ORIGINAL ARTICLE

RYR1-related myopathies: a wide spectrum of phenotypes throughout life

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Background and purpose: Although several recent studies have implicated *RYR1* mutations as a common cause of various myopathies and the malignant hyperthermia susceptibility (MHS) trait, many of these studies have been limited to certain age groups, confined geographical regions or specific conditions. The aim of the present study was to investigate the full spectrum of *RYR1*-related disorders throughout life and to use this knowledge to increase vigilance concerning malignant hyperthermia.

Methods: A retrospective cohort study was performed on the clinical, genetic and histopathological features of all paediatric and adult patients in whom an *RYR1* mutation was detected in a national referral centre for both malignant hyperthermia and inherited myopathies (2008–2012).

Results: The cohort of 77 non-related patients (detection rate 28%) included both congenital myopathies with permanent weakness and 'induced' myopathies such as MHS and non-anaesthesia-related episodes of rhabdomyolysis or hyperCKemia, manifested throughout life and triggered by various stimuli. Sixty-one different mutations were detected, of which 24 were novel. Some mutations are present in both dominant (MHS) and recessive modes (congenital myopathy) of inheritance, even within families. Histopathological features included an equally wide spectrum, ranging from only subtle abnormalities to prominent cores.

Conclusions: This broad range of *RYR1*-related disorders often presents to the general paediatric and adult neurologist. Its recognition is essential for genetic counselling and improving patients' safety during anaesthesia. Future research should focus on *in vitro* testing by the *in vitro* contracture test and functional characterization of the large number of *RYR1* variants whose precise effects currently remain uncertain.

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Introduction

Mutations in the skeletal muscle ryanodine receptor (*RYR1*) gene have emerged as a common cause of inherited neuromuscular disease, including malignant hyperthermia susceptibility (MHS). The *RYR1* gene encodes the principal sarcoplasmic reticulum calcium release channel (RyR1), with a crucial role in excitation–contraction coupling. The fundamental role of *RYR1* in normal muscle homeostasis and functioning is reflected in the wide range of both dominant and recessive disorders associated with *RYR1* mutations.

Whilst the clinical and pathological features of *RYR1*-related disorders with mainly autosomal-dominant (AD) inheritance – central core disease (CCD) and the MHS trait based on a positive *in vitro* contracture test (IVCT) – have been recognized for a long time [1], the full clinico-pathological spectrum of autosomal-recessively (AR) inherited *RYR1*-related myopathies has only emerged in recent years and continues to expand. More recently recognized recessive *RYR1*-related myopathies include forms of multiminicore disease (MmD) [2], centronuclear myopathy (CNM) [3] and congenital fibre type disproportion [4], but also presentations with only subtle or a combination of histopathological features [4–10]. In addition, King–Denborough syndrome (KDS) [11], exertional rhabdomyolysis [12] and late-onset axial myopathy [13] appear to be specific myopathic manifestations of malignant hyperthermia (MH) related *RYR1* mutations.

As a result of the increased availability of both diagnostic *RYR1* sequencing and exome sequencing, more and more patients with *RYR1* mutations are identified and referred for pre-anaesthetic screening and genetic counselling of family members. At the same time, the wide spectrum of *RYR1*-related myopathies includes many individuals without a (family) history of MH. In fact, MHS can be considered as a multifactorial event with low penetrance reflecting the outcome of an unfortunate combination of different factors. As a result, becoming familiar with the wide *RYR1* spectrum is of great value for all (paediatric) neurologists and anaesthesiologists to recognize patients at risk for MHS.

The aim of the present study was therefore to investigate the full genetic, histopathological and clinical spectrum of *RYR1*-related disorders at all ages in the entire Dutch population. Based on the unique experience of our national referral centre for both MH and congenital myopathies this has not been limited to certain age groups, confined geographical regions or specific conditions as seen in most recent studies [13–17].

Patients and methods

General

The human genetics department (Radboud University Medical Centre) and the Malignant Hyperthermia Investigation Unit Nijmegen (together the national referral centre for both MH and congenital myopathies) perform *RYR1* sequence analysis for the entire Dutch population.

Patients

This study was set up as an observational cohort study with a cross-sectional design. It was approved by the local research medical ethics committee. Between 2008 and 2012, DNA samples from 272 non-related patients were tested [225 with congenital myopathy, hyperCKemia or rhabdomyolysis; 47 with a (family) history of MH and a positive IVCT]. In all of them, the (family) history, physical examination, and/or results of ancillary investigations had suggested one of the *RYR1*-recognized phenotypes. One or more *RYR1* mutations were detected in 77 patients, 16 of which with undetermined significance. In the other 195 patients no mutation was found. Clinical and genetic data of all these 77 non-related patients were collected by one of the neurologists of the national referral centre for both MH and congenital myopathies, with the help of the network of neuromyologists and clinical geneticists in the Netherlands.

In vitro contracture studies

The halothane–caffeine IVCT was performed according to the European Group protocol for investigation of MHS on freshly biopsied muscle tissue of the quadriceps muscle [18].

Molecular genetic studies

The coding regions (exons 1–106) of the *RYR1* gene, including splice sites, were screened at the genomic level by standard Sanger sequencing. Relatives were investigated for the presence of the familial mutations only. Western blotting of RYR1 protein extracted from muscle and densitometric analysis were performed as described previously [19]. MHS patients were first tested by multiplex ligation-dependent probe amplification (MLPA) using two kits containing the first 27 functionally characterized MH mutations (www.emhg.org) supplemented with six frequently occurring, non-functionally characterized mutations in Europe. In the case of no mutation, sequencing was performed. Pathogenicity of mutations was estimated

Table 1 Phenotype and molecular features of *RYR1*-related myopathies: overview of the modes of inheritance

	Number of probands[a] (%)	Number of affected relatives with mutation(s)	Number of asymptomatic carriers	Individual mutations	
				Total	Novel
Putatively dominant mode of inheritance	49 (64)	116		37	8
Putatively recessive mode of inheritance	12 (16)	1	13	15 (4)	8
Unknown mode of inheritance/pathogenicity	16 (21)		7	9 (4)	8
Total	77 (100)	117	20	61	24

[a]The additional number of novel mutations for the mutations with recessive and unknown mode of inheritance, which are also reported as mutations with a dominant mode of inheritance in this cohort, are given in parentheses.

Table 2 Phenotype and molecular features of *RYR1*-related myopathies: overview of the phenotypes

Phenotype	AD families (1–49)[a]	AR families (50–61)[a]	Sporadic patients (62–77)[a]	Total (%) (1–77)[a]
Age at presentation, median (range), years	12 (0–60)	3 (0–27)	2 (0–50)	8 (0–60)
Age at genetic diagnosis, median (range), years	30 (0–68)	28 (2–47)	26 (8–64)	29 (0–68)
Age at longest follow-up, median (range), years	30 (6–70)	29 (12–55)	24 (9–67)	28 (0–70)
MH during anaesthesia	28 (2)[b]	(3)[b]	1	29 (38)[b]
EIR/rhabdomyolysis	6	1	2	9 (12)
HyperCKemia			1	1 (1)
Axial myopathy	2			2 (3)
CCD	11	7	5	23 (30)
Fetal akinesia	1			1 (1)
MmD		3	4	7 (9)
NM			1	1 (1)
CNM		1		1 (1)
KDS			1	1 (1)
CM n.o.s.	1		1	2 (3)
Total (%)	49 (64)	12 (15)	16 (21)	77 (100)

AD, autosomal-dominant; AR, autosomal-recessive; CCD, central core disease; CM n.o.s., congenital myopathy not otherwise specified; CNM, centronuclear myopathy; EIR, exercise-induced rhabdomyolysis; KDS, King–Denborough syndrome; MH, malignant hyperthermia; MmD, multiminicore disease; NM, nemaline myopathy.
[a]Numbers refer to the index patient nr's in the text and tables.
[b]The additional number of probands with an MH during anaesthesia and also CCD is given in parentheses; this occurred in two AD and three AR CCD probands; they have been classified and counted as CCD.

by frequency in large cohorts, recurrence, the literature and conservation of the involved amino acids (EVS/ClinSeq/EMHG).

Clinical studies

Information on (family) history, initial presentation, and most predominant symptoms and signs was obtained through the neurologists and geneticists of the national neuromuscular network. HyperCKemia was defined as creatine kinase (CK) > 1.5 × the upper limit of normal. Results of ancillary investigations (electromyography and muscle imaging) and of cardiorespiratory screening were also collected.

Histological studies

The reports of standard histological and histochemical stains and, if available, electron microscopy were reviewed. Needle or open muscle biopsies were taken from the quadriceps muscle, in one of the neuromuscular referral centres in the Netherlands. In seven patients sequential biopsies could be performed. Western blotting was performed in three patients.

