

# DOMINANT AND RECESSIVE RYR1 MUTATIONS IN ADULTS WITH CORE LESIONS AND MILD MUSCLE SYMPTOMS

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**ABSTRACT:** *Introduction:* Ryanodine receptor gene (*RYR1*) mutations have been associated with central core disease (CCD), multiminicore/minicore/multicore disease (MmD), and susceptibility to malignant hyperthermia (MH). *Methods:* Patients with muscle symptoms in adulthood, who had features compatible with CCD/MmD, underwent clinical, histological, and genetic (*RYR1* and *SEPNI* genes) evaluations. Published cases of CCD and MmD with adult onset were also reviewed. *Results:* Eight patients fulfilled the criteria for further analysis. Five *RYR1* mutations, 4 of them unreported, were detected in 3 patients. Compound heterozygosity was proven in 1 case. *Conclusions:* To our knowledge, this is the only report of adult onset associated with recessive *RYR1* mutations and central core/multiminicores on muscle biopsy. Although adult patients with CCD, MmD, and minimally symptomatic MH with abnormal muscle biopsy findings usually have a mild clinical course, differential diagnosis and carrier screening is crucial for prevention of potentially life-threatening reactions to general anesthesia.

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**C**entral core disease (CCD; MIM #117000) is a congenital myopathy in which type I skeletal muscle fibers exhibit amorphous areas with sarcomere disorganization (cores) that lack mitochondria and oxidative enzyme activity along the length of the myofiber.<sup>1</sup> CCD typically presents in infancy with hypotonia and motor developmental delay. Orthopedic complications are common, and susceptibility to malignant hyperthermia (MH) is a frequent association,<sup>2</sup> because CCD and MH are allelic conditions. They both result predominantly from dominant mutations in the skeletal muscle ryanodine receptor gene (*RYR1*) that encodes the principal sarcoplasmic reticulum calcium release channel.

Multiminicore/minicore/multicore disease (MmD; MIM #117000, #602771, and #255320) can be distinguished from CCD based on histological findings and

by characteristic clinical features in some cases. Histologically, type I and type II muscle fibers are characterized by multiple cores that do not extend along the entire length of the myofiber and short areas of sarcomeric disorganization lacking mitochondria.<sup>1</sup> MmD was initially associated with recessive mutations in the selenoprotein N1 gene (*SEPNI*),<sup>3</sup> but there have been increasing reports of recessive mutations in the *RYR1* gene being related to this entity,<sup>1,4–7</sup> and in some cases also with enhanced susceptibility to MH.<sup>8–11</sup> The onset of MmD is usually in infancy or childhood with hypotonia or proximal weakness, and only a limited number of cases have been reported with onset in adult life.<sup>12–15</sup> These are occasionally associated with progressive muscle weakness and respiratory or cardiac failure.

MH is a pharmacogenetic disorder in which susceptible individuals develop generalized muscle contracture, followed by a hypermetabolic state due to massive calcium release from the sarcoplasmic reticulum when they are exposed to inhaled general anesthetics or to the depolarizing muscle relaxant succinylcholine. Abnormal muscle biopsy findings, namely central cores and multiminicores, have been described in MH,<sup>1,8,11</sup> and a clear separation between the CCD, MmD, and MH is not always possible. Thus, it is always advisable to consider the possibility of MH whenever cores, multiminicores, or both features are found on muscle biopsy.

We report a series of adult patients with muscle symptoms, histological alterations compatible with cores or multiminicores, and distinct genetic inheritance patterns. Our aim is to describe clinical, histological, and genetic features of these patients and also to review the previously reported cases in the literature in order to improve the awareness of adult-onset presentations of *RYR1* mutations and, subsequently, prevent life-threatening anesthetic complications.

