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ScienceDirect

Neuromuscular Disorders 25 (2015) 397–402

www.elsevier.com/locate/nmd

Case report

A novel large deletion in the RYR1 gene in a Belgian family with late-onset and recessive core myopathy

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Received 4 September 2014; received in revised form 20 January 2015; accepted 25 January 2015

Abstract

We report a *novel* and particularly unusual type of mutation, namely, *large deletion* in the RYR1 gene, in a Belgian family with myopathy: Patients were found to be compound heterozygous and presented a clinico-pathological phenotype characterized by *late-onset* and *recessive* myopathy with cores. We depict the clinical, electrophysiological, pathological and molecular genetic characteristics of family members.

To date, large deletions in the RYR1 gene have been reported in only two cases. Both involved different mutations and, in sharp contrast to our cases, presented with a very early-onset, neonatal, and a very severe or lethal phenotype. Overview of reported clinico-pathologic phenotypes, also highlights the rarity of combined late-onset/recessive co-occurrence in this group of myopathies with cores. Finally, this report underlines the broadening spectrum in this group of myopathologic disorders and highlights the concept of 'RYR1-associated/related core myopathies'.

Keywords: RYR1 gene; Core myopathy; Mutation; Deletion; Pathology; Central core disease

1. Introduction

Mutations in the gene encoding the skeletal muscle ryanodine receptor type-1 (RYR1) protein (which is an intracellular calcium-release channel, crucial for excitation/contraction coupling in muscle tissues), are known to be linked to a group of myopathies in which the forerunner was central core disease (CCD) which is typically of neonatal-onset and autosomal dominant inheritance.

Identification of mutations on both alleles [1] permitted the detection of an increasing number of recessive forms of these myopathic disorders [2–4]. These recessive cases sometimes present less characteristic histopathological features and a varied clinical picture. The clinical, pathological and genetic spectra of this group of disorders are thus expanding, and this highlights the essence of the emerging broad-spectrum designation "RYR1-related myopathies" [5,6].

Lately, rare "late-onset" cases (mostly mild), and other contrastingly severe and rapidly fatal cases (mainly neonatal) have been depicted. Heterogeneity is thus increasing.

To-date, only 2 *large* genomic-deletions in *RYR1* have been reported: both presented with a severe or lethal phenotype, and were in a *neonatal* context of congenital myopathy [7,8].

Here, we report a *novel* large deletion in the *RYR1* gene in a Belgian family with *late-onset* and *recessive* myopathy with cores; we depict the clinico-pathological phenotype in affected patients and highlight the rarity of combined late-onset/recessive co-occurrence.

2. Case reports

Two sisters in a Caucasian Belgian family (pedigree; Fig. 1) presented a progressively deteriorating muscular disorder.

The proband (III.3), a 58-year-old woman, had long-lasting limbs weakness and movement-limitations, with deteriorating ability to perform physical activities, including difficulties in driving and in carrying-out daily activities (bathing, manipulating domestic objects, etc.). Past-history revealed normal development during infancy/childhood and early adolescence until age 15. Afterwards, she experienced sporting-difficulties and weakened handgrip over objects. Weakness

