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Mutations in *RYR1* are a common cause of exertional myalgia and rhabdomyolysis

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Abstract

Mutations in the skeletal muscle ryanodine receptor (*RYR1*) gene are a common cause of neuromuscular disease, ranging from various congenital myopathies to the malignant hyperthermia (MH) susceptibility trait without associated weakness.

We sequenced *RYR1* in 39 unrelated families with rhabdomyolysis and/or exertional myalgia, frequent presentations in the neuromuscular clinic that often remain unexplained despite extensive investigations. We identified 9 heterozygous *RYR1* mutations/variants in 14 families, 5 of them (p.Lys1393Arg; p.Gly2434Arg; p.Thr4288_Ala4290dup; p.Ala4295Val; and p.Arg4737Gln) previously associated with MH. Index cases presented from 3 to 45 years with rhabdomyolysis, with or without exertional myalgia ($n = 12$), or isolated exertional myalgia ($n = 2$). Rhabdomyolysis was commonly triggered by exercise and heat and, less frequently, viral infections, alcohol and drugs. Most cases were normally strong and had no personal MH history. Inconsistent additional features included heat intolerance, and cold-induced muscle stiffness. Muscle biopsies showed mainly subtle changes. Familial *RYR1* mutations were confirmed in relatives with similar or no symptoms. These findings suggest that *RYR1* mutations may account for a substantial proportion of patients presenting with unexplained rhabdomyolysis and/or exertional myalgia. Associated clinico-pathological features may be subtle and require a high degree of suspicion. Additional family studies are paramount in order to identify potentially MH susceptible relatives.

Keywords: Skeletal muscle ryanodine receptor (*RYR1*) gene; Congenital myopathies; Malignant hyperthermia (MH) susceptibility; Rhabdomyolysis; Calcium homeostasis

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1. Introduction

Rhabdomyolysis is a syndrome characterized by muscle breakdown as the common endpoint of multifactorial etiologies and accounts for up to 7% of all cases of acute renal failure (ARF). The reported annual incidence is 26,000 cases in the United States alone [1]. Exertional myalgia with or without rhabdomyolysis is a common presenting complaint in neurological practice. The underlying cause remains often elusive, particularly in the absence of associated weakness and once an underlying metabolic disorder has been excluded [2].

Mutations in the skeletal muscle ryanodine receptor (*RYR1*) gene have recently emerged as one of the most common causes of inherited neuromuscular disease, associated with a wide clinical spectrum ranging from the malignant hyperthermia susceptibility (MHS) trait, a pharmacogenetic hypermetabolic reaction to volatile anesthetics and muscle relaxants [for review [3]], to various congenital myopathies [4]. Whilst muscle pain is a commonly associated complaint in *RYR1*-related myopathies, rhabdomyolysis and/or exertional myalgia as the sole presenting feature of *RYR1* mutations have only been reported in isolated cases in the anesthesia literature [5–9].

Here we report clinical, histopathological and genetic findings from 14 families presenting with rhabdomyolysis and/or exertional myalgia but no or only little associated weakness. *RYR1* mutations were identified in all index cases but also in some relatives with only subtle or no symptoms.

2. Patients and methods

2.1. Patients

Patients were selected from a cohort of 39 families where one or more family member had presented with rhabdomyolysis and/or exertional myalgia to a tertiary neurological centre in the United Kingdom or The Netherlands. All index cases had been comprehensively investigated for common etiologies of rhabdomyolysis and/or exertional myalgia, including disorders of lipid metabolism, glycogenoses, mitochondrial or other metabolic myopathies, but no underlying cause had been identified prior to *RYR1* sequencing. Detailed medical histories and neurological examinations were obtained from all index cases and their relatives in whom *RYR1* mutations were identified. The study was approved and performed under the ethical guidelines issued by our institutions for clinical studies, with written informed consent obtained from all subjects (or, where applicable, their legal guardians) for genetic studies. A signed consent form was obtained for photos of any recognizable patient.

2.2. Muscle histology and biochemistry

All index cases from the 14 families had muscle biopsies, except in Family 13 where the muscle biopsy was obtained from the similarly affected father of the index case. All muscle biopsies were taken from the quadriceps. We reviewed the standard histological (hematoxylin and eosin, H&E; Gomori trichrome, GT; periodic acid-Schiff, PAS) and histochemical (nicotinamide adenosine dinucleotide-tetrazolium reductase, NADH-TR; myosin adenosine triphosphatase, ATPase, preincubated at pH 9.4, 4.6 and 4.3; cytochrome C oxidase, COX) stains in all patients. In addition, electron microscopy images were obtained from 4 biopsies.