## METHODS

We analyzed muscle biopsies performed in the Neuropathology Laboratory of Santa Maria Hospital between 2002 and 2007 in order to select adult

**Abbreviations:** CCD, central core disease; CK, creatine phosphokinase; COX, cyclooxygenase; E-C, excitation-contraction; HGVS, Human Genome Variation Society; MH, malignant hyperthermia; MmD, multiminicore/minicore/multicore disease; NADH-TR, nicotinamide adenine dinucleotide plus hydrogen-terazolium reductase; *RYR1*, ryanodine receptor 1 gene; PAS, periodic acid-Schiff; SDH, succinate dehydrogenase; SECIS, selenocysteine insertion sequence; *SEPNI*, selenoprotein N1 gene

**Key words:** adult presentation, central core disease, malignant hyperthermia, multiminicore disease, *RYR1*

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patients with nonspecific muscle complaints and histopathological findings suggestive of CCD/MmD. All biopsies performed during this period in adults due to nonspecific muscle complaints and without a final diagnosis were reanalyzed ( $n = 309$ ).

**Muscle Histology Studies.** Specimens were obtained by an open muscle biopsy of the left deltoid. They were frozen in isopentane cooled in liquid nitrogen and stained using standard techniques as described by Dubowitz and Sewry.<sup>16</sup> In addition, 6-mm sections were immunolabeled with antibodies to desmin (mouse monoclonal; Dako) and  $\alpha\beta$ -crystallin (rabbit polyclonal; Chemicon).

**Clinical Reassessment.** Patients included in the study were clinically re-evaluated, and data were collected according to the following items: disease onset (after 18 years of age); neuromuscular symptoms and laboratory findings of muscle weakness, muscle atrophy, myalgia, and abnormal creatine kinase (CK) levels; history of previous incidents related to anesthetic procedures (malignant hyperthermia); and family history. All patients had a history of normal motor development. Signs or symptoms compatible with muscle involvement before adulthood represented an exclusion criterion.

**Literature Review.** A literature review was performed, using a PubMed search, to find reports written in English of patients who presented with adult-onset CCD or MmD.

**Genetic Analysis.** Due to the exceptionally large size of the *RYR1* gene, mutation screening was performed in a two-step strategy. All cases were initially subjected to direct genomic sequencing in the three mutational hotspots (exons 1–17, 39–52, and 92–105). The remaining *RYR1* exons were then sequenced in patients 2, 4, 5, 7, and 8. Briefly, genomic DNA was polymerase chain reaction (PCR)-amplified using intronic M13-tailed primers. Amplicons were then sequenced (BigDye Terminator Cycle Sequencing Kit, version 1.1; Applied Biosystems, Foster City, California) and analyzed on a genetic analyzer (ABI 3130xl; Applied Biosystems). Sequence analysis was aided by SeqScape v2.5 software (Applied Biosystems). Variant nomenclature was according to the recommendations of the Human Genome Variation Society (HGVS),<sup>17</sup> using the reference cDNA sequence filed under GenBank Accession No. NM\_000540.2. For undocumented variants, a population screen was performed on at least 150 anonymous control samples. To further validate the pathogenicity of unreported missense substitutions, phylogenetic conservation analysis was performed using ryanodine receptor I sequences from several organisms

(list of sequence accession numbers in Supplementary Material) aligned by ClustalX software (version 2.0.12).<sup>18</sup> In patients 5, 7, and 8, cDNA analysis was conducted to further characterize novel variants. RNA was extracted from muscle biopsies and converted to cDNA used for PCR amplification and subsequent sequencing. Analysis of the *SEPNI* gene was conducted in *RYR1* mutation-negative cases, namely 4, 5, 7, and 8, by direct genomic sequencing of all exons and surrounding intronic sequences and the selenocysteine insertion sequence (SECIS) element located in the 3' untranslated region.

## RESULTS

**Clinical and Histological Findings.** Tables 1 and 2 show the clinical and histological characteristics of the patients included in this study. One patient had proximal lower limb weakness, and the remaining 7 patients complained of myalgia. Four patients had elevated CK levels, between 421 and 619 IU/L (normal reference values: 32–294 IU/L). Three patients had personal or family history of serious adverse reactions to anesthetic procedures (patients 1, 2, and 6), leading to death in 1 case (patient 2). Two patients with identified mutations had associated rheumatological complaints: generalized arthralgia and fatigue in patient 1, and systemic lupus erythematosus in patient 3.

