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Clinical, laboratory and anatomopathological evaluation of patients with RyR1 mutations

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Clinical, laboratory and anatomopathological evaluation of patients with RyR1 mutations

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Abstract

Introduction: The ryanodine receptor type 1 (RyR1) is an intracellular ion channel present in both cardiac and skeletal muscle and has an important role in the excitation-contraction coupling. Mutations in the *RyR1* gene underlie several debilitating and/or life threatening muscle conditions: central core disease, susceptibility to malignant hyperthermia, multiminicore disease and centronuclear myopathy. Their diagnosis was suggested by an appropriate clinical symptomatology, "specific" pathological findings and confirmed by a positive molecular result.

Objectives: To describe the clinical, laboratory, anatomopathological and genetic findings of a group of patients with *RyR1* gene mutations followed at the Neuromuscular Disease Unit of the Neurology Department of Coimbra's University and Hospital Centre.

Material and Methods: The medical files of patients with confirmed pathogenic *RyR1* gene mutations were reviewed for demographic, historical and clinical data. Muscle strength of the cervical, upper and lower limbs was graduated according to the MRC scale. The Gowers' Manoeuver was performed on each patient. Serum creatine kinase, forced vital capacity and electromyography were also analysed. Four muscle biopsies were available.

Results: Seven patients, three females and four males, from five unrelated families were included. There was no familial consanguinity. The actual mean age is 41,28 years, the symptoms began mainly in the first decade of life and the disease was slowly progressive. All patients have independent ambulation, with three of them reporting delayed attainment of motor skills and two of these presented an abnormal, myopathic gait. One patient is asymptomatic. Muscle weakness, either proximal (three patients), global (two patients) or in the lower limbs (one patient) was evident in the other six patients. The mean CK value was 1111.25 U/L. The muscular biopsies showed morphologic findings compatible with central core disease (two patients), multiminicore disease

(one patient) and centronuclear disease (one patient). All patients had a molecular study confirming a pathogenic mutation in the *RyR1* gene. The mutations were clustered in hotspot 1 or 3. Five patients had one heterozygous mutation and two patients were compound heterozygous.

Conclusions: Our study provides further evidence that *RyR1* related myopathies are very heterogeneous. It was also recognised that one single mutation may be associated with more than one disease. A new pathogenic mutation was identified. Clinical, histopathological and molecular features are essential to better understand genotype-phenotype correlation.

Resumo

Introdução: O receptor da rianodina tipo 1 (RyR1) é um canal iónico intracelular, localizado no músculo cardíaco e no músculo esquelético, que tem um papel importante no acoplamento excitação-contração. Mutações no gene *RyR1* são responsáveis por várias doenças musculares debilitantes e/ou ameaçadoras da vida: doença de central core, susceptibilidade à hipertermia maligna, miopatia multiminicore e miopatia centronuclear. O seu diagnóstico é sugerido por uma sintomatologia clínica própria e características patológicas "específicas" e confirmado por um resultado molecular positivo.

Objectivos: Apresentar os resultados clínicos, laboratoriais, anatomopatológicos e genéticos de um grupo de doentes com mutações no gene *RyR1* seguidos na Unidade de Doenças Neuromusculares do Serviço de Neurologia do Centro Hospitalar e Universitário de Coimbra.

Material e métodos: Os processos clínicos de doentes com mutações patogénicas confirmadas do gene *RyR1* foram revistos e os dados demográficos, históricos e clínicos foram registados. A força muscular da região cervical, membros superiores e inferiores foi graduada de acordo com a escala MRC. A manobra de Gowers foi realizada em todos os doentes. A creatina cinase sérica, a capacidade vital forçada e a electromiografia foram também analisados. Estavam disponíveis quatro biópsias musculares.