2.3. Molecular genetic studies

The entire *RYR1* coding regions (exons 1–106) including splice sites were screened at the genomic level in all patients except Patient 8.II.1 who was only investigated for a *RYR1* mutation previously identified in a relative with a MH history. Where DNA was available, relatives of the probands were investigated for the presence of the *RYR1* mutation identified by direct sequencing. Novel *RYR1* variants identified were investigated by in silico analysis using Alamut v1.5 (Interactive Biosoftware) as previously described [4]. We also checked the frequency of the *RYR1* mutations and variants identified in our cohort in the 1000 genomes project (www.1000genomes.org) and the exome variant server (EVS) (http://evs.gs.washington.edu/EVS) datasets.

2.4. Haplotyping at the RYR1 locus

Haplotyping of unrelated patients carrying the recurrent *RYR1* p.Thr4288_Ala4290dup *RYR1* duplication was carried out using a panel of highly polymorphic microsatellite repeat markers located in and around the *RYR1* locus as previously described [10].

3. Results

3.1. Clinical features

The main clinical findings from the 14 families presenting with rhabdomyolysis and/or exertional myalgia where *RYR1* mutations were identified are summarized in Table 1. More detailed histories for each of the 14 families are provided in Supplemental file S1. Family 7 is shown in Fig. 1.

We identified *RYR1* mutations in a total of 24 individuals from 14 families, 14 index cases and 10 relatives in families 1, 3, 5, 7, 10, 11 and 13. The 14 index cases presented with isolated exertional myalgia ($n = 2$; Family 11 and 14), or rhabdomyolysis with or without exertional myalgia ($n = 12$; all other families). In contrast, out of the 10 relatives investigated, only 3 presented with rhabdomyolysis with or without exertional myalgia, 4 with isolated exertional myalgia and 3 were asymptomatic. There was a male preponderance ($n = 15$) amongst the 21 symptomatic individuals and the first episode of

rhabdomyolyis occured from early childhood to 45 years of age. Rhabdomyolysis was most commonly triggered by intense exercise ($n = 15$) and, less frequently, heat ($n = 2$), intercurrent infection ($n = 2$) and alcohol intake ($n = 1$); in some individuals only a combination of factors, namely exercise, heat and/or alcohol, appeared to have triggered an event. Of note, two individuals (6.I.1 and 12.II.1) were on Olanzapine treatment and another (4.I.1) was retrospectively diagnosed with hypothyroidism, untreated at the time of presentation. Out of the 15 individuals (both index cases and relatives) who experienced rhabdomyolysis events, in 5 those were isolated and in 10 those were recurrent. Rhabdomyolysis events or exertional myalgia typically occurred at an interval, often more than 24 h, after sustained exercise. In cases where exertional myalgia occurred earlier during exercise there was no "second wind" phenomenon. During episodes of rhabdomyolysis, serum creatine kinase (CK) levels increased markedly, up to 378,900 IU/l, often requiring forced diuresis and intensive care support. However, only 3 patients (3.I.1, 12.I.1 and 13.I.1) required prolonged haemodialysis. Baseline CK levels measured at an interval ranged from 50 IU/l to 1171 IU/l.

Signs and symptoms of mild proximal muscle weakness were detected in 4 unrelated individuals only on careful assessment at presentation; one patient (7.I.1) who had normal strength at the initial assessment developed weakness only over time. Other individuals were normally strong or even particularly athletic, engaging in a wide range of sport activities. Whilst facial or extraocular muscle involvement was not a typical feature, ptosis was present in two families (7, 8), necessitating corrective surgery in two individuals. In only two families (Family 11 and Family 14) had a neuromuscular condition been suspected prior to presentation with exertional myalgia and/or rhabdomyolysis. Other, inconsistently associated clinical features included signs of heat intolerance ($n = 6$) as well as muscle stiffness, mainly in response to cold ($n = 3$).