Results

Inheritance and molecular genetics findings

In 77 non-related patients one or more *RYR1* mutations were detected, in 63 by sequencing and in 14 by MLPA analysis. Subsequently, 117 affected and 20 asymptomatic family members (carriers) were identified. The mutation detection rate was 28% (77/272). In total, 61 different mutations were detected throughout the *RYR1* sequence, of which 24 were novel. Inheritance was AD in 49 families (64%) and AR in 12 families (15%). In 16 families, the pathogenicity of

Table 3 Phenotype and molecular features of *RYR1*-related myopathies: specific molecular features in individual patients with putatively dominant inheritance, putatively recessive inheritance and of patients with uncertain pathogenicity or uncertain mode of inheritance

Patient no.	Sex, age at diagnosis	Phenotype	Family members tested (additional no. of affected family members with same mutation)	Coding DNA mutation and exon (ref: NM_000540.2)	Deduced mRNA or protein change	Frequency in controls or presence in EMHG (EVS/ ClinSeq/EMHG)	References/conservation
Patients with putatively dominant inheritance							
1	F, 46	MH during anaesthesia (family)	Symptomatic uncle (positive IVCT heterozygous); large MH family (5)	c.38T>G exon 1	p.(Leu13Arg)	−/−/−	Snoeck *et al.* 2004 [28]: AD in MHS; mutation not yet known (this patient) Ibarra *et al.* 2006 [29]: AD in MHS
2	M, 20	EIR	Asymptomatic father has same mutation (1)	c.957 + 5_957 + 29del intron 10	r.(spl?)	na	Dlamini *et al.* 2013 [12]: AD in EIR (this patient)
3	M, 15	MH during anaesthesia	Family members carriers; no IVCT performed (5)	c.1021G>A exon 11	p.(Gly341Arg)	−/−/y	Robinson *et al.* 2006 [6]: AD in MHS
4	F, 35	MH during anaesthesia (family)	Sister of mother: MH during anaesthesia; cousin fatal MH during anaesthesia (9)	c.1021G>A exon 11	p.(Gly341Arg)	−/−/y	Robinson *et al.* 2006 [6]: AD in MHS
5	F, 34	MH during anaesthesia	Symptomatic mother and daughter (MH) heterozygous (2)	c.1840C>T exon 17 c.14364 + 1G>T intron 99	p.(Arg614Cys) r.(spl?)	0.002/−/y −/−/−	Gilllard *et al.* 1991 [30]: AD in MHS Laquérriere *et al.* 2014 [31]: AR in arthrogryposis multiplex congenita
6	F, 44	MH during anaesthesia	Symptomatic relatives (positive IVCT) have same mutation (2)	c.1840C>T exon 17	p.(Arg614Cys)	−/−/y	Gillard *et al.* 1991 [30]: AD in MHS
7	M, 37	MH during anaesthesia	Symptomatic relative (positive IVCT) has same mutation (1)	c.1840C>T exon 17	p.(Arg614Cys)	−/−/y	Gilllard *et al.* 1991 [30]: AD in MHS
8	M, 62	MH during anaesthesia	Five sibs have mutation in exon 17; symptomatic son, daughter and four sibs (positive IVCT, MH during anaesthesia) have the mutation in exon 50; two sibs have both mutations (13)	c.1840C>T exon 17 c.8026C>T exon 50	p.(Arg614Cys) p.(Arg2676Trp)	−/−/y −/−/−	Gilllard *et al.* 1991 [30]: AD in MHS Guis *et al.* 2004 [32]: AD in MHS
9	M, 30	CCD	Mildly symptomatic father (proximal weakness, cramps) heterozygous (1)	c.2654G>A exon21	p.(Arg885His)	0.0002/−/−	This report; mammals, frog and fish
10[a]	M, 68	MH during anaesthesia; EIR	Symptomatic sibs (positive IVCT; heterozygous for both mutations) (5)	c.4178A>G exon 29 c.14210G>A exon 98	p.(Lys1393Arg) p.(Arg4737Gln)	0.01/−/− −/−/−	Broman *et al.* 2009 [33]: AD in MHS Vukcevic *et al.* 2010 [34]: AD in MHS
11	F, 64	CCD	Symptomatic daughter (heterozygous) (1)	c.5194G>A exon 34	p.(Glu1732Lys)	−/−/−	This report; mammals, frog and fish
12	M, 61	EIR	Asymptomatic daughters (no mutations)	c.6385G>A exon 39	p.(Asp2129Asn)	−/−/−	Dlamini *et al.* 2013 [12]: AD in EIR (this patient)
13	M, 34	EIR	Asymptomatic parents not tested	c.6394G>A exon 39	p.(Gly2132Ser)	−/−/−	Dlamini *et al.* 2013 [12]: AD in EIR (this patient)

(continued)

Table 3 *(Continued)*

Patient no.	Sex, age at diagnosis	Phenotype	Family members tested (additional no. of affected family members with same mutation)	Coding DNA mutation and exon (ref: NM_000540.2)	Deduced mRNA or protein change	Frequency in controls or presence in EMHG (EVS/ ClinSeq/EMHG)	References/conservation
14	M, 19	MH during anaesthesia; EIR	Symptomatic father (positive IVCT) same mutation (2)	c.6617C>T exon 40	p.(Thr2206Met)	−/−/y	Robinson *et al.* 2006 [6]: AD in MHS
15	M, 24	MH during anaesthesia	Mother and sib positive IVCT (heterozygous) (2)	c.6710G>A exon 41	p.(Cys2237Tyr)	−/−/−	Klingler *et al.* 2014 [35]: AD in MHS (this patient)
16	F, 46	CCD	Symptomatic family members (MHS) (heterozygous) (2)	c.6863T>C exon 42	p.(Leu2288Ser)	−/−/−	This report; mammals, frog and fish
17[b]	F, 48	CCD	Symptomatic daughter (MH reaction during anaesthesia) and son (EIR) have same mutation in exon 43 (2)	c.7018T>C exon 43 c.7760A>G exon 48	p.(Phe2340Leu) p.(Tyr2587Cys)	−/−/− −/−/−	This report; mammals, frog and fish This report; mammals, frog and fish
18	M, 24	MH during anaesthesia	Mother positive IVCT (heterozygous) (2)	c. 7025A>G exon 43	p.(Asn2342Ser)	0.001/−/−	Robinson *et al.* 2006 [6]: AD in MHS
19	M, 38	MH during anaesthesia	Symptomatic daughter (hyperlordosis and toewalking) not tested; symptomatic sibs and father (positive IVCT) not tested	c.7035C>A exon 44	p.(Ser2345Arg)	−/−/−	This report; mammals, frog and fish
20	F, 29	MH during anaesthesia (family)	Symptomatic mother (fatal MH) (1)	c.7048G>A exon 44	p.(Ala2350Thr)	−/−/y	Sambuughin *et al.* 2001 [36]: AD in MHS
21	M, 10	MH during anaesthesia	Symptomatic father (positive IVCT) same mutation (1)	c.7048G>A exon 44	p.(Ala2350Thr)	−/−/y	Sambuughin *et al.* 2001 [36]: AD in MHS
22	M, 36	EIR	Unknown	c.7277A>G exon 45	(p.Tyr2426Cys)	−/−/−	Dlamini *et al.* 2013 [12]: AD in EIR (this patient)
23	F, 16	EIR	Symptomatic mother (EIR; heterozygous); symptomatic brother (hyperCKemia; heterozygous) (2)	c.7300G>A exon 45	p.(Gly2434Arg)	−/−/y	Robinson *et al.* 2006 [6]: AD in MHS
24	F, 28	MH during anaesthesia	Symptomatic father and uncle (IVCT positive) same mutation (4)	c.7300G>A exon 45	p.(Gly2434Arg)	−/−/y	Keating *et al.* 1994 [37]: AD in MHS
25	F, 6	CM n.o.s.	Asymptomatic father same mutation; paternal grandfather had CCD but has not been tested	c.7354C>T exon 46	p.(Arg2452Trp)	−/−/−	Robinson *et al.* 2006 [6]: AD in MHS
26	F, 22	MH during anaesthesia	Asymptomatic father same mutation (2)	c.7361G>A exon 46	p.(Arg2454His)	−/−/y	Barone *et al.* 1999 [38]: AD in MH
27	M , 7	MH during anaesthesia	Possibly symptomatic father (sudden unexplained death) not tested	c.7523G>A exon 47	p.(Arg2508His)	−/−/−	Wu *et al.* 2006 [39]: AD in MHS

(continued)