Resultados: Foram incluídos sete doentes, três do sexo feminino e quatro do sexo masculino, pertencentes a cinco famílias não relacionadas entre si. Não havia história de consanguinidade familiar. A média das idades actuais é de 41,28 anos, os sintomas começaram maioritariamente na primeira década de vida e a doença foi lentamente progressiva. Todos os doentes têm marcha independente, três manifestaram um atraso na aquisição das competências motoras e dois destes apresentam uma marcha anormal e miopática. Um doente é assintomático. Os restantes seis

apresentam fraqueza muscular proximal (três doentes), generalizada (dois doentes) ou nos membros inferiores (um doente). O valor médio de CK foi de 1111.25 U/L. As biópsias musculares mostraram características morfológicas compatíveis com doença de central core (dois doentes), miopatia multiminicore (um doente) e miopatia centronuclear (um doente). Todos os doentes tinham um estudo genético que confirmava a existência de uma mutação patogénica no gene *RyR1*. As mutações estavam agrupadas no Hotspot 1 ou 3. Cinco doentes tinham uma mutação heterozigótica e dois eram heterozigóticos compostos.

Conclusão: O nosso estudo evidencia que as miopatias relacionadas com o gene *RyR1* são muito heterogéneas. Também foi reconhecido que uma única mutação pode estar associada a mais do que uma doença. Uma nova mutação não descrita na literatura foi identificada. As características clínicas, anatomopatológicas e moleculares são essenciais para se compreender melhor a correlação genótipo-fenótipo.

Introduction

The RyRs are the major known intracellular ion channels localised on the membrane of the sarcoplasmic and endoplasmic reticulum in both cardiac and skeletal muscle, respectively (1). They are capable of creating rapid transient increase in cytosolic calcium levels, being therefore essential in the excitation-contraction coupling (2).

RyR1 is one of the three isoforms of the ryanodine receptor (RyR), none of which is tissue specific. However, RyR1 is mainly expressed in the skeletal muscle, RyR2 exists predominantly in the cardiac muscle and RyR3 was first identified in the brain. The RyR1 is composed by a membrane domain localised in the COOH-terminal (C-terminal) of the protein and a cytoplasmic domain which is closer to the NH2-terminal (N-terminal) (3).

Dysregulation and impaired RyR1 channel function can cause several non dystrophic inherited neuromuscular disorders, including congenital myopathies, such as central core disease (CCD), multiminicore disease (MmD), congenital neuromuscular disorder with uniform fibre type 1 (CNMDU1), atypical periodic paralyses (APP), centronuclear myopathy (CNM) and the core-rod myopathy. They are also responsible for susceptibility to malignant hyperthermia (MHS) and some cases of hyperCKemia and exertional myalgias. The *RyR1* mutations linked to MHS and CCD are typically located in one of three 'hot spot' regions of the protein: the N-terminal (exons 1-17) (hotspot 1), the central region (exons 39-46) and the C-terminal (exons 90-104) (hotspot 3) portions (3).

CCD is a congenital myopathy defined by the histopathological finding of central cores, which represent areas lacking mitochondria and oxidative enzymatic activity longitudinally extended throughout the muscle fibre (4). Most patients have a mild phenotype characterised by a static or slowly progressive hypotonia and generalised proximal muscle weakness from birth, or, less often,

starting in later ages. Usually there is reduced muscle bulk and a delayed attainment of motor skills. Its inheritance is primarily autosomal dominant with *RyR1* missense mutations (5).

Malignant hyperthermia is a pharmacogenetic condition in which genetically susceptible individuals develop generalised muscle contractures followed by a hypermetabolic state when they are exposed to certain triggers such as inhaled general anaesthetics (e.g. isoflurane, halothane, sevoflurane, desflurane), the depolarising muscle relaxant succinylcholine, and rarely to exercise and heat (6). MHS type 1 is associated with *RyR1* mutations and is usually inherited in an autosomal dominant way.

MmD is pathologically defined by the presence of multiple "minicores" on muscle biopsy. Minicores are typically unstructured small cores which only cover a portion of the longitudinal axis of the muscle fibre. Classically it presents with severe axial muscle weakness, scoliosis and major respiratory involvement and when associated with a *RyR1* mutation it may include distal weakness and wasting, external ophthalmoplegia and hip girdle affection (7).

CNM is a rare heterogenous neuromuscular condition defined by numerous central nuclei on muscle biopsy, and associated with generalised congenital weakness (including facial muscles) (8).

