Congenital myopathies: disorders of excitation–contraction coupling and muscle contraction

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Abstract | The congenital myopathies are a group of early-onset, non-dystrophic neuromuscular conditions with characteristic muscle biopsy findings, variable severity and a stable or slowly progressive course. Pronounced weakness in axial and proximal muscle groups is a common feature, and involvement of extraocular, cardiorespiratory and/or distal muscles can implicate specific genetic defects. Central core disease (CCD), multi-minicore disease (MmD), centronuclear myopathy (CNM) and nemaline myopathy were among the first congenital myopathies to be reported, and they still represent the main diagnostic categories. However, these entities seem to belong to a much wider phenotypic spectrum. To date, congenital myopathies have been attributed to mutations in over 20 genes, which encode proteins implicated in skeletal muscle Ca^{2+} homeostasis, excitation–contraction coupling, thin–thick filament assembly and interactions, and other mechanisms. *RYR1* mutations are the most frequent genetic cause, and CCD and MmD are the most common subgroups. Next-generation sequencing has vastly improved mutation detection and has enabled the identification of novel genetic backgrounds. At present, management of congenital myopathies is largely supportive, although new therapeutic approaches are reaching the clinical trial stage.

The congenital myopathies are a genetically heterogeneous group of early-onset neuromuscular disorders characterized by variable degrees of muscle weakness and distinctive structural abnormalities in muscle biopsy samples. The conditions that have been identified to date are mostly disorders of muscle excitation–contraction coupling (ECC) or of proteins primarily involved in sarcomeric filament assembly and interaction. However, recent findings suggest other less common pathogenic mechanisms. The concept of congenital myopathies was established in the 1950s and 1960s, when the application of histochemical and ultrastructural techniques to diseased muscle identified histopathological features that were considered to be pathognomonic. Recognition of these features — namely, central cores, multi-minicores, central nuclei and nemaline rods — resulted in the designation of four novel disease entities, central core disease (CCD)[1], multi-minicore disease (MmD)[2], centronuclear myopathy (CNM)[3] and nemaline myopathy[4], which still represent the main diagnostic categories.

Considerable progress has been made in understanding the phenotypic spectrum, diagnosis and management of the congenital myopathies. In addition to primary myopathic features, non-neuromuscular manifestations are observed in several forms, pointing to a role for the defective proteins in non-skeletal muscle tissues[5]. Muscle imaging, in particular, muscle MRI, has emerged as a powerful tool for deep phenotyping[6]. Presentations late in adulthood have now been recognized[7,8], and owing to improved standards of care, even patients with severe early-onset forms increasingly transition from paediatric to adult neurology services.

Since the identification of dominant mutations in the skeletal muscle ryanodine receptor 1 (*RYR1*) gene as the cause of malignant hyperthermia in 1991 and CCD in 1993[9,10], mutations in more than 20 genes have been identified in patients with congenital myopathies. Introduction of next-generation sequencing (NGS) techniques into routine clinical diagnosis[11] has resulted in an improved detection rate for mutations in genes such as *RYR1*, nebulin (*NEB*) and titin (*TTN*). Owing to their large size, these genes were previously only studied by Sanger sequencing in a few patients. Novel NGS techniques have led to the recognition that different mutations in the same gene can give rise to various histopathological phenotypes, and that mutations in different

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Key points

- Congenital myopathies are clinically and genetically heterogeneous conditions characterized by muscle weakness and distinctive structural abnormalities in muscle biopsy samples
- Clinically, congenital myopathies have a stable or slowly progressive course, and the severity varies depending on the causative mutation
- More than 20 genes have been implicated in congenital myopathies
- The most commonly affected genes encode proteins involved in skeletal muscle Ca^{2+} homeostasis, excitation–contraction coupling and thin–thick filament assembly and interactions
- Management of congenital myopathies is largely supportive, although experimental therapeutic approaches are reaching the clinical trial stage

genes can cause the same histopathological feature, often owing to functional associations between the defective proteins. Moreover, it has become increasingly clear that many congenital myopathies are characterized by non-specific or complex pathological abnormalities rather than a 'pure' muscle pathology picture. A classification based on predominant histopathological and associated clinical features is still useful; however, it is also helpful to consider these conditions according to the main underlying disease mechanisms.

In this Review, we summarize genetic, clinical and pathological features of the main congenital myopathies. Common pathogenic mechanisms, diagnostic and current management approaches, and principles of therapy development will be outlined.

Classification and epidemiology

Data concerning the precise epidemiology of the congenital myopathies are limited and are mostly focused on the four main pathological variants: CCD, MmD, CNM and nemaline myopathy. The key characteristics of these entities are detailed below and illustrated in FIG. 1.

CCD — initially described in the 1950s[1] — and MmD[2] are often referred to as the 'core myopathies'[12], and their names are derived from the histochemical appearance of focally reduced oxidative enzyme activity, which corresponds to myofibrillar changes on ultrastructural examination. CCD is characterized by centrally located, well-demarcated cores that run along the fibre axis for a substantial distance on longitudinal sections, whereas MmD is defined by multiple cores of less well-defined appearance and more-limited length.

The hallmark of CNM is the presence of fibres with centralized nuclei, which show variations in terms of numbers and associated features between muscles and genetic backgrounds. Nemaline myopathy is characterized by the presence of numerous nemaline rods that stain red with Gomori trichrome and can be confirmed by electron microscopy[13].

The overall prevalence of these congenital myopathy variants has been estimated at 1 in 26,000[14]. Nemaline myopathy was originally considered to be the most frequent form, but emerging data suggest that congenital myopathies with cores (CCD and MmD) represent the most common subgroup. Marked genetic heterogeneity is now acknowledged, as detailed below. *RYR1* seems to be the gene most frequently involved in congenital myopathies, in particular, CCD and MmD. Recessive *NEB* mutations and de novo dominant mutations in *ACTA1*, which encodes skeletal muscle α-actin, are the most common known causes of nemaline myopathy, whereas X-linked recessive mutations in the myotubularin gene (*MTM1*) are believed to be the most common cause of CNM. Mutations in *TTN* are increasingly recognized and may be involved in a substantial proportion of currently unresolved congenital myopathies as well as other neuromuscular disorders, including muscular dystrophies[15]. The genes implicated in the congenital myopathies are listed in TABLE 1, and the key clinicopathological features associated with the most common genetic backgrounds are summarized in TABLE 2. Characteristic histopathological features are illustrated in FIG. 1.

Clinicopathology and genetics

Congenital myopathies with cores

In view of their pathological and genetic overlap, CCD, MmD and malignant hyperthermia are discussed together in this section.

CCD is closely associated with dominant *RYR1* mutations, whereas MmD is genetically more heterogeneous. Most cases of MmD have been attributed to recessive mutations in *RYR1*[16–18], *SEPN1* (also known as *SELENON*)[19] or — less frequently — *MYH7*[20]. Histopathological features consistent with MmD have also been described in some patients with recessive mutations in *MEGF10*, which encodes multiple epidermal growth factor-like domains protein 10[21–24]. Cores or minicores in muscle biopsy samples can also be prominent in *TTN*-related myopathies[25], often in conjunction with other myopathic and dystrophic features, and might occur in other neuromuscular disorders.

Clinically, CCD due to dominant *RYR1* mutations[12] is usually a mild condition, although early severe presentations, often associated with de novo inheritance, have been recorded[26]. Extraocular muscles are usually spared, and facial, bulbar and respiratory involvement is typically mild. Congenital dislocation of the hips and scoliosis are common. Most patients achieve independent ambulation and have a static or only slowly progressive course.

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The clinical features of MmD are more variable[12]. *SEPN1*-related myopathies[19,27] are characterized by marked weakness, early spinal rigidity, scoliosis and respiratory impairment. Patients with recessively inherited *RYR1*-related core myopathies exhibit a distribution of weakness and wasting that resembles the *SEPN1*-related form but have additional extraocular muscle involvement and, with few exceptions, lack severe respiratory impairment[17,18]. Various combinations of scoliosis, spinal rigidity, multiple (mainly distal) contractures and associated cardiomyopathy can occur in *TTN*-related and *MYH7*-related forms[20,25]. *MEGF10*-related myopathies have a wide clinical spectrum, ranging from a severe early-onset myopathy with areflexia, respiratory distress and dysphagia (termed EMARDD)[21,23,24] to adult-onset cases with minicores in muscle biopsy samples[22]. Muscle MRI can help to differentiate genetically distinct core myopathies[28,29].

Dominant *RYR1*-related CCD is allelic to the malignant hyperthermia susceptibility (MHS) trait — a pharmacogenetic predisposition to malignant hyperthermia and severe adverse reactions to volatile anaesthetics and muscle relaxants[30] — and some CCD-associated *RYR1* mutations also carry an increased risk of MHS. The association between MHS and recessive *RYR1*-related MmD is less well established; however, some cases of MmD have been attributed to compound heterozygosity for dominant MHS-associated *RYR1* mutations and missense, nonsense or other loss-of-function mutations[18,31,32].

RYR1-related King–Denborough syndrome (KDS) is an MHS-associated myopathy characterized by dysmorphic facial features, short stature, spinal rigidity, scoliosis and various histopathological features[33]. Another recently recognized myopathy with similar clinicopathological features is Native American myopathy (NAM), originally described in the Lumbee population of North Dakota and caused by homozygosity for a founder mutation (Trp284Ser) in *STAC3*, which encodes SH3 and cysteine-rich domain-containing protein 3[34].

MHS-associated *RYR1* mutations have also been identified as a common cause of exertional myalgia and rhabdomyolysis (ERM) in otherwise healthy individuals with various muscle biopsy findings[35]. Of note, exertional myalgia can be prominent in CCD[36], and mild to moderate creatine kinase elevations (up to 1,000 international units (IU)/l), which are unusual in the context of other congenital myopathies, are not uncommon. MHS-associated *RYR1* mutations can also give rise to late-onset axial myopathy in previously healthy (or even athletic) individuals[37,38].