Table 3 *(Continued)*

Patient no.	Sex, age at diagnosis	Phenotype	Family members tested (additional no. of affected family members with same mutation)	Coding DNA mutation and exon (ref: NM_000540.2)	Deduced mRNA or protein change	Frequency in controls or presence in EMHG (EVS/ ClinSeq/EMHG)	References/conservation
28	M , 12	CCD	Symptomatic (EIM) mother (heterozygous) (1)	c.7645_7650dup exon 48	p.(Ala2549_ Leu2550dup)	na	Böhm *et al.* 2013 [40]: AR in CCD
29	M , 44	Rhabdo-myolysis	Asymptomatic parents/sibs not tested	c.10219G>T exon 67	p.(Ala3407Ser)	−/−/−	Molenaar *et al.* 2014 [41]: AD in rhabdomyolysis (this patient)
30	M, 47	Axial myopathy	Asymptomatic father (heterozygous) (1)	c.10621G>A	p.(Ala3541Thr) exon 71	0.0001/−/−	Løseth *et al.* 2013 [13]: AD in axial myopathy (this patient)
31	M, 2	CCD	*De novo* mutation	c.11905C>A exon 86	p.(Gln3969Lys)	−/−/−	This report; mammals, frog and fish
32	F, 4	CCD	Symptomatic (CCD) father and sib same mutation (2)	c.12819_12830del exon 91	p.(Glu4276_ Ala4279del)	na	This report; not conserved
33[c]	M, 59	CCD	Three symptomatic sibs, two symptomatic daughters, two symptomatic nephews (heterozygous) (7)	c.13940T>C exon 95	p.(Leu4647Pro)	−/−/−	Kreava *et al.* 2013 [42]: AD in CCD
34	M, 17	MH during anaesthesia	Symptomatic father (postoperative death) not tested; asymptomatic mother and sister same mutation (2)	c.14477C>T exon 100	p.(Thr4826Ile)	−/−/y	Brown *et al.* 2000 [43]: AD in MH
35	M, 30	MH during anaesthesia	Symptomatic father (positive IVCT) heterozygous; symptomatic nephew (hyperCKemia) heterozygous; asymptomatic brother and sister heterozygous (4)	c.14545G>A exon 101	p.(Val4849Ile)	−/−/−	Robinson *et al.* 2006 [6]: AD in MHS Jungbluth *et al.* 2002 [44]: AR in CCD
36	F, 43	MH during anaesthesia (family)	Unknown	c.14545G>A exon 101	p.(Val4849Ile)	−/−/−	Robinson *et al.* 2006 [6]: AD in MHS Jungbluth *et al.* 2002 [44]: AR in CCD Lose *et al.* 2013 [13]: AD in axial myopathy
37	M, 47	Axial myopathy	Symptomatic sister has same mutation (1)	c.14545G>A exon 101	p.(Val4849Ile)	−/−/−	Robinson *et al.* 2006 [6]: AD in MHS Jungbluth *et al.* 2002 [44]: AR in CCD Løseth *et al.* 2013 [13]: AD in axial myopathy (this patient)
38	F, 37	MH during anaesthesia	Symptomatic relatives (positive IVCT) have same mutation (5)	c.14545G>A exon 101	p.(Val4849Ile)	−/−/−	Robinson *et al.* 2006 [6]: AD in MHS Jungbluth *et al.* 2002 [44]: AR in CCD

(continued)

Table 3 *(Continued)*

Patient no.	Sex, age at diagnosis	Phenotype	Family members tested (additional no. of affected family members with same mutation)	Coding DNA mutation and exon (ref: NM_000540.2)	Deduced mRNA or protein change	Frequency in controls or presence in EMHG (EVS/ ClinSeq/EMHG)	References/conservation
39	M, 37	MH during anaesthesia	Symptomatic relatives (positive IVCT) have same mutation (5)	c.14545G>A exon 101	p.(Val4849Ile)	−/−/−	Robinson *et al.* 2006 [6]: AD in MHS Jungbluth *et al.* 2002 [44]: AR in CCD
40	F, 41	MH during anaesthesia	Symptomatic relatives (positive IVCT) have same mutation (2)	c.14545G>A exon 101	p.(Val4849Ile)	−/−/−	Robinson *et al.* 2006 [6]: AD in MHS Jungbluth *et al.* 2002 [44]: AR in CCD
41	M, 16	MH during anaesthesia	Symptomatic relatives (positive IVCT) have same mutation (4)	c.14545G>A exon 101	p.(Val4849Ile)	−/−/−	Robinson *et al.* 2006 [6]: AD in MHS Jungbluth *et al.* 2002 [44]: AR in CCD
42	M, 67	MH during anaesthesia (family)	Symptomatic relatives (MH during anaesthesia in son; positive IVCT) have same mutation (2)	c.14545G>A exon 101	p.(Val4849Ile)	−/−/−	Robinson *et al.* 2006 [6]: AD in MHS Jungbluth *et al.* 2002 [44]: AR in CCD
43	M, 9	MH during anaesthesia	Symptomatic relatives (positive IVCT) have same mutation (3)	c.14545G>A exon 101	p.(Val4849Ile)	−/−/−	Robinson *et al.* 2006 [6]: AD in MHS Jungbluth *et al.* 2002 [44]: AR in CCD
44	M, 10	MH during anaesthesia	Symptomatic relatives (positive IVCT) have same mutation (6)	c.14545G>A exon 101	p.(Val4849Ile)	−/−/−	Robinson *et al.* 2006 [6]: AD in MHS Jungbluth *et al.* 2002 [44]: AR in CCD
45	F, 0	Fetal akinesia	Asymptomatic parents not tested	c.14549A>G exon 101	p.(Tyr4850Cys)	–	Bharucha-Goebel *et al.* 2013 [14]: fetal akinesia (*de novo*)
46	F, 2	CCD	*De novo* mutation	c.14581C>T exon 101	p.(Arg4861Cys)	−/−/y	Davis *et al.* 2003 [45]: AD in CCD
47	M, 10	CCD	Asymptomatic parents not tested	c.14581C>T exon 101	p.(Arg4861Cys)	−/−/y	Davis *et al.* 2003 [45]: AD in CCD
48	F, 14	CCD	Symptomatic mother and brother not tested	c.14582G>A exon 101	p.(Arg4861His)	−/−/y	Monnier *et al.* 2001 [46]: AD in CCD
49	M, 8	MH during anaesthesia	Symptomatic mother (cramps, hyperCKemia, positive IVCT) has same mutation (1)	c.15060G>T exon 106	p.(Trp5020Cys)	−/−/−	Klingler (2014) Orphanet J Rare Dis 9: 8
Patients with putatively recessive inheritance							
50	M, 12	CCD	Asymptomatic parents heterozygous (2): Father, mutation in exon 14; mother, mutations in exon 33, 67 and 86	c.1501del (p) exon 14 c.4711A>G (m exon 33) c.10097G>A (m exon 67) c.11798A>G (m) exon 86	p.(His501 fs) p.(Ile1571Val) p.(Arg3366His) p.(Tyr3933Cys)	−/−/− 0.002/−/− 0.001/−/− 0.001/−/−	This report; mammals, frog and fish Tammaro *et al.* 2011 [47]: AD in MHS Klein *et al.* 2012 [22]: AR in CM Duarte *et al.* 2011 [10]: AR in MmD Gillies *et al.* 2008 [48]: AD in MHS Klein *et al.* 2012 [22]: AR in CM
51	F, 23	CCD; MH during anaesthesia	Mother: mutation in exon 20 (1); father not tested	c.2513T>C (m) exon 20 c.14545G>A exon 101	p.(Leu838Pro) p.(Val4849Ile)	−/−/− −/−/−	This report; mammals, frog and fish Jungbluth *et al.* 2002 [44]: AR in CCD Carpenter *et al.* 2009 [23]: AD in MHS

(continued)