In all of them serum creatine kinase concentration may be normal or elevated and electromyography may confirm the presence of myopathy.

The diagnosis requires a typical clinical and laboratory presentation together with a pathologic muscle biopsy showing suggestive features (central cores, minicores, rods, numerous central nuclei or exclusivity of type 1 muscle fibres) and a genetic testing identifying a pathogenic mutation in the *RyR1* gene.

We will present the clinical, laboratory, anatomopathological and genetic findings of a small group of patients with *RyR1* gene mutations followed at the Neuromuscular Disease Unit (NDU) of the Neurology Department of the Coimbra's University and Hospital Centre (CHUC).

Material and Methods

The medical files of the NDU were screened for patients with a diagnosis of muscle disease caused by pathogenic *RyR1* gene mutations and seven patients were found. Four of them had a muscle biopsy result and all of them had a confirmatory genetic study. The study protocol included:

1 - Demographic and historical features: age, sex, race, familial consanguinity, family history of *RyR1* gene mutations, skeletal deformities present at birth (congenital hip dislocation, scoliosis, joint contractures and hyperlaxity), age of first symptoms, age of walking onset, progression of the disease and associated medical conditions including episodes of malignant hyperthermia.

2 - Actual symptoms: complaints of muscle weakness (including extra ocular, facial and bulbar muscles), cramps, exercise-induced myalgia, gait difficulties, cardiac and respiratory complaints.

3 - Clinical evaluation: Evaluation of cranial muscles for ophthalmoparesis, ptosis, palate paresis, and facial paresis. Manual muscle testing (MMT) was done and graded according to the MRC scale, where 5 is normal and 0 is absence of any voluntary muscle activation. The following movements were evaluated: neck - flexion and extension; upper limbs - abduction, adduction, flexion and extension of the arm, flexion and extension of the hand and fingers and finger abduction; lower limbs - flexion and extension of the thigh, flexion and extension of the leg and ankle and plantar and dorsal flexion of the foot. Muscle tonus and bulk were evaluated and gait was observed. The Gowers' manoeuver was performed on every patient.

4 - Laboratory evaluation: The serum creatine kinase (CK) level on each patient's medical file was recorded.

5 - Medical Tests: Forced vital capacity (FVC) had been assessed in four patients and its value registered. The results of the electrocardiogram (ECG), electromyography (EMG) and magnetic resonance imaging scans (MRI) that had been performed by some patients were also registered.

6 - Muscle biopsy evaluation: A total of four muscle biopsies were available. They were performed at the Neuropathology Department of the CHUC. The technique used for processing, analysing and grading histopathologic findings was already described in detail previously (9).

7 - Genetic studies: The genetic studies were performed at the Medical Genetic Centre Doutor Jacinto Magalhães, Porto, Portugal, according to a procedure already explicated in detail previously (9).

Results

1- Demographical and historical features (Table 1)

The seven patients, three females and four males, represented five unrelated families and were all caucasians. Consanguinity was not present in any family.

Regarding family history of muscle disease caused by *RyR1* gene mutations, patient B was son of patient C, who declared that his mother presented with myalgia and cramps in the lower limbs but was not genetically studied. Patient E, patient's D mother, had another son, with a confirmed *RyR1* gene mutation and her father had similar symptoms, but never performed a genetic study.

Patient F was diagnosed with scoliosis at birth. Other skeletal deformities such as congenital hip dislocation, joint contractures and hyperlaxity were not reported by any of the patients.

Three patients reported the onset of symptoms during childhood and two on the second decade of life. Three patients presented with delayed attainment of motor skills.

All patients reported slow progression of the disease.

One episode of malignant hyperthermia characterised by rhabdomyolysis, lower limbs myalgia and paraparesis after exposure to inhaled general anaesthetics was described by patient C. Two other patients (B and E) had already undergone surgery with general anaesthetics reporting no complications.

Allergic rhinitis was reported by four patients, three of whom had also asthma. One had had an episode of pulmonary embolism, another patient presented with hypertension and another one with an uterine fibroleiomyoma.