Centronuclear myopathy

CNM[39] is associated with X-linked recessive mutations in the myotubularin gene *MTM1* (a condition termed X-linked myotubular myopathy or XLMTM)[40], autosomal dominant mutations in dynamin 2 (*DNM2*)[41] and amphiphysin II (*BIN1*)[42], and autosomal recessive mutations in *RYR1*[43], *BIN1*[44] and *TTN*[45]. Recessive mutations in *SPEG* have been identified in a small number of families[46], and dominant mutations in *CCDC78*[47] were found in one isolated pedigree. Heterozygous missense variants in *MTMR14*, which have been identified in two patients with CNM, might represent a genetic modifier of other genetic backgrounds[48].

In *MTM1*-related CNM, the central nuclei are usually spaced out along the long fibre axis, whereas in *DNM2*-related cases, these nuclei can form chains. In the rare *BIN1*-related cases, the central nuclei can form clusters. In patients with *MTM1*-related CNM, typical features of the muscle fibres include central areas of increased oxidative enzyme activity and a pale peripheral halo. These features, along with the presence of central nuclei, are also seen in congenital myotonic dystrophy. Strictly centralized nuclei are more common than multiple internalized nuclei in the *MTM1*-related, *DNM2*-related and *BIN1*-related forms of CNM[40,41,44], whereas multiple internalized nuclei are more common in *RYR1*-related and *TTN*-related cases[43]. A radial distribution of sarcoplasmic strands that stain positively with NADH tetrazolium reductase and periodic acid–Schiff is often seen in *DNM2*-related CNM[41]. 'Necklace' fibres are often seen in patients carrying milder *MTM1* mutations or in female carriers of *MTM1* mutations[49], and occasionally in patients carrying *DNM2* mutations[50]. Ultrastructural triad abnormalities are observed in most forms of CNM[51].

From a clinical perspective, extraocular muscle involvement is a consistent feature of most forms of CNM[52], the exceptions being the *TTN*-related, *SPEG*-related and *CCDC78*-related forms. The most severe form, XLMTM, typically manifests in affected males with profound hypotonia, weakness and contractures at birth, as well as bulbar and respiratory involvement that almost always necessitates ventilation for survival. Although the provision of constant respiratory support improves life expectancy in patients with XLMTM, some long-term survivors experience complications[53], probably related to the ubiquitous role of the defective protein.

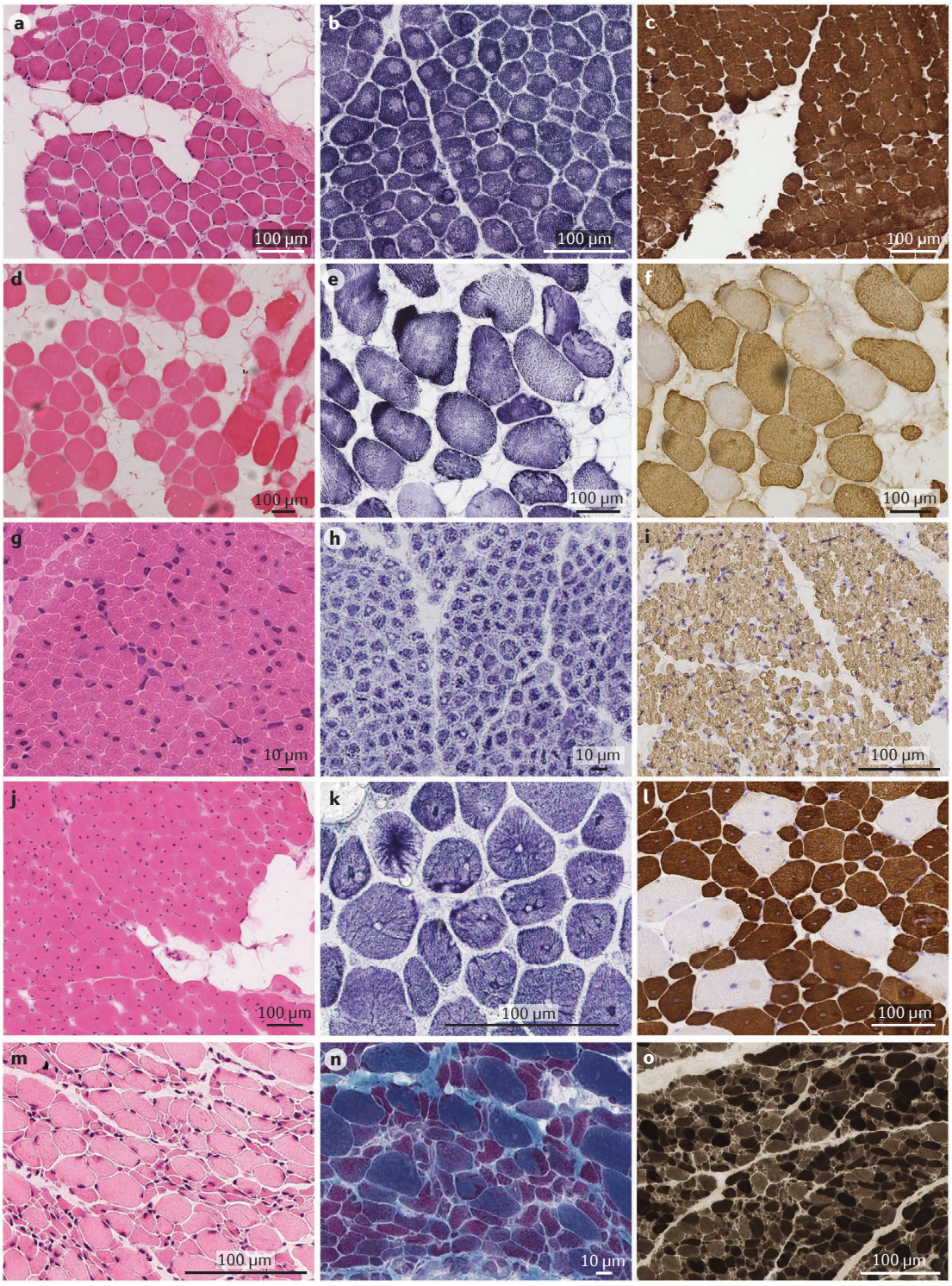
Dominantly inherited CNM associated with mutations in *DNM2* is frequently a relatively mild condition[41,54], although more-severe de novo cases have been recorded[55,56]. Additional characteristic features of this condition include distal weakness, calf muscle hypertrophy, exertional myalgia and/or fatigue, PNS or CNS involvement, and multisystem features such as neutropenia or cataracts. The peripheral axonal neuropathy Charcot–Marie–Tooth disease, dominant intermediate B (CMTDIB) is allelic to *DNM2*-related CNM[57].

Recessively inherited and — less frequently — dominantly inherited as well as milder forms of *BIN1*-related CNM have been reported in a few families[42,44,58]. Recessively inherited CNM due to *RYR1* mutations[43] shows considerable clinical overlap with other forms of recessively inherited *RYR1*-related myopathy (see above). Mutations in *TTN* are often associated with dysmorphic facial features, scoliosis, spinal rigidity and contractures[45], showing some overlap with Emery–Dreifuss muscular dystrophy and the KDS spectrum. Cardiac involvement has been reported only in the *TTN*-related and *SPEG*-related forms of CNM.

Nemaline myopathy

Nemaline myopathy has been associated with mutations in more than ten genes to date, most commonly, recessive mutations in *NEB*[59,60] and — usually de novo — dominant mutations in *ACTA1*[61]. Rarer causes of nemaline myopathy, some of which are limited to single families, include dominant mutations in tropomyosin 3 (*TPM3*)[62], tropomyosin 2 (*TPM2*)[63] and *KBTBD13*[64] and recessive mutations in *ACTA1*[65], *TPM3*[66], *TPM2*[67], *TNNT1*[68], *CFL2*[69], *KBTBD13*[64], *KLHL40*[70], *KLHL41*[71], *LMOD3*[72], *MYPN*[73,74] and *MYO18B*[75].

The number and distribution of nemaline rods vary among muscles and patients. Rods are believed to be derived from Z-lines and may show continuity with these structures. The rods are mainly cytoplasmic but can also be nuclear, particularly in *ACTA1*-related



nemaline myopathy[76], where additional actin accumulation and compensatory expression of cardiac actin can be observed. Nemaline rods are usually seen in both type I and type II muscle fibres, except in patients with *TPM3* mutations, where they are limited to type I fibres. Numerous small rectangular rods in fibres with sparse myofibrils are a feature of *KLHL40*-related nemaline myopathy[70].

Clinically, nemaline myopathy is highly variable and is conventionally classified by age of onset and severity. Severe, often lethal cases within the fetal akinesia spectrum have been reported in association with recessive mutations in *KLHL40*[70], *KLHL41*[71], *LMOD3*[72] and *MYO18B*[75], whereas the typical congenital form characterized by infantile onset, hypotonia and often disproportionate bulbar involvement is most commonly due to recessive *NEB* mutations[77]. Dominant — frequently de novo — *ACTA1* mutations are often associated with severe congenital presentations, but milder cases have been reported[65,78–80]. *KBTBD13*-related nemaline myopathy is an unusual form characterized by progressive proximal and neck weakness, gait abnormalities, poor exercise tolerance and peculiar slowness of movement[81]. Extraocular muscle involvement is seen in only a fraction of patients with *KLHL40*, *KLHL41* and *LMOD3* mutations. Cardiomyopathy is sometimes seen in *MYPN*-associated and *MYO18B*-associated nemaline myopathy[74,75]. Marked distal involvement is observed in numerous forms of nemaline myopathy, and many of the causative genes have also been implicated in distinct distal arthrogryposis syndromes[82]. Muscle MRI might help to distinguish different genetic forms of nemaline myopathy[83].