Only one of the individuals (3.I.1) included in our study had experienced a clinical MH reaction, whilst MHS was confirmed on IVCT in two others (3.I.2, 8.I.1). There had been suspected MH events in families 8 and 14 but no further detail could be obtained. Of note, in one family (Family 7) there was a history of sudden unexpected death in several young adults and one infant. Other medical problems in our series included an unexplained increased bleeding tendency in family 7, psychiatric problems in patients 6.I.1 and 12.I.1, and hypothyroidism in patient 4.I.1.

Four patients had muscle ultrasound of the lower limb, demonstrating selective involvement characterized by more pronounced involvement of the vasti compared to the rectus femoris (7.I.1, 11.II.1) and a normal appearance in

Table 1
Main clinical features at the time of presentation in individuals presenting with exertional myalgia and/or rhabdomyolysis associated with mutations in the skeletal muscle ryanodine receptor (*RYR1*) gene. MHS, malignant hyperthermia susceptibility, ND, no data. Index cases are indicated in bold.

Family	Case	Sex	Exertional myalgia	Rhabdomyolysis			CK (IU/l)		MHS	Heat intolerance	Stiffness	Weakness
				Episodes	Nr	Trigger	Max	Min				
1	I.1	M	Yes	No	0	Exercise	ND	ND	No	No	No	No
	I.2	M	Yes	Yes	>5	Exercise	ND	ND	No	No	No	No
	II.1	M	Yes	Yes	>5	Exercise	8866	1130	No	No	No	No
2	**I.1**	M	Yes	Yes	>3	Exercise, Heat, Alcohol	3849	96	No	Yes	No	Yes
3	**I.1**	M	Yes	Yes	>2	Exercise	4800	65	Yes	No	No	No
	I.2	M	Yes	No	0	Exercise	ND	ND	Yes*	No	No	No
4	**I.1**	M	Yes	Yes	1	Exercise**	12670	769	No	No	No	No
5	I.1	M	No	No	0	Not applicable	ND	ND	No	No	No	No
	II.1	M	Yes	Yes	>3	Exercise	12,000	299	No	No	No	No
6	**I.1**	M	No	Yes	2	Exercise**	44,500	150	No	Yes	No	No
7	**I.1**	F	Yes	Yes	3	Exercise	56,900	185	No	Yes	Yes	No
	II.1	M	No	No	0	Not applicable	2100	1171	No	No	No	No
	II.2	F	Yes	Yes	2	Exercise	378,900	184	No	Yes	Yes	No
8	**II.1**	M	No	Yes	1	Exercise	14,765	157	Yes*	No	No	No
9	**I.1**	M	Yes	Yes	>3	Exercise	ND	N	No	Yes	No	Yes
10	I.1	F	Yes	No	0	Not applicable	ND	ND	No	No	No	No
	II.1	M	Yes	Yes	1	Exercise, Heat	1553	N	No	Yes	No	No
	II.2	F	No	No	0	Not applicable	ND	ND	No	No	No	No
11	I.1	F	Yes	No	0	Not applicable	ND	50	No	No	No	No
	II.1	M	Yes	No	0	Not applicable	ND	N	No	No	No	Yes
12	**II.1**	M	Yes	Yes	>10	Exercise**	234,417	120	No	No	No	No
13	I.1	M	No	Yes	1	Infection	ND	ND	No	No	No	No
	II.1	F	No	Yes	1	Infection	179,600	40	No	No	No	No
14	**II.1**	F	Yes	No	0	Not applicable	274	260	No*	No	Yes	Yes

* Patient 3.I.1 had a clinical malignant hyperthermia (MH) reaction, whilst MHS was confirmed in patients 3.I.2 and 8.II.1 on in vitro contracture testing but neither had a clinical MH history; patient 14.II.1 had a family but no personal history of MH, and no IVCT was performed.
** Patient 4.I.1 had a history of hypothyroidism, and patients 6.I.1 and 12.II.1 were on Olanzapine treatment at the time of presentation.