Table 3 *(Continued)*

Patient no.	Sex, age at diagnosis	Phenotype	Family members tested (additional no. of affected family members with same mutation)	Coding DNA mutation and exon (ref: NM_000540.2)	Deduced mRNA or protein change	Frequency in controls or presence in EMHG (EVS/ ClinSeq/EMHG)	References/conservation
52	M, 27	EIR; heat stroke	Asymptomatic parents heterozygous (2)	c.2488C>T (p) exon 22	p.(Arg830Trp)	0.001/−/−	This report; moderately conserved (Arg in mammals; His in frog and fish)
				c.10219G>A (m) exon 67	p.(Ala3407Thr)	−/−/−	Molenaar *et al.* 2014 [41]: AD in rhabdomyolysis
53	F, 51	MmD	Asymptomatic parents heterozygous (2); less severely affected daughter heterozygous for maternal mutations (1)	c.4711A>G (p) exon 33	p.(Ile1571Val)	0.002/−/−	Tammaro *et al.* 2011 [47]: AD in MHS Klein *et al.* 2012 [22]: AR in CM
				c.10097G>A (p) exon 67	p.(Arg3366His)	0.001/−/−	Duarte *et al.* 2011 [10]: AR in MmD
				c.11798A>G (p) exon 86	p.(Tyr3933Cys)	0.001/−/−	Gillies *et al.* 2008 [48]: AD in MHS Klein 2012 *et al.* [22]: AR in CM
				c.12629A>G (m) exon 91	p.(Lys4210Arg)	0.0003/−/−	This report; mammals, frog and fish
				c.14723A>G (m) exon 102	p.(Asp4908Gly)	−/−/−	This report; mammals, frog and fish
54	M, 30	CCD	Asymptomatic parents heterozygous (2): father, mutation in exon 33, 67 and 86; mother, mutation in exon 100	c.4711A>G (p) exon 33	p.(Ile1571Val)	0.002/−/−	Tammaro *et al.* 2011 [47]: AD in MHS
				c.10097G>A (p) exon 67	p.(Arg3366His)	0.001/−/−	Klein *et al.* 2012 [22]: AR in CM
				c.11798A>G (p) exon 86	p.(Tyr3933Cys)	0.001/−/−	Duarte *et al.* 2011 [10]: AR in MmD
				c.14416A>G (m) exon 100	p.(Asn4806Asp)	−/−/−	Gillies *et al.* 2008 [48]: AD in MHS Klein *et al.* 2012 [22]: AR in CM
							Klein *et al.* 2012 [22]: AR in CM
55	M, 16	CCD	Asymptomatic parents not tested	c.4711A>G exon 33	p.(Ile1571Val)	0.002/−/−	Tammaro *et al.* 2011 [47]: AD in MHS
				c.10097G>A exon 67	p.(Arg3366His)	0.001/−/−	Klein *et al.* 2012 [22]: AR in CM
				c.11798A>G exon 86	p.(Tyr3933Cys)	0.001/−/−	Duarte *et al.* 2011 [10]: AR in MmD
				c.14545G>A exon 101	p.(Val4849Ile)	−/−/−	Gillies *et al.*2008 [48]: AD in MHS Klein *et al.* 2012 [22]: AR in CM Jungbluth *et al.* 2002 [44] : AD in CCD Carpenter *et al.* 2009 [23]: AD in MHS
56	M, 47	MmD	Asymptomatic parents and symptomatic sister not tested	c.4711A>G exon 33	p.(Ile1571Val)	0.002/−/−	Tammaro *et al.* 2011 [47]: AD in MHS
				c.10097G>A exon 67	p.(Arg3366His)	0.001/−/−	Klein *et al.* 2012 [22]: AR in CM
				c.11798A> G exon 86	p.(Tyr3933Cys)	0.001/−/−	Duarte *et al.* 2011 [10]: AR in MmD
				c.14545G>A exon 101	p.(Val4849Ile)	−/−/−	Gillies *et al.* 2008 [48]: AD in MHS Klein *et al.* 2012 [22]: AR in CM Jungbluth *et al.* 2002 [44]: AD in CCD Carpenter *et al.* 2009 [23]: AD in MHS
57	M, 33	CCD; MH during anaesthesia	Asymptomatic parents heterozygous (2)	c.6617C>T (m) exon 40	p.(Thr2206Met)	−/−/y	Robinson *et al.* 2006 [6]: AD in MHS
				c.9001−2A>G intron 59	r.spl?	−/−/−	This report

(continued)

Table 3 *(Continued)*

Patient no.	Sex, age at diagnosis	Phenotype	Family members tested (additional no. of affected family members with same mutation)	Coding DNA mutation and exon (ref: NM_000540.2)	Deduced mRNA or protein change	Frequency in controls or presence in EMHG (EVS/ ClinSeq/EMHG)	References/conservation
58	F, 39	CCD	Asymptomatic parents not tested	c.6617C>T exon 40	p.(Thr2206Met)	−/−/y	Robinson *et al.* 2006 [6]: AD in MHS Amburgey *et al.* 2013 [16]: AR in MmD
				c.13525_13531dup exon 93	p.(Lys4511 fs)	na	This report
59	F, 2	CCD; MH during anaesthesia	Asymptomatic parents heterozygous (2)	c.7523G>A (m) exon 47 c.8327C>T (p) exon 53	p.(Arg2508His) p.(Ser2776Phe)	−/−/y 0.001/−/−	Wu *et al.* 2006 [39]: AD in MHS Dowling *et al.* 2011 [11]: AD in KDS
60	F, 14	MmD	Asymptomatic parents not tested	c.7858C>T exon 49 c.10616G>A exon 71	p.(Gln2620*) p.(Arg3539His)	−/−/− 0.003/0.002/−	This report Monnier *et al.* 2008 [9]: AR in CM
61	M, 29	CNM	Asymptomatic parents not tested	c.10616G>A exon 71 c.14804−1G>A intron 102	p.(Arg3539His) r.spl?	0.003/0.002/− −/−/−	Monnier *et al.* 2008 [9]: AR in CM Monnier *et al.* 2008 [9]: AR in CM
Patients with uncertain pathogenicity or uncertain mode of inheritance							
62	F, 25	CCD	Asymptomatic parent heterozygous (1)	c.2603G>A exon 21	p.(Arg868His)	−	This report; mammals, frog and fish
63	F, 20	MmD	Asymptomatic mother no mutations; asymptomatic father not tested	c.2682G>A exon 21 c.7209C>T exon 44	r.spl? r.(spl?)	− −	This report; in canonical donor splice site; possible exon 21 skipping (in frame) This report; a weak donor is created 5 bp upstream
64	F, 22	MmD	Asymptomatic mother heterozygous for both mutations (1)	c.3145G>A (m) exon 24 c.9811G>A (m) exon 66	p.(Gly1049Ser) p.(Glu3271Lys)	− −	Klein *et al.* 2012 [22]: AD in CCD This report; mammals, frog and fish
65	M, 8	MmD	Asymptomatic parents not tested	c.6820G> exon 42T	p.(Asp2274Tyr)	−	This report; mammals, frog and fish
66[d]	M, 38	CCD	Asymptomatic mother (heterozygous) (1)	c.7025A>G exon 43	p.(Asn2342Ser)	0.001/−/−	Robinson *et al.* 2006 [6]: AD in MHS
67	M, 64	CCD	Unknown	c.7025A>G exon 43	p.(Asn2342Ser)	0.001/−/−	Robinson *et al.* 2006 [6]: AD in MHS
68	F, 19	CCD	Asymptomatic mother (heterozygous) (1)	c.7025A>G exon 43	p.(Asn2342Ser)	0.001/−/−	Robinson *et al.* 2006 [6]: AD in MHS
69	M, 21	KDS	Asymptomatic parents not tested	c.7523G>A exon 47	p.(Arg2508His)	−	Wu *et al.* 2006 [39]: AR in CCD
70	M, 34	Rhabdomyolysis	Statin-induced rhabdomyolysis; asymptomatic mother has same mutation (1)	c.8327C>T exon 53	p.(Ser2776Phe)	0.001/−/−	Dowling *et al.* 2011 [11]: AD in KDS
71	M, 46	MH during anaesthesia (family)	Symptomatic sibs (MH reaction during anaesthesia or positive IVCT) (heterozygous) (5)	c.10616G>A exon 71	p.(Arg3539His)	0.003/0.002/−	Monnier *et al.* 2008 [9]: AR in CM
72	M, 26	NM	Asymptomatic parents not tested	c.10616G>A exon 71	p.(Arg3539His)	0.003/0.002/−	Monnier *et al.* 2008 [9]: AR in CM
73[e]	F, 38	HyperCKaemia	Symptomatic brother no mutation	c.10616G>A exon 71	p.(Arg3539His)	0.003/0.002/−	Monnier *et al.* 2008 [9]: AR in CM

(continued)

Table 3 *(Continued)*

Patient no.	Sex, age at diagnosis	Phenotype	Family members tested (additional no. of affected family members with same mutation)	Coding DNA mutation and exon (ref: NM_000540.2)	Deduced mRNA or protein change	Frequency in controls or presence in EMHG (EVS/ ClinSeq/EMHG)	References/conservation
74	F, 46	CCD	Symptomatic son not tested	c.10616G>A exon 71	p.(Arg3539His)	0.003/0.002/–	Monnier *et al.* 2008 [9]: AR in CM
75	F, 18	Rhabdomy-olysis	Asymptomatic father carrier (1)	c.12282 + 57_12282 + 60 delins238 intron 89	r.spl?	–	This report
76[f]	M, 35	MmD	Asymptomatic mother (heterozygous) (1)	c.13867G>A exon 95	p.(Asp4623Asn)	–	This report; moderately conserved (Asp or Glu in mammals, frog and zebrafish)
77	M, 9	CM n.o.s.	Asymptomatic parents not tested	c.14770_14772del exon 102	p.(Phe4924del)	na	This report; mammals, frog and fish

