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Congenital myopathies – Clinical features and frequency of individual subtypes diagnosed over a 5-year period in the United Kingdom

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Abstract

The congenital myopathies are a group of inherited neuromuscular disorders mainly defined on the basis of characteristic histopathological features. We analysed 66 patients assessed at a single centre over a 5 year period. Of the 54 patients where muscle biopsy was available, 29 (54%) had a core myopathy (Central Core Disease, Multi-minicore Disease), 9 (17%) had Nemaline Myopathy, 7 (13%) had Myotubular/Centronuclear Myopathy, 2 (4%) had Congenital Fibre Type Disproportion, 6 (11%) had isolated type 1 predominance and 1 (2%) had a mixed Core–Rod Myopathy. Of the 44 patients with a genetic diagnosis, *RYR1* was mutated in 26 (59%), *ACTA1* in 7 (16%), *SEPN1* in 7 (16%), *MTM1* in 2 (5%), *NEB* in 1 (2%) and *TPM3* in 1 (2%). Clinically, 77% of patients older than 18 months could walk independently. 35% of all patients required ventilatory support and/or enteral feeding. Clinical course was stable or improved in 57/66 (86%) patients, whilst 4 (6%) got worse and 5 (8%) died. These findings indicate that core myopathies are the most common form of congenital myopathies and that more than half can be attributed to *RYR1* mutations. The underlying genetic defect remains to be identified in 1/3 of congenital myopathies cases.

Keywords: Congenital myopathies; RYR1; Core myopathy; Muscle ultrasound; NEB; MTM1; SEPN1; ACTA1; TPM3

1. Introduction

Congenital myopathies (CM) are a group of inherited neuromuscular diseases with early onset, mainly defined by the predominant histopathological features which include central cores, multiple minicores, nemaline rods and central nuclei [1,2]. Based on these features, individual congenital myopathies such as Central Core Disease (CCD) [3], Multi-minicore Disease (MmD) [4], Nemaline Myopathy (NM) [5] and Centronuclear Myopathy (CNM) [6] were reported in the 1950s and 1960s. However, with recent molecular genetic advances it has become increasingly obvious that different genetic CMs can share pathological findings, complicating the correlation between pathological diagnosis and genetic findings. Moreover, it has become equally clear that many individuals with a genetically confirmed CM have only non-specific histopathological features.

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The frequency of single CMs entities is not known. Epidemiological data in the literature are scarce and either focused on single CMs subgroups or limited to geographical regions [7–9]. Whilst some of these studies have suggested NM as the most frequent CM [10,11], two recent studies indicate CCD [12] and other forms related to mutations in the skeletal muscle ryanodine receptor (*RYR1*) gene as the most common subgroup [9]. The true prevalence of CMs is likely to be underestimated, due to a substantial proportion of patients with mild clinical and/or non-specific histopathological features, or at the other end early fatal variants, and the complexity of systematically studying all CMs genes, which include some of the largest genes involved in neuromuscular disorders.

The primary goal of the present study was to establish relative frequencies of individual CMs variants, classified according to the predominant histopathological feature and, where available, genetic diagnosis. The secondary goal was to evaluate the clinical profiles of the CMs cases assessed at our centre.

2. Patients and methods

2.1. Patients

We studied retrospectively the clinical, histological and genetic data of 66 patients affected with CM who had been referred to the Dubowitz Neuromuscular Centre in London, UK, during the years 2005–2009. The Dubowitz Neuromuscular Centre is nationally commissioned by the United Kingdom National Health Service to assess and diagnose patients from England, Northern Ireland and Scotland affected by congenital muscular dystrophies and congenital myopathies. Patients can be referred for clinical assessment, or the referring clinician can forward muscle biopsies and/or DNA for further testing. The Diagnostic DNA Laboratory at Guy's Hospital, affiliated to the Centre, offers genetic screening of most of the currently known CMs genes.

We included only patients who had been clinically assessed at the Dubowitz Neuromuscular Centre and for whom a diagnosis of CM could be established, based on clinical features and the presence of suggestive histopathological features, an established genetic diagnosis or an affected relative. The clinical diagnosis of a congenital myopathy was made in individuals with essentially static weakness, affecting predominantly proximal and axial muscles, often of congenital or early childhood onset, with normal or mildly elevated serum CK, and in whom other conditions such as muscular dystrophies, myofibrillar myopathies, congenital myasthenic syndromes and neurogenic conditions had been excluded by the appropriate investigations. The pathological categories considered were those related to the predominance of specific structural changes according to criteria suggested by Dubowitz and Sewry [2] and included congenital myopathies (i) with cores (core myopathies) (Central Core Disease, CCD, and Multi-minicore Disease, MmD), (ii) with central nuclei (Centronuclear Myopathy, CNM), (iii) with nemaline rods (Nemaline Myopathy, NM), (iv) fibre type disproportion (Congenital Fibre Type Disproportion, CFTD). We also included patients with (v) type 1 fibre predominance or uniformity, as this abnormality has previously been associated with mutations in CMs genes, and (vi) mixed structural pathological features, for example a combination of cores and rods.

2.2. Muscle imaging

Muscle ultrasound was performed with a 7.5 MHz PVG 7205 transducer (Toshiba CAPASEE II) for qualitative assessment of the lower limb muscles, mainly the quadriceps, the calves, and in some cases the upper limb muscles, mainly the deltoid, biceps and triceps brachii muscles. The examination was considered abnormal in the presence of increased and/or reduced muscle volume and/or abnormal muscle echogenicity, according to criteria proposed by Heckmatt et al. [13,14].

2.3. Muscle biopsy

Skeletal muscle biopsies were investigated according to standard histopathological and immunohistochemical procedures and reviewed by one of the authors (CAS) [2]. Fibre typing was demonstrated by immunolabelling of fast and slow isoforms of myosin heavy chain. Analysis by electron microscopy was used in selected cases to characterize better certain structural abnormalities, in particular rods and cores [2].