Fig. 1. In Family 7, both mother (7.I.1, on the right) and daughter (7.II.2, centre) presented with recurrent episodes of rhabdomyolysis and were found to carry the same heterozygous *RYR1* missense mutation, p.Gly2434Arg. Her brother (7.II.1, on the left) carrying the same *RYR1* substitution had marked muscle hypertrophy and baseline hyperCKemia but had never experienced any rhabdomyolysis episodes despite a vigorous exercise regime. All 3 family members had ptosis, progressive and requiring surgical correction in the mother who also developed limb girdle weakness in her 5th decade.

two patients (7.II.1, 1.II.1) each. Muscle MRI findings in 8 patients ranged from normal ($n = 4$) to muscle atrophy with mild increases in signal intensity ($n = 2$) and variable degrees of muscle hypertrophy without increases in signal intensity ($n = 2$) (Fig. 2).

3.2. Muscle histology

Muscle biopsies were available for review from 13 index cases and two similarly affected relatives (7.II.2, 13.I.1). The main histopathologic findings are summarized in Supplemental Table 1 and typical features are illustrated in Fig. 3. In most patients there were non-specific but unequivocal changes, comprising increased variability in fiber size, increased internal nucleation and unevenness or core-like structures on oxidative stains. Highly atrophic ("pinprick") fibers expressing fetal myosin were observed in few patients where additional immunohistochemical stains were performed, however, this had not been done systematically, reflecting different practices at different centres.

3.3. Molecular genetic analysis and haplotyping at the RYR1 locus

The main genetic findings in our cohort are summarized in Table 2. Out of the 9 mutations identified, 4 (p.Lys1393Arg; p.Gly2434Arg, p.Ala4295Val and p.Arg4737Gln) have been previously implicated in the MHS trait [11–14]. One *RYR1* variant (p.Thr4288_Ala4290dup) identified in 4 unrelated Afro-Carribbean families in our series has been previously found in an African–American army recruit presenting with an isolated rhabdomyolysis episode and confirmed MH susceptibility [8]; this individual also harbored two additional *RYR1* variants and the authors concluded that in the absence of functional data and segregation analysis it was difficult to be certain which of the 3 variants was associated with ERM, MHS or both phenotypes. In our series, some but not all patients heterozygous for p.Thr4288_Ala4290dup had additional *RYR1* variants of uncertain significance, suggesting the possibility of a synergistic or dosage effect as seen with some MHS mutations [15]. Although the recurrent p.Thr4288_Ala4290dup duplication was only identified in families of African or Afro-Caribbean origin, haplotyping studies did not support a founder effect on the level investigated (data not shown). Although not previously reported, the p.Tyr2426Cys variant identified in Family 6 localizes to a known MHS mutational hotspot, in close proximity to the previously reported *RYR1* MHS mutation p.Gly2434Arg [12], also identified in Family 7 and Family 8 in our cohort, and p.Arg2433Gly, also previously implicated in ERM in one patient [5]. However, formal in vitro contracture testing (IVCT) testing in patient 6.I.1 did not suggest MHS associated with this variant.

The *RYR1* variant identified in families 13 and 14 (p.Ala4295Val) has been previously reported in a large Korean MH pedigree [13], however, as it always occurred