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http://dx.doi.org/10.1016/j.nmd.2015.01.016
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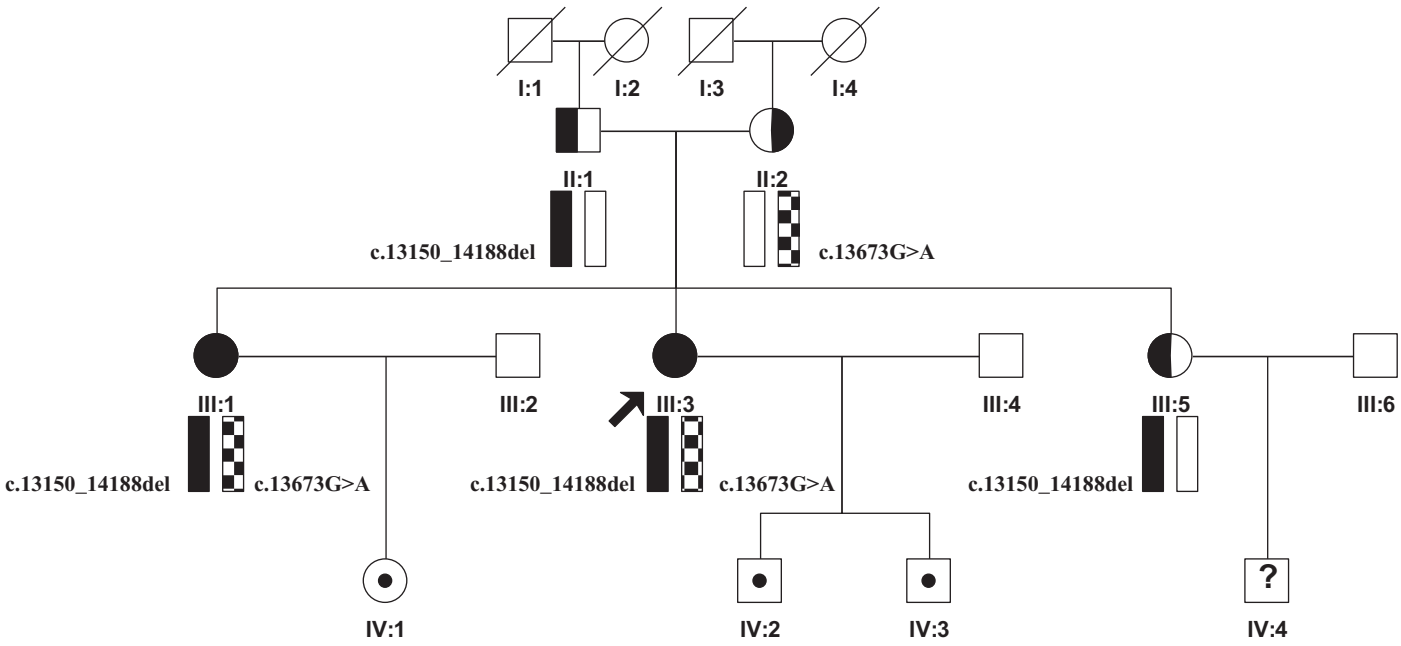

Fig. 1. Family pedigree and segregation-study results. Only individuals who harbored compound heterozygous mutations (2 daughters, namely III.1 and III.3) had myopathy. The non-consanguineous parents (II.1 and II.2) and a third daughter (III.5) were simple heterozygous carriers and were asymptomatic. Besides, three other *obligatory* carriers, namely, the two sons of the proband (patient III.3; arrowed), and the daughter of her affected sister (patient III.1), were to date (in their 3rd and 4th decades of life) also asymptomatic. This mode is therefore compatible with *recessive* inheritance. The two patients (III.1 and III.3), and the asymptomatic sister (III.5) inherited the large deletion from their asymptomatic father (II.1). Husbands (III.2, III.4, and III.6) of the 3 sisters were not related to the family and were in good health.

increased slowly but progressively, with difficulties in raising hands above head-level (combing), and in rising-up from a lying position. At 40, she was unable to run and developed difficulties in rising-up from a chair. She stopped working because of physical infirmity. Surgery/anesthesia (for unrelated conditions) did not cause adversities.

Clinical examination (age 58) showed waddling-gait, lumbar hyperlordosis, bilateral ptosis and marked facial-temporal atrophy.

There was notable weakness in pelvic and shoulder-girdles' muscles with marked difficulties in rising-up from a chair. Walking-tolerance was 100 m.

MRCMS-scores (right/left) were: shoulder abduction: 3-/3-, forearm flexion: 3/3, forearm extension: 4/4, wrist flexion: 4/4, wrist extension: 3/3, fingers flexion: 4/4, fingers abduction: 3/3, fingers extension: 3/3, thigh flexion: 2/2, thigh extension: 3/3, thigh abduction: 3/3, leg flexion: 4/4, leg extension: 3/3, foot flexion: 4/4, and foot extension: 4/4. There were, besides, mild contractures in fingers, and in wrist- and ankle-joints, without evidence of notable ligamentous laxity. Grip-force (using Jamar Dynanometer) measured 6 kg bilaterally. Tendon-reflexes were absent in all limbs. There was no calve-muscles hypertrophy. There were no sensory complaints or dysphagia. Blood- and CK tests were unremarkable.

On 2 examinations (5-year interval), CT/MRI-imaging showed muscle-atrophy with notably-diffuse fatty-involution of particularly glutei-, thigh- and leg-muscles on both sides (milder in *rectus femoris* and *gracilis*). Shoulder muscles seemed also affected. These muscle-imaging findings, in particular relative sparing of the *rectus femoris* and *gracilis*, are very much in keeping with what has been reported in RYR1-related myopathies [9].