Table 1. Demographic and historical features

Patient	Sex F/M	Actual age (years)	Family history	Walking Onset	Age of Symptoms Onset	Progression of Disease	Previous episode of MH	Other Medical Conditions
A	M	48	No	N	20	Slow	No	Pulmonary embolism
B	M	27	Yes	N	-	-	No - S	Asthma, Allergic Rhinitis, Gastritis
C	M	55	Yes	N	childhood	Slow	Yes	Asthma, Allergic Rhinitis
D	F	24	Yes	L	childhood	Slow	No	Hypertension
E	F	42	Yes	L	20	Slow	No - S	Uterine Fibroleiomyoma
F	F	33	No	L	childhood	Slow	No	Allergic Rhinitis
G	M	60	No	N	-	Slow	No	Asthma, Allergic Rhinitis

F: female; M: male; N: normal; L: late; S: already exposed to surgery with inhaled general anaesthetics

2 - Actual symptoms (Table 2)

One patient (B) was asymptomatic and performed the genetic test due to positive family history.

Complaints suggestive of proximal weakness were reported by four patients and of generalised weakness by the remaining two. Three patients referred fatigue as a main complaint. Only one patient reported symptoms of facial weakness. No patient reported weakness of the extra ocular muscles or symptoms of bulbar muscle involvement. Three patients referred exercise-induced myalgia and only one (patient C) referred occasional cramps. Ambulation impairment was referred by patient A and F. Respiratory and cardiac symptoms were not referred by any patient.

Table 2. Actual Symptoms

Patient	Muscle weakness	Fatigue	Extra ocular muscles weakness	Facial muscles weakness	Bulbar weakness	Cramps	Exercise-induced myalgia	Gait difficulties	Cardiac and Respiratory complaints
A	Proximal	**Yes**	No	No	No	No	No	**Yes**	No
B	-	No	No	No	No	No	No	No	No
C	Proximal	No	No	No	No	Yes	Yes	No	No
D	Global	**Yes**	No	No	No	No	Yes	No	No
E	Proximal	No	No	No	No	No	Yes	No	No
F	Lower limbs	No	No	**Yes**	No	No	No	**Yes**	No
G	Global	**Yes**	No	No	No	No	No	No	No

3 - Clinical evaluation (Table 3)

Patient B presented a normal clinical evaluation.

Facial paresis was present in three patients namely left facial paresis in patient D and bilateral facial paresis in patients E and F. No patient presented with ophthalmoparesis, ptosis, palate paresis or hypotonia.

Only two patients had reduced muscular strength. Patient E presented with cervical and proximal upper and lower limb (arm abduction/adduction and thigh flexion/extension) muscular weakness G4-/5 (MRC) and patient F presented with a proximal upper and lower limb muscular weakness G4/5 (MRC).

Patient A and F presented with myopathic gait. Patient A had generalised muscular hypertrophy, bilateral Achilles tendon retraction and lumbar hyperlordosis. Patient C presented with calf hypertrophy. No patient had hypotonia.

Four patients (A, D, E and F) had a positive Gowers' manoeuver.

Table 3. Clinical evaluation

Patient	Ophtalmo-paresis	Ptosis	Palate paresis	Facial paresis	Muscle weakness	Tonus and bulk	Gait	Gowers' Manoeuvre
A	No	No	No	No	-	Generalised hypertrophy	Myopathic	**Positive**
B	No	No	No	No	-	-	-	Negative
C	No	No	No	No	-	Calf hypertrophy	-	Negative
D	No	No	No	**Yes**	-	-	-	**Positive**
E	No	No	No	**Yes**	G4-/5: C/UL/LL	-	-	**Positive**
F	No	No	No	**Yes**	G4/5: UL/LL	-	Myopathic	**Positive**
G	No	No	No	No	-	-	-	Negative

C: cervical; UL: upper limb; LL: lower limb

4 - Laboratory evaluation

Five patients had an available CK level. Four presented with elevated values. The mean value of those four patients was 1111.25 UI/L with significant variability between the highest and the lowest values, 2457 UI/L and 300 UI/L, respectively. Furthermore, patient C presented with a CK value of 22478 UI/L during the episode of MH.