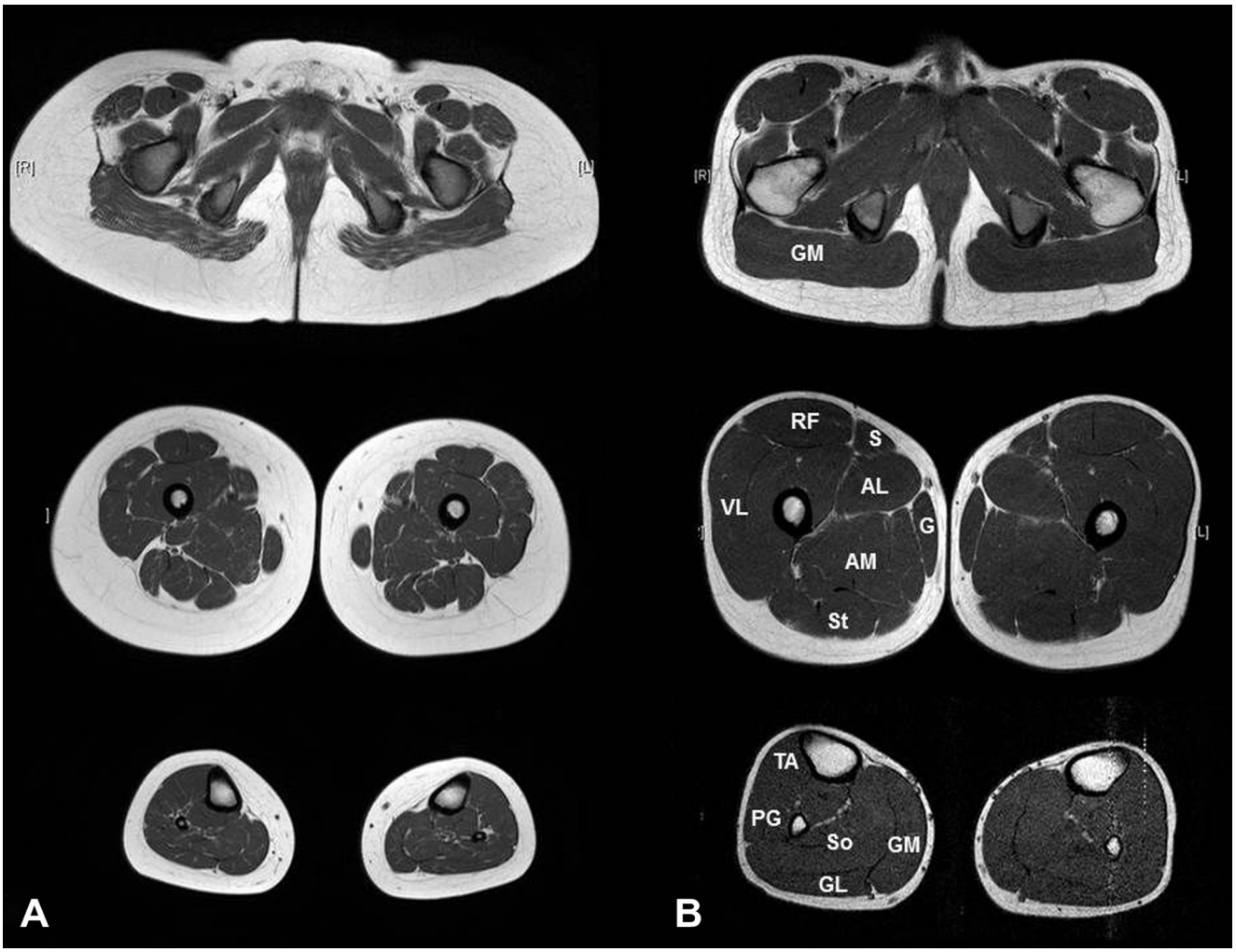

Fig. 2. T1-weighted muscle MRI, transverse sections from the pelvis (top row), the thigh (middle row) and the calves (bottom row) from Patients 9.I.1 (A, at 7 years of age) and 10.II.I (B, at 15 years of age) harboring the same *RYR1* p.Thr4288_Ala4290 duplication. In (A), there is generalized atrophy pronounced in the pelvis and thigh with mild increase in abnormal signal. In (B), muscle bulk is generally hypertrophic with normal signal. GM, gluteus maximus; VL, vastus lateralis; RF, rectus femoris; AL, adductor longus; AM, adductor magnus; G, gracilis; S, sartorius; TA, tibialis anterior; PG, peroneal group; So, soleus; GM, gastrocnemius medialis; GL, gastrocnemius lateralis.

in conjunction with Arg2435His in the Korean family it may only be pathogenic as part of a composite genotype. Interestingly, also in Family 13 and 14 p.Ala4295Val occurred in conjunction with a second *RYR1* variant, p.Pro1787Leu, suggesting the possibility of a composite effect also in these families. p.Pro1787Leu has been previously reported as a polymorphism [14] but is frequently found in patients with *RYR1*-related myopathies (unpublished observation), suggesting at least a modifying effect of this variant. In Family 3, two *RYR1* mutations previously associated with the MHS trait, p.Lys1393Arg [11] and p.Arg4737Gln, [14] were identified.

None of the *RYR1* mutations/variants identified in our cohort was found in the 1000 genomes project (www.1000genomes.org) or the exome variant server (EVS) (http://evs.gs.washington.edu/EVS/) datasets, with the notable exception of p.Lys1393Arg whose frequency in the general population based on these data appears to be around 1%. However, previous functional studies on B-lymphocytes carrying p.Lys1393Arg [16] have demonstrated increased resting calcium levels as well as an increased 4-cmc induced calcium release, suggesting that p.Lys1393Arg is indeed functionally relevant. MH susceptibility has also been demonstrated on IVCT testing in patients heterozygous for p.Lys1393Arg [17].