◀ Fig. 1 | **Muscle pathology in congenital myopathies.** Tissue samples from a child with dominant *RYR1*-related central core disease (parts **a**–**c**). Muscle shows myopathic fibre size variation and marked perimysial fatty infiltration (part **a**). Most fibres contain a single central or eccentric core with a well-delineated zone of diminished or absent oxidative staining; some fibres also show a rim of increased oxidative staining surrounding the core lesion (part **b**). Fibres are uniformly type I (part **c**). Tissue samples from an adolescent with recessive *SEPN1*-related multi-minicore disease (parts **d**–**f**). Muscle shows myopathic fibre size variation and perimysial fatty infiltration (part **d**). Fibre typing is preserved, with a predominance of type I fibres (darker staining), and both type I and type II fibres display foci of diminished or absent oxidative staining (multi-minicores) and, occasionally, larger lesions (parts **e**,**f**). Tissues samples from a patient with *MTM1*-related centronuclear myopathy (CNM) (parts **g**–**i**). Samples from a male neonate with severe X-linked recessive myotubular myopathy show many fibres with centrally placed nuclei (part **g**). Most fibres display pale peripheral haloes (part **h**), and type I fibres predominate (part **i**). Tissues samples from an adult with *DNM2*-related CNM (parts **j**–**l**). Muscle shows a marked increase in central nucleation and perimysial fatty infiltration (part **j**). Many fibres display 'radial strands' emanating from a centrally placed nucleus (part **k**). Type I fibre predominance and hypotrophy create fibre size disproportion; central nuclei are present in both fibre types (part **l**). Tissue samples from a severely affected neonate with de novo dominant *ACTA1*-related nemaline myopathy (parts **m**–**o**). Muscle shows myopathic fibre size variation with the appearance of two fibre populations: smaller type I and larger type II fibres (part **m**). Numerous thread-like inclusions are seen in both fibre sizes and appear red with the modified Gomori trichrome stain (part **n**) and eosinophilic with haematoxylin and eosin (part **m**). Pale-stained type I fibres are often more severely affected than type II fibres, showing atrophy or hypotrophy (part **o**). Muscle biopsy samples were stained with haematoxylin and eosin (parts **a**,**d**,**g**,**j**,**m**), NADH tetrazolium reductase (parts **b**,**e**,**h**,**k**) and modified Gomori trichrome (part **n**), as well as stains for slow myosin heavy chain (parts **c**,**f**,**i**,**l**) and myosin ATPase at pH 4.6 (part **o**).

Other congenital myopathies

In recent years, we have witnessed an expansion of the phenotypic spectrum associated with the known congenital myopathy-associated genes, as well as the description of novel conditions that share some of the clinical and muscle biopsy findings of the better-characterized entities without reaching a comparable level of histopathological 'purity'. These congenital myopathies with nonspecific, multiple (structural) and unusual or other features are summarized in the sections that follow.

Congenital myopathies with nonspecific features. Marked type I fibre predominance or uniformity is common in all congenital myopathies and can be the sole presenting feature[84]. Type I predominance and atrophy were also reported in one consanguineous family with clinical features of a congenital myopathy and recessive mutations in 3-hydroxyacyl-CoA dehydratase 1 (*HACD1*)[85]. Recessive mutations in the corresponding canine gene cause a form of CNM in dogs[86,87], although increased numbers of central nuclei are not a feature in humans with *HACD1* mutations. Congenital fibre type disproportion, in which type I fibres are substantially smaller than type II fibres, is another common feature that has been reported in association with mutations in *TPM3*[88,89], *RYR1*[90], *ACTA1*[91], *SEPN1*[92] and *MYH7*[93], with or without additional structural abnormalities.

Congenital myopathies with multiple structural abnormalities. Congenital myopathies with multiple structural abnormalities, which were already recognized in the pre-molecular era[94], have now been largely genetically resolved and are often attributed to previously identified genetic backgrounds. The common occurrence of cores and rods (core–rod myopathy) has been ascribed to mutations in *RYR1*, *ACTA1* and *NEB*, whereas the combination of cores and central nuclei is seen with *RYR1*, *TTN*, *CCDC78*, *DNM2* and *SPEG* mutations.

Novel entities that lack a single predominant histopathological abnormality and, therefore, do not readily fit into the conventional classification are increasingly recognized. *CACNA1S*-related myopathy[95] is characterized by marked neonatal hypotonia, generalized weakness with pronounced axial involvement, and variable extraocular, bulbar and respiratory features. This condition is caused by recessive and dominant mutations in *CACNA1S*, which encodes voltage-dependent L-type Ca^{2+} channel subunit-α1S (Cav1.1), the pore-forming subunit of the voltage sensing L-type Ca^{2+} channel dihydropyridine receptor (DHPR) in skeletal muscle. Allelic *DHPR* mutations were previously associated with dominantly inherited forms of periodic paralysis (and, in rare cases, MHS phenotypes)[96,97]. Characteristic histopathological features of *CACNA1S*-related myopathy include sarcoplasmic reticulum (SR) dilatation, increased numbers of internal nuclei, and myofibrillar disorganization resembling minicores.

Recessively inherited *PYROXD1*-related congenital myopathy[98] is an early-onset myopathy of moderate severity characterized by slowly progressive generalized

Table 1 | **Genes implicated in congenital myopathies and related conditions**

Gene symbol	Chromosomal location	Protein	Condition	Inheritance
Sarcoplasmic reticulum Ca^{2+} release, excitation–contraction coupling and/or triadic assembly				
RYR1[a]	19q13.1	Ryanodine receptor 1 (skeletal)	CCD[a]	AD, AR
			MmD[a]	AD, AR
			CNM	AR
			CFTD	AR
			KDS	AR, AD
STAC3	12q13.3	SH3 and cysteine-rich domain-containing protein 3	NAM	AR
ORAI1	12q24.31	Ca^{2+}-release-activated Ca^{2+} channel protein 1	TAM	AD
STIM1	11p15.4	Stromal interaction molecule 1	TAM	AD
			Stormorken syndrome	AD
MTM1[a]	Xq28	Myotubularin	XLMTM	X-linked
BIN1[a]	2q14	Amphiphysin II	CNM[a]	AR, AD
DNM2[a]	19p13.2	Dynamin 2	CNM	AD
SPEG	2q35	Striated muscle preferentially expressed protein kinase	Congenital myopathy with central nuclei and cardiomyopathy	AR
CCDC78	16p13.3	Coiled-coil domain-containing protein 78	Congenital myopathy with cores and central nuclei	AD
CACNA1S	1q32	Voltage-dependent L-type Ca^{2+} channel subunit-α1S	Congenital myopathy with EOM	AD, AR
SEPN1[a]	1p36.13	Selenoprotein N	MmD[a]	AR
			CFTD	AR
Thin–thick filament assembly and/or interaction, myofibrillar force generation and protein turnover				
NEB[a]	2q22	Nebulin	Nemaline myopathy[a]	AR
ACTA1[a]	1q42.1	Actin, α-skeletal muscle	Nemaline myopathy[a]	AD, AR
			CFTD	AD, AR
			Cap myopathy	AD, AR
TNNT1	19q13.4	Troponin T, slow skeletal muscle	Nemaline myopathy	AR
TPM2[a]	9p13	Tropomyosin β-chain	Nemaline myopathy[a]	AD
			Cap myopathy	AD
			DA1A	AD
			DA2B	AD
			Escobar syndrome	AR
TPM3[a]	1q21.2	Tropomyosin α3-chain	Nemaline myopathy[a]	AD
			CFTD	AD
			Cap myopathy	AD
MYH2	17p13.1	Myosin 2	Congenital myopathy with EOM	AD, AR
MYH3	17p13.1	Myosin 3	DA2A, DA2B and DA8	AD
MYH7	14q12	Myosin 7	CFTD	AD
			MmD	AR
			MSM	AR
MYH8	17p13.1	Myosin 8	Trismus–pseudocamptodactyly syndrome	AD
			Carney complex	AD
KBTBD13	15q22.31	Kelch repeat and BTB domain-containing protein 13	Nemaline myopathy with cores	AD

Table 1 (cont.) | **Genes implicated in congenital myopathies and related conditions**

Gene symbol	Chromosomal location	Protein	Condition	Inheritance
Thin–thick filament assembly and/or interaction, myofibrillar force generation and protein turnover (cont.)				
KLHL40[a]	2p22.1	Kelch-like protein 40	Nemaline myopathy[a]	AR
KLHL41	2q31.1	Kelch-like protein 41	Nemaline myopathy	AR
LMOD3	3p14.1	Leiomodin 3	Nemaline myopathy	AR
MYBPC3	11p11.2	Myosin binding protein C, cardiac-type	Congenital myopathy with cardiomyopathy	AR
MYPN	10q21.3	Myopalladin	Nemaline myopathy with cardiomyopathy	AR
TTN[a]	2q31	Titin	CNM[a]	AR
			MmD	AR
Other cellular processes or unknown protein functions				
CFL2	14q12	Cofilin 2	Nemaline myopathy with cores	AR
CNTN1	12q11–q12	Contactin 1	Congenital myopathy lethal	AR
ECEL1	2q37.1	Endothelin-converting enzyme-like 1	DA5	AR
PIEZO2	18p11.21–p22	Piezo-type mechanosensitive ion channel component 2	Marden–Walker syndrome	AD
			DA3	AD
			DA5	AD
			DA with impaired proprioception	AR
MEGF10	5q23.2	Multiple epidermal growth factor-like domains protein 10	Congenital myopathy with minicores	AR
			Congenital myopathy with areflexia, respiratory distress and dysphagia	AR
HACD1	10p12.33	Very-long-chain (3R)-3-hydroxyacyl-CoA dehydratase 1	Congenital myopathy (nonspecific)	AR
SCN4A	17q23.3	Sodium channel, protein type 4 subunit-α	Congenital myopathy (nonspecific)	AR
TRIM32	9q33.2	E3 ubiquitin–protein ligase TRIM32	Sarcotubular myopathy	AR
PYROXD1	12p12.1	Pyridine nucleotide-disulfide oxidoreductase domain-containing protein 1	Congenital myopathy (nonspecific)	AR

AD, autosomal dominant; AR, autosomal recessive; CCD, central core disease; CFTD, congenital fibre type disproportion; CNM, centronuclear myopathy; DA, distal arthrogryposis; EOM, extraocular muscle involvement; KDS, King–Denborough syndrome; MmD, multi-minicore disease; MSM, myosin storage myopathy; NAM, North American myopathy; TAM, tubular aggregate myopathy; XLMTM, X-linked myotubular myopathy. [a]Genes most commonly implicated in the classic structural congenital myopathies, and the corresponding conditions.

weakness, facial and bulbar involvement, and increased internalized nuclei and myofibrillar disorganization in muscle biopsy samples.