Needle EMG showed fine polyphasic potentials (myogenic pattern). Insertional activity was normal with no spontaneous activity. Nerve-conduction studies were unremarkable. Cardiac echography was unremarkable; ECG showed mild right-bundle-block. Respiratory functions tests suggested a mild restrictive picture.

Muscle-biopsy (*Tibialis anterior*; Fig. 2), was crucial: Histo-morphology showed variation in fiber-diameter with atrophic and severely hypertrophied muscle-fibers, few splittings, slight endomysial fibrosis, mild fatty-infiltration, internal nuclei, and occasional nuclear-clumps/chains-of-nuclei. Frozen-sections showed few rimmed-vacuoles with occasional granular inclusions. Oxidative-enzymes staining showed defective histochemical reactivity in numerous fibers, consisting of mainly "cores" and core-like defects. Besides, there were "mini-cores", "moth-eaten", and occasional "pale" fibers. Cox-negative fibers were not prominent. Lack-of-distinction between muscle-fibers' types appearing like type-1-uniformity, was noticeable. PAS, modified-Gomori, ORO, acid-phosphatase and red-Congo stains, and, immuno-histochemical-staining for Desmin, were unremarkable. Electron-microscopy showed focal myofibrillar-disorganization with loss of normal-striation, typical of "streaming". These foci were partly devoid of mitochondria, and were compatible with "cores".

Altogether, these histo-morphological features (Fig. 2) suggested core-related myopathy. Remarkably, four years earlier,