AD, autosomal-dominant; AR, autosomal-recessive; CCD, central core disease; CM, congenital myopathy; CM n.o.s., congenital myopathy not otherwise specified; CNM, centronuclear myopathy; EIM, excercise-induced myalgia; EIR, exercise-induced rhabdomyolysis; IVCT, *in vitro* contracture test; KDS, King–Denborough syndrome; MH, malignant hyperthermia; MHS, malignant hyperthermia susceptibility; MmD, multiminicore disease; NM, nemaline myopathy. The grey rows depict the noven mutations. Mutation nomenclature is according to HGVS guidelines (www.HGVS.org). Numbers of patients are in parentheses, in bold and italic. The following diagnostic categories were used to classify the phenotypes of the patients referred for *RYR1* mutational analysis: CCD, MmD, CNM, NM, CM n.o.s., KDS, MHS and fetal akinesia syndrome. EIR was defined as a potentially lethal clinical syndrome that results from acute muscle fibre necrosis with leakage of muscle constituents into the blood, reflected by an acute rise of serum CK [>10 times the upper limit of normal (ULN)] and hyperCKemia as an increase of CK >1.5 times ULN. One family with MHS was previously described by Snoeck (patient 1), six families with exertional rhabdomyolysis by Dlamini *et al.* [12] (patients 2, 10, 12, 13, 22 and 23) and three families with axial myopathy by Løseth *et al.* [13] (patients 30, 37 and 66).[a]Dominant segregation for MHS. The mutation in exon 17 was detected in the proband, her mother and her daughter. The second mutation was detected only in the proband. This second mutation in intron 99 is in the canonical splice site and is therefore likely to be pathogenic. The index patient had muscle cramps, which were not present in her mother or daughter. [b]Dominant segregation for MHS. The mutation in exon 43 is present in the daughter with the MH episode, and in the son with exertional hyperCKemia and myalgia (CK 3600–4000 U/l). The mutation in exon 48 was detected in the asymptomatic son (CK 200 U/l). [c]Also hereditary motor and sensory neuropathy with an *MFN2* mutation (c.2113G>A; p.Val705Leu). [d]Also paroxysmal extreme pain disorder with an *SCN9A* mutation (c.1007A>C; p.Asn336Thr). [e]This mutation causes AR inheritance (in the literature and in our database: twice in combination with a nonsense/frame-shift (Table 3) and once without detection of a second mutation). These three families have this mutation, and a second mutation if recessive may have been missed. If dominant, they are not causative, but possibly carriers. [f]Also a single *SEPN1* mutation in the patient, and not in his asymptomatic mother (c.293_301del; p.?) .

the mutation and the inheritance pattern were undetermined (21%). Median age at onset was 8 years; and median age at genetic diagnosis was 29 years, reflecting the delay in genetic confirmation due to the limited availability of *RYR1* sequencing until 2008. A summary of the mutations and phenotypes is shown in Tables 1, 2 and 3. Table 4 presents an overview of the clinical features, and Table 5 of the histopathological findings of the whole cohort.

Mutations in both dominant (AD) and recessive (AR) disease were found across the gene. Since *RYR1*-related myopathies can be inherited in a dominant or recessive manner, testing of sporadic patients leading to identification of heterozygous mutations is inconclusive without testing of parents or family members. Furthermore, the inheritance of *RYR1*-related myopathies may be non-penetrant [6] or complicated by an epigenetic mechanism, such as maternal allele silencing [20]. Additionally, recessive inheritance and failure to detect a second mutation (such as a larger deletion not detected by sequencing, or intronic or promoter mutations) cannot be excluded. For that reason, the pathogenicity of the mutation or the exact inheritance pattern could not be ascertained in all patients. Specific features of dominant and recessive *RYR1*-related disorders and of patients harbouring *RYR1* mutations with uncertain inheritance pattern or pathogenicity are presented in Data S1.

In vitro contracture test findings

The MH susceptible status of patients referred with a past MH reaction or an *RYR1* mutation was determined by the IVCT based on European Malignant Hyperthermia Group (EMHG) diagnostic criteria; the results are shown in Data S2.

Clinical findings

Malignant hyperthermia susceptibility by a positive IVCT was the initial manifestation in 29 (38%) patients. Rhabdomyolysis was the predominant symptom in six (8%) families, five with AD inheritance (patients 2, 12, 13, 22 and 23) and in one patient with AR inheritance (patient 52). Triggers in three non-exercise related cases were viral infections [patients 29 (AD) and 75, CK 13 000 and 521 500 U/l respectively] and statin use (patient 70, CK 6055 U/l), the latter two patients with *RYR1* mutations of uncertain pathogenicity (patients 70 and 75). Together, these 'induced myopathies' accounted for 51% of all patients.

The other half of the cohort (49%) consisted of patients with permanent weakness: CCD and MmD were found in 23 (30%) and seven (9%) patients; less

Table 4 Overview of the clinical features of the various phenotypes

Phenotype	Number of patients (%)	Muscle weakness (%)	Facial weakness (%)	Ptosis/ ophthalmoplegia (%)	Spine/joints (contractures, joint hypermobility) (%)	Myalgia/ muscle cramps (%)	Impaired mobility at follow-up (age) (%)	Cardiac dysfunction (%)	Respiratory weakness (%)	Rhabdomyolysis (%)
MH during anaesthesia	29 (38)	1 (3)	0	0	0	5 (17)	1 (3)	1 (3)	0	2 (7)
EIR/rhabdomyolysis	9 (12)	2 (22)	0	2 (22)	0	4 (44)	3 (33)	1 (11)	0	9 (100)
HyperCKemia	1 (1)	0	0	0	0	0	0	0	0	1 (100)
Axial myopathy	2 (3)	2 (100)	0	0	0	2 (100)	2 (100)	0	0	1 (50)
CCD	23 (30)	23 (100)	10 (43)	4 (14)	11 (48)	6 (26)	19 (83)	2 (9)	5 (22)	0
Fetal akinesia	1 (1)	1 (100)	1 (100)	0	1 (100)	–	1 (100)	1 (100)	1 (100)	–
MmD	7 (9)	7 (100)	5 (71)	3 (43)	6 (86)	1 (14)	7 (100)	1 (14)	3 (43)	0
NM	1 (1)	1 (100)	1 (100)	0	1 (100)	1 (100)	0	0	0	0
CNM	1 (1)	1 (100)	1 (100)	1 (100)	1 (100)	0	1 (100)	0	1	0
KDS	1 (1)	1 (100)	1 (100)	0	1 (100)	0	1	0	0	0
CM n.o.s.	2 (3)	2 (100)	1 (50)	0	1 (50)	1 (50)	2 (50)	0	0	0
Total (%)	77 (100)	41 (53)	25 (35)	10 (13)	22 (29)	20 (26)	36 (47)	5 (6)	9 (12)	13 (17)

CCD, central core disease; CM n.o.s., congenital myopathy not otherwise specified; CNM, centronuclear myopathy; EIR, exercise-induced rhabdomyolysis; KDS, King–Denborough syndrome; MH, malignant hyperthermia; MmD, multiminicore disease; NM, nemaline myopathy. Clinical features of individual patients are presented in Data S2.

Table 5 Overview of the histopathological features

Phenotype	Number of patients (% of all patients)	Number of histopathological analyses performed (% of patients of that phenotype)	Number of biopsies with increase of internal nuclei (% of biopsies of that phenotype)	Number of biopsies with increase of fibre size variation (% of biopsies of that phenotype)	Number of biopsies with fibre type I predominance (% of biopsies of that phenotype)	Number of biopsies with minicores (LM and/or EM) (% of biopsies of that phenotype)	Number of biopsies with central cores (LM and/or EM) (% of biopsies of that phenotype)	Number of biopsies with unevenness of oxidative staining (% of biopsies of that phenotype)
MH during anaesthesia	29 (38)	18 (62)	13 (72)	8 (44)	3 (17)	1 (6)	2 (11)	9 (50)
EIR/rhabdomyolysis	9 (12)	8 (89)	6 (66)	3 (33)	3 (33)	0	1 (11)	2 (22)
HyperCKemia	1 (1)	0						
Axial myopathy	2 (3)	2 (100)	2 (100)	1 (50)	1 (50)	0	0	1 (50)
CCD	23 (30)	22 (96)	18 (82)	17 (77)	12 (55)	0	16 (73)	5 (23)
Fetal akinesia	1 (1)	0						
MmD	7 (9)	7 (100)	6 (86%)	7 (100)	4 (57)	7 (100)	0	0
NM	1 (1)	1 (100)	1 (100)	1 (100)	0	0	0	1 (100)
CNM	1 (1)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0	1 (100)
KDS	1 (1)	1 (100)	1 (100)	1 (100)	1 (100)	0	1 (100)	1 (100)
CM n.o.s.	2 (3)	2 (100)	1 (50)	1 (50)	1 (50)	0	0	0
Total (%)	77 (100)	62 (81)	49 (79)	40 (65)	26 (42)	9 (15)	20 (32)	16 (26)

CCD, central core disease; CM, congenital myopathy; CM n.o.s., congenital myopathy not otherwise specified; CNM, centronuclear myopathy; EIR, exercise-induced rhabdomyolysis; EM, electron microscopy; KDS, King–Denborough syndrome; LM, light microscopy; MH, malignant hyperthermia; MmD, multiminicore disease; NM, nemaline myopathy. Histopathological features of individual patients are presented in Data S3.

frequent phenotypes included fetal akinesia syndrome (patient 45), KDS (patient 69), nemaline myopathy (patient 72; mutations in *ACTA1, TNNT1, TPM2, TPM3* and *CFL2* were excluded), CNM (patient 61), congenital myopathy not otherwise specified (CM n.o.s., patients 25 and 77) and axial myopathy (patients 30 and 37) (Fig. 1, Data S2). Data of cardiac and respiratory evaluation and detailed results of ancillary investigations and of genotype–phenotype correlations are available online (Data S2).