4. Discussion

A link with exertional rhabdomyolysis (ERM) has been suggested in the anesthesia literature almost since the recognition of the malignant hyperthermia susceptibility (MHS) trait as a distinct entity, but overall is considered rare. ERM and MHS are both syndromes characterized by an uncontrolled rise in intracellular skeletal muscle calcium [1] as the pivotal mechanism leading from accelerated mechanical, chemical or oxidative stress to

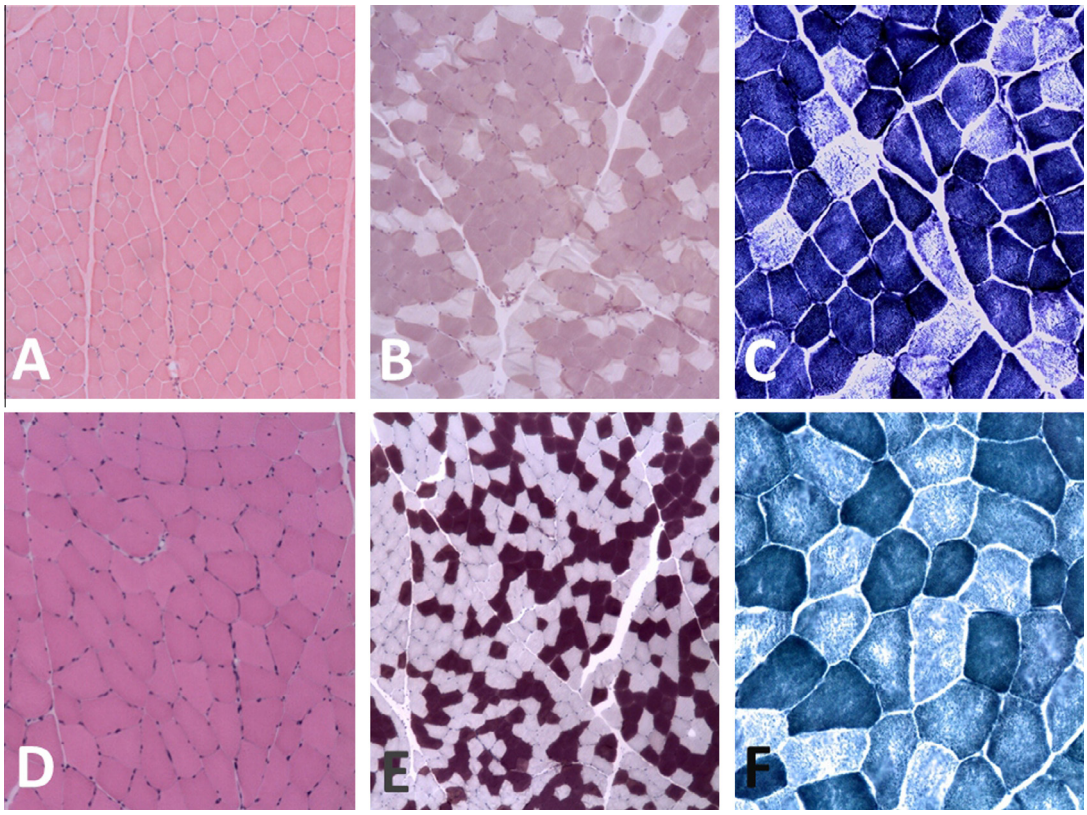

Fig. 3. Quadriceps biopsies from Patients 9.I.1 (A–C) and 10.II.1 (D–F), transverse sections, H&E (A,D), ATPase pre-incubated at a pH of 4.2 (B,E) and NADH-TR (C,F). In Patient 9.I.1, there is increased variability in fiber size (A) and prominence of type 1 fibers (B,C). In Patient 10.II.1, H&E (D) and ATPase (E) stains appear normal but there is unevenness on oxidative stains and a few core-like structures (F).

Table 2
Skeletal muscle ryanodine receptor (*RYR1*) gene variations in individuals presenting with exertional myalgia and/or rhabdomyolysis. MHS, malignant hyperthermia, ERM, exertional rhabdomyolysis.