2.4. Molecular genetic studies

Genomic DNA was extracted from peripheral blood leukocytes according to standard procedures and the PCR-amplified exons of the genes investigated were sequenced from genomic DNA. The following genes were analysed based on their recognised involvement in the CMs: skeletal muscle ryanodine receptor (RYR1) gene, selenoprotein N (SEPN1) gene, skeletal muscle alpha actin (ACTA1) gene, tropomyosin 2 (TPM2) gene, tropomyosin 3 (TPM3) gene, myotubularin (MTM1) gene, amphiphysin 2 (BIN1) gene and dynamin 2 (DNM2) gene. One patient had a deletion in MTM1 gene detected by MLPA (multiplex ligand-dependent probe amplification) (SALSA MLPA kit P309-A1 MTM1, MRC, Holland) not further investigated. Considering the large size of the gene, for nebulin (NEB) we screened only for the common c.7431+1916_7536+372del of intron 54 and exon 55. Analysis of the rest of the gene is not available as yet in an accredited UK laboratory. The choice of specific genetic tests was mainly informed by the presence of suggestive clinical and histopathological

features and, where available, muscle imaging features. In the majority of cases, genetic testing was limited to the most likely candidates based on our current understanding of these conditions. In addition, dystrophia myotonica-protein kinase (DPMK) expansions were excluded in all children whose muscle biopsy suggested CNM, considering the recognised histopathological overlap of this condition with DM1. Pathogenicity evaluation of previously unreported genetic variants was carried out using Alamut v1.5, a bioinformatics software incorporating several prediction algorithms and variant scoring methods.

3. Results

3.1. Patients

According to the selection criteria, 66 patients were included in the study, of whom 29 were females (43.9%) and 37 were males (56.1%). The mean age at onset was 0.8 (1.5 years, ranging from 0 to 7 years. The mean age (±SD) at first evaluation was 4.7 (4.5 years, ranging from birth to 18.6 years. Of the 66 patients, 44 had a genetic diagnosis (66.7%), whereas the remaining 22 (33.3%) were included according to clinical and histological findings only.

3.2. Histopathological features

Muscle biopsy samples for histological diagnosis were available for 54 (81.8%) of the 66 patients. The mean age at muscle biopsy was 3.84 ± 3.9 years, ranging from 1 week to 13.4 years. Core myopathy was the most frequent histopathological diagnosis, in 29/54 patients (53.7%), followed by NM in 9/54 patients (16.7%), myotubular/centronuclear myopathy in 7/54 patients (13%), congenital fibre type disproportion (FTD) in 2/54 patients (3.7%) and rod–core myopathy in 1/54 patients (1.8%). The relative prevalence of specific histopathological diagnoses is illustrated in Fig. 1A. In 6/54 patients (11.1%), only nonspecific myopathic findings were found, in particular predominance of type 1 fibres; in 4 of them pathogenic RYR1 mutations were identified. Twenty-two of 54 patients who underwent muscle biopsy (40.7%) had no molecular genetic diagnosis. Key histopathological, clinical and genetic findings in CMs patients without and with genetic diagnoses are summarised in Tables 1 and 2, respectively.

3.3. Genetic findings

A genetic diagnosis could be established in 44 (66.7%) out of 66 patients. Amongst those 44 patients, 26 (59.1%) were mutated in *RYR1*, 7 (15.9%) in *SEPN1*, 7 (15.9%) in *ACTA1*, 2 (4.5%) in *MTM1*, 1 (2.3%) in *TPM3*, and 1 (2.3%) in *NEB*. The relative prevalence of specific genetic diagnoses is illustrated in Fig. 1B. A total of 51 different mutations was identified, 4 of which not reported previously, including 2 *RYR1*, 1 *ACTA1*, and 1 *NEB* mutations. Of the 26 patients with *RYR1* mutations, 15 (57.7%) had compound heterozygous mutations

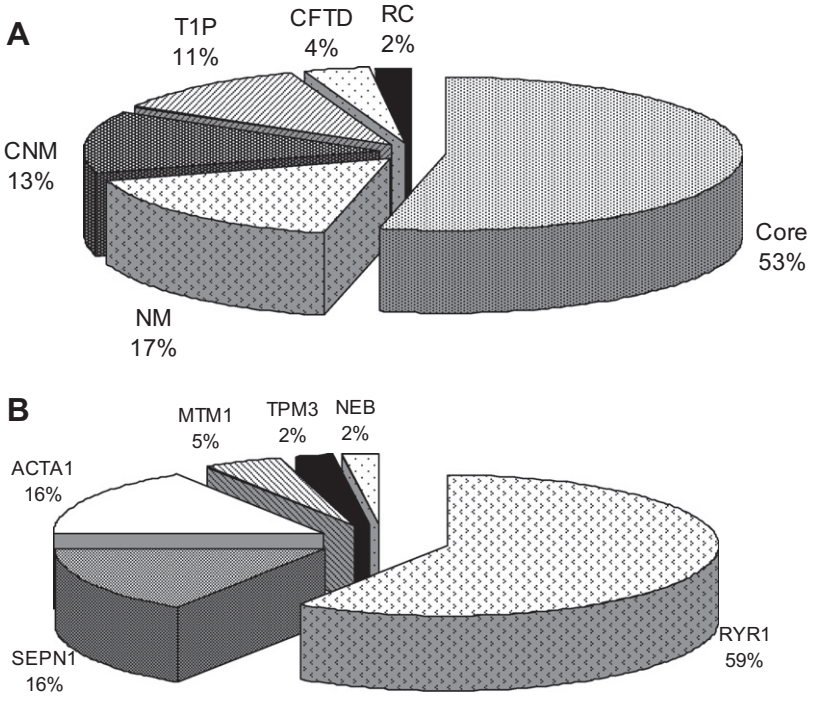

Fig. 1. Relative prevalence of histopathological phenotypes (A) and genetic backgrounds (B) identified in our cohort of patients with congenital myopathies. CNM = myotubular/centronuclear myopathy; CORE = core myopathy; FTD = fibre type disproportion; NM = nemaline myopathy; RC = rod–core myopathy; T1P = type 1 predominance.

Table 1
Clinical and histopathological features from congenital myopathy patients without genetic diagnosis.