The onset of symptoms ranged from birth to late adulthood in patients with AD mutations and in the first to second decade in those with AR mutations. In the families with AD inheritance, 12 patients presented with CCD or a CM n.o.s., 10 in the first two decades and two in adulthood. Fourteen MHS patients presented in the first decade and four in the second. Rhabdomyolysis or hyperCKemia presented at various ages and patients had often been asymptomatic until the occurrence. The eldest patient in the cohort with AD mutations was 60 years of age at presentation with exercise-induced rhabdomyolysis (EIR) (patient 12). Amongst the patients with AR mutations, onset was predominantly in the first decade.

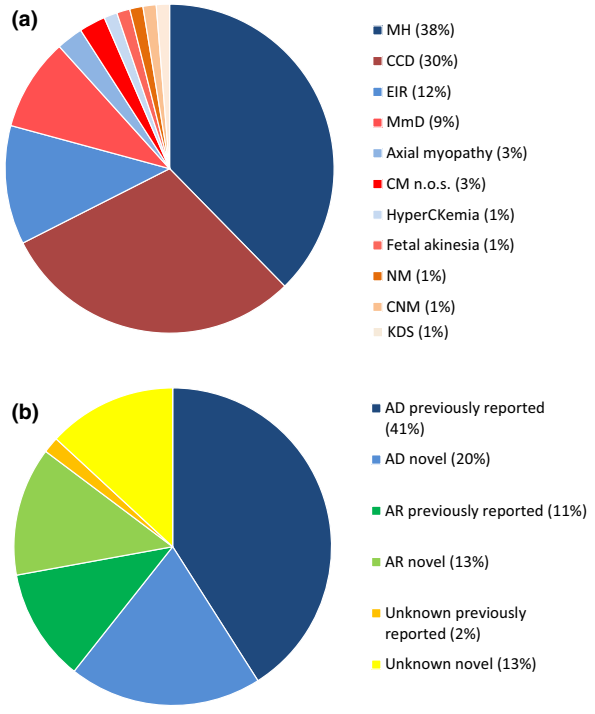

Figure 1 Overview of phenotype and molecular features of *RYR1*-related myopathies. (a) Distribution of phenotypes. (b) Distribution of AD mutations, AR mutations and mutations with unknown heredity, specified as mutations previously defined and novel mutations.

Medical and family history

Cryptorchidism occurred in seven patients (16% of male patients) and inguinal hernia in three (7%). In two of them an MH reaction occurred during the orchidopexy (patients 27 and 57). Pervasive developmental disorder not otherwise specified and attention deficit hyperactivity disorder had been diagnosed in one patient previously (patient 28), and one patient had a history of psychosis and drug abuse (patient 22). Another patient and his affected sister with axial myopathy had Gilles de la Tourette syndrome (patient 37). In two AD CCD families, the family history revealed MHS (patients 16 and 17).

Neuromuscular features

Mild proximal weakness in legs was reported in one MHS patient (patient 5), in two with rhabdomyolysis (patients 29 and 52) and in all patients with other phenotypes. Facial weakness occurred in all patients with nemaline myopathy (NM), CNM, fetal akinesia and KDS, in five with MmD, and in one with CM n.o.s. Ptosis and/or ophthalmoplegia occurred in the patient with CNM, in three patients with MmD, in four with CCD, all with (presumed) AR inheritance. Ptosis without ophthalmoplegia occurred in two patients with exertional rhabdomyolysis. Skeletal manifestations were common amongst the patients with congenital myopathies: they were present in all patients with NM, CNM, fetal akinesia and KDS, in six MmD patients, in one patient with CM n.o.s. and in 11 CCD patients. Muscle cramps and/or myalgia occurred in both patients with axial myopathy, in four patients with rhabdomyolysis, in five MHS patients, and to a lesser extent in CCD and MmD: six and one patient respectively.

Mobility was normal in almost all MHS families. Most patients with congenital myopathies with AD inheritance had difficulty running but retained normal walking ability. Mobility in patients with recessive mutations was relatively preserved, but patients were generally more severely affected compared to the AD group. One patient with AR CNM (patient 61) used an outdoor wheelchair, and another AR EIR patient was wheelchair-bound after a spinal cord lesion following a heat stroke (patient 52). Amongst the sporadic patients, three patients were wheelchair-bound: one with CCD (patient 68), one with NM (patient 72) and one with recurrent episodes of rhabdomyolysis (patient 75). Furthermore, the patient histories showed that muscle strength was largely stable in most patients. Some of the clinical features are depicted in Fig. 2.

Histopathological findings

Muscle biopsies were performed in 62 patients. In 18 of 29 patients with an MH reaction (including the

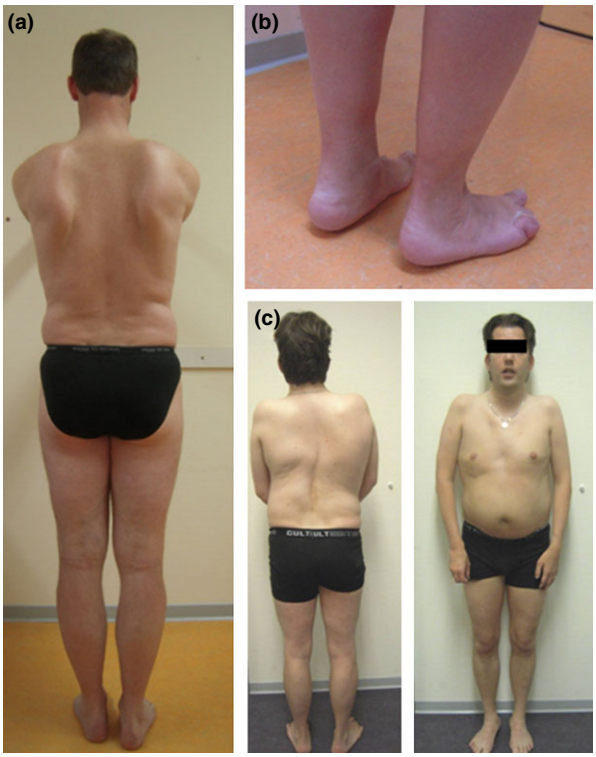

Figure 2 Some aspects of the clinical spectrum of *RYR1*-related myopathies. (a) Axial myopathy in patient 37: wasting of the paravertebral muscles. (b) Ankle contractures in patient 53 with MmD. (c) Scoliosis, elbow contractures and elongated face in patient 76 with MmD.

relatives of eight children with an MH reaction during anaesthesia), muscle tissue for histological analysis was obtained during the IVCT. Furthermore, diagnostic biopsies were performed in all patients with MmD (n = 7), CM n.o.s. (n = 2), axial myopathy (n = 2), and NM, CNM and KDS (n = 1 each), in 22 of the 23 patients with CCD, and in eight of the nine patients with rhabdomyolysis.

Overall findings were an increased prevalence of internal nuclei (n = 49; 79% of 62 biopsies), increased variation of fibre size diameter (n = 40; 65%), fibre type I predominance (n = 26; 42%), central cores (n = 19; 31%), minicores (n = 9; 15%) and (mild) unevenness of oxidative enzyme staining without cores (n = 16; 26%). Biopsies of patients with MH during anaesthesia showed a low prevalence of central or minicores (11% and 6% respectively), but frequent mild myopathic features [increase of internal nuclei (n = 13; 72%) or increased fibre size variation (n = 8; 44%)]. Unevenness of oxidative staining, considered the less severe form of cores, was observed in nine patients (50%). Specific histopathological features in the various phenotypes are discussed below; features of individual patients are presented in Data S3 and Fig. 3.