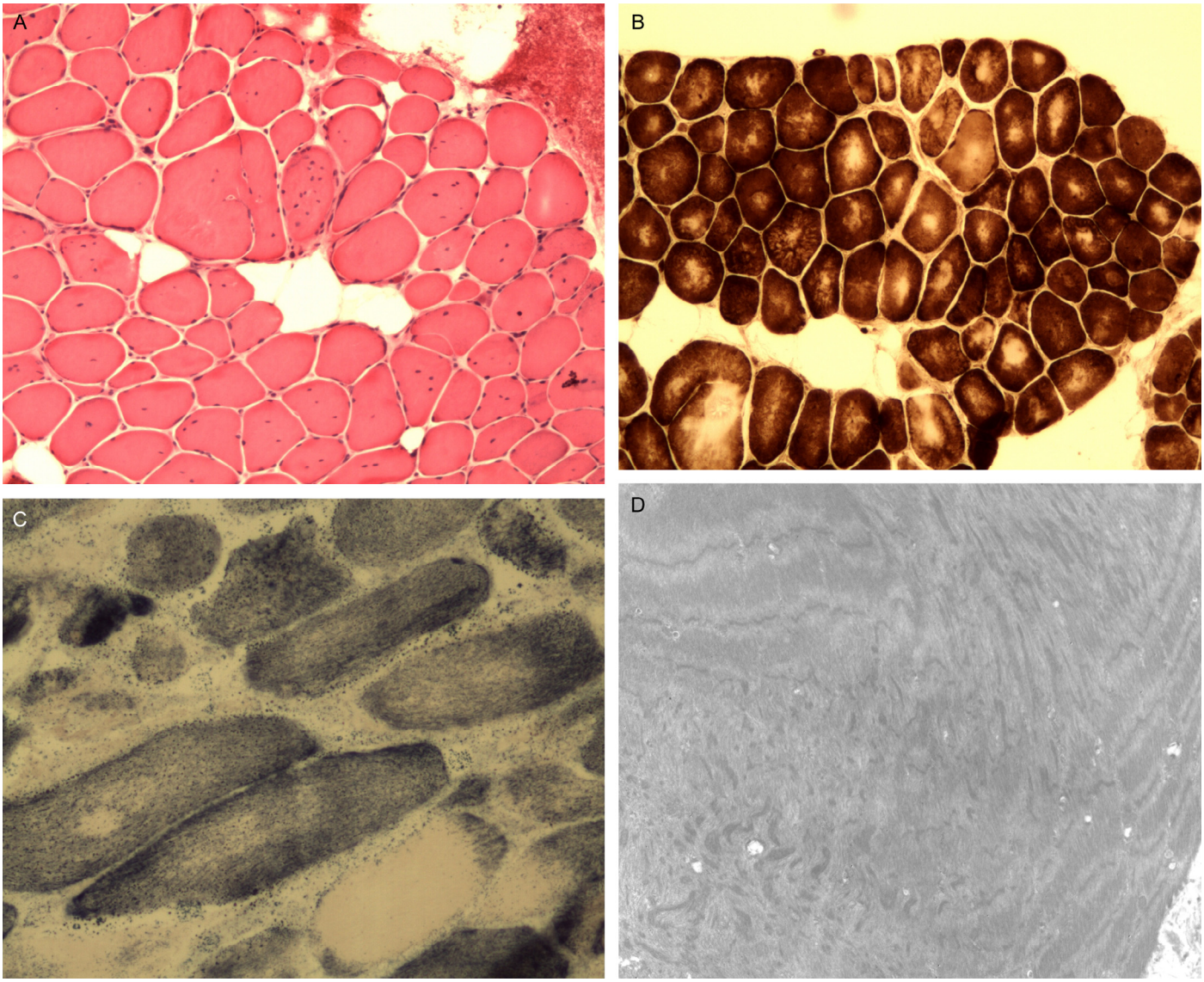

Fig. 2. Principal histopathological features of the muscle biopsy (index case). (A) Hematoxyline–eosine staining showing marked fiber-size variability and atrophic fibers, increased internal nuclei, occasional fiber-splitting, rare rimmed-vacuoles, focal fatty infiltration and mild endomysiale fibrosis. (B) Cox, and, (C) 'SDH' oxidative enzymes stainings reveal a spectrum of defective-reactivity in numerous fibers, namely cores and core-like defects, pale fibers, and other staining defects. Other/abnormal forms were also present (not shown). (D) Ultrastructural view of a core showing severe myofibrillar disruption and pronounced accumulation of smeared Z-line material (×4400). Original magnification: ×80; A and B (horizontal sections), ×200; C (longitudinal/oblique section), ×4400; D (electron microscopy).

biopsy (*vastus lateralis*) did not reveal "Cores" or lack of fiber-types distinction.

These findings prompted genetics explorations/family-counseling. We therefore resorted to *RYR1* gene-analyses that comprised leukocyte genomic DNA-sequencing, followed by muscle cDNA studies. MLPA was carried out on cDNA to look for potential genomic rearrangements.

The two sons (IV.2 and IV.3), aged 35 and 19 respectively, were healthy.

Patient III.1 (the oldest sister), was 62 years. She complained of progressive limbs'-weakness and chronic lumbar-backache, and presented a clinical picture not much different from her sister excepting milder waddling-gait, asymmetric scapular-winging and no apparent facial weakness or ptosis/ophthalmoplegia. Cardiac echography was normal. ECG showed discrete aspecific repolarization-defects.

Her daughter (IV.1; aged 35) had no complaints.

The youngest sister (III.5), aged 55, looked healthy. She had a healthy 31 year-old son (IV.4).

The *parents* (II.1 and II.2), aged 95 and 86 respectively, did not have related-abnormalities. Surgery/anesthesia (for unrelated conditions) did not cause adversities.

3. Molecular-genetics and *RYR1*-gene segregation studies (Fig. 1)

DNA from cases II.1, II.2, III.1, III.3, and III.5 was extracted from peripheral blood. Also, RNA was extracted from frozen muscle (proband), reverse-transcribed and

overlapping fragments were amplified [10]. cDNA-PCR-products were directly sequenced using the ABI PRISM Big Dye Terminator Cycle Sequencing v3.0 reaction kit and were analyzed on an AB1 3100 DNA Analyzer. The P281/282 MLPA kit allowed evaluating exonic deletion-size. Long-range PCR-amplifications were performed on gDNA and cDNA using QIAgen HotStar Taq polymerase (QIAGEN, USA) and primers encompassing the c.13150–14188 cDNA and g.132385_144834 gDNA sequences.

Mutation-numbering was based on cDNA sequence (Genbank NM_000540.2) and on gDNA sequence (Ensembl ENST00000359596).

Results disclosed compound heterozygous (Bi-allelic) mutations; a substitution and a deletion of the *RYR1*-gene, in both symptomatic sisters.

The first detected variant was a *c.13673G > A* change substituting an arginine-residue by glutamine (p.Arg4558Gln) in exon 94. This was found in the proband, initially appearing as homozygous in both cDNA and gDNA. This missense-mutation was present in the mother but was absent in the father; this suggested presence of deletion in exon 94 on the paternal allele. Quantification of exon 94 was performed using P281/P282 MLPA kit which includes copy number quantification of 14 *RYR1* exons spread over the gene including exons 90, 94 and 99. Heterozygous deletion of exon 94 was confirmed while exons 90 and 99 were not deleted in the affected sisters. Long-range PCR associated sequencing characterized the junction point of the deletion at cDNA level (c.13150_14188del) and further at gDNA level (g.132385_144834del). The 12.5 kb genomic-deletion started in exon 91 and ended inside exon 98. At cDNA level, the deletion induced a frame-shift (involving deletion of 1039 bp of transcript) leading to a premature stop codon (p.Pro4384GlyfsX20). This would result in the loss of the trans-membrane domain of the calcium channel. The paternal genomic-rearrangement was also present in the unaffected sister (III.5). As suggested by cDNA analysis, the deleted transcript was apparently stable. However, expression of the mutated protein would imply defective functioning since it would lack the calcium-channel and the trans-membrane region required for proper anchoring to sarcoplasmic membrane [11].