No.	Histological diagnosis	Loci excluded	Sex	Onset	Age	Function	Weakness	EOM	Bulbar	Scoliosis	Spinal rigidity
1	CNM	*MTM1*	M	Birth	1 m	NA	Axial/proximal	–	+	–	–
2	CNM	*RYR1*, *MTM1*, *DNM2*	F	Birth	2.1 y	Independent walking, stairs	Axial/proximal	–	+	+	–
3	CNM	*MTM1*, *DNM2*, *BIN1*	M	Birth	2.1 y	Sitting	Axial/proximal	+	+	+	–
4	CNM	*MTM1*	M	Birth	10 d	NA	Facial/bulbar/ respiratory	–	+	–	–
5	CORE	*RYR1*, *SEPN1*	M	1 y	5.4 y	Independent walking, stairs	Axial/proximal	–	–	–	–
6	CORE	*RYR1*, *SEPN1*	F	Birth	13.1 y	Independent walking, stairs	Axial/proximal	–	+	+	–
7	CORE	*RYR1*, *SEPN1*	M	1.5 y	5.4 y	Independent walking, stairs	Axial/proximal	–	–	–	–
8	CORE	*RYR1*, *SEPN1*	M	6 m	4.3 y	Independent walking, stairs	Axial/proximal	–	–	–	–
9	CORE	*RYR1*, *SEPN1*	F	Birth	8.5 y	Independent walking, stairs	Axial/proximal	+	+	–	–
10	CORE	*RYR1*	M	Birth	1.8 y	Supported sitting	Axial/proximal	–	+	+	–
11	CORE	*RYR1*, *SEPN1*, *DNM2*, *MTM1*, *BIN1*	M	Birth	8 y	Independent walking, stairs	Axial	–	–	–	–
12	CORE	*RYR1*, *SEPN1*, *ACTA1*	F	Birth	4.1 y	Sitting	Axial/proximal	–	+	–	+
13	CORE	*RYR1*, *SEPN1*, *MTM1*, *BIN1,DNM2*	M	Birth	4.6 y	Independent walking, stairs, running	Axial	–	+	–	+
14	CORE	*RYR1*, *SEPN1*, *ACTA1*	F	5 y	12.6 y	Independent walking, stairs, running	Axial/proximal	–	–	–	–
15	CORE	*RYR1*, *MTM1*, *DNM2*	M	Birth	4.1 y	Sitting	Axial/proximal	+	+	–	–
16	CORE	*SEPN1*, *ACTA1*	M	8 m	10.7 y	Independent walking, stairs, running	Axial/proximal	–	–	–	+
17	CORE	*RYR1*	M	Birth	12.1 y	Independent walking, stairs, running	Axial/proximal	–	–	–	–
18	CORE	*RYR1*	F	Birth	2.6 y	Sitting	Axial/proximal	–	–	–	–
19	NM	*ACTA1*	F	Birth	0.5 y	NA	Axial	–	+	–	–
20	NM	*ACTA1*	M	Birth	12 d	NA	Axial/proximal	–	+	–	–
21	T1P	*RYR1*, *SEPN1*, *TPM3*	F	9 m	5.5 y	Independent walking, stairs, running	Axial/proximal	–	–	–	–
22	T1P	*SEPN1*, *TPM3*	M	Birth	3.5 y	Independent walking, stairs, running	Axial/proximal	–	+	–	+

Age = age at most recent follow-up; Function = functional abilities at most recent follow-up; EOM = extraocular muscles; CNM = myotubular/centronuclear myopathy; CORE = core myopathy; NM = nemaline myopathy; T1P = type 1 predominance; NA = not applicable; ND = no data. Shaded areas indicate sibling pairs.

consistent with recessive inheritance, whereas single heterozygous mutations were detected in 11 (42.3%) patients, five of them with a family history consistent with autosomal-dominant inheritance. All mutations identified are listed in Table 3.

A total of 13 familial cases (19.7%) from 6 different families were investigated, of which 11 (84.6%) were patients with mutations in the RYR1 gene (2 with dominant and 9 with recessive mutations) and 2 (15.4%) were sibling patients with histopathological features of a core myopathy but no mutation identified.

3.4. Clinical features

3.4.1. Onset and presentation

Of the 66 patients, 39 (59.1%) presented at birth or in first month of life, 13 (19.7%) between 1 and 12 months of age, 12 (18.2%) between 1 and 5 years of age and 2 (3%) after 5 years of age.

Within the group with congenital onset (within the first month of life), the majority presented with hypotonia ($n = 29$; 74.4%), whereas isolated swallowing or respiratory problems ($n = 6$; 15.4%), hip dislocation ($n = 2$; 5.1%) and arthrogryposis ($n = 2$; 5.1%) were less frequent presentations. Of the 29 patients with congenital hypotonia, 12 (38%) required nasogastric feeding and 13 (44.9%) required assisted ventilation.

Of the 13 patients who presented between 1 and 12 months of age, 11 (84.6%) displayed delayed motor milestones, 1 (7.1%) had isolated nasal speech and 1 (7.1%) marked isolated facial weakness. In the subgroup with onset between 1 and 5 years of age, all patients showed walking or running difficulties, which were also the presenting features in the remaining 2 patients presenting after 5 years of age.

Table 2
Clinical and histopathological features from congenital myopathy patients with genetic diagnosis.