Muscle biopsy studies showed nonspecific findings in all patients, namely variation in fiber size and central nuclei. Type 1 fiber predominance was present in 6 cases. Enzymatic reactions [nicotinamide adenine dinucleotide plus hydrogen (NADH)-tetrazolium reductase, succinate dehydrogenase (SDH), and cyclooxygenase (COX)] disclosed areas of absent activity characteristic of cores and/or multimimicore (Fig. 1). Central accumulation of periodic acid-Schiff (PAS)-stained material was visualized in 6 cases (patients 1, 2, 3, 5, 6, and 7). In addition, desmin immunocytochemical studies revealed antibody deposition colocalized with cores in 3 patients (patients 2, 3, and 5).

**Genetic Studies.** Five heterozygous missense mutations, four of which were previously unreported, were detected in patients 1, 3, and 6 (Table 2).

In patient 1, two heterozygous mutations were identified: c.6612C>G (p.His2204Gln) and c.14228G>A (p.Gly4743Asp) in exons 40 and 98, respectively. These novel mutations were not detected in 300 control alleles, and ryanodine receptor 1 protein alignments suggested that the alterations affected highly conserved amino acids (Fig. 2A and B). Several family members of patient 1 were also studied (respective genotypes shown in Fig. 3). The brother of patient 1 (individual II.3),

**Table 1.** Clinical and analytical data of patients.

Patient	Age*/gender	Spine/joint malformations	Muscle weakness	Muscle atrophy	Myalgia	Malignant hyperthermia	CK <sup>†</sup> (IU/L)	Additional features
1	43 y /F	Generalized arthralgia	—	—	+	Adverse reaction to succinylcholine during caesarean section	456	Fatigue, male brother with MH during surgery and elevated CKs
2	48 y /F	Pes planus	—	Calf hypertrophy	+	—	601	AST and ALT elevation, son died during surgical procedure
3	55 y / F	—	—	—	+	Not submitted to anesthetic procedures before RYR1 study	264	SLE myopathic EMG fatigue
4	58 y / M	Pes cavus	LL proximal	—	—	—	270	Myopathic EMG
5	23y / M	—	—	—	+	—	N	—
6	59 y / M	—	—	—	+	Cardiorespiratory arrest and rhabdomyolysis during anesthetic induction	619	Fatigue
7	49 y/ M	—	—	—	+	—	421	—
8	56 y/ M	—	—	—	+	—	N	—

y, years; m, male; F, female; LL, lower limbs; +, presence; —, absence; MH, malignant hyperthermia; N, normal; EMG, electromyogram; AST, aspartate aminotransferase; ALT, alanine transaminase; SLE, systemic lupus erythematosus.

\*At first symptoms.

<sup>†</sup>Highest value during follow-up.

who shows clinical signs compatible with MmD, has the same *RYR1* genotype. The patient's sons, brother, and nephews, who harbor only one of the

mutations, are clinically asymptomatic. These results are suggestive of an autosomal-recessive pattern of inheritance for adult-onset MmD in this family.

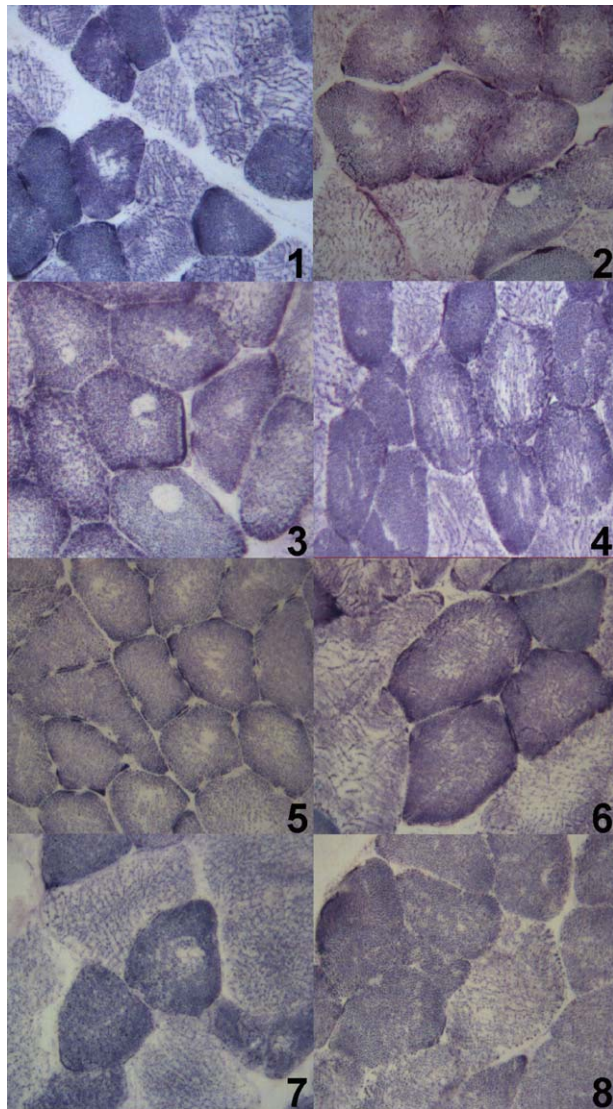
**Table 2.** Histological and molecular data of patients..