Observations in MHS, (exercise induced) rhabdomyolysis and hyperCKemia

Overall, the 18 biopsies in patients or relatives with MHS showed myopathic features: increase in the number of fibres with internal nuclei (n = 13; 72% of 18 biopsies), increase of fibre size variation (n = 8; 44%), fibre type I predominance (n = 3; 17%), and features suggestive of other *RYR1*-related myopathies – central cores (n = 3; 17%), multiple minicores (n = 2; 11%) and unevenness of oxidative staining (n = 10; 56%). Strikingly, some biopsies exhibited mild increase of lipid vacuoles: in rhabdomyolysis (patient 29), EIR (patient 52) and in CCD (patient 54). In the first two patients, both with rhabdomyolysis, this initially suggested a metabolic myopathy. After genetic testing (metabolic screening and *CPT2* in patient 29, and *CPT1, CPT2* and *VLCAD* in patient 52), this was considered unlikely. In patient 52, it was later considered as secondary to long-term propofol administration.

Observations in CCD, MmD and other congenital myopathies

Histopathological studies were performed in 22 of the 23 patients with CCD, showing myopathic changes more frequently than in the group of MHS and rhabdomyolysis: increase in the number of fibres with internal nuclei (n = 18; 82% of 23 biopsies), increase of fibre size variation (n = 17; 77%) and fibre type I predominance (n = 24; 55%). Central cores were observed in 16 patients and one daughter and unevenness of oxidative staining in five (73% and 23% respectively). In one CCD patient neither cores nor unevenness was reported, but other typical features were encountered: increase of internal nuclei and of fibre size variation and type I predominance; electron microscopy was not performed. In the seven patients with MmD the myopathic changes were even more prevalent: increase in the number of fibres with internal nuclei (n = 6; 86%), increase of fibre size variation (n = 7; 100%) and fibre type I predominance (n = 4; 57%). Multiple minicores were seen in all patients with MmD and unevenness of oxidative staining in none. Both myopathic changes and cores or unevenness of oxidative staining occurred in almost all patients with the other congenital phenotypes (CM n.o.s., NM, CNM, fetal akinesia and KDS).

Progress of histological changes

In seven patients, serial biopsies were performed: in a patient with MH (patient 8) at age 41 and 56 years; in patients with CCD at age 27 and 28 years (9), at age 0 and <10 years (patient 47), at age 8 and 10 years (patient 57), and at ages 53, 54, 63 and 64 years

(patient 67); and in patients with MmD at age 20 and 49 years (patient 53), and at 0, 24, 34 and 35 years (patient 76). Initial histological changes in the biopsy were not always typical for a specific core myopathy, but later biopsies frequently exhibited more typical diagnostic features. The subsequent biopsies in patient 67 show the gradual development of cores (Fig. 3a). The first biopsy of patient 76 in childhood suggested CNM, whereas the biopsies taken in adulthood were suggestive of MmD.

Mitochondrial and inflammatory changes

Cox-deficient or ragged red fibres were seen in four patients (patients 9, 13, 30 and 67). In two of them

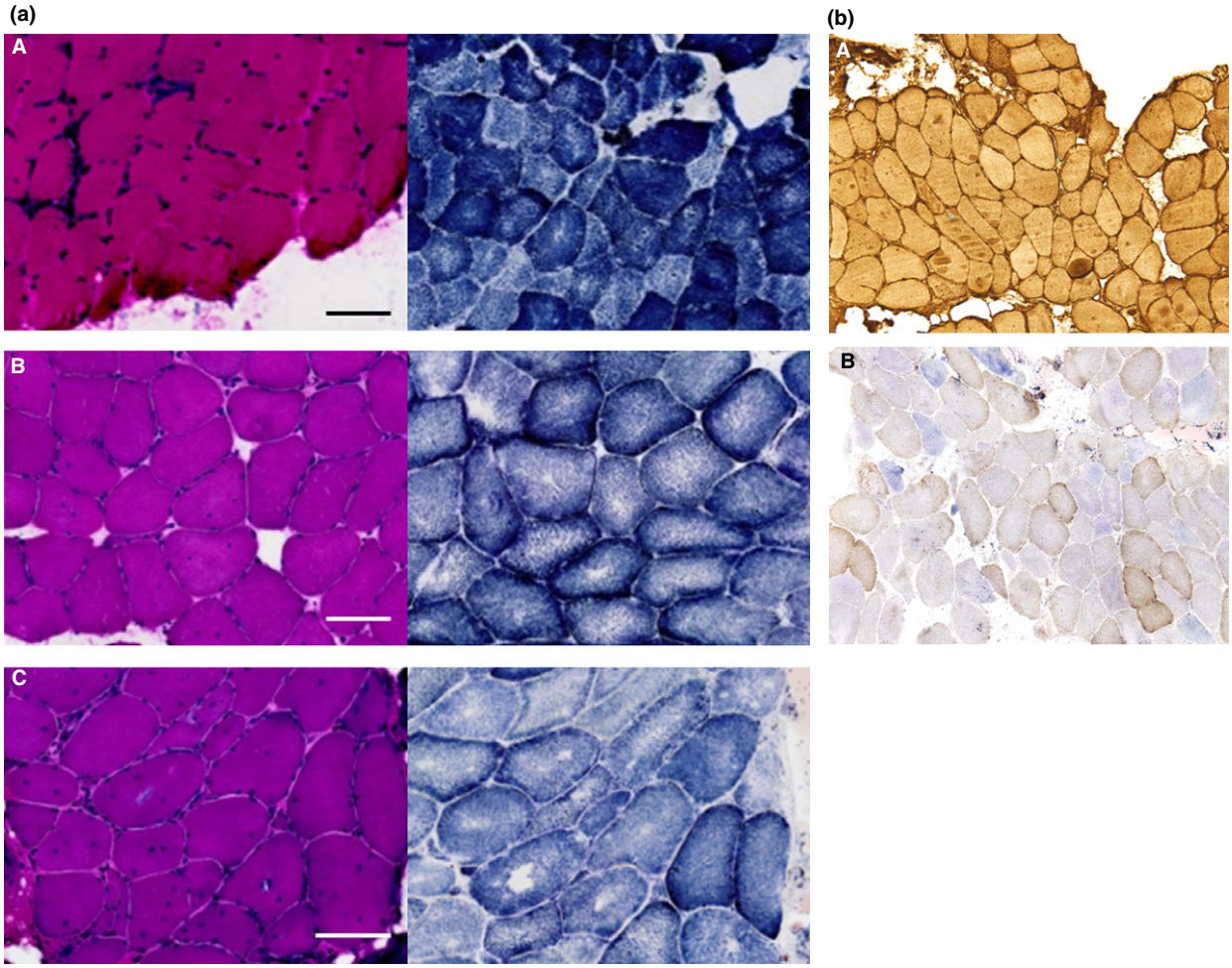

Figure 3 (a) Development of cores in subsequent biopsies in CCD. Gradual development of cores in subsequent biopsies in a patient with CCD (c.7025A>G (Asn2342Ser) (patient 67). HE and NADH staining. Bar 100 μm. The initial histological changes in the biopsy were not typical for a specific core myopathy, but later biopsies exhibited more diagnostic features. Row A, biopsy at age 53 showing a cellular infiltrate (focally invasive). In retrospect, very mild unevenness of oxidative staining can be detected, although this was not classified as such at that time. Row B, biopsy at age 63, without infiltrates, and with increase of myopathic changes and of the unevenness of oxidative staining. Row C, biopsy at age 64, with increase of myopathic features and evident cores. (b) Mitochondrial and inflammatory changes in CCD. HLA-ABC staining and combined COX-SDH staining in the third biopsy of the same patient (patient 67; age 63). Bar 100 μm. Cox-deficient or ragged red fibres were seen in four patients (patients 9, 13, 30 and 67). In two of them (patients 9 and 67), biochemical analysis showed reduced ATP production and mildly reduced oxidation rates, but normal activities of respiratory chain enzymes, complex V and citrate synthase. No mitochondrial DNA changes were detected. In two muscle biopsy specimens inflammatory changes were seen with T-cell infiltrates and increased staining for MHC class 1 (patients 12 and 67). Row A, HLA-ABC staining was positive, compatible with an inflammatory myopathy. Row B, the combined COX-SDH staining showed a number of COX-negative fibres. Biochemical analysis of muscle showed reduction of ATP production (10.5; reference value 15.4–30.2 ATP / h / mU CS (mU citrate synthase) without reduced activity of respiratory chain enzymes, complex V and citrate synthase. No mitochondrial DNA changes were detected in full mtDNA testing.

(patients 9 and 67), biochemical analysis showed reduced ATP production and mildly reduced oxidation rates, but normal activities of respiratory chain enzymes, complex V and citrate synthase. No mitochondrial DNA changes were detected. In two muscle biopsy specimens inflammatory changes were seen with T-cell infiltrates and increased staining for MHC class 1 (patients 12 and 67) (Fig. 3b).