No.	Gene	Histological diagnosis	Sex	Onset	Age	Function	Weakness	EOM	Bulbar	Scoliosis	Spinal rigidity
23	*ACTA1*	NM	F	3 w	10.4 y	Independent walking, stairs, running	Axial/proximal	–	+	–	+
24	*ACTA1*	NM	F	Birth	2.5 y	Independent walking, stairs	Axial/proximal	–	+	–	–
25	*ACTA1*	NM	F	Birth	4.7 y	Supported standing	Axial/proximal	–	+	+	–
26	*ACTA1*	NM	M	Birth	2 m	NA	Axial/proximal	–	+	–	–
27	*ACTA1*	NM	F	Birth	2.4 y	Independent walking, stairs, running	Axial/proximal	–	+	–	–
28	*ACTA1*	NM	M	Birth	1 w	NA	Axial/proximal	–	+	–	–
29	*ACTA1*	NM	F	Birth	5 m	NA	Axial/proximal	–	+	–	–
30	*MTM1*	CNM	M	Birth	1.1 y	NA	Axial	+	+	–	–
31	*MTM1*	CNM	M	Birth	2 m	NA	Axial/proximal	+	+	–	–
32	*NEB*	RC	M	1 y	7.7 y	Independent walking, stairs	Distal	–	–	+	+
33	*RYR1*	CORE	M	2 y	12.1 y	Independent walking, stairs, running	Axial/proximal	–	–	–	–
34	*RYR1*	T1P	M	Birth	4.1 y	Independent walking, stairs, running	Proximal	+	–	–	–
35	*RYR1*	T1P	M	Birth	1.6 y	Supported standing	Axial/proximal	–	+	–	–
36	*RYR1*	CORE	M	6 m	6.8 y	Supported walking	Axial/proximal	+	+	–	+
37	*RYR1*	CORE	M	1 y	14.3 y	Independent walking, stairs, running	Axial/proximal	–	–	–	–
38	*RYR1*	ND	F	6 m	18.6 y	Supported walking	Axial/proximal	–	–	+	–
39	*RYR1*	CORE	F	1.5 y	4.1 y	Independent walking, stairs	Axial/proximal	–	–	+	–
40	*RYR1*	FTD	F	Birth	2.7 y	Independent walking, stairs	Axial/proximal	–	+	–	–
41	*RYR1*	T1P	M	1.5 y	14.4 y	Independent walking, stairs, running	Axial	–	+	–	+
42	*RYR1*	CORE	M	1.5 y	7.4 y	Independent walking, stairs, running	Axial	–	–	–	+
43	*RYR1*	CNM	F	Birth	4.4 y	Independent walking, stairs, running	Axial/proximal	+	+	–	–
44	*RYR1*	ND	M	Birth	2.1 y	Independent walking, stairs, running	Axial	–	+	–	–
45	*RYR1*	CORE	M	2 y	8 y	Independent walking, stairs	Axial/proximal	–	–	–	–
46	*RYR1*	CORE	M	Birth	1 m	NA	Axial	–	+	+	–
47	*RYR1*	CORE	M	Birth	3.4 y	Supported standing	Axial/proximal	+	+	+	–
48	*RYR1*	ND	F	3 y	10.5 y	Independent walking, stairs, running	Axial/proximal	+	–	–	–
49	*RYR1*	CORE	F	Birth	7.4 y	Supported walking	Axial/proximal	–	–	–	+
50	*RYR1*	ND	M	4 m	1.7 y	Independent walking, stairs, running	Axial/proximal	–	–	–	–
51	*RYR1*	ND	M	2 y	3.7 y	Independent walking, stairs, running	Axial	–	–	–	–
52	*RYR1*	ND	F	7 y	15.7 y	Independent walking, stairs, running	Proximal	–	–	–	–
53	*RYR1*	ND	F	2 y	14.6 y	Independent walking, stairs, running	Proximal	–	–	–	–
54	*RYR1*	ND	F	2 y	3.6 y	Independent walking, stairs	Proximal	–	–	–	–
55	*RYR1*	ND	F	Birth	14.7 y	Independent walking, stairs, running	Axial/proximal	–	+	–	–
56	*RYR1*	CORE	F	Birth	6.6 y	Independent walking, stairs, running	Axial/proximal	–	–	+	+
57	*RYR1*	CORE	M	Birth	10.1 y	Independent walking, stairs, running	Axial	–	–	–	+
58	*RYR1*	T1P	F	Birth	12.5 y	Independent walking, stairs, running	Axial/proximal	–	+	–	+
59	*SEPN1*	ND	F	4 m	4.3 y	Independent walking, stairs	Axial	–	–	–	+
60	*SEPN1*	ND	F	7 y	8.5 y	Independent walking, stairs, running	Axial	–	+	–	+
61	*SEPN1*	CORE	F	4 m	8 y	Independent walking, stairs running	Axial	–	–	–	+
62	*SEPN1*	CORE	M	6 m	2.5 y	Independent walking, stairs	Axial/proximal	–	+	+	–
63	*SEPN1*	ND	M	1 m	6.4 y	Wheelchaired for most of time	Axial/proximal	–	+	–	+
64	*SEPN1*	CORE	F	4.5 y	5.1 y	Independent walking, stairs, running	Axial/proximal	–	–	–	+
65	*SEPN1*	CORE	M	6 m	7.2 y	Independent walking, stairs, running	Axial/proximal	–	+	–	+
66	*TPM3*	FTD	M	Birth	7.8 y	Independent walking, stairs	Axial/proximal	–	+	+	+

Age = age at most recent follow-up; Function = functional abilities at most recent follow-up; EOM = extraocular muscle; CNM = myotubular/centronuclear myopathy; CORE = core myopathy; FTD = fibre type disproportion; NM = nemaline myopathy; RC = rod–core myopathy; T1P = type 1 predominance; NA = not applicable; ND = no data. Shaded areas indicate sibling pairs.

Within the group with congenital onset, mutations in RYR1 ($n = 12$; 30.8%), ACTA1 ($n = 6$; 15.4%) and MTM1 ($n = 2$; 5.1%) were the most common identifiable causes. The two patients with late-onset beyond 5 years of age had mutations in SEPN1 and RYR1, respectively.

3.4.2. Functional abilities

Nine patients (13.6%) were less than 18 months at the last follow-up. Of the remaining 57 patients, 44 (77.2%) were independently ambulant, 3 (5.3%) were able to walk with support and 1 (1.7%) required a wheelchair for longer distances. Amongst the 4 patients requiring support for walking or wheelchair assistance, 3 had RYR1 mutations, one had a SEPN1 mutation. Nine of the 57 patients (15.8%) were never able to walk, with functional abilities ranging from supported standing ($n = 3$) to sitting with or without support ($n = 6$). Five of these 9 patients had a core myopathy, of which only 1 genetically confirmed with recessive RYR1 mutations; however in this patient, physical disability was confounded by a hemiparesis secondary to a perinatal middle cerebral artery infarct (patient No. 47). The functional abilities of each individual patient are summarised in Tables 1 and 2.

Table 3
Mutations identified in patients with congenital myopathies.