5 - Medical tests

Patients A, D, E and G had their FVC accessed. Patients A and D presented with a slightly reduced FVC - 81.6% and 69.9% respectively. Patient D also showed a decrease of 8.6% in the supine lying position. Patients E and G had normal values - 91.9% and 95.0% respectively.

Only patient G had performed an ECG and it was normal.

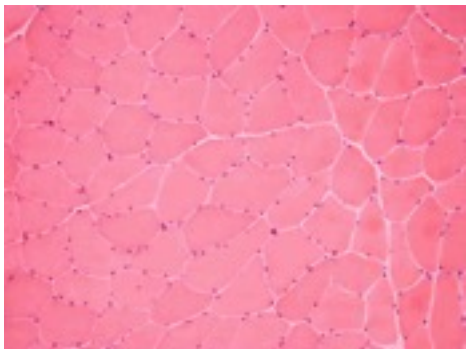
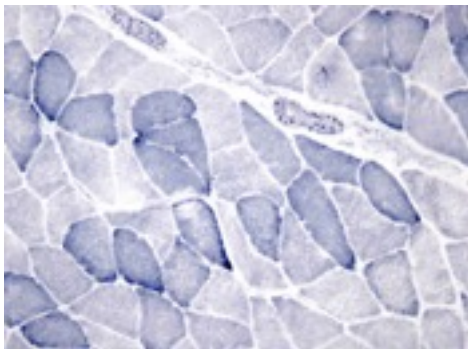
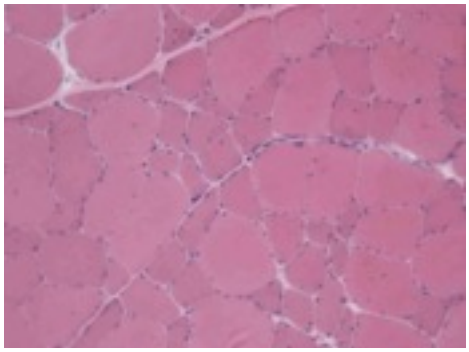
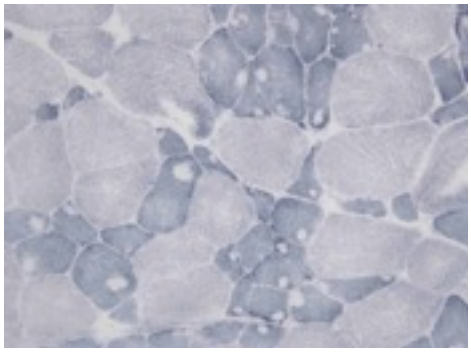
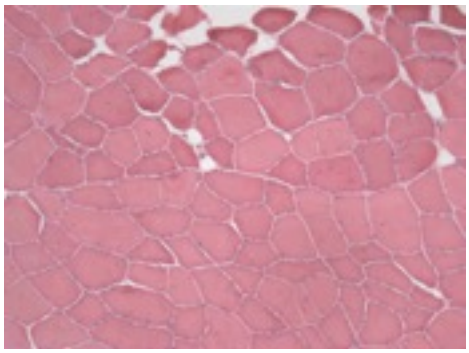
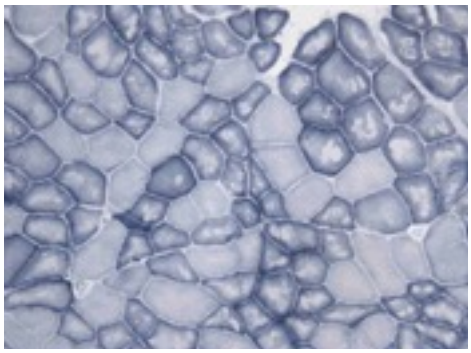
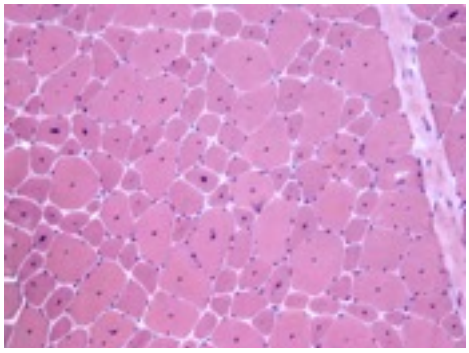
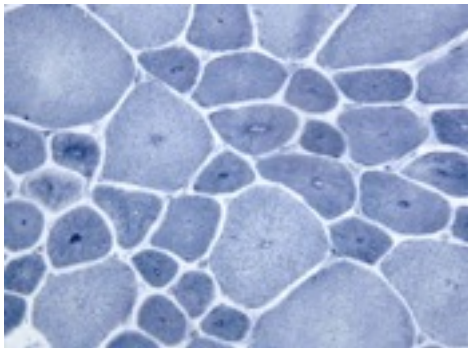
Three patients had performed an EMG, one of which was myopathic (patient F).