Hereditary myosin myopathies (myosinopathies[99]) comprise distinct distal arthrogryposis syndromes caused by dominant mutations in *MYH3* and *MYH8* (which encode two developmental myosin heavy chain isoforms), as well as congenital myopathies of variable onset and severity caused by dominant and recessive mutations in *MYH2* and *MYH7*. *MYH7* mutations are also implicated in Laing distal myopathy and myosin storage myopathy. In addition to the variable presence of cores in muscle biopsy samples, recessive *MYH2*-related myopathies[100–102] are characterized by marked reduction or absence of type IIA fibres[99,103], whereas accumulation of slow myosin (so-called 'hyaline bodies') can be seen in some *MYH7*-related cases. Other features that may be seen in *MYH7*-related and *MYH2*-related myopathies include increased connective tissue, internal nuclei, rimmed vacuoles, and ring and lobulated fibres[20,93,99,103]. In the context of overlapping histopathological features, the presence of extraocular muscle involvement might cause diagnostic confusion with recessive *RYR1*-related MmD.

Two other conditions combining ocular involvement, contractures within the distal arthrogryposis spectrum and features of a congenital myopathy are recessively inherited *ECEL1*-related congenital myopathy[104–108] and dominantly inherited *PIEZO2*-related congenital myopathy[109] (also classified as distal

arthrogryposis type 5), both of which are associated with cores and increased internal nuclei in muscle biopsy samples.

A recessive congenital myopathy due to homozygous or compound heterozygous mutations in *SCN4A*, a gene previously associated with dominantly inherited myotonia and periodic paralysis, was recently described[110]. This condition has a wide clinical spectrum, from severe in utero (often early lethal) presentations to neonatal-onset conditions of variable severity. The phenotype is mainly characterized by hypotonia, facial and neck weakness, respiratory and swallowing difficulties and early-onset spinal deformities, but interestingly, it is not associated with clinical or electrophysiological evidence of myotonia. Mutations in the same gene are associated with a presentation featuring severe neonatal laryngospasm[111]. Histopathologically, *SCN4A*-related congenital myopathy is characterized by a combination of increased fibre size heterogeneity and variable increases in fatty tissue and tends to lack more-distinct structural abnormalities[110]. In fact, many of the genetic backgrounds implicated in congenital myopathies — in particular, mutations in *RYR1*, *TTN* and *DNM2* — are associated with marked increases in fat and connective tissue, mimicking a congenital muscular dystrophy[112,113].

Congenital myopathies with unusual or other features. Some of the genes that are associated with nemaline myopathy — namely, *TPM2, TPM3, ACTA1, NEB* and *MYPN* — have also been implicated in rare myopathies with unusual histopathological features, such as cap myopathy and zebra body myopathy[73,114–116].

STIM1-related and *ORAI1*-related congenital myopathies[117] caused by dominant gain-of-function mutations result in either tubular aggregate myopathy — a slowly progressive myopathy with varying degrees of extraocular muscle involvement, exertional myalgia and variable calf hypertrophy — or York platelet and Stormorken syndromes, related disorders that form a clinical continuum and are characterized by congenital myopathy, pupillary and platelet abnormalities and variable multisystem involvement. Recessive inheritance of loss-of-function mutations in *ORAI1* and *STIM1* leads to various combinations of severe combined immunodeficiency,

Table 2 | **Features associated with different genetic backgrounds in congenital myopathies**

Feature	*RYR1* autosomal dominant	*RYR1* autosomal recessive	*SEPN1*	*TTN*	*MTM1*	*DNM2*	*NEB*	*ACTA1*	*KLHL40*
Epidemiology									
Frequency of mutations	+++	+++	++	++	++	+	++	++	+
Onset									
Infancy	++	+++	+	+++	+++	+	+++	++	+++
Childhood	+++	++	+++	+	+	+	+	++	+
Adulthood	++	+	–	–	–	+++	–	–	–
Clinical features									
Extraocular muscle involvement	+	+++	–	–	+++	+++	–	–	++
Bulbar involvement	+	+++	++	++	+++	++	++	++	+++
Distal involvement	–	+	–	++	+	+++	++	+	+
Respiratory involvement	+	++	+++	++	+++	+	++	++	+++
Cardiac involvement	–	+	+[a]	+++[b]	–	–	–	+	–
Contractures	+	+	+	+++	+++	++	++	++	+++
Histopathology									
Cores	+++	+++	+++	++	–	+	+	+	–
Central nuclei	++	++	–	+++	+++	+++	–	–	–
Nemaline rods	+	+	–	+	–	–	+++	+++	+++
Fibre type disproportion	+	+++	+	+	+	–	–	+	–
Connective tissue and/or fat infiltration	++	++	++	+++	–	+	–	–	–
Imaging									
Muscle MRI (specificity for genetic defect)	+++	++	++	–	+	+++	+++	+	–

Key: –, not reported; +, infrequent; ++, common; +++, very common. [a]Right ventricular impairment secondary to respiratory involvement. [b]Includes both congenital cardiac defects and acquired cardiomyopathies.

ectodermal dysplasia and congenital myopathy, a combination that was reported in the pre-molecular era in association with minicores in muscle biopsy samples[118].

'Triadin knockout syndrome', which is caused by compound heterozygosity for *TRDN* null mutations, is a recessive cardiac arrhythmia syndrome with various clinical and histopathological features of a congenital myopathy, the latter features being characterized by focal dilatation and degeneration of the lateral SR cisternae[119,120]. The reason for the highly variable penetrance of the myopathy associated with *TRDN* mutations remains unknown.

Mutations in *TRIM32* are associated with limb-girdle muscular dystrophy type 2H and sarcotubular myopathy, and mutations in *TRIM54* and *TRIM63* are associated with microtubular abnormalities and myosin-containing inclusions[121]. These observations illustrate the increasingly fluid boundaries between congenital myopathies and other neuromuscular disorders, in particular, myofibrillar, protein aggregate and vacuolar myopathies.

Pathogenesis

The vast majority of the proteins implicated in congenital myopathies have been associated with primary or secondary defects of muscle ECC, intracellular Ca^{2+} homeostasis and disturbed sarcomeric assembly and function (FIG. 2), although other mechanisms are emerging.

ECC, muscle contraction and relaxation

ECC is the process whereby an electrical signal generated by a neuronal action potential is converted into a chemical gradient — that is, an increase in myoplasmic Ca^{2+} — leading to muscle contraction. The two main players in skeletal muscle ECC are RYR1 and DHPR (FIG. 2). RYR1 is located on the SR junctional face membrane, and DHPR is located on the plasmalemma and transverse tubules (T-tubules) — plasmalemmal invaginations that run deep into the muscle fibre. ECC is extremely rapid, occurring within a few milliseconds, and relies on a highly defined subcellular architecture, with each DHPR positioned opposite an RYR1, and every other RYR1 tetramer facing four DHPRs arranged in a characteristic checkerboard shape called a tetrad.

In addition to its principal regulation through direct interaction with DHPR, RYR1 is regulated by Ca^{2+} and Mg^{2+} and is subjected to post-translational modifications such as phosphorylation, sumoylation and nitrosylation, which affect the channel open probability. The junctional SR membrane contains RYR1 as well as many other smaller proteins, including the structural proteins triadin and junctin (also known as aspartyl/asparaginyl β-hydroxylase), junctional SR protein 1 (JP45), the high-capacity, low-affinity Ca^{2+} binding protein calsequestrin[122,123] (in an area adjacent to RYR1), and other proteins with roles in the fine regulation of SR Ca^{2+} release or in maintaining the structural integrity of the Ca^{2+} release machinery[122,124–131].

Following its release from the SR, Ca^{2+} binds to troponin C and interacts directly with thin filaments. As a consequence, muscle contraction occurs in the sarcomere, a structure that is principally composed of parallel thick and thin filaments. Sarcomeric regulation of contraction involves structural changes in the thin filament complex (composed of actin, tropomyosin and troponin), triggered by Ca^{2+} binding to troponin. The simplest model for the regulation of the sarcomere by Ca^{2+} is based on steric blocking, whereby tropomyosin prevents myosin from binding to the actin filament to generate force. Binding of Ca^{2+} to troponin triggers a chain reaction that results in azimuthal movements of tropomyosin around the filament to unmask binding sites on actin for myosin — the molecular motor and also the main component of thick filaments — allowing force production and motion[132]. These contractile proteins and related isoforms are differently expressed in slow-twitch and fast-twitch muscles to fulfil different functional demands[132].

Termination of the contraction cycle and muscle relaxation is achieved by RYR1 closure and activation of sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA), the protein component that is responsible for pumping the Ca^{2+} back into the SR[133]. SERCA activity is modulated by two small regulatory proteins, sarcolipin and phospholamaban[134–136].

Although skeletal muscle ECC can occur in the presence of extracellular Ca^{2+} in the nanomolar range, a wide consensus exists that Ca^{2+} entry from the extracellular space is essential to ensure prolonged muscle activity. Two main mechanisms of Ca^{2+} entry have been identified in skeletal muscle: excitation-coupled Ca^{2+} entry via DHPR, which is activated by a train of action potentials or prolonged membrane depolarization; and store-operated Ca^{2+} entry via stromal interaction molecule 1 (encoded by *STIM1*) and Ca^{2+}-release-activated Ca^{2+} channel protein 1 (ORAI1), which is triggered by depletion of endoplasmic reticulum and SR Ca^{2+} stores[137–141].