Segregation studies suggested recessive trait for both mutations whereas both patients were compound heterozygous, while 3 carriers (parents and their third daughter) were heterozygous (Fig. 1).

A brief overview of this study was presented in an abstract form [12].

4. Discussion

Large deletions in the *RYR1*-gene have been reported in only 2 cases; both presented with very early-onset (neonatal) and severe or lethal phenotype [7,8].

Here, we report a *novel* **large deletion** in the *RYR1*-gene in a Belgian family with *late-onset* core myopathy and *recessive* inheritance.

Patients were compound heterozygous involving additionally a "missense" mutation (p.Arg4558Gln). The latter has only been reported in two other families with core-related myopathy; a Brazilian family [13] and a second (unrelated) Belgian family [3]. In both, inheritance was recessive, but phenotype was *early-onset*.

The premature STOP codon (p.Pro4384GlyfsX20) associated with the large genomic rearrangement would result in loss of the trans-membrane calcium-channel and the C-terminal domain.

The *missense*-mutation (p.Arg4558Gln) targets a conserved arginine residue that maps to the M5 transmembrane portion of the calcium-channel [14]. The novel large-deletion (c.13150_14188del) we report herein would result in the synthesis of an apparently stable/truncated mRNA. Translation of the transcript would result in the synthesis of a non-functional RYR1 lacking the transmembrane domain required for proper anchoring onto the sarcoplasmic reticulum membrane. This would result in a premature degradation of the aberrant subunits and the ultimate expression of the Gln4558-RYR1 in a hemizygous state in affected patients.

In such a context whereby null-mutation affects one allele, phenotype-variability would depend on the nature of mutations present on the second allele [3]. Interestingly, one of the only 2 other cases of p.Arg4558Gln mutation, which was associated with a null-mutation [3], also presented a *mild* form of core myopathy {though onset was earlier (childhood)}: p.Arg4558Gln mutation could thus underlie mild phenotype.

It is probable that, in a context of a quantitative defect of RYR1, onset and evolution of myopathy would depend on the level of expression and stability of the transcript carrying the missense mutation and would consequently depend on the individual genetic background. Furthermore, RYR1 is associated with numerous regulatory proteins whose levels and functions might vary depending on the individuals' genetic-background. The precise pathogenic mechanism underlying age-of-onset in such circumstances thus awaits further elucidations.

Reports with elaborate genetic and clinico-pathological data on *late-onset* (beyond early childhood) phenotype and RYR1 mutations with confirmed core myopathy are rare; Table 1 shows that among these cases, only 2 (no. IX.1 and XIII.1; with proven myopathy) were *recessive*. This overview, thus, highlights the rarity of recessive co-occurrence in late-onset RYR1-associated core-myopathies.

Phenotype/genotype variations are thus increasingly emerging in RYR1-related core-myopathies; the majorities are point-mutations although *small* deletions have also been detected [23]. The *large deletion* we report herein is unique, and the combined late-onset/recessive co-occurrence is exceptionally rare (Table 1).

It is note-worthy that the large size of the *RYR*-gene makes molecular studies laborious, often relying on genomic sequencing of exons. Our report highlights that the detection of such rare mutations (large genomic rearrangements), could be missed unless resorting to muscle tissue for complementary explorations like cDNA and protein analyses.

Histo-pathologically, a wide array of abnormalities could be seen [24], and cores might be atypical, absent, or evolve with time. Besides, differential muscle-involvement could be observed.

Table 1
Overview of verified cases of late-onset RYR1-associated core myopathy.