Family	Case	Ethnicity	Sex	*RYR1* mutation	Reference	Previously reported phenotype
1	I.1	Caucasian	M	c.957+5_957+29del	Not previously reported	
	I.2	Caucasian	M	c.957+5_957+29del	Not previously reported	
	II.1	Caucasian	M	c.957+5_957+29del	Not previously reported	
2	I.1	Caucasian	M	p.Lys1393Arg	[11]	MHS
3	I.1	Caucasian	M	p.Lys1393Arg	[11]	MHS
				p.Arg4737Gln	[11]	MHS
	I.2	Caucasian	M	p.Lys1393Arg	[11]	MHS
				p.Arg4737Gln	[14]	MHS
4	I.1	Caucasian	M	p.Asp2129Asn	Not previously reported	
5	I.1	Caucasian	M	p. Gly2132Ser	Not previously reported	
	II.1	Caucasian	M	p. Gly2132Ser	Not previously reported	
6	I.1	Afro-Caribbean	M	p.Tyr2426Cys	Not previously reported	
7	I.1	Caucasian	F	p.Gly2434Arg	[12]	MHS
	II.1	Caucasian	M	p.Gly2434Arg	[12]	MHS
	II.2	Caucasian	F	p.Gly2434Arg	[12]	MHS
8	II.1	Caucasian	M	p.Gly2434Arg	[12]	MHS
9	I.1	African	M	p.Thr4288_Ala4290dup	[8]	ERM, MHS
10	I.1	Afro-Caribbean	F	p.Thr4288_Ala4290dup	[8]	ERM, MHS
	II.1	Afro-Caribbean	M	p.Thr4288_Ala4290dup	[8]	ERM, MHS
	II.2	Afro-Caribbean	F	p.Thr4288_Ala4290dup	[8]	ERM, MHS
11	I.1	African	F	p.Thr4288_Ala4290dup	[8]	ERM, MHS
	II.1	African	M	p.Thr4288_Ala4290dup	[8]	ERM, MHS
12	II.1	African	M	p.Thr4288_Ala4290dup	[8]	ERM, MHS
13	I.1	Caucasian	M	p.Ala4295Val	[13]	MHS
	II.1	Caucasian	F	p.Ala4295Val	[13]	MHS
14	II.1	Caucasian	F	p.Ala4295Val	[13]	MHS

irreversible muscle breakdown. Moreover, ERM is often associated with profound hyperpyrexia, muscle stiffness and metabolic derangements closely resembling malignant hyperthermia (MH) [18]. A link between *RYR1*-related MH and ERM is also supported by observations in porcine [19], equine [20] and murine [21,22] *RYR1* mutants.

Exertional myalgia and rhabdomyolysis have been reported in several individuals with in vitro evidence of MHS [5–9,23–27], only few of them genetically resolved. Our findings based on complete *RYR1* sequencing suggest that *RYR1* mutations may account for more than a third of otherwise unexplained rhabdomyolysis events.

RYR1 mutations/variants identified in our cohort have either been previously reported in MH susceptible individuals [8,11–13], or localize to known MH mutational hotspots. In addition to *RYR1* mutations localizing to the central MH domain, we identified a recurrent exon 91 p.Thr4288_Ala4290dup variant, previously reported in an African-American army recruit with ERM and MHS [8], localizing to the *RYR1* C-terminus. Although without functional data and extended segregation analyses the precise pathogenicity of the exon 91 p.Thr4288_Ala4290dup variant has to remain uncertain, identification of this variant (notably absent from the 1000genomes dataset) in 4 unrelated families presenting with ERM indicates a possible causal association.

Of note, only one patient in our cohort had a personal MH history, in keeping with the previously reported discrepancy between the presumed high prevalence of *RYR1*–related MHS mutations, estimated at 1 in 2000 in some populations, and the relatively low incidence of clinically manifest MH reactions, estimated at between 1 in 80,000–100,000 [3].