No.	Gene	Mutation(s)
23	*ACTA1*	c.1111A>C (p.Ile369Leu) exon 7
24	*ACTA1*	c.553C>G (p.Arg185Gly) exon 4
25	*ACTA1*	c. 802T>C (p.Phe268Leu) exon 5
26	*ACTA1*	c.109G>T (p.Val37Leu) exon 2
27	*ACTA1*	c.422T>C (p.Val141Ala) exon 3
28	*ACTA1*	c.211A>T (p.IIe71Phe) exon 3
29	*ACTA1*	c.821C>A (p.Ala274Glu) exon 6
30	*MTM1*	c.1132G>A (p.Gly378Arg) exon 11
31	*MTM1*	Deletion of exon 1 + part of 5′ UTR (the promoter region of the gene)
32	*NEB*	Exon 55 deletion (p.Arg2478_Asp2512del) + c.24267_24270dup (p.Val8091fs) exon 171
33	*RYR1*	c.12861_12869dup (p.Thr4288_Ala4290dup) exon 91
34	*RYR1*	c.2677G>A (p.Gly893Ser) exon 21 + c.4024A>G (p.Ser1342Gly) exon 28
35	*RYR1*	c.13513G>C (p.Asp4505His) exon 92
36	*RYR1*	c.11798A>G (p.Tyr3933Cys) exon 86 + c.12687G>T (p.Lys4229Asn) exon 91
37	*RYR1*	c.14749T>C (p.Phe4917Leu) exon 102
38	*RYR1*	c. 14588–14605del (p.Phe4863-Asp4869delinsTyr) exon 101
39	*RYR1*	c.7354C>T (p.Arg2452Trp) exon 46
40	*RYR1*	c.10348–6C>G (p.His3449 fs) intron 68 + c.14524G>A (p.Val4842Met) exon 101 + c.2113G>C (p.Gly705Arg) exon 18
41	*RYR1*	Homozygous c.11315G>A (p.Arg3772Gln) exon 79
42	*RYR1*	Homozygous c.11315G>A (p.Arg3772Gln) exon 79
43	*RYR1*	c.2060_2061delTC (p.Leu687fs) exon 18 + c.4405C>T (p.Arg1469Trp) exon 30
44	*RYR1*	c.2060_2061delTC (p.Leu687fs) exon 18 + c.4405C>T (p.Arg1469Trp) exon 30
45	*RYR1*	c14582G>A (p.Arg4861His) exon 101
46	*RYR1*	c.3877C>A (p.Pro1293Thr) exon 28 + c.14939C>T (p.Thr4980Met) exon 104
47	*RYR1*	c.5030A>G (p.Asn1677Ser) exon 34 + c.11752A>C (p.Thr3918Pro) exon 85
48	*RYR1*	c.10343C>T (p.Ser3448Phe) exon 68 + c.14365–2A>T (p. Ser4789-Lys4822del) exon 100
49	*RYR1*	c.14423T>A (p.Phe4808Tyr) exon 100
50	*RYR1*	c.13912G>A (p.Gly4638Ser) exon 95
51	*RYR1*	c.13912G>A (p.Gly4638Ser) exon 95
52	*RYR1*	c.11798A>G (p.Tyr3933Cys) exon 86 + c.13892A>G (p.Tyr4631Cys) exon 95
53	*RYR1*	c.11798A>G (p.Tyr3933Cys) exon 86 + c.13892A>G (p.Tyr4631Cys) exon 95
54	*RYR1*	c.11798A>G (p.Tyr3933Cys) exon 86 + c.13892A>G (p.Tyr4631Cys) exon 95
55	*RYR1*	c.14558C>T (p.Thr4853Ile) exon 101
56	*RYR1*	c.14582G>A (p.Arg4861His) exon 101
57	*RYR1*	c.3381 + 1G>A Intron 25 + c.2635G>A (p.Glu879Lys) exon 21
58	*RYR1*	c.3381 + 1G>A Intron 25 + c.2635G>A (p.Glu879Lys) exon 21
59	*SEPN1*	c.802C>T (p Arg268Cys) exon 6 + c.1397G>A (p.Arg466Gln) exon 11
60	*SEPN1*	Homozygous c.943G>A (p.Gly315Ser) exon 7
61	*SEPN1*	Homozygous c.13_22dup10 (p.GIn8 fs) exon 1
62	*SEPN1*	Homozygous c. 1282–2A>C intron 9
63	*SEPN1*	Homozygous c.820G>C (p.Ala274Pro) exon 6
64	*SEPN1*	c.1A>G (p.Met1Val) exon 1 + c.883G>A (p.Glu295Lys) exon 7
65	*SEPN1*	Homozygous c.943G>A (p.Gly315Ser) exon 7
66	*TPM3*	c.521A>C (p.Glu174Ala) exon 5

Shaded areas indicate sibling pairs.

3.4.3. Distribution of weakness and wasting

The clinical phenotype was characterised by predominant weakness of axial and proximal limb muscles in 47/66 patients (71.2%), whereas 13/66 patients (19.7%) showed a predominantly axial phenotype (neck and/or trunk muscles) together with an only modest involvement of the proximal limb muscles. In 4/66 patients (6.1%), only proximal limb muscle weakness was observed, all of them with recessive mutations in the RYR1 gene. One (1.5%) patient mutated in NEB and with histological diagnosis of rod–core myopathy had a predominantly distal muscle involvement, although additional mild to moderate distal muscle weakness was detected in 43/66 patients (65.1%). Patient No. 4 with genetically unresolved CNM had lower limb contractures but only facial and profound bulbar and respiratory weakness, which led to death at 1 month of age.

Disorders of ocular motility and/or eyelid ptosis were observed in 19/63 patients (30.2%) where this information was available; opthalmoparesis ($n = 10$) in particular was only observed in patients with hemizygous *MTM1* (100%) or compound heterozygous *RYR1* (5 of 15 patients, 33.3%) mutations but not with any other genetic background.

3.4.4. Respiratory and bulbar involvement

Nineteen of 66 patients (28.8%) received assisted ventilation, including 5 (7.6%) on invasive and 14 (21.2%) on non-invasive ventilation (NIV). Within the group on invasive ventilation, 2 (3.0%) had been intubated and

Table 4
Assisted ventilation (A/V) and gastrostomy/jejunostomy (G/J) according to genetic and histological diagnosis.

