



## Clinical Observations

## Ryanodine Myopathies Without Central Cores—Clinical, Histopathologic, and Genetic Description of Three Cases



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## ABSTRACT

**BACKGROUND:** Mutations in ryanodine receptor 1 gene (*RYR1*) are frequent causes of myopathies. They classically present with central core disease; however, clinical variability and histopathologic overlap are being increasingly recognized. **PATIENTS:** Patient 1 is a 15-year-old girl with mild proximal, four-limb weakness from age 5, presenting with a progressive scoliosis starting at age 10. Patient 2 is an 18-year-old girl with progressively worsening muscle hypotrophy and mild proximal, four-limb weakness. She developed a rapidly progressive scoliosis from age 11 and needed surgical treatment at age 14 years. Patient 3 is an 11-year-old boy with moderate proximal limb weakness and progressive neck flexor weakness, first noticed at age 2. Muscle biopsies revealed type 1 fiber predominance (Patients 1 and 2) or abnormal type 1 fiber uniformity (Patient 3). Different *RYR1* variants were identified in all patients. In Patients 1 and 3, these changes were validated as being pathogenic. **CONCLUSIONS:** These patients illustrate early-onset, progressive myopathies with predominant axial involvement. Histopathologic findings were abnormal but not specific for a diagnosis, particularly central core myopathy. Genetic testing helped broaden the range of phenotypes included in the *RYR1*-related myopathies. Our patients reinforce the need to recognize the broad histopathologic variability of *RYR1*-related myopathies and sometimes lack of pathognomonic findings that may reduce the diagnostic threshold of this disease. We suggest that the predominance of type 1 fibers and involvement of axial muscles may be an important element to consider the *RYR1* gene as candidate.

**Keywords:** ryanodine receptor, *RYR1*, myopathy, muscle biopsy

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### Introduction

The ryanodine receptor 1 gene (*RYR1*) encodes the main  $Ca^{2+}$  channel in the skeletal muscle's sarcoplasmic reticulum, having a crucial role in muscle excitation-contraction mechanisms.<sup>1</sup> More than 300 distinct mutations in *RYR1* (19q13.1) have been reported.<sup>2</sup> Classically, they have been

associated with autosomal dominant central core disease (MIM #117000) and malignant hyperthermia susceptibility. Clinical features have been described as globally nonprogressive and include congenital hypotonia and minor weakness with proximal lower limb predominance (pelvic girdle). Sometimes, axial involvement without respiratory compromise, motor milestones delay, or skeletal malformations (hip dislocation, scoliosis, and foot deformities) may be observed.<sup>3</sup> Throughout the years, other phenotypes have been attributed to *RYR1* mutations, including King Denborough syndrome, limb-girdle myopathy, core-rod myopathy, bent spine myopathy, exertional or drug-associated myalgia, and rhabdomyolysis and asymptomatic creatine kinase elevation. A marked interpersonal and

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even intrafamilial variation in phenotype has been evident in previous reports.<sup>3–6</sup> Typical muscle histopathology includes type 1 muscle fibers with central amorphous areas of sarcomeric disorganization characterized by the absence of mitochondria and of oxidative enzymatic activity (“cores”).<sup>7</sup> In recent years, there has been an increased awareness of a marked clinical variability and histopathologic overlap with other myopathies, precluding a direct diagnosis.<sup>3,8</sup> These phenotypes include myopathies with multiminicores and atypical cores, centrally placed nuclei, cores and nemaline rods, congenital fiber-type disproportion, and marked predominance or uniformity of type 1 fibers.<sup>7,8</sup>

Although *RYR1* gene analysis was initially restricted to hotspot regions because of its large size (106 exons), sequencing all the exonic regions of the gene is now recommended, especially when patients have atypical muscle phenotypes, which are difficult to correlate with the genotype, or in sporadic cases. The broader analysis of *RYR1* gene has brought the molecular diagnosis of these patients to a new level of complexity because several new sequence variants are often found, frequently described as unclassified variants, making the genetic diagnosis problematic.

We report three independent pediatric patients with early-onset myopathies with nonspecific histopathology and highlight some clinical characteristics that can lead to the diagnosis; in two instances, mutations were identified in *RYR1* gene.

