, *Muller M*, *Schumann G*, *et al*. Consensus of DGKL and VDGH for interim reference intervals on enzymes in serum. J Lab Med 2005; 29: 301-8.
9. *Dubowitz V*. Muscle Biopsy: a Practical Approach. 2nd ed. London: Baillière Tindal; 1985 .
10. *Lowry OH*, *Rosebrough NJ*, *Farr AL*, *Randall RJ*. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193: 265-75.
11. *Sobreira C*, *Hirano M*, *Shanske S*, *et al*. Mitochondrial encephalomyopathy with coenzyme Q10 deficiency. Neurology 1997; 48: 1238-43.
12. *Birch-Machin MA*, *Briggs HL*, *Saborido AA*, *Bindoff LA*, *Turnbull DM*. An evaluation of the measurement of the activities of complexes I-IV in the respiratory chain of human skeletal muscle mitochondria. Biochem Med Metab Biol 1994; 51: 35-42.
13. *Naslavsky MS*, *Yamamoto GL*, *Ferreira de Almeida T*, *et al*. Exomic variants of an elderly cohort of Brazilians in the ABraOM database. Hum Mutat 2017. DOI:10.1002/humu.23220.
14. *Dlamini N*, *Voermans NC*, *Lillis S*, *et al*. Mutations in RYR1 are a common cause of exertional myalgia and rhabdomyolysis. Neuromuscul Disord 2013; 23: 540-8.
15. *Malandrini A*, *Orrico A*, *Gaudiano C*, *et al*. Muscle biopsy and in vitro contracture test in subjects with idiopathic hyperCKemia. Anesthesiology 2008; 109: 625-8.
16. *Silvestri NJ*, *Wolfe GI*. Asymptomatic/pauci-symptomatic creatine kinase elevations (hyperckemia). Muscle Nerve 2013; 47: 805-15.
17. *Sano K*, *Miura S*, *Fujiwara T*, *et al*. A novel missense mutation of RYR1 in familial idiopathic hyper CK-emia. J Neurol Sci 2015; 356: 142-7.
18. *Vladutiu GD*, *Isackson PJ*, *Kaufman K*, *et al*. Genetic risk for malignant hyperthermia in non-anesthesia-induced myopathies. Mol Genet Metab 2011; 104: 167-73.
19. *Timmins MA*, *Rosenberg H*, *Larach MG*, *Sterling C*, *Kraeva N*, *Riazi S*. Malignant hyperthermia testing in probands without adverse anesthetic reaction. Anesthesiology 2015; 123: 548-56.
20. *Sambuughin N*, *Capacchione J*, *Blokhin A*, *Bayarsaikhan M*, *Bina S*, *Muldoon S*. The ryanodine receptor type 1 gene variants in African American men with exertional rhabdomyolysis and malignant hyperthermia susceptibility. Clin Genet 2009; 76: 564-8.
21. *Larach MG*, *Brandom BW*, *Allen GC*, *Gronert GA*, *Lehman EB*. Cardiac arrests and deaths associated with malignant hyperthermia in North America from 1987 to 2006: a report from the North American Malignant Hyperthermia Registry of the Malignant Hyperthermia Association of the United States. Anesthesiology 2008; 108: 603-11.
22. *Rueffert H*, *Wehner M*, *Ogunlade V*, *Meinecke C*, *Schober R*. Mild clinical and histopathological features in patients who carry the frequent and causative malignant hyperthermia RyR1 mutation p.Thr2206Met. Clin Neuropathol 2009; 28: 409-16.
23. *MacLennan DH*. Ca2+ signalling and muscle disease. Eur J Biochem 2000; 267: 5291-7.
24. *Brewster LM*, *Mairuhu G*, *Sturk A*, *van Montfrans GA*. Distribution of creatine kinase in the general population: implications for statin therapy. Am Heart J 2007; 154: 655-61.
25. *Kimura L*, *Ribeiro-Rodrigues EM*, *De Mello Auricchio MT*, *Vicente JP*, *Batista Santos SE*, *Mingroni-Netto RC*. Genomic ancestry of rural African-derived populations from Southeastern Brazil. Am J Hum Biol 2013; 25: 35-41.
26. *Coelho DB*, *Morandi RF*, *Melo MA*, *Andrade RL*, *da Paixão RC*, *Silami-Garcia E*. Analysis of the trends of creatine kinase levels during the preseason of a professional soccer team. Rev Bras Cineantropom Desempenho Hum 2014; 16: 129-35.
27. *Reijneveld JC*, *Notermans NC*, *Linssen WH*, *Wokke JH*. Benign prognosis in idiopathic hyper-CK-emia. Muscle Nerve 2000; 23: 575-9.
28. *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.
29. *Islander G*, *Rydenfelt K*, *Ranklev E*, *Bodelsson M*. Male preponderance of patients testing positive for malignant hyperthermia susceptibility. Acta Anaesthesiol Scand 2007; 51: 614-20.
30. *Snoeck M*, *Engelen BGM*, *Küsters B*, *et al*. RYR1-related myopathies: a wide spectrum of phenotypes throughout life. Eur J Neurol 2015; 22: 1094-112.
31. *Sunohara N*, *Takagi A*, *Nonaka I*, *Sugita H*, *Satoyoshi E*. Idiopathic hyperCKemia. Neurology 1984; 4: 544-7.
32. *Kasi PM*. Malignant hyperthermia and idiopathic hyperCKemia. Case Rep Med 2011. DOI:10.1155/2011/194296.
33. *D'Arcy CE*, *Bjorksten A*, *Yiu EM*, *et al*. King-denborough syndrome caused by a novel mutation in the ryanodine receptor gene. Neurology 2008; 71: 776-7.
34. *Orlov D*, *Keith J*, *Rosen D*, *Croul S*, *Kraeva N*, *Riazi S*. Analysis of histomorphology in malignant hyperthermia-susceptible patients. Can J Anesth 2013; 60: 982-9.
35. *Mezin P*, *Payen JF*, *Bosson JL*, *Brambilla E*, *Stieglitz P*. Histological support for the difference between malignant hyperthermia susceptible (MHS), equivocal (MHE) and negative (MHN) muscle biopsies. Br J Anaesth 1997; 79: 327-31.

36. *Kozak-Ribbens G*. Mitochondrial disordens in malignant hyperthermia. In: Moro M, Kikuchi H, Yuge O, editors. malignant hyperthermia. Tokyo: Springer; 1994. p. 1129-36.
37. *Yuen B*, *Boncompagni S*, *Feng W*, *et al*. Mice expressing T4826I-RYR1 are viable but exhibit sex- and genotype-dependent susceptibility to malignant hyperthermia and muscle damage. FASEB J 2012; 26: 1311-22.
38. *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.
39. *Levano S*, *Vukcevic M*, *Singer M*, *et al*. Increasing the number of diagnostic mutations in malignant hyperthermia. Hum Mutat 2009; 30: 590-8.