Patient	Muscle histology	<i>RYR1</i> genotype*			<i>SEPN1</i> genotype
		Variant/pathogenicity	Exon	Predicted polypeptide change	
1	Central cores, multiple minicores, type I fiber predominance	c.6612C>G / mut. c.14228G>A / mut. (only hotspots screened)	40 98	p.His2204Gln, p.Gly4743Asp	Not performed
2	Central and eccentric cores, type I fiber predominance	No mut. detected	—	—	Not performed
3	Central and peripheral cores, predominantly on type I fibers; type I fiber predominance	c.479A>G / mut. <sup>19</sup> (only hotspots screened)	6	p.Glu160Gly	Not performed
4	Central cores, multiple minicores, type I fiber predominance	No mut. detected	—	—	No mut. detected
5	Central and eccentric cores, type I fiber predominance	No mut. detected	—	—	No mut. detected
6	Multiminicores, type I fiber predominance	c.10097G>A / mut. c.11798A>G / mut. (only hotspots screened)	6786	p.Arg3366His, p.Tyr3933Cys	Not performed
7	Multiminicores, normal fiber type differentiation	c.13477C>G / poly. c.14505G>A / poly.	92100	p.Pro4493Ala—	No mut. detected
8	Multiminicores, normal fiber type differentiation	c.5360C>T / poly.,1,20 c.6178G>T / poly.1,20	3438	p.Pro1787Leu, p.Gly2060Cys	No mut. detected

mut., mutation; poly., polymorphism.

\*Variants detected in *RYR1* coding sequence, reference sequence accession number NM\_000540.2.





**FIGURE 1.** Skeletal muscle sections of the 8 patients studied, stained with succinate dehydrogenase, revealing core and/or multimimicore lesions. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

Patient 3 had the heterozygous mutation c.479A>G (p.Glu160Gly) in exon 6. This variation was previously reported to be associated with an MH/CCD phenotype.<sup>19</sup>

In patient 6, two heterozygous missense variants were detected: c.10097G>A (p.Arg3366His) in exon 67 and c.11798A>G (p.Tyr3933Cys) in exon 86. Both variants were considered pathogenic, because they were not detected in control samples and predictably altered conserved residues (Fig. 2C and D). Because no relatives were available for study, mutation allelism could not be confirmed or excluded in this case.

In the remaining patients (2, 4, 5, 7, and 8), the *RYR1* gene was fully sequenced, but no causative mutations were identified. Seven *RYR1* polymorphisms were also detected in this study. These

included two previously reported missense polymorphisms c.5360C>T and c.6178G>T<sup>1,20</sup> in patient 8, and a novel missense change c.13477C>G (p.Pro4493Ala) in patient 7. The latter was also detected in heterozygosity in 1 control sample (1 of 300 alleles) and affects a non-conserved amino acid. The remainder were a silent change (c.14505G>A) in exon 100 and three intronic base changes: c.3765+130A>G (intron 27), c.5548-76G>A (intron 34), and c.10627-45A>T (intron 71). These four variants were further analyzed at the cDNA level, but none appeared to influence mRNA splicing.