### Patient Descriptions

#### Patient 1

A 15-year-old girl with a family history of “lower limb weakness” (affecting her mother, maternal uncle, and grandfather) was first observed in the Neuropediatrics Department at age 11 with a slight delay in motor milestones but stable development up to 10 years of age. At this point, she started developing a scoliosis that has progressed to a severe form. Surgical treatment has been proposed but not yet performed. Physical examination revealed a right ptosis, bilateral facial palsy, and arched palate. She had considerable axial weakness with neck flexor involvement, dorso-lumbar scoliosis (Fig 1A), and a moderate, proximal, nonprogressive upper and lower limb weakness. A muscle biopsy performed when she was 6 years old revealed normal muscle fiber size variability and preserved internal structure but marked type 1 fiber predominance (Fig 2, P1). Molecular study of the *RYR1* gene revealed the

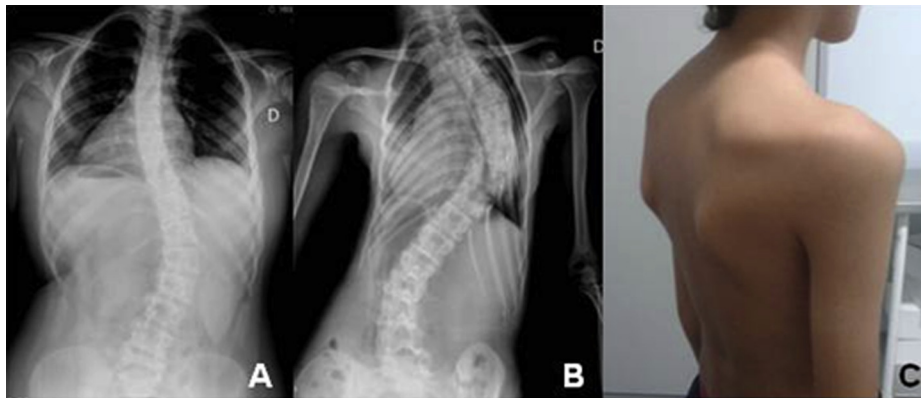
presence of a heterozygous mutation c.14677C>T (p.Arg4893Trp) in exon 102 (hotspot 3) (Fig 3). This mutation was first identified by Monnier et al. in two distinct families with autosomal dominant (AD) transmission. Similarly, the mutation was also found in our patient's mother and uncle suggesting an AD inheritance pattern.

#### Patient 2

An 18-year-old girl with an unremarkable family history had been monitored by the Neuropediatrics Department from 12 years of age. She was described as hypotonic since birth and had mildly delayed motor milestones. Progressive fatigue and weakness after physical activity was evident since early childhood, and at age 11, she began developing a rapidly progressive, severe scoliosis requiring surgical correction and noninvasive ventilation by age 14. On examination, she had global muscle hypotrophy and low weight and stature. She also has an arched palate and axial weakness with involvement of neck flexors, narrow thorax, and dorso-lumbar scoliosis (Fig 1B). Low-grade proximal and slowly progressive upper and lower limb weakness with joint retractions has been observed during follow-up. Muscle biopsy at 12 years of age revealed increased variability in fiber diameter with frequent fiber atrophy and occasional fiber hypertrophy. There was some endomysial fibrosis and adipose substitution but with preserved internal structure (Fig 2, P2). Marked type 1 fiber predominance was also noted. Molecular study of the *RYR1* gene revealed a heterozygous missense variant with unknown significance c.7843C>T (p.Arg2615Cys) in exon 49 (hotspot 2) (Fig 3). This previously unreported variant affects a highly conserved residue. *RYR1* exonic regions were fully sequenced, and her asymptomatic father was found to carry the same variant. The muscle complementary DNA study, restricted to the *RYR1* transcript region where the variant is located, demonstrated biallelic expression because the c.7843C>T was detected in heterozygosity.