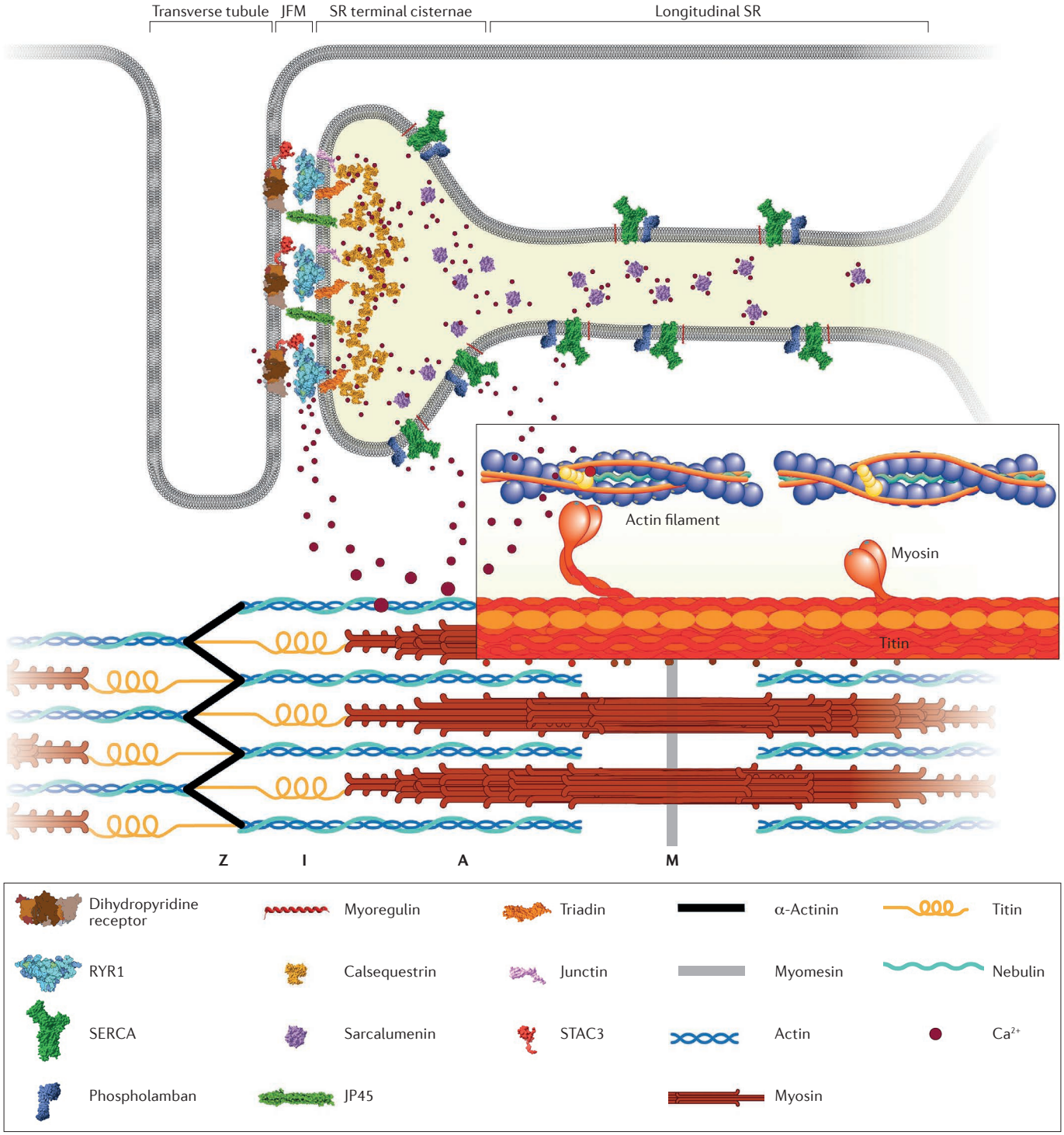
ECC and Ca^{2+} homeostasis abnormalities

Mutations in *RYR1* are the most common cause of primary defects of ECC and Ca^{2+} homeostasis[7,18,142,143]. Functional studies utilizing cellular and animal models[144,145] indicate that excessive Ca^{2+} release and lower RYR1 activation thresholds are consequences of dominantly inherited MHS-associated *RYR1* mutations, whereas both SR Ca^{2+} store depletion with a resulting increase in cytosolic Ca^{2+} levels (the 'leaky channel' hypothesis) and disturbed ECC (the 'excitation–contraction uncoupling' hypothesis) have been proposed as mechanisms underlying dominantly inherited CCD[142]. On the basis of the limited studies performed so far, quantitative reduction of RYR1 channels is a more likely mechanism than qualitative RYR1 dysfunction in recessive *RYR1*-related myopathies[146–148]. Primary or secondary reductions in Cav1.1 protein levels are seen in recessive *RYR1*-related and *CACNA1S*-related congenital myopathies[95,146]; the latter conditions also feature disturbed ECC and, consequently, reduced depolarization-induced SR Ca^{2+} release in myotubes and mature muscle fibres. *STAC3*, the gene that is homozygously mutated in NAM, encodes a protein that targets Cav1.1 to the T-tubules and, thus, participates in voltage-induced Ca^{2+} release[149,150]. Disturbances in voltage-induced Ca^{2+} release are likely

to be involved in the recently described triadin knockout syndrome[119], although the basis for the highly variable penetrance of skeletal muscle features in this condition is currently uncertain.

Dominant mutations in *STIM1* and *ORAI1* are associated with distinct alterations in store-operated Ca^{2+} influx, resulting in increased resting Ca^{2+} levels due to mediation of Ca^{2+} influx by constitutively active molecules independently of SR Ca^{2+} levels[140,151]. By contrast, recessive *ORAI1* mutations, which lead to reduced ORAI1 expression, result in impaired Ca^{2+} influx[152].

Secondary defects of ECC and Ca^{2+} homeostasis, probably due to RYR1 redox modifications, have been demonstrated in *SEPN1*-mutated myotubes and in the *Sepn1*-knockout mouse model[153,154]. Many of the genes implicated in CNM, including *MTM1*[40], *DNM2*[41] and



BIN1[44], encode proteins that have an important role in intricately linked intracellular membrane trafficking pathways. Mutations in these genes might indirectly affect muscle Ca^{2+} handling and ECC, probably secondary to abnormalities of triad assembly and the ECC machinery[155]. Although such abnormalities have been demonstrated in mouse models of both *DNM2*-related and *MTM1*-related myopathies[156], a recent study on *MTM1*-mutated human myoblasts failed to demonstrate any alterations in ECC and Ca^{2+} release, indicating that these alterations may reflect long-term effects in vivo[157].

The pathogenicity of *TTN* mutations is probably multifactorial and is likely to involve several mechanisms implicated in ECC, including calpain 3-mediated RYR1 recruitment to the triad and obscurin-mediated interactions between the T-tubules, the SR and the sarcomere.

Sarcomeric abnormalities

The majority of the genes implicated in nemaline myopathy to date, including *NEB*[59], *ACTA1*[61], *TPM2*[63], *TPM3*[62] and *TNNT1*[68], are involved in thin filament assembly and interactions. Pathogenic mutations in the two most commonly mutated genes, *NEB* and *ACTA1*, have been extensively studied[158]. Dominant *ACTA1* mutations exert a dominant-negative effect on muscle function that is mediated through lowered Ca^{2+} sensitivity, whereas recessive *ACTA1* mutations abolish functional protein expression, with the phenotype severity probably depending on the expression of compensatory proteins such as actin, α-cardiac muscle 1 (ACTC1)[159,160]. In rare cases, *ACTA1* mutations result in increased muscle contractility[161,162].

NEB mutations affect the specific function of nebulin in thin filament regulation and force generation[163]. The effects of various nemaline myopathy-associated mutations on the interactions of nebulin with actin and tropomyosin, thin filament length and force generation were demonstrated in two in vitro studies[164,165]. *MYO18B*, which was found to be mutated in one family with a severe form of nemaline myopathy[75], encodes an unconventional myosin protein with a more general role in sarcomeric assembly and maintenance[166,167].

KBTBD13, *KLHL40*, *KLHL41* and *LMOD3*, which have recently been implicated in nemaline myopathy, encode a group of Kelch and Kelch-like proteins that are not primary thin filament components but are involved in muscle quality control processes[168] and may, thus, affect myofibrillar assembly and function indirectly. Evidence for a direct interaction between Kelch-like protein 40 (KLHL40), nebulin and leiomodin 3 has been provided[169].

The myosinopathies[99] — disorders of the thick filaments — are likely to cause muscle disease through two principal mechanisms: disturbed thick filament interaction and function and, particularly in *MYH7*-related congenital myopathies[99], aggregation of abnormal protein.

Other pathogenic mechanisms

Some of the proteins implicated in congenital myopathies are specifically involved in ECC and Ca^{2+} homeostasis, whereas others are thought to have additional roles in and beyond muscle. Selenoprotein N (encoded by *SEPN1*) belongs to a family of proteins that contain selenium in the form of the 21st amino acid, selenocysteine. In muscle, this protein has been specifically implicated in myogenesis — a role that it shares with the protein encoded by *MEGF10*, which is mutated in a rare form of MmD[23] — and redox regulation[170,171]. The important role of normally functioning redox regulation for muscle health is also illustrated by the identification of recessive mutations in the oxidoreductase-encoding gene *PYROXD1* as a cause of early-onset congenital myopathies[98].

Reflecting their essential roles in intricately linked intracellular membrane trafficking pathways, mutations in the CNM-associated genes *MTM1*, *DNM2* and *BIN1* have been associated with a wide range of downstream effects, including defects in mitochondria, the intermediate filament protein desmin, satellite cell activation and the neuromuscular junction[155]. Abnormalities of muscle membrane systems have also been described in association with canine *HACD1*-related CNM[86,87], a naturally occurring animal model of a nonspecific congenital myopathy that has also been described in humans[172].