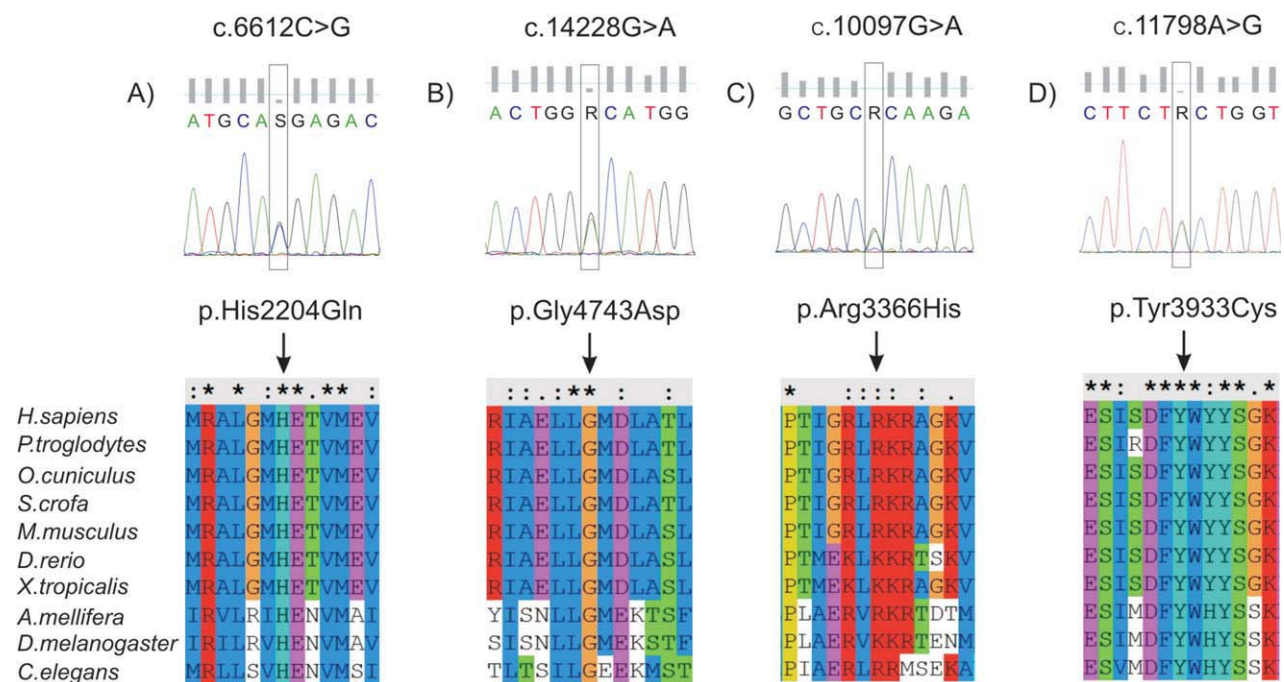
The *SEPN1* gene was also screened for pathogenic changes in patients 4, 5, 7, and 8, but no causative mutations were detected.

**Late-Onset CCD/MmD Literature Review.** From the literature review, cases were selected for comparative evaluation. HyperCKemia<sup>13,15,21-23</sup> and proximal muscle weakness<sup>12,13,23,24</sup> were the most frequently reported features. Myalgia was described less often.<sup>14,21,23</sup> One case was associated with scoliosis,<sup>24</sup> and another was characterized by an axial myopathy with prominent involvement of the spine extensors.<sup>22</sup> The most consistent findings in muscle biopsies were type I fiber predominance, increased number of central nuclei, and the absence of enzymatic staining on SDH and NADH-tetrazolium reductase. Labeling of desmin in the core region was the most frequent immunohistochemical finding. A detailed description of these patients is presented in Table 3.

## DISCUSSION

In this work we have presented a clinical, histological, and genetic report on patients in whom cores or multimimicores were detected on muscle biopsies performed due to muscle complaints appearing during adulthood. Although most patients did not meet all the diagnostic criteria of adult-onset CCD or MmD, muscle complaints were considered significant enough to justify a muscle biopsy, where central cores or multimimicores were found. These findings oriented genetic studies and helped prevent additional anesthetic complications.