Genetic or histological diagnosis	Mean age at onset (range)	AV	Mean age AV (range)	G/J	Mean age G/J (range)
ACTA1	3 d (birth-21)	6 (85.7%)	1.6 y (birth-7.5)	4 (57.1%)	7.5 m (4–12)
SEPN1	24 m (1–84)	2 (28.6%)	3.5 y (1–6)	1 (14.3%)	1 y
RYR1	12 m (birth-84)	3 (11.5%)	4 m (birth-10)	4 (15.4%)	13 m (10–16)
MTM1	Birth	1 (50%)	1 m	1 (50%)	6 m
TPM3	Birth	1 (100%)	2 y	0	
NEB	1 y	1 (100%)	6.3 y	0	
CORE	7 m (birth-60)	1 (7.1%)	4 m	1 (7.1%)	5 m
NM	Birth	1 (50%)	Birth	1 (50%)	10 m
CNM	Birth	3 (75%)	Birth	2 (50%)	9 m (7–11)

Histopathological diagnosis only has been indicated for patients without genetic confirmation. AV = assisted ventilation and includes NIV, ventilation through tracheostomy or intubation; CNM = myotubular/centronuclear myopathy; CORE = core myopathy; NM = nemaline myopathy (note that none of the 2 patients with predominance of fibre type I and without genetic diagnosis underwent AV or G/J).

ventilated from birth before demise at 10 and 15 days of life, respectively; 3 (4.5%) were still alive and had been ventilated via tracheostomy since the first month of life. Within the group on NIV, this was started at a mean age of 1.8 ± 2.7 years (range 0–7.5 years). Another 11 (16.7%) patients, including 4 with *SEPN1* mutations, showed abnormal respiratory function but were not on NIV yet. Table 4 shows the data on assisted ventilation, according to histological and genetic diagnosis.

Swallowing problems were observed in 37/66 patients (56.1%) and were present without exception in patients with a histopathological diagnosis of NM, MTM/CNM and CFTD. In addition, dysphagia was also observed in 11/29 patients with a histopathological diagnosis of a core myopathy, 3 of them with (recessive) *RYR1* mutations, and 2 with *SEPN1* mutations. In 8 patients, 3 of them with *RYR1* mutations, swallowing problems were present after birth but gradually resolved over time, usually within months, but only after the age of 7 years in one patient with a *SEPN1* mutation. Weight measurements at different ages were available in 60/66 patients. Fifteen (25%) of these patients were underweight (below 3rd centile) and 3 (5%) were overweight (above the 97th centile). No swallowing problem was reported in 6 of the 15 underweight patients (40%), in whom low weight was also compounded by the reduced muscle mass. Gastrostomy (or, less frequently, jejunostomy) was performed in 14 (21.2%) of patients, at an average age of 9.5 ± 3.9 months (range 4–16 months). In 8 (57.1%) of these patients, fundoplication was also performed. Nasogastric feeding (NGF) was initiated prior to gastrostomy/jejunostomy insertion in all patients and was commenced from birth in 9/14 patients (range 0–10 months). In the 14 patients requiring gastrostomy or jejunostomy insertion, weight measurements prior to the procedure were below the 3rd centile in 7 patients. After gastrostomy/jejunostomy insertion, weight increased across centiles in 8/14 patients (57.1%) and continued to increase along the predicted centile in 4/14 patients (28.6%).

3.4.5. Orthopaedic complications

Scoliosis was present in 13 (19.7%) out of 66 patients. Seven of these patients had a histopathological diagnosis of a core myopathy, 4 with confirmed *RYR1* and 1 with confirmed *SEPN1* mutation. Of the 13 patients with scoliosis, scoliosis surgery was indicated in two, being performed in one (Patient No. 47) with improvement of sitting position, but other patients were either too young or the scoliosis too mild to be considered for surgical correction. Spinal rigidity was observed in 20 patients (30.3%), of whom 11 (55%) had core myopathy, 6 with *RYR1* and 2 with *SEPN1* mutations. Only 3 (15%) of the 20 patients with spinal rigidity also had scoliosis, 1 mutated in the *RYR1* gene, 1 in *NEB* and 1 in *TPM3*.

3.4.6. Clinical course

During the 5-year follow-up period, 5 patients (7.6%) died, 4 of them within the first 2 months of life and one at 6 years of age. Amongst the 5 patients who died, 3 had a histopathological diagnosis of NM (one due to a confirmed *ACTA1* mutation) and two a histopathological diagnosis of CNM, both of them genetically unresolved; all these patients had severe respiratory insufficiency: three required NIV, one was intubated, and one had an abnormal overnight oxygen saturation study. Amongst the remaining 61 patients 57/66 (86.4%) were stable or improved over time, whilst 4/66 (6.1%) had progressive worsening over the years.

3.4.7. Muscle imaging

Muscle ultrasound was performed in 44/66 patients (66.7%) and showed increased echogenicity in 37/44 (84.1%) patients. Results for the different genetic and histological diagnosis are shown in Fig. 2. More specifically, muscle ultrasound was abnormal in 23/25 patients (92%) with core myopathy, of whom 8 were mutated in *RYR1* and 3 in *SEPN1*. Muscle ultrasound specificity was 26.3% in patients with core myopathy, including those mutated in *RYR1* and *SEPN1*, whilst positive predictive value was 62.2%. The values of sensitivity and specificity in patients mutated in *RYR1*,

including those without histological diagnosis of core myopathy, were respectively 77.8% and 11.5%, whilst positive predictive value was 37.8%.

4. Discussion

Studies on large cohorts of CMs patients that include several different CMs entities are scarce. Here, we report a retrospective study describing a cohort of 66 patients diagnosed at a National Referral centre over a period of 5 years with distinct CMs entities. Genetic characterisation was achieved in 44 patients (66.7%), whilst the remaining 22 patients (33.3%) only had a clinical and histopathological diagnosis. The proportion of genetically resolved patients was higher than in other recently reported series, probably reflecting the fact that all patients were assessed at the same centre but also the exclusion of those with non-specific histopathological abnormalities, except isolated type 1 uniformity or predominance, a feature previously associated with mutations in genes implicated in more distinct CMs [15]. Indeed, the identification of RYR1 mutations in 4 patients with isolated type 1 uniformity or predominance suggest that RYR1 mutations are a relatively common cause of these histological findings, as had already been indicated in isolated cases [15,16]. As similar cases due to the lack of more specific structural abnormalities are not necessarily classified as congenital myopathies, the true incidence of CMs may be higher than previously reported [9,12]. Inability to detect the underlying genetic cause in one third of patients with histopathological features of a CM suggests either further genetic heterogeneity, or the presence of mutations in known genes not identifiable by conventional Sanger sequencing approaches, or both. It should also be emphasised that in this study we did not systematically screen all know CMs genes in all patients, and that sequencing of the giant nebulin gene was not comprehensive.