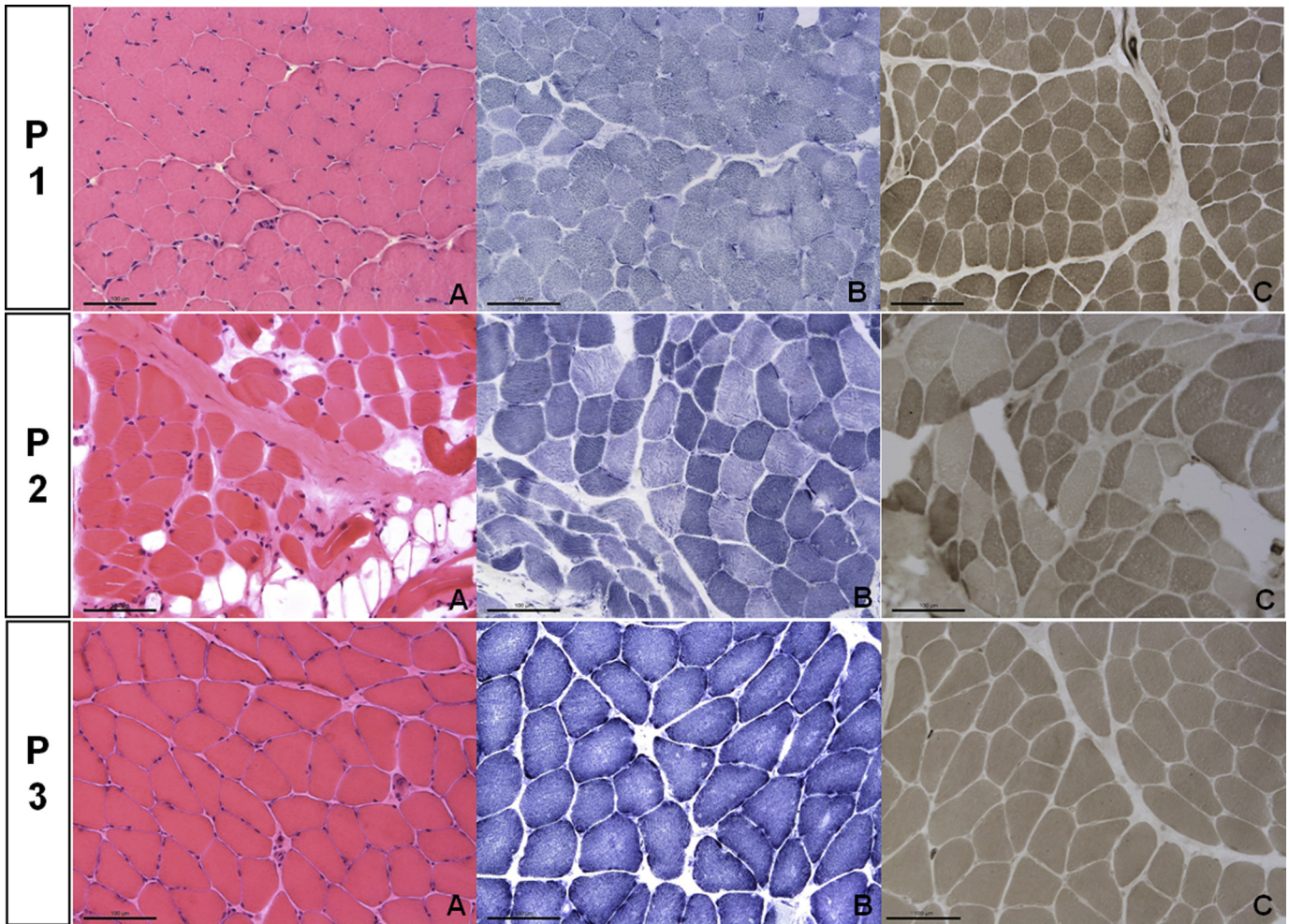
#### Patient 3

An 11-year-old boy with an unremarkable family history, first observed in the Neuropediatrics Department at age 10, exhibited a minor delay in motor milestones from 2 years of age. On physical examination, he presented with a bilateral facial palsy, arched palate, and axial weakness with neck flexor weakness and a narrow thorax (Fig 1C). He had a moderate-grade proximal and nonprogressive upper and lower limb weakness. His muscle biopsy, performed at 10 years of age had normal fiber size variability with global preservation of internal structure and only slight central dimming on oxidative stain but no cores. He had abnormal type 1 fiber uniformity (Fig 2, P3). Molecular *RYR1* revealed three sequence variants: c.4711A>G (p.Ile1571Val) in exon 33; c.10097G>A (p.Arg3366His) in exon 67; c.11798G>A; p.Tyr3933Cys) in exon 86. These variants have been previously described in the same allele.<sup>3</sup> A heterozygous mutation c.14537C>T (p.Ala4846Val) in exon 101



**FIGURE 1.**

Pattern with marked axial involvement: (A) Patient 1 vertebral column x-ray revealing dorso-lumbar scoliosis; (B) Patient 2 vertebral column x-ray revealing severe dorso-lumbar scoliosis; (C) Patient 3 lateral profile with narrow thorax.



**FIGURE 2.** Muscle biopsy histopathologic features. (A) Hematoxylin and eosin stain; (B) Nicotinamide adenine dinucleotide stain; and (C) Adenosine triphosphatase 4,35 stain. P1: (A) normal muscle fiber size variability; (B and C) internal structure is normal, but marked type 1 fiber predominance is observed; P2: (A) some atrophy and occasional fiber hypertrophy and endomysial fibrosis with scanty adipose replacement; (B and C) normal internal structure with type 1 fiber predominance; P3: (A) normal fiber size variability; (B and C) internal structure is normal and slight central dimming on oxidative stain, but no cores are observed, and type 1 fiber uniformity. Scale bar: 100  $\mu$ m.

(hotspot 3) was also present, previously reported as pathologic<sup>9</sup> (Fig 3). The parents are awaiting screening to assess the possibility of autosomal recessive inheritance.