The CNM-associated genes *MTM1* and *DNM2* have also been implicated in pathways that affect muscle protein turnover and/or muscle growth and atrophy pathways. In zebrafish and mouse models of myotubularin deficiency, disturbances of the autophagy pathway, associated with F-box only protein 32 upregulation and atrophy, have been reported[173–175]. Abnormalities of autophagosome maturation and autophagic flux have also been described in a mouse model of *DNM2*-related CNM in association with marked muscle atrophy and weakness[176]. Several mechanisms might affect

◀ Fig. 2 | **Proteins involved in congenital myopathies.** The figure shows the subcellular localization of the main proteins implicated in skeletal muscle excitation–contraction coupling (ECC) and thin–thick filament interaction and assembly. Genes encoding components of the ECC machinery and the thin and thick filaments of skeletal muscle are commonly mutated in congenital myopathies. The transverse tubules are invaginations of the plasma membrane where the dihydropyridine receptor complex (containing SH3 and cysteine-rich domain-containing protein 3 (STAC3)) is located. This membrane compartment faces the sarcoplasmic reticulum (SR) junctional face membrane (JFM), which contains ryanodine receptor 1 (RYR1) as well as junctional SR protein 1 (JP45) and the structural proteins triadin and aspartyl/asparaginyl β-hydroxylase (junctin). Calsequestrin bound to Ca^{2+} forms a mesh-like structure within the lumen of the SR terminal cisternae. JP45 also interacts with calsequestrin via its lumenal carboxy-terminal domain. Ca^{2+} release into the cytosol results in sarcomeric shortening through specific interactions between thin and thick filaments, in particular, the sliding of actin past myosin filaments. ECC is terminated through SR Ca^{2+} reuptake through sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA) Ca^{2+} pumps. SERCAs are present in the terminal cisternae as well as the longitudinal SR and are regulated by phospholamban, myoregulin and sarcolipin. The Ca^{2+}-buffering protein sarcalumenin is also located in the longitudinal SR and terminal cisternae and is involved in regulating SERCA activity. A, A-band; I, I-band; M, M-band; Z, Z-line. Image courtesy of Christoph Bachmann, Departments of Anesthesia and Biomedicine, Basel University Hospital, Basel, Switzerland. 3D representations from RSCB PDB: calsequestrin, PDB ID 2VAF (REF. 227); dihydropyridine receptor, PDB ID 4MS2 (REF. 228); phospholamban, PDB ID 1N7L (REF. 229); RYR1, PDB ID 4UWA (REF. 230); SERCA, PDB ID 1SU4 (REF. 231); STAC3, PDB ID 2DB6 (http://www.rcsb.org/pdb/explore.do?structureId=2db6).

autophagy and other degradation pathways in *TTN*-related CNM. These mechanisms include abrogation of calpain 3-mediated protein turnover (in the case of C-terminal-truncating *TTN* mutations), perturbation of the link between titin and the ubiquitin ligase myospryn[177], and perturbation of the link between the titin kinase domain and the autophagy cargo adaptors NBR1 (next to BRCA1 gene 1 protein NBR1) and sequestosome 1 (SQSTM1) by M-band-disrupting *TTN* mutations[25]. Intriguingly, the typical histopathological features of CNM have also been reported in primary disorders of autophagy[178,179], further supporting a close link between defective autophagy and abnormal nuclear positioning.

A novel epigenetic mechanism involving alterations of muscle-specific microRNAs, increased DNA methylation and increased expression of class II histone deacetylases has been reported in *RYR1*-related myopathies[180] and might also be relevant for other congenital myopathies[157].

The mechanisms through which mutations in *ECEL1*, *PIEZO2* and *SCN4A* cause specific early-onset congenital myopathies are currently uncertain.

Diagnostic approach

The International Standard of Care Committee for Congenital Myopathies has provided a structured diagnostic approach to the congenital myopathies[181]. Many features, including axially pronounced weakness and hypotonia, are consistent but nonspecific on clinical assessment, whereas others — in particular, the degree of distal, extraocular muscle, cardiac and respiratory involvement — can indicate specific genetic backgrounds.

Useful laboratory investigations include measurement of serum creatine kinase levels, which are typically normal or slightly elevated, and assays for acetylcholine receptor antibodies to exclude autoimmune myasthenic conditions[182]. Neurophysiological studies, such as electromyography and nerve conduction studies, are useful mainly for excluding congenital neuropathies, myotonic disorders[111] and congenital myasthenic syndromes[183]. Muscle imaging[6], in particular, muscle ultrasonography as a screening test and muscle MRI for a more detailed assessment, can reveal diagnostic patterns of selective muscle involvement. Assessment of muscle biopsy samples with a standard panel of histological, histochemical and immunohistochemical stains[13] will confirm the specific congenital myopathy and exclude distinct conditions with overlapping pathological features, such as the congenital muscular dystrophies[184], myofibrillar myopathies[185] and autophagic vacuolar myopathies[186]. Electron microscopy helps to clarify the pathognomonic structural abnormalities that are seen with light microscopy.

Concomitant analysis of multiple congenital myopathy-associated genes through NGS is rapidly becoming the preferred diagnostic approach. Functional studies will be increasingly relevant for pathogenicity assessment of variants in large genes such as *TTN*, *NEB* and *RYR1*, as variants of uncertain significance are not uncommon in these genes, even in healthy control populations.

Management and therapy development

Supportive management

Supportive management (outlined in detail elsewhere[187]) is based on a multidisciplinary approach. Regular physiotherapy and provision of orthotic support is beneficial to prevent contracture development and to maintain mobility. Patients with dysarthria and/or feeding difficulties will benefit from regular speech and language therapy. In some cases, bulbar involvement and poor weight gain may necessitate gastrostomy. Regular monitoring of respiratory function (including sleep studies) and proactive respiratory management (including timely noninvasive ventilation and cough assistance techniques) are essential, particularly in conditions where substantial respiratory involvement — often disproportionate to the degree of limb-girdle weakness — is recognized. Regular cardiac monitoring is crucial for patients with congenital myopathies that are consistently associated with cardiomyopathy (in particular, the *TTN*-related and *MYH7*-related forms) and also for individuals in whom the genetic defect is uncertain. Given the often-complex comorbidities associated with congenital myopathies, orthopaedic surgery, most notably to treat scoliosis, should be undertaken at a tertiary neuromuscular centre. MHS must be anticipated in the anaesthetic management of patients with *RYR1* or *STAC3* mutations and in those with unresolved genetic backgrounds.

Mechanism-based therapies

Mechanism-based therapies for the congenital myopathies that are already available or currently in development have been reviewed in detail elsewhere[188].

Genetic therapies. Owing to their enormous size, most of the genes commonly implicated in congenital myopathies are not amenable to virus-based gene transfer approaches. However, delivery of *MTM1* via an adeno-associated virus 8-based vector has been demonstrated to improve the clinicopathological phenotype in *Mtm1*-deficient mice and a canine model of XLMTM[172,189].

Restoration of the mRNA reading frame is theoretically applicable to various congenital myopathies in which nonsense mutations are implicated. Exon skipping has been successfully applied in vitro to remove a pseudo-exon from the mRNA of a child with a recessive *RYR1*-related myopathy[190]. Considering that carriers of truncating *RYR1* mutations are asymptomatic[190,191], selective silencing of the mutant gene could be a feasible therapeutic strategy for dominant *RYR1*-related myopathies in the future. Pharmacological suppression of stop codons[192] by compounds such as ataluren is a potential approach in congenital myopathies that involve nonsense mutations, although it is currently uncertain whether such an approach will increase normal protein levels sufficiently to restore structural integrity and function, and the effects on the many loss-of-function variants in the human genome have yet to be determined[193].

Downregulation or upregulation of genes that act in convergent pathways could be a relevant approach for various forms of CNM. Studies have demonstrated

that downregulation of dynamin 2[194] or targeting of class II and III phosphoinositide 3-kinases in muscle[195] can rescue the phenotype in XLMTM animal models, suggesting that pharmacological modification of intricately linked pathways is a potential treatment modality for XLMTM and, possibly, other forms of CNM. Upregulation of cardiac actin might be a feasible therapeutic approach for patients with *ACTA1* null mutations[196,197].

Enzyme replacement therapy. Enzyme replacement therapy is currently relevant only to XLMTM caused by loss of myotubularin function. In *Mtm1*-knockout mice, improvements in contractile function and histopathological features were observed following short-term myotubularin enzyme replacement[198].

Pharmacological therapies. Pharmacological therapies that are potentially applicable to congenital myopathies can be grossly divided into three principal approaches: direct modification of altered protein function (for example, modification of RYR1 release in *RYR1*-related myopathies); enhancement of thin–thick filament interactions (for example, in some nemaline myopathies); and therapies aimed at nonspecifically ameliorating downstream effects of the specific gene mutation.

Modification of RYR1 Ca^{2+} release by use of the specific RYR1 antagonist dantrolene[199] is the established emergency treatment for malignant hyperthermia and has also been used effectively in a few patients with *RYR1*-related ERM[35,200] and CCD[201,202]. Other compounds with the potential to treat excessive SR Ca^{2+} release and/or increased SR Ca^{2+} leakage are the calstabin-stabilizing 1,4-benzothiazepine derivatives JTV519 and S107[203,204] and the AMP-activated protein kinase activator 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR)[205,206]. However, the safety profiles of these compounds in humans and their roles in *RYR1*-related myopathies associated with reduced rather than increased Ca^{2+} conductance are currently uncertain.

Enhancement of filament interactions and promotion of force production[207,208], either by slowing the rate of Ca^{2+} release from troponin C or by directly targeting myosin molecules, are potentially valuable approaches for some nemaline myopathies. However, concerns remain regarding fibre type specificity and potential cardiac adverse effects of the molecules that are being utilized.

Modification of the downstream effects of specific gene mutations encompasses various approaches. Inhibition of myostatin, an important negative regulator of muscle fibre size[209], might be applicable to congenital myopathies in which fibre atrophy is prominent. Following observations of increased oxidative stress and a favourable response to these compounds in animal models[154,210,211], antioxidants such as *N*-acetylcysteine are currently being investigated in clinical trials concerning *RYR1*-related and *SEPN1*-related myopathies. In light of the neuromuscular junction and transmission abnormalities in CNM, *RYR1*-related MmD and *KLHL40*-related nemaline myopathy[212–215], acetylcholinesterase inhibitors have been used with some benefit in a small number of patients. Two other compounds, salbutamol for core myopathies[216–218] and — supported by preclinical data from a relevant animal model[219] — L-tyrosine in nemaline myopathy[220], have shown apparent benefits in open-label pilot studies.