With regards to specific subtypes, core myopathies, found in 29/66 patients, were the most frequent histopathological diagnosis, corresponding to findings in other recent, but regionally more limited studies [9,12].

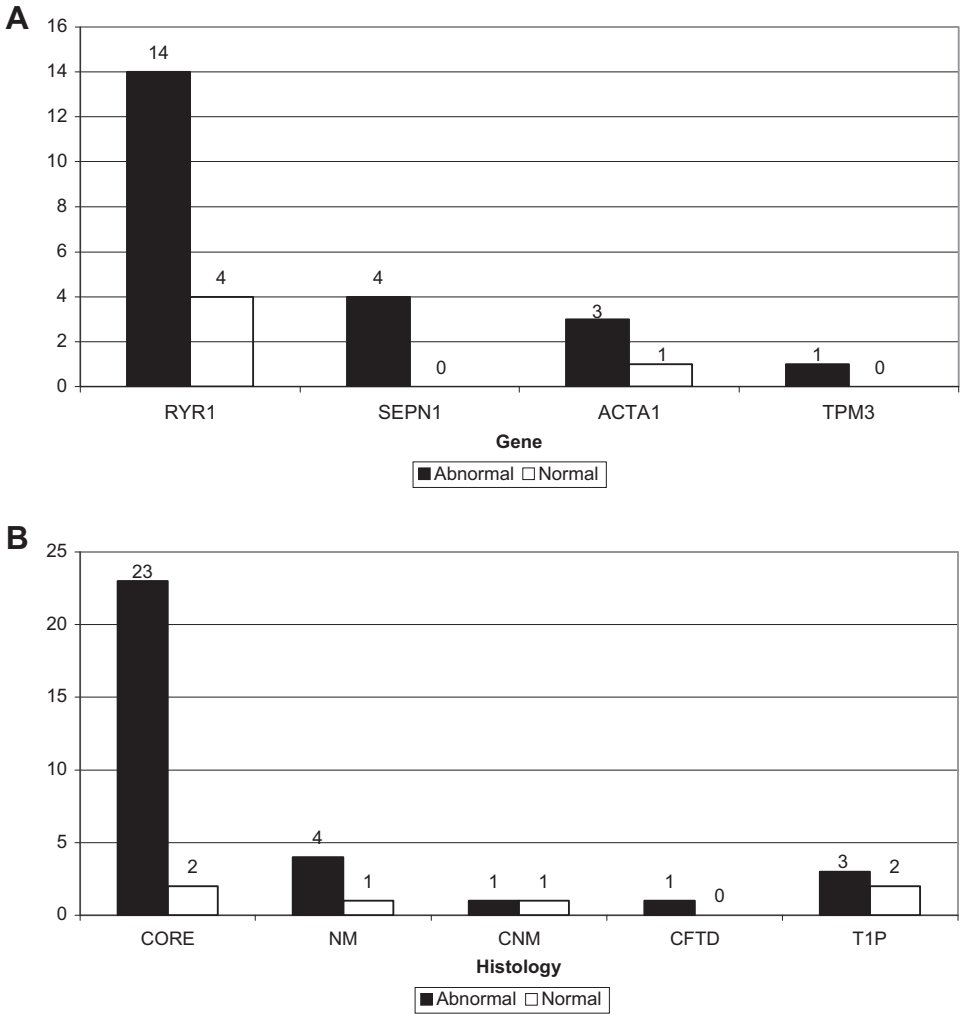

Fig. 2. Summary of muscle ultrasound findings according to genetic mutations (A) and histological features (B). Note that none of the patients ($n = 3$) with MTM1 or NEB mutation underwent muscle ultrasound. CNM = myotubular/centronuclear myopathy; CORE = core myopathy; FTD = fibre type disproportion; NM = nemaline myopathy; RC = rod–core myopathy; T1P = type 1 predominance.

Congenital myopathies with nemaline rods, diagnosed in 9/66 patients, were the second most common form, in contrast to some earlier reports where NM was considered the most frequent subgroup, probably reflecting an ascertainment bias [10,11]. The relative prevalence of congenital myopathies with central nuclei (CNM/MTM), not previously investigated with regards to the CMs group, was 10.6% ($n = 7/66$), in keeping with the low incidence of XLMTM, the most common form, estimated at 2/100,000 male births per year in France [17]. Only one patient had a diagnosis of a core–rod myopathy, indicating that truly dual pathology as previously reported in few genetically resolved cases is a relatively rare occurrence within the CMs cohort [18,19].

Genetic characterisation achieved in the majority of patients also allowed for better determination of the relative frequencies of single genetic entities, with RYR1 mutations accounting for almost two third of the genetically diagnosed patients. Mutations in SEPN1 and ACTA1 were the other most common identifiable genetic causes but were overall much less frequent, at 15.9% and 4.5%, respectively. The large proportion of RYR1 mutations identified in our series reflects the wider availability of complete sequencing of this very large gene, in contrast to earlier approaches where genetic analysis was limited to few mutational hotspot regions. Whilst CCD, the first RYR1-related myopathy to be identified, has been mainly associated with dominant RYR1 mutations in the C-terminal mutational hotspot [20,21], compound heterozygosity for recessive RYR1 mutations distributed throughout the RYR1 coding sequence is the most common genetic mechanism underlying the much wider range of CMs now recognised as related to RYR1 involvement [22,23]. Other recent studies focusing on RYR1-related myopathies alone have indicated that cases with recessive inheritance account for about half of all cases [22,24] and appear to be more commonly associated with certain histopathologic phenotypes such as MmD [25], CNM [26] or CFTD [27].