Adult patients with CCD/MmD/MH and/or abnormal muscle biopsy findings usually have mild phenotypes (myalgia, mild CK elevation, and discrete proximal and/or axial weakness), but we also found reports of progressive disease with respiratory failure<sup>13</sup> and hypertrophic cardiomyopathy.<sup>12</sup> The association with joint deformities in 2 of our patients was also reported in the literature. When axial muscles are involved, scoliosis is a common finding.<sup>22,23,25</sup> Unexplained joint deformities in patients with muscle complaints should therefore raise the suspicion of CCD or MmD.



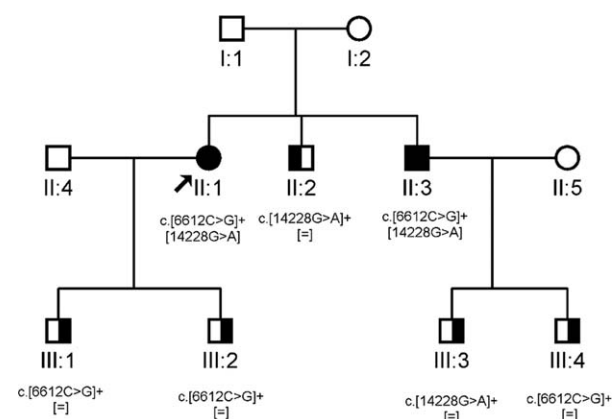
Rheumatological disorders, as found in our patients 1 and 3, have also been described previously in association with these myopathies,<sup>26</sup> although the physiopathological relation between them remains unexplained. In our series, muscle pain (myalgia or cramps) was one of the most frequent clinical signs and, in some cases, the only complaint pointing toward a muscle disorder.

Two patients had both central cores and multi-minicores on the biopsy, and one of these had *RYR1* mutations. A continuum between the histopathological appearance of CCD and MmD related to *RYR1* mutations has been suggested,<sup>11,15</sup> with evolution over time, as observed on consecutive muscle biopsies of the same patient.<sup>7,28</sup>

The histological features found in this study are similar to those described in the literature (see Table 2). The scarcity of clinical findings contrasted with the marked morphological changes, namely type 1 fiber predominance, central nuclei, variability in fiber size, and cores and multinicores on SDH and NADH staining. Immunohistochemistry with desmin can be useful to show the presence of cores, as was demonstrated by the colocalization of cores and desmin accumulation. The disturbance of desmin may be related to the ultrastructural appearance of misaligned myofibrils, because desmin links the myofibrils at the Z-line.<sup>27,28</sup>

In our group of patients, five missense mutations were identified in the *RYR1* gene. We could

not find a clear clinical or histological distinction between patients with identified *RYR1* mutations and patients with no causative changes in this gene. Four of these mutations were not previously reported: c.6612C>G (p.His2204Gln), c.10097G>A (p.Arg3366His), c.11798A>G (p.Tyr3933Cys), and c.14228G>A (p.Gly4743Asp). None of these variants were detected in control samples, and all predictably affected conserved residues in the ryanodine receptor 1 (RyR1) protein. Only mutation p.Gly4743Asp, found in patient 1, is located in the principal CCD mutational hotspot (C-terminal residues 4647–4914).<sup>25</sup> It has been suggested that