Patient A had undergone a pelvic and lower limb MRI which showed adipose tissue infiltration in almost every muscle with predilection for the gluteal within the hip, the vasti within the thigh and the soleus and gastrocnemius within the lower leg. The iliopsoas and both obturator externus and internus within the hip and the adductor muscles of the thigh were spared.

6 - Muscular Biopsy Evaluation (Table 4)

The four biopsies, all performed in the deltoid muscle and processed at the Neuropathology Department, revealed three different patterns of muscle disorder. Muscle of patients C and G showed findings compatible with CCD. In patient A the biopsy was suggestive of MmD and in patient F muscle had CNM pathologic findings associated with mitochondrial pathology.

Table 4. Muscular Biopsies

Patient	H-E	SDHase
C		
	Fig 1. H-E (X200) Fibre atrophy/hypertrophy	**Fig 2.** SDHase (X200) Central cores, without oxidative enzimatic activity
G		
	Fig 3. H-E (X200) Fibre atrophy/hypertrophy	**Fig 4.** SDHase (X200) Central cores, without oxidative enzimatic activity
A		
	Fig 5. H-E (X100) Fibre atrophy/hypertrophy	**Fig 6.** SDHase (X100) Multiple minicores, without oxidative enzimatic activity
F		
	Fig 7. H-E (X200) Fibre atrophy/hypertrophy; central nuclei	**Fig 8.** SDHase (X400) Central nuclei

7 - Genetic study

All patients had a molecular study confirming the diagnosis of muscle disease caused by *RyR1* gene mutations. The mutations were clustered either between exon 9 and 17 or between exon 90 and 99. Five patients had one heterozygous mutation and two patients were compound heterozygous. Only two types of mutations were identified: 8 missense and 1 frameshift (hypomorphic). Patients B and C (son and father) presented the same mutation - c.1840C>T in exon 17 in an heterozygous state. This mutation is associated with MHS. Patients D and E (daughter and mother) also presented the same mutation - c.12623A>G in exon 90 in a heterozygous state. Both mutations of patient F - c. 12860_12869delinsT and c.12956G>A in exon 90 – were not previously reported in the literature (10).

Table 4. Genetic Study

Patient	Location and type of mutation	Consequences at protein level
A	exon 15 c.1628T>C	p.Leu543Ser
B	exon 17 heterozygous c.1840C>T	p.Arg614Cys
C	exon 17 heterozygous c.1840C>T	p.Arg614Cys
D	exon 90 heterozygous c.12623A>G	p.Gln4280Arg
E	exon 90 heterozygous c.12623A>G	p.Gln4280Arg
F	exon 90 c.12860_12869delinsT / exon 90 c.12956G>A	p.Ala4287_Ala4290delinsVal/p.Arg4319Gln
G	exon 9 p.Arg245His / exon 99 p.Thr4823Met	p.Arg245His/p.Thr4823Met

Discussion

Mutations in the *RyR1* gene underlie several debilitating diseases which in this case series included CCD, MHS, MmD and CNM. Muscle biopsy features are essential for a diagnosis of the above mentioned conditions (11).

Dominant *RyR1* mutations are most often associated with CCD and MHS. The possibility of genetic analysis of the entire *RyR1* gene allowed identification of recessive mutations causing MmD and CNM. In this study dominantly acting mutations were found in five patients. Two patients were compound heterozygous for *RyR1* gene mutations.