For those disease entities where misfolded proteins or domains have unequivocal primary roles in the disease process (for example, titin in autosomal recessive MmD with heart disease), compounds that act as chemical chaperones show promise. A pharmacochaperone approach, using the small amphipathic compound 4-phenylbutyrate, was shown to alleviate some of the pathological features in a mouse model of *PLEC*-associated epidermolysis bullosa simplex with muscular dystrophy[221], although it is uncertain whether the observed effect was attributable to stabilization of misfolded mutant protein or its clearance through induction of autophagy by the drug[222,223]. A beneficial effect of 4-phenylbutyrate has also been suggested in a mouse model of *RYR1*-related myopathy[224]. The range of chemical chaperones is increasing rapidly[225], but the half-maximal inhibitory concentration — a measure of the ability of a compound to inhibit a specific function — is often low[226], and the development of more target-specific compounds might make this approach more effective and applicable.

Conclusions and outlook

Widespread clinical implementation of NGS has rapidly expanded the genetic and clinicopathological spectrum of the congenital myopathies. In addition to the classic entities CCD, MmD, CNM and nemaline myopathy, the congenital myopathies now encompass a wide range of early-onset, non-dystrophic neuromuscular disorders with various combinations of structural defects. Congenital myopathies due to mutations in *RYR1*, the most common genetic cause, form a continuum with intermittent induced myopathies, such as malignant hyperthermia and exertional rhabdomyolysis, in otherwise healthy individuals. Other forms of congenital myopathy overlap substantially with the distal arthrogryposis and protein aggregation myopathy spectrum, particularly in cases where sarcomeric proteins are implicated.

The unravelling of the underlying molecular mechanisms has advanced not only our understanding of the congenital myopathies but also our knowledge of normal muscle physiology and homeostasis. Although the primary genetic defects and principal pathogenic mechanisms have largely been elucidated, downstream effects on muscle growth and atrophy pathways, the role of genetic and other modifiers, and the molecular basis of the common histopathological features remain uncertain.

Specific therapies for congenital myopathies, utilizing genetic, enzyme replacement and pharmacological approaches, are currently being developed or are already reaching the clinical trial stage, emphasizing the need for comprehensive natural history studies concerning these clinically variable conditions.

1. Magee, K. R. & Shy, G. M. A new congenital non-progressive myopathy. *Brain* **79**, 610–621 (1956).
2. Engel, A. G., Gomez, M. R. & Groover, R. V. Multicore disease. A recently recognized congenital myopathy associated with multifocal degeneration of muscle fibers. *Mayo Clin. Proc.* **46**, 666–681 (1971).
3. Spiro, A. J., Shy, G. M. & Gonatas, N. K. Myotubular myopathy. Persistence of fetal muscle in an adolescent boy. *Arch. Neurol.* **14**, 1–14 (1966).
4. Shy, G. M., Engel, W. K., Somers, J. E. & Wanko, T. Nemaline myopathy. a new congenital myopathy. *Brain* **86**, 793–810 (1963).
5. Lopez, R. J. et al. An *RYR1* mutation associated with malignant hyperthermia is also associated with bleeding abnormalities. *Sci. Signal.* **9**, ra68 (2016).
6. Jungbluth, H. Myopathology in times of modern imaging. *Neuropathol. Appl. Neurobiol.* **43**, 24–43 (2017).
7. Snoeck, M. et al. *RYR1*-related myopathies: a wide spectrum of phenotypes throughout life. *Eur. J. Neurol.* **22**, 1094–1112 (2015).
8. Jungbluth, H. & Voermans, N. C. Congenital myopathies: not only a paediatric topic. *Curr. Opin. Neurol.* **29**, 642–650 (2016).
9. Quane, K. A. et al. Mutations in the ryanodine receptor gene in central core disease and malignant hyperthermia. *Nat. Genet.* **5**, 51–55 (1993).
10. Fujii, J. et al. Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. *Science* **253**, 448–451 (1991).
11. Biancalana, V. & Laporte, J. Diagnostic use of massively parallel sequencing in neuromuscular diseases: towards an integrated diagnosis. *J. Neuromuscul. Dis.* **2**, 193–203 (2015).
12. Jungbluth, H., Sewry, C. A. & Muntoni, F. Core myopathies. *Semin. Pediatr. Neurol.* **18**, 239–249 (2011).
13. Dubowitz, V., Sewry, C. A. & Oldfors, A. *Muscle Biopsy: a Practical Approach* 4th edn (Saunders, 2013).
14. Amburgey, K. et al. Prevalence of congenital myopathies in a representative pediatric United States population. *Ann. Neurol.* **70**, 662–665 (2011).
15. Hackman, P., Udd, B., Bonnemann, C. G., Ferreiro, A. & Titinopathy Database Consortium. 219th ENMC International Workshop Titinopathies International database of titin mutations and phenotypes, Heemskerk, The Netherlands, 29 April – 1 May 2016. *Neuromuscul. Disord.* **27**, 396–407 (2017).
16. Jungbluth, H. et al. Autosomal recessive inheritance of *RYR1* mutations in a congenital myopathy with cores. *Neurology* **59**, 284–287 (2002).
17. Jungbluth, H. et al. Minicore myopathy with ophthalmoplegia caused by mutations in the ryanodine receptor type 1 gene. *Neurology* **65**, 1930–1935 (2005).
18. Klein, A. et al. Clinical and genetic findings in a large cohort of patients with ryanodine receptor 1 gene-associated myopathies. *Hum. Mutat.* **33**, 981–988 (2012).
19. Ferreiro, A. et al. Mutations of the selenoprotein N gene, which is implicated in rigid spine muscular dystrophy, cause the classical phenotype of multiminicore disease: reassessing the nosology of early-onset myopathies. *Am. J. Hum. Genet.* **71**, 739–749 (2002).
20. Cullup, T. et al. Mutations in *MYH7* cause multi-minicore disease (MmD) with variable cardiac involvement. *Neuromuscul. Disord.* **22**, 1096–1104 (2012).
21. Takayama, K. et al. Japanese multiple epidermal growth factor 10 (MEGF10) myopathy with novel mutations: a phenotype–genotype correlation. *Neuromuscul. Disord.* **26**, 604–609 (2016).
22. Liewluck, T. et al. Adult-onset respiratory insufficiency, scoliosis, and distal joint hyperlaxity in patients with multiminicore disease due to novel *Megf10* mutations. *Muscle Nerve* **53**, 984–988 (2016).
23. Logan, C. V. et al. Mutations in *MEGF10*, a regulator of satellite cell myogenesis, cause early onset myopathy, areflexia, respiratory distress and dysphagia (EMARDD). *Nat. Genet.* **43**, 1189–1192 (2011).
24. Boyden, S. E. et al. Mutations in the satellite cell gene *MEGF10* cause a recessive congenital myopathy with minicores. *Neurogenetics* **13**, 115–124 (2012).
25. Chauveau, C. et al. Recessive *TTN* truncating mutations define novel forms of core myopathy with heart disease. *Hum. Mol. Genet.* **23**, 980–991 (2014).
26. Romero, N. B. et al. Dominant and recessive central core disease associated with *RYR1* mutations and fetal akinesia. *Brain* **126**, 2341–2349 (2003).
27. Scoto, M. et al. *SEPN1*-related myopathies: clinical course in a large cohort of patients. *Neurology* **76**, 2073–2078 (2011).
28. Klein, A. et al. Muscle MRI in congenital myopathies due to ryanodine receptor type 1 gene mutations. *Arch. Neurol.* **68**, 1171–1179 (2011).
29. Jungbluth, H. et al. Magnetic resonance imaging of muscle in congenital myopathies associated with *RYR1* mutations. *Neuromuscul. Disord.* **14**, 785–790 (2004).
30. Rosenberg, H., Davis, M., James, D., Pollock, N. & Stowell, K. Malignant hyperthermia. *Orphanet J. Rare Dis.* **2**, 21 (2007).
31. Zhou, H. et al. Characterization of recessive *RYR1* mutations in core myopathies. *Hum. Mol. Genet.* **15**, 2791–2803 (2006).
32. Kraeva, N. et al. Compound *RYR1* heterozygosity resulting in a complex phenotype of malignant hyperthermia susceptibility and a core myopathy. *Neuromuscul. Disord.* **25**, 567–576 (2015).
33. Dowling, J. J. et al. King–Denborough syndrome with and without mutations in the skeletal muscle ryanodine receptor (*RYR1*) gene. *Neuromuscul. Disord.* **21**, 420–427 (2011).
34. Horstick, E. J. et al. Stac3 is a component of the excitation–contraction coupling machinery and mutated in Native American myopathy. *Nat. Commun.* **4**, 1952 (2013).
35. Dlamini, N. et al. Mutations in *RYR1* are a common cause of exertional myalgia and rhabdomyolysis. *Neuromuscul. Disord.* **23**, 540–548 (2013).
36. Bethlem, J., van Gool, J., Hulsmann, W. C. & Meijer, A. E. Familial non-progressive myopathy with muscle cramps after exercise. A new disease associated with cores in the muscle fibres. *Brain* **89**, 569–588 (1966).
37. Løseth, S. et al. A novel late-onset axial myopathy associated with mutations in the skeletal muscle ryanodine receptor (*RYR1*) gene. *J. Neurol.* **260**, 1504–1510 (2013).
38. Jungbluth, H. et al. Late-onset axial myopathy with cores due to a novel heterozygous dominant mutation in the skeletal muscle ryanodine receptor (*RYR1*) gene. *Neuromuscul. Disord.* **19**, 344–347 (2009).
39. Jungbluth, H., Wallgren-Pettersson, C. & Laporte, J. Centronuclear (myotubular) myopathy. *Orphanet J. Rare Dis.* **3**, 26 (2008).
40. Laporte, J. et al. A gene mutated in X-linked myotubular myopathy defines a new putative tyrosine phosphatase family conserved in yeast. *Nat. Genet.* **13**, 175–182 (1996).
41. Bitoun, M. et al. Mutations in dynamin 2 cause dominant centronuclear myopathy. *Nat. Genet.* **37**, 1207–1209 (2005).
42. Böhm, J. et al. Adult-onset autosomal dominant centronuclear myopathy due to *BIN1* mutations. *Brain* **137**, 3160–3170 (2014).
43. Wilmshurst, J. M. et al. *RYR1* mutations are a common cause of congenital myopathies with central nuclei. *Ann. Neurol.* **68**, 717–726 (2010).
44. Nicot, A. S. et al. Mutations in amphiphysin 2 (*BIN1*) disrupt interaction with dynamin 2 and cause autosomal recessive centronuclear myopathy. *Nat. Genet.* **39**, 1134–1139 (2007).
45. Ceyhan-Birsoy, O. et al. Recessive truncating titin gene. *TTN*, mutations presenting as centronuclear myopathy. *Neurology* **81**, 1205–1214 (2013).
46. Agrawal, P. B. et al. SPEG interacts with myotubularin, and its deficiency causes centronuclear myopathy with dilated cardiomyopathy. *Am. J. Hum. Genet.* **95**, 218–226 (2014).
47. Majczenko, K. et al. Dominant mutation of *CCDC78* in a unique congenital myopathy with prominent internal nuclei and atypical cores. *Am. J. Hum. Genet.* **91**, 365–371 (2012).
48. Tosch, V. et al. A novel PtdIns3P and PtdIns(3,5)P2 phosphatase with an inactivating variant in centronuclear myopathy. *Hum. Mol. Genet.* **15**, 3098–3106 (2006).
49. Bevilacqua, J. A. et al. "Necklace" fibers, a new histological marker of late-onset *MTM1*-related centronuclear myopathy. *Acta Neuropathol.* **117**, 283–291 (2009).
50. Liewluck, T., Lovell, T. L., Bite, A. V. & Engel, A. G. Sporadic centronuclear myopathy with muscle pseudohypertrophy, neutropenia, and necklace fibers due to a *DNM2* mutation. *Neuromuscul. Disord.* **20**, 801–804 (2010).
51. Toussaint, A. et al. Defects in amphiphysin 2 (BIN1) and triads in several forms of centronuclear myopathies. *Acta Neuropathol.* **121**, 253–266 (2011).
52. Romero, N. B. Centronuclear myopathies: a widening concept. *Neuromuscul. Disord.* **20**, 223–228 (2010).
53. Herman, G. E., Finegold, M., Zhao, W., de Gouyon, B. & Metzenberg, A. Medical complications in long-term survivors with X-linked myotubular myopathy. *J. Pediatr.* **134**, 206–214 (1999).
54. Bohm, J. et al. Mutation spectrum in the large GTPase dynamin 2, and genotype–phenotype correlation in autosomal dominant centronuclear myopathy. *Hum. Mutat.* **33**, 949–959 (2012).
55. Bitoun, M. et al. Dynamin 2 mutations cause sporadic centronuclear myopathy with neonatal onset. *Ann. Neurol.* **62**, 666–670 (2007).
56. Jungbluth, H. et al. Centronuclear myopathy with cataracts due to a novel dynamin 2 (*DNM2*) mutation. *Neuromuscul. Disord.* **20**, 49–52 (2010).
57. Zuchner, S. et al. Mutations in the pleckstrin homology domain of dynamin 2 cause dominant intermediate Charcot–Marie–Tooth disease. *Nat. Genet.* **37**, 289–294 (2005).
58. Jungbluth, H., Wallgren-Pettersson, C. & Laporte, J. F. 198th ENMC International Workshop: 7th Workshop on Centronuclear (Myotubular) Myopathies, 31st May – 2nd June 2013, Naarden, The Netherlands. *Neuromuscul. Disord.* **23**, 1033–1043 (2013).
59. Pelin, K. et al. Mutations in the nebulin gene associated with autosomal recessive nemaline myopathy. *Proc. Natl Acad. Sci. USA* **96**, 2305–2310 (1999).
60. Pelin, K. et al. Nebulin mutations in autosomal recessive nemaline myopathy: an update. *Neuromuscul. Disord.* **12**, 680–686 (2002).
61. Nowak, K. J. et al. Mutations in the skeletal muscle α-actin gene in patients with actin myopathy and nemaline myopathy. *Nat. Genet.* **23**, 208–212 (1999).
62. Laing, N. G. et al. A mutation in the α tropomyosin gene *TPM3* associated with autosomal dominant nemaline myopathy. *Nat. Genet.* **9**, 75–79 (1995).
63. Donner, K. et al. Mutations in the β-tropomyosin (*TPM2*) gene — a rare cause of nemaline myopathy. *Neuromuscul. Disord.* **12**, 151–158 (2002).
64. Sambuughin, N. et al. Dominant mutations in KBTBD13, a member of the BTB/Kelch family, cause nemaline myopathy with cores. *Am. J. Hum. Genet.* **87**, 842–847 (2010).
65. Laing, N. G. et al. Mutations and polymorphisms of the skeletal muscle α-actin gene (*ACTA1*). *Hum. Mutat.* **30**, 1267–1277 (2009).
66. Lehtokari, V. L. et al. Identification of a founder mutation in *TPM3* in nemaline myopathy patients of Turkish origin. *Eur. J. Hum. Genet.* **16**, 1055–1061 (2008).
67. Monnier, N. et al. Absence of β-tropomyosin is a new cause of Escobar syndrome associated with nemaline myopathy. *Neuromuscul. Disord.* **19**, 118–123 (2009).
68. Johnston, J. J. et al. A novel nemaline myopathy in the Amish caused by a mutation in troponin T1. *Am. J. Hum. Genet.* **67**, 814–821 (2000).
69. Agrawal, P. B. et al. Nemaline myopathy with minicores caused by mutation of the *CFL2* gene encoding the skeletal muscle actin-binding protein, cofilin-2. *Am. J. Hum. Genet.* **80**, 162–167 (2007).
70. Ravenscroft, G. et al. Mutations in *KLHL40* are a frequent cause of severe autosomal-recessive nemaline myopathy. *Am. J. Hum. Genet.* **93**, 6–18 (2013).
71. Gupta, V. A. et al. Identification of *KLHL41* mutations implicates BTB-Kelch-mediated ubiquitination as an alternate pathway to myofibrillar disruption in nemaline myopathy. *Am. J. Hum. Genet.* **93**, 1108–1117 (2013).
72. Yuen, M. et al. Leiomodin-3 dysfunction results in thin filament disorganization and nemaline myopathy. *J. Clin. Invest.* **124**, 4693–4708 (2014).
73. Lornage, X. et al. Recessive *MYPN* mutations cause cap myopathy with occasional nemaline rods. *Ann. Neurol.* **81**, 467–473 (2017).
74. Miyatake, S. et al. Biallelic mutations in *MYPN*, encoding myopalladin, are associated with childhood-onset, slowly progressive nemaline myopathy. *Am. J. Hum. Genet.* **100**, 169–178 (2017).
75. Malfatti, E. et al. A premature stop codon in *MYO18B* is associated with severe nemaline myopathy with cardiomyopathy. *J. Neuromuscul. Dis.* **2**, 219–227 (2015).