**Table 3.** Reported cases of central core and multiminicore disease with adult presentation..

Age/ gender	Myalgia	Spine/joint malformation	Muscle weakness	MH	CK*(IU/L)	Additional features	Muscle, histology	<i>RYR1</i> genotype	Reference
46 y / M	+	—	Neck flexors, UL proximal, LL proximal	—	209	Myopathic EMG	Marked type 1 fiber predominance, central cores on oxidative staining.	Not reported	23
29 y / M	—	—	Generalized, predominantly proximal	—	N	High-arched palate, biventricular enlargement, myopathic EMG	Marked type 1 fiber predominance, multiminicores	Not reported	12
43 y / F	—	—	Facial, truncal diaphragm, UL proximal, LL proximal	—	500	Fatigue, cardiorespiratory failure	Selective type I fiber atrophy, multiminicores	Not reported	13
46 y / M	—	—	—	—	Varying elevation	Fatigue, myopathic EMG	Multiminicores, slight type 1 fiber predominance, central cores	c.1201C>T (exon 12), p.Arg401Cys <sup>†</sup>	15
47 y / F	+	—	—	—	N	Exertional fatigue, myopathic EMG	Type I fiber predominance, multiminicores and central cores	Not reported	14
45 y / M	—	Scoliosis	UL proximal	—	N	Severe restriction of pulmonary function	Single and central cores	Not reported	24
58 y / M	+	—	LL asymmetric	—	Mildelevation	—	Central cores	Not reported	21
77 y / M	—	Axial myopathy	Prominent involvement of spine extensors	—	499	Myopathic EMG (in the lumbar and lower thoracic paraspinal muscles)	Marked type 1 fiber predominance, central and eccentric cores	c.119G>T (exon 2), p.Gly40Val	22

y, years; M, male; F, female; LL, lower limbs; UL, upper limbs; +, presence; -, absence; EMG, electromyogram; N, normal; MH, malignant hyperthermia.

\*Highest value during follow-up.

<sup>†</sup>*RYR1* partially sequenced (34 exons).

mutations located in the C-terminal region are associated with specific clinical findings, such as infantile hypotonia, delayed motor development, and limb muscle weakness.<sup>29</sup> In contrast, the majority of CCD patients with at least one mutation located outside this region present milder musculoskeletal abnormalities, such as joint contractures and scoliosis.<sup>29</sup> Our work corroborates these observations, considering the mutation spectrum in this particular cohort with mild clinical presentation and late onset. The second mutation detected in patient 1 (p.His2204Gln) is located in hotspot 2, more precisely between amino acids 1924 and 2446, a region found to be critical for excitation-contraction (E-C) coupling.<sup>30</sup> Two mutations were also detected in patient 6: p.Arg3366His, in the vicinity of the previously reported mutation p.Lys3367Arg identified in a Japanese patient with

MH and CCD,<sup>31</sup> and p.Tyr3933Cys. The latter affects a residue located within the *RyR* and *IP3R* homology-associated domain. This domain is found in ryanodine receptors and inositol 1,4,5-trisphosphate receptors, which comprise a superfamily of homotetrameric ligand-gated intracellular Ca<sup>2+</sup> channels.<sup>32</sup>

It was perhaps unexpected to find that 2 of the 3 characterized patients (1 and 6) each had two missense mutations. In the first case, family studies were conducted, and results are compatible with an autosomal-recessive pattern of inheritance. The mutations were seen to be allelic, and only the 2 individuals with both alterations had clear clinical signs of the disease. In terms of muscle histology, patient 1 presented both central cores and multiminicores. MH susceptibility was not confirmed experimentally, but she experienced an adverse



reaction to succinylcholine during an anesthetic intervention. CCD and MmD were initially thought to be inherited in an autosomal-dominant manner. However, there have been reports of at least 18 families with compound heterozygosity, as proven by haplotyping and/or segregation analysis.<sup>4,7,30,33–36</sup> Nevertheless, the majority of the previously reported *RYR1* recessive cases had a neonatal or childhood onset. Our report adds some more complexity to this picture and widens the phenotypic variability. Accordingly, because *RYR1* is a large gene, and mutation screening is often limited to the hotspot regions, it was previously recognized that autosomal-recessive cases are probably underestimated in the literature.<sup>35</sup>

Although the genetic cause of the phenotype in some patients remains to be established, either due to *RYR1* mutations, which are undetectable by routine methods, or to defects in other genes, our findings support the idea that *RYR1* mutation screening should be considered in cases with adult-onset, mild muscle symptoms and histological findings compatible with CCD or MmD. Considering these inclusion criteria, the detection of *RYR1* mutations in 3 of our 8 patients (38%) seems sufficiently relevant to validate our study approach.

The first two authors (S.T.D. and J.O.) contributed equally to this work. We thank Dr. José Pedro Vieira for helpful manuscript review.

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