Discussion

General observations

The main finding is the marked genetic, clinical and histological heterogeneity of *RYR1*-related myopathies, even within families, in a large cohort of patients and the high percentage of novel mutations (38%). The cohort included an equal proportion of congenital myopathies with permanent weakness [23 patients with CCD (30%), seven with MmD (9%), two with axial myopathy (3%) and six with other congenital myopathies (8%)] and 'induced myopathies', comprising anaesthesia-triggered myopathic reactions (38%; MH) and non-anaesthesia-related episodes of rhabdomyolysis or hyperCKemia (13%) (Fig. 1). Amongst the 23 CCD patients, five also manifested MH during anaesthesia (two with AD and three with AR inheritance). The cohort is unique since it represents all *RYR1*-related myopathies diagnosed in the Dutch population of 16 million over a 4-year period (2008–2012), whereas previous studies mostly focused on specific phenotypes, certain age groups or geographically more restricted regions. This allows the relative contributions of these presentations to the overall spectrum of *RYR1*-related disorders to be estimated for the first time.

The substantial overlap between the MH phenotype and recessively inherited *RYR1*-related myopathies, even within families, has important implications for anaesthetic management and family counselling. Furthermore, the triggers inducing hyperCKemia and rhabdomyolysis varied widely, including exercise, statins (similar to recent observations in mouse models [21]) and viral infections. These observations emphasize that muscle breakdown in *RYR1*-related myopathies is the common end-point of multifactorial aetiologies.

In vitro contracture test findings

Patients with an MHS or MHE (malignant hyperthermia equivocal, reacting positive either to halothane or caffeine only) result should not get 'trigger anaesthesia' using volatile anaesthetics and/or succinylcholine. To patients (or family members) with an *RYR1* mutation and a malignant hyperthermia negative (MHN) IVCT result (patients 22, 52, 76) our advice is to use volatile anaesthetics only for induction or short procedures (e.g. 15 min) and never use succinylcholine. All patients with an *RYR1* mutation should be regarded clinically as MHS until a normal IVCT result has decreased the risk of an MH reaction during or after trigger anaesthesia.

Inheritance and molecular aspects

Mutations in dominant *RYR1*-related myopathies (37 in total, eight novel) were typically missense mutations, whilst the recessive forms were caused by combinations of null mutations with missense mutations or combinations of two or more missense mutations (15 in total, eight novel) in both alleles, as has been observed in other recent series [22]. In addition, in 16 families, the role of the *RYR1* mutations remained unclear (nine in total, eight novel). Two recurrent pathogenic alleles were observed, the p.(Val4849Ile) mutation seen in one-third of the MHS families and possibly representing a founder mutation in the Dutch area, and a recurrent allele carrying three different missense mutations [p.(Ile1571Val), p.(Arg3366His) and p.(Tyr3933Cys)] detected in five different AR families. This combination of mutations on one allele has been observed before [22] and is very likely to be pathogenic because it is quite frequent in the Dutch population with recessive *RYR1*-related myopathy. It is unclear whether one of the mutations is causative or whether a combination of two or all three is causative.

In six out of 12 families with a recessive myopathy, one of the alleles carried a mutation associated with MHS (p.(Thr2206Met); p.(Arg2508His); p.(Val4849Ile); and the recurrent allele with three missense mutations). In two other AR families, both alleles carried a mutation(s) associated with MHS (p.(Val4849Ile) and the recurrent allele with three missense mutations. The observation of two dominant *RYR1* mutations in *trans* causing a recessive *RYR1*-related myopathy has been described before [9,23] and should prompt segregation analysis and appropriate MH counselling in those families.

Clinical findings

The distribution of phenotypes indicates the relative contribution of specific presentations to the overall spectrum of *RYR1*-related disorders, presenting at different ages. AR inherited congenital myopathies, MHS, and AD inherited congenital myopathies

present in childhood. In adolescence and adulthood, predominantly AD inherited congenital myopathies are observed. Later in life, MH reactions, non-anaesthesia-related rhabdomyolysis and axial myopathy are manifested. Often, patients with 'adult-onset' CCD reported difficulties with sports in childhood, likely to be early manifestations of their condition. A previous review showed similar age of onset distribution for MHS individuals, with approximately 50% of MH reactions presenting before the age of 15 years [24]. Furthermore, this wide spectrum is in line with the wide variety of clinical and histopathological features of *RYR1*-related myopathies presented in the recent consensus statement of the International Standard of Care Committee for Congenital Myopathies [25].

The medical history revealed a high prevalence of cryptorchism and inguinal hernias in males, which in some led to an MH reaction during surgery. The prevalence of a history of cryptorchism in our cohort is more frequent than the prevalence of cryptorchism in the general population (1%) [24].

The spectrum of neuromuscular features in this cohort shows a predominance of cramps, myalgia and incidental ptosis in the patients with 'induced' myopathies (amongst which is MHS), and frequent facial weakness, ptosis and ophthalmoplegia and skeletal features in the patients with AR congenital myopathies. Both patients with axial myopathies suffered from myalgia and cramps, providing further evidence for their close association with the MHS/EIR-related mutational spectrum.

Cardiac function was abnormal in a minority of patients. The occurrence of a cardiomyopathy in three patients and the sudden unexplained death in family members of three other patients may reflect undiagnosed coronary involvement (as *RYR1* is expressed in smooth muscles) or the presence of another undiagnosed cause of (cardio)myopathy [26,27]. Respiratory function was reduced in 10 of 16 patients tested (63%: 24%–70% of predicted vital capacity; predominantly in patients with AD and AR early-onset congenital myopathies), requiring non-invasive positive pressure ventilation in one patient. This suggests that vital capacity should be measured regularly in the patients with these phenotypes, and certainly prior to anaesthesia.

Single or recurrent CK elevations were detected in 46 patients (72%), more frequently in AD than in AR families [33/38 (89%) vs. 6/11 (55%)]. Electromyography was only infrequently performed and showed aspecific myopathic features in most, and as such is not specific for *RYR1* as the causative gene. Muscle imaging showed myopathic changes (atrophy, fatty infiltration) in mostly pronounced involvement of axial, shoulder girdle and upper leg muscles as previously reported.

Histopathological findings

The wide histopathological range suggests that also on the histopathological level *RYR1*-related late-onset 'induced' and early-onset congenital myopathies are part of a similar spectrum: in the first group, mild myopathic changes and unevenness of oxidative staining occur, whereas in the congenital myopathies the central cores and multiple minicores are detected more frequently. The subsequent biopsies showed evolution of changes over time, in some cases initially without features typical for *RYR1*-related myopathies, but also findings sometimes suggesting a different (e.g. mitochondrial or inflammatory) aetiology. These might reflect the fact that *RYR1* mutations cause secondary mitochondrial changes or that dual pathology occurs.

Limitations of this study

One limitation of this study is its design as a cohort study, in which diagnostic protocols might have differed slightly amongst centres. Nevertheless, with help of the well-organized network of neuromyologists and clinical geneticists in the Netherlands, data from all patients were collected. Furthermore, the 'induced' phenotypes might not have been recognized to the same extent in all centres throughout the study due to higher awareness of *RYR1* mutations in these phenotypes in the national referral centre for both MH and congenital myopathies. This study cannot be used to draw conclusions about the incidence of *RYR1*-related disorders in the Netherlands, since presumably DNA tests were ordered abroad before the introduction of *RYR1* DNA analysis in the Netherlands in 2008 and due to a backlog of patients who had the clinical diagnosis of MHS or a congenital myopathy and were only genetically tested after 2008. Nevertheless, this is the most complete cohort of *RYR1*-related disorders reported so far, and the only one demonstrating the full spectrum of these recognized conditions in a single country.

Concluding remarks

Thus *RYR1*-related myopathies are genetically, histopathologically and clinically more diverse than previously considered and may manifest throughout life, with a wide range from early-onset myopathies to rhabdomyolysis triggered by various stimuli in otherwise healthy individuals. A high percentage of novel mutations ($n = 24$; 38%) was detected and it was shown that some mutations may cause both dominant

and recessive modes of inheritance, causing challenges for genetic counselling. Ongoing diagnostic investigation and future research should focus on *in vitro* testing by IVCT and functional characterization of the large number of *RYR1* variants whose precise effects currently remain uncertain. Wide recognition of this continuum is essential for counselling and improving patient safety during anaesthesia.

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Disclosure conflicts of interest

The authors declare no financial or other conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Specific genetic features of *RYR1*-related disorders.
Data S2. Clinical findings in *RYR1*-related myopathies.
Data S3. Histopathological findings in *RYR1*-related myopathies.
Data S4. Abbreviations used in text (online only), tables and figure legends.

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