Family. Patient number	Clinico-pathological findings			Inheritance	*RYR1* mutation(s)	References
	Weakness	Age of onset	Pathology (cores)			
I.1	+	Adolescence	NA	AD	c.12639del9; p.R4214_F4216del	Ref [10].
I.2	+	Adolescence	+	AD	c.12639del9; p.R4214_F4216del	Ref [10].
I.3	+	Adolescence	+	AD	c.12639del9; p.R4214_F4216del	Ref [10].
II.1	+	Adolescence	+	AD	c.14473C > T; p.R4825C	Ref [10].
II.2	+	Adolescence	+	AD	c.14473C > T; p.R4825C	Ref [10].
III.1	+	30	NA	AD	c.14818G > A (somatic mosaic); p.A4940T	Ref [15].
IV.1	+	38	+	AD	c.14677C > T; p.R4893W	Ref [15].
V.1	−; atrophy	35	+	Sporadic; apparently AD	c.1201C > T; p.R401C	Ref [16].
VI.1	+	Adolescence	+	AD	c.7522C > T; p.R2508C	Ref [17].*
VII.1	+	30	+	AD	c.[7304G>A+12891C>T]; p.[R2435H + A4295V]	Ref [18].
VII.2	+	24	+	AD	c.[7304G>A+12891C>T]; p.[R2435H + A4295V]	Ref [18].
VIII.1	+	Adulthood	+	Sporadic; no SS	c.119G > T; p.G40V	Ref [19].
IX.1	−; myalgia	43	+	AR	c.[6612C>G+14228G>A]; p.[H2204Q + G4743D]	Ref [20].
X.2	−; myalgia	59	+	Sporadic; no SS	c.[10097G>A+ 11798A>G]; p.[R3366H + Y3933C]	Ref [20].
XI.1	−; camptocormia	Adulthood	+	Sporadic; no SS	c.9713A > G; p.E3238G	Ref [21].
XI.2	+	Adulthood	+	Sporadic; no SS	c.10354A > C; p.K3452Q	Ref [21].
XII.1	−; camptocormia	Adulthood	+	Sporadic; no SS mention!	c13513G > C; p.D4505H	Ref [21].
XIII.1	+	Adulthood	+	AR; sporadic	c.[8888T>C+8888T>C]; p.[L2963P + L2963P]	Ref [22].

−: no muscle weakness; +: muscle weakness present; AD: autosomal dominant; AR: autosomal recessive; NA: not available, SS: segregation study.
* This author also refers to five patients who were screened for malignant hyperthermia only (because of positive family history) but with no core myopathy.

Cores can also occur in other conditions (including neurogenic atrophy), and in association with mutations in other genes (ACTA1, SEPN1, and MYH7). Finally, among RYR1-related core-myopathies, "typical CCD" is seemingly the only entity that presents uniformity in inheritance- and histopathology-patterns.

RYR1 pathology is known to be linked to malignant hyperthermia (MH). None of our patients were IVCT-tested, but among members of this family who were subjected to general anesthesia, none developed clinically-evident MH. However, we do not know the type of anesthesia used, whereas the main risk factors are the type of anaesthetics and/or muscle-relaxants used.

Interestingly, one of the exceptionally-rare patients with a nul-mutation who reportedly had the RYR1 p.Arg4558Gln mutation [3; case no. 8], was IVCT-tested and shown to be MHN. This observation suggests that even when expressed in a hemizygous state, the mutation would not present a MH risk. It is therefore unlikely for the mutation when expressed in heterozygous states to present MH risk. However, it remains highly recommended that whenever functional assay (to evaluate MH risk) could not be secured, concerned individuals should best be referred to specialized MH-centers.

In summary, we report a *novel* and rare (large) *RYR1*genomic-*deletion* in a Belgian family remarkably characterized by *late-onset* and *recessive* core myopathy.

The lack of phenotype/genotype correlation and the growing number of atypical cases can cause diagnostic-difficulties; this should raise alertness about RYR1-pathology, and should favor new methods of molecular-investigations that allow simultaneous-exploration of large panels of genes. Besides, careful history-taking often reveals that "late-onset" myopathies often comprise manifestations (albeit subtle) that could date back to quite-long periods whereas many patients report evidence of childhood-onset weakness (for example, never being good at sports, and/or having other physical-effort limitations) when specifically asked for.

Acknowledgements

Authors are very grateful to the family that supported this research work. Authors thank Dr. Catherine Fourneau (Brussels) for valued comments on parts of the manuscript, and Mr. Jean-Yves Bonnet, Mr. Abdelrahmanne Bouysantiman, and Ms, Nathalie Vennik for excellent technical assistance. Comments and suggestions made by the Editor-in Chief and the Reviewers greatly improved our article; to them we express our gratitude. Non-Targeted research-financing (HK) was partly provided by "Fondation Brugmann" (a non-profit University-Hospital Funding Body).

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