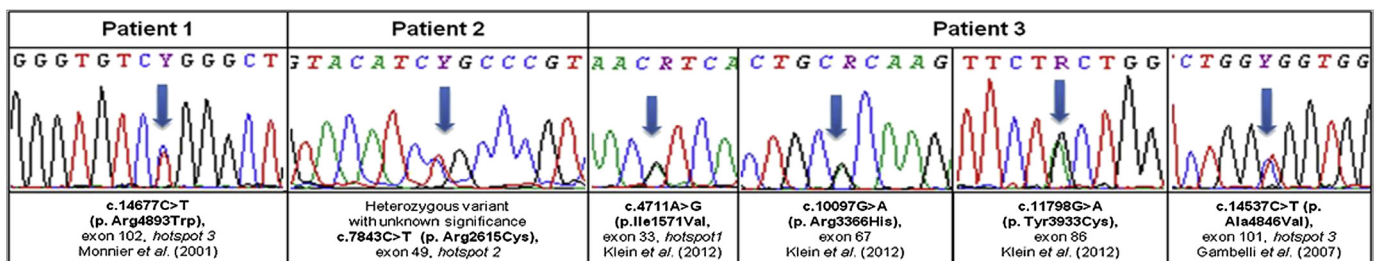
All analytical studies were normal in the three cases, including muscle creatine kinase.

**Discussion**

Our patients illustrate early-onset myopathies with prominent progressive axial involvement, variable clinical

severity, and progressive course. The first two patients rapidly evolved to severe scoliosis during the second decade of life. Patient 3 has not developed scoliosis, having only marked neck flexor weakness, but he is the youngest of the three individuals, having only started the second decade of life. Histopathologic examination revealed changes suggestive of but not classical for *RYR1*-related myopathy.

All patients had marked type 1 fiber predominance without typical “cores,” a feature recently recognized to be



**FIGURE 3.** Molecular study of *RYR1* gene: image reveals sequences’ electropherograms containing mutations (all variants are heterozygous).

associated with *RYR1* myopathies.<sup>3,10</sup> Noncore *RYR1*-related myopathies may be more frequent than initially thought and are probably underdiagnosed. Amburgey et al.<sup>4</sup> stated that 49% of recessive cases in their study (n = 106) were noncore myopathies. In these patients, histopathologic presentation evolves during the course of the disease and the typical central cores may only appear in later stages.<sup>4,7,11</sup> Patient 3 may be an example of such an evolving course where there seems to be a central dimming on oxidative stains, without a clear core formation. Endomysial fibrosis and adipose substitution, as found in the biopsy of Patient 2, are characteristics more commonly associated with dystrophies and are less specific traits, but they have also been described in *RYR1*-related myopathies.<sup>7,12</sup>

Concerning molecular analysis, Patients 1 and 3 have *RYR1* variants previously recognized as pathogenic. However, Patient 2, the most severely affected of our three cases, has a heterozygous missense variant of unknown significance (p.Arg2615Cys) in hotspot 2 of the ryanodine receptor gene. This variant, previously unreported in the literature and in sequence variant databases, affects a highly conserved residue and is of paternal origin. *RYR1* exonic regions were fully sequenced, and no other mutation was found. Although the patient's father is an asymptomatic carrier of the same mutation, we cannot completely exclude its pathologic role, because high inter- and intra-familial phenotype variability, ranging from asymptomatic to severely affected individuals, has been described with other mutations in this disease.<sup>13</sup> Epigenetic allele silencing of the *RYR1* gene due to imprinting could also provide an explanation for our case.<sup>14</sup> However, this hypothesis was excluded because we have detected biallelic expression of *RYR1* gene in complementary DNA obtained from muscle, demonstrating heterozygous expression. In the first case, transmission is compatible with an autosomal dominant inheritance, in contrast with Patient 3, where autosomal recessive inheritance is likely. This assumption was made because this sporadic case has four missense variants, three of which have been previously described in the same allele, and where p.Ile1571Val and p.Tyr3933Cys were considered as mutations.<sup>3</sup>

Our report highlights the phenotypic variability of *RYR1* myopathies, which may lack pathognomonic histopathologic findings, or these may overlap with other non-*RYR1* myopathies. This is a serious obstacle in the diagnostic process, where muscle biopsy is considered the “gold standard”. Our cases illustrate the paradigm of some patients that in the past would go years without a specific diagnosis, until they developed histopathologic pathognomonic features. Genetic testing has helped to solve many of these difficult diagnoses. However, in the case of *RYR1* genetic analysis, there are still

some difficulties to overcome specially when dealing with sequence variants with unknown clinical significance. The development of new approaches based on next generation sequencing should increase the diagnostic yield in this field and may bring some additional clues to the apparent genotype and/or phenotype discrepancies reported in some patients.

We suggest that the predominance of type 1 fibers and involvement of axial muscles may be an important element to consider the *RYR1* gene as candidate.

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