Despite being the most common identifiable genetic causes, mutations in the RYR1 and, less frequently, the SEPN1 gene accounted for only half of all cases within the core myopathy group, emphasizing that cores on muscle biopsy are a non-specific feature but also indicating further genetic heterogeneity. Whilst recent findings suggest that large genomic rearrangements involving the RYR1 locus may account for a proportion of cases where no RYR1 mutation has been identified on routine sequencing [28], it is likely that the majority of patients who remain genetically uncharacterised have mutations in other genes.

Within the NM group, 7/9 patients were mutated in ACTA1 and 2/9 did not have a genetic diagnosis. These findings are in contrast to other studies focussing on NM only and including larger numbers of patients, where NEB mutations accounted for 50% and ACTA1 mutations accounted for 20% of cases [29–31], but are in keeping with findings in other NM cohorts including severe-early onset cases [30,32] where ACTA1 mutations were also found to be frequent. Considering that our approach to NEB sequencing was limited due to the large size of the gene, it is possible that our only two genetically unresolved NM patients are also related to NEB mutations.

Of the 3/7 genetically confirmed patients within the CNM/MTM group, two males had MTM1 mutations whereas one patient had recessive RYR1 mutations. Whilst MTM1 mutations have been considered the most common genetic cause of CNM/MTM, RYR1 mutations have been more recently implicated in congenital myopathies with central nuclei but may be more prevalent in certain populations due to the presence of founder mutations [26].

In our cohort, CFTD was detected in two patients, one with a TPM3 mutation and one with recessive RYR1 mutations. Fibre type disproportion is an additional pathological feature in several CMs, but when present in isolation is most commonly due to mutations in TPM3, in up to 25% of cases in one series [33]. Recessive RYR1 mutations have been identified in 5 patients with isolated fibre type disproportion, also a frequent additional feature in RYR1-related CNM [26], suggesting a histopathological continuum of recessively inherited RYR1-related myopathies. Other known genetic causes of CFTD include mutations in ACTA1 or SEPN1 [34,35], none identified in patients from our cohort with this histopathological appearance.

The only patient with dual pathology, a core–rod myopathy, had compound heterozygous NEB mutations, previously only reported once in the literature [36]. Core–rod myopathies have previously been mainly attributed to heterozygous dominant RYR1 mutations in a number of patients [18,19].

We also aimed to establish possible clinical correlations for our cohort of CMs patients as a group and for the specific genetic defects identified most frequently.

As a group, amongst the 61 surviving patients followed over the 5 year period, 57 (93.4%) remained stable or improved, whilst 4 (6.6%) worsened, suggesting only slow disease progression in CMs and an overall favourable prognosis. Nineteen patients needed assisted ventilation and another 11 showed abnormal respiratory function, emphasizing the importance of close monitoring of respiratory function in the CMs, including annual overnight oxygen saturation studies, especially in patients under the age of 5 years in whom pulmonary function tests are not practicable but in whom NIV may already be necessary. In most cases, gastrostomy/jejunostomy insertion was needed earlier than assisted ventilation and had a demonstrable positive effect in more than half of all patients, with a documented substantial increase in weight.

Clinical correlations with specific genetic defects were not consistent with a few notable exceptions: despite

congenital onset in half of all cases, patients with RYR1 mutations followed a milder clinical course and in some cases there was mild but definite improvement over time. However, as our observations concerned a predominantly paediatric cohort we cannot comment on worsening of symptoms later in adulthood, as has been reported in some instances [37–39]. None of our patients with dominant RYR1 mutations had ophthalmoparesis, in contrast to 5 of the 15 patients with recessive RYR1 mutations, corresponding to earlier suggestions that extraocular muscle involvement is closely associated with recessively inherited RYR1 mutations [22,24]. Respiratory involvement was less pronounced in RYR1-related compared to SEPN1-related myopathies, which were consistently associated with abnormal pulmonary function tests and/or overnight oxygen saturation studies in accordance to what has been recently reported [40].

Our study suggests that ACTA1 mutations are not only a relatively common cause of NM but also more frequently associated with early-onset severe disease, as has been suggested in some earlier studies [30,32]. In particular, cases with ACTA1-related NM had consistent and substantial respiratory impairment, with 6 of the 7 patients mutated in ACTA1 needing some form of ventilatory support. In addition, almost one third of the 14 patients requiring gastrostomy/jejunostomy insertion belonged to the ACTA1 group, despite the fact that ACTA1 mutated patients only represented 10.6% of all patients with CMs. It is important to acknowledge that patients with mild forms of ACTA1-related NM have also been reported [29,30,35]; it is therefore likely that our findings are biased towards the severe end of the spectrum based on the pattern of referral to our Paediatric Centre. Regarding patients with NEB-related NM, we only screened for a single common exon deletion, and identified compound heterozygous NEB mutations in one isolated case with rod–core myopathy. In contrast to the only other previously published case [36], our patient with NEB-related rod–core myopathy had later disease onset and a relatively milder course, achieving and maintaining independent walking and not requiring NIV before the age of 6 years.

In addition to clinical features, we also looked at the use of muscle imaging as an adjunct in the diagnosis of the CMs. Muscle ultrasound was performed systematically at the first patient evaluation. We found muscle imaging using ultrasound useful in the diagnostic workup in 37 of the 44 patients investigated. Muscle US is readily available and particularly helpful in patients under the age of 5 years. Similar to our findings, where 92% of core myopathy patients showed abnormal muscle ultrasound, several studies demonstrated that muscle ultrasound is a highly sensitive technique for the detection of some neuromuscular diseases [13,14,41]. Whilst muscle MRI is also a very useful imaging modality in CMs, as previously demonstrated by us and others [42–45]; however in contrast to muscle US it cannot be performed without sedation before the age of 5 years, and it is not available in all centres.

In conclusion, this is the first study detailing the relative prevalence of specific genetic defects in a large cohort of patients with CMs assessed at one single national referral centre. With regards to the most common genetic backgrounds, our study indicates disease-specific gene profiles that may be used both for improved diagnosis and for providing a personalised approach to the medical treatment. Around 1/3 of congenital myopathies remain currently genetically unresolved, indicating the need for further studies exploring their genetic basis.

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