Dominant mutations causing CCD are commonly clustered in the C-terminal domain (12) but a clear clustering for recessive mutations is not well established. In our study, patients F and G presented with C terminal recessive *RyR1* gene mutations.

Dominant mutations causing MHS are normally confined to the N-terminal domain. Moreover patients suffering from CCD who have pathogenic variants in the N-terminal domain may have a higher probability of MHS than those with pathogenic variants in the C-terminal domain (13). Patient C, who was the only patient in our study with a previous episode of MH, had muscle biopsy features of CCD and a mutation in the N-terminal domain.

All patients in our study had common features of a *RyR1*-mutation-associated condition such as: a mild phenotype characterised by a slowly progressive proximal muscle weakness, in some cases a delayed attainment of motor skills and variable involvement of facial and neck muscles. The extraocular muscles are often spared in the autosomal dominant form (14) which was the case in our patients. The CK levels may be mildly elevated, which happened in more than half of the patients and electromyography may confirm the presence of myopathy (14) which was the fact in one patient.

One patient showed features of MmD in the muscle biopsy and presented a dominant *RyR1* gene mutation. Patients with multiple minicores in muscle biopsy may present with severe axial muscle weakness (the classic phenotype associated with SEPN1 mutation), major respiratory involvement, varying degrees of external ophthalmoplegia as well as antenatal onset of hip-girdle weakness and arthrogryposis (15), which did not occur in the patient with such pathological findings. This patient presented however with myopathic gait which may be associated with the pelvic girdle weakness commonly observed in this disease. A feature not previously described was the presence of generalised muscle hypertrophy presented by this patient.

Another patient, who presented with a bilateral facial paralysis, had characteristics of CNM in the muscle biopsy and was a compound heterozygous for *RyR1* gene mutations. This disease is frequently associated with ptosis, restriction of eye movements and generalised weakness (8). A recent study correlating genotype and phenotype of recessive *RyR1*-related myopathies reported that non-core myopathies were more likely to have at least one hypomorphic mutation (a mutation that causes a partial loss of gene function), especially when compared to CCD (16). Our only patient presenting with a confirmed diagnosis of a non-core myopathy was also the only case with a hypomorphic mutation.

The mutations observed in patients B and C are associated with type 1 MHS (17). Both patients were subjected to surgeries with inhaled general anaesthetics in different occasions of their lives and only one possible episode of MH was reported. This points out the heterogenous manifestations of this disease. Patient C also presented with central cores in the muscle biopsy which supports the fact that CCD and MHS are often linked, and the same mutation can sometimes be found to cause both (17).

Patient B never performed a muscular biopsy because the molecular study was done after his father was diagnosed. Clinically, CCD has a variable expression even among family members (14) which

would explain his absence of symptoms. Patients with dominant CCD typically present with hypotonia, proximal and mild facial weakness, often marked joint laxity, and ortophedic complications such as congenital hip dislocation and scoliosis, but these features were not observed in this patient – only a mild calf hypertrophy.

Allergic rhinitis was reported by four patients (two with dominant *RyR1* gene mutations and another two were compound heterozygous). This association has not been described in the literature.

Furthermore, in a recent study with a large multi centre cohort of patients with *RyR1* gene mutations there was a tendency for earlier and more severe presentation associated with recessive mutations when compared to most dominant ones (18). In our study no phenotype was considered severe.

Our findings reinforced the wide spectrum of clinical and histopathological features of *RyR1*-related myopathies.

Conclusions

Following an era of trying to split the *RyR1* mutations into different clinical, pathological and imaging groups there is now a tendency to see similar phenotypes and muscle biopsies related to different mutations. This study supports this fact.

It also provides further evidence that *RyR1* related myopathy has a very heterogenous clinical, pathological and imaging spectrum, even within families, and there is no single assessment tool capable of evaluating all patients. It also shows the importance of collecting imaging and pathology data to better understand the diseases. Functional and molecular studies are also essential to understand the effect of the combination of the mutations on the phenotype and the reasons of heterogeneity.

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