Whilst exercise was the most common identifiable trigger, rhabdomyolysis events in our cohort were often only triggered by a combination of stimuli, for example exercise, heat and alcohol, corresponding to recent findings in animal models of MHS [21,22]. In addition to well recognized triggers such as exercise or heat, we identified one family where rhabdomyolysis had been triggered by intercurrent infection (Family 13), previously only reported in one large Maori MHS pedigree [28]. In a clinical setting, the well-recognized association of intercurrent viral infection and rhabdomyolysis will often be attributed to subclinical myositis rather than an underlying genetic defect, particularly when occurring in isolated cases, and a genetic background in this family had indeed only be suspected because of multigenerational involvement. Two patients (6.I.1, 12.II.1) were on Olanzapine treatment at the time of presentation with rhabdomyolysis, a previously reported association [29–31] whose pharmacogenetic basis has so far remained elusive. *RYR1* mutations have been recently implicated in other acquired myopathies, induced by both medical [32] and recreational [33] drug use. In another patient (4.I.1), co-existing hypothyroidism is likely to have exacerbated muscle symptoms.

Presentation at an interval after sustained exercise distinguishes *RYR1*-related rhabdomyolysis and exertional myalgia, for example from the glycogenoses, where early-onset of symptoms and a second wind phenomenon are typical. In contrast to the few previously published cases [5–9], marked hyperpyrexia, muscle rigidity or more profound metabolic derangements were not consistently associated with the rhabdomyolysis events observed in our cohort. This discrepancy may reflect an ascertainment bias, as those presenting with predominant hyperpyrexia are more likely to be referred to specialized MH units whereas those presenting with rhabdomyolysis without hyperpyrexia are more likely to be reviewed at a neuromuscular or neurological centre. Acute renal failure requiring dialysis was only rarely observed, in keeping with the recent observation that this complication seems to be less common in rhabdomyolysis due to exertion rather than other causes [34].

Associated neuromuscular features were highly variable in our cohort, even in carriers of the same *RYR1* mutation. A substantial number of unrelated individuals were very athletic with hypertrophic muscle bulk, a feature also observed in the porcine MH model and other patients with MHS [18], but only four unrelated individuals (2.I.1, 9.I.1, 11.II.1, and 14.II.1) had moderate proximal muscle weakness. Possible explanations for this marked inter- and intrafamilial variability include the presence of additional *RYR1* variations or variations concerning proteins involved in the same regulatory pathway. Multiple *RYR1* variants have been reported in other *RYR1*-related myopathies [8,35,36], occasionally on the same allele [37], whilst variations in different genes involved in calcium homeostasis have been found in individuals with ERM [9], suggesting a synergistic effect. Of note, in one patient (7.I.1) harboring a known MHS mutation weakness developed only in the 5th decade, in line with recent observations in humans [38] and MHS animal models [22]. Additional clinical features indicative of *RYR1* involvement were increased sweating and heat intolerance, also features in some animal models of MHS [21]. Exertional myalgia, a prominent feature of *RYR1*-related myopathies recognized already before their genetic resolution [39], was almost invariably present and the sole presenting feature in two families (11,14).

Other findings included mild to moderate CPK elevations in some individuals (up to 1171 IU/l at an interval), a potential source of diagnostic confusion in the distinction from other neuromuscular disorders. Muscle MRI findings were mainly non-specific where performed, in contrast to other *RYR1*-related myopathies where muscle MRI is often highly informative [40]. Histopathological changes such as increased variability in fiber size, increased internal nucleization, type 1 predominance and unevenness of oxidative stains, were non-specific but overall consistent with a *RYR1*-related myopathy. Highly atrophic ("pinprick") fibers expressing

fetal myosin were observed in few patients, however, more systematic and comparative immunohistochemical studies will be required to further evaluate if this is a consistent finding specific for *RYR1*-related myopathies with otherwise only subtle muscle biopsy abnormalities.

In conclusion, *RYR1* mutations ought to be considered in the investigation of patients presenting with unexplained rhabdomyolysis and/or exertional myalgia as they may account for a substantial proportion of cases. As *RYR1* screening is still laborious and often reveals variations of uncertain significance, requests should be based on a careful assessment, taking into account suggestive anamnestic and clinical features such as dominant inheritance, heat intolerance, prominent muscle bulk or weakness. When muscle biopsy is considered this should be done in conjunction with an MHS diagnostic IVCT and include comprehensive myosin immunohistochemistry, where available. The presence of cores or other suggestive histopathological features on muscle biopsy, or the demonstration of MH susceptibility should prompt *RYR1* mutation screening. Considering the marked intrafamilial variability associated with specific *RYR1* mutations, other family members ought to be assessed carefully in order to identify individuals at potential risk for MH reactions.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.nmd.2013.03.008.

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