76. Domazetovska, A. et al. Intranuclear rod myopathy: molecular pathogenesis and mechanisms of weakness. *Ann. Neurol.* **62**, 597–608 (2007).
77. Ryan, M. M. et al. Nemaline myopathy: a clinical study of 143 cases. *Ann. Neurol.* **50**, 312–320 (2001).
78. Feng, J. J. & Marston, S. Genotype–phenotype correlations in *ACTA1* mutations that cause congenital myopathies. *Neuromuscul. Disord.* **19**, 6–16 (2009).
79. Witting, N., Werlauff, U., Duno, M. & Vissing, J. Prevalence and phenotypes of congenital myopathy due to α-actin 1 gene mutations. *Muscle Nerve* **53**, 388–393 (2016).
80. Jungbluth, H. et al. Mild phenotype of nemaline myopathy with sleep hypoventilation due to a mutation in the skeletal muscle α-actin (*ACTA1*) gene. *Neuromuscul. Disord.* **11**, 35–40 (2001).
81. Sambuughin, N. et al. KBTBD13 interacts with Cullin 3 to form a functional ubiquitin ligase. *Biochem. Biophys. Res. Commun.* **421**, 743–749 (2012).
82. Davidson, A. E. et al. Novel deletion of lysine 7 expands the clinical, histopathological and genetic spectrum of TPM2-related myopathies. *Brain* **136**, 508–521 (2013).
83. Jungbluth, H. et al. Magnetic resonance imaging of muscle in nemaline myopathy. *Neuromuscul. Disord.* **14**, 779–784 (2004).
84. Sato, I. et al. Congenital neuromuscular disease with uniform type 1 fiber and *RYR1* mutation. *Neurology* **70**, 114–122 (2008).
85. Muhammad, E. et al. Congenital myopathy is caused by mutation of *HACD1*. *Hum. Mol. Genet.* **22**, 5229–5236 (2013).
86. Maurer, M. et al. Centronuclear myopathy in Labrador retrievers: a recent founder mutation in the *PTPLA* gene has rapidly disseminated worldwide. *PLoS ONE* **7**, e46408 (2012).
87. Walmsley, G. L. et al. Progressive structural defects in canine centronuclear myopathy indicate a role for HACD1 in maintaining skeletal muscle membrane systems. *Am. J. Pathol.* **187**, 441–456 (2017).
88. Clarke, N. F. et al. Mutations in *TPM3* are a common cause of congenital fiber type disproportion. *Ann. Neurol.* **63**, 329–337 (2008).
89. Munot, P. et al. Congenital fibre type disproportion associated with mutations in the tropomyosin 3 (*TPM3*) gene mimicking congenital myasthenia. *Neuromuscul. Disord.* **20**, 796–800 (2010).
90. Clarke, N. F. et al. Recessive mutations in *RYR1* are a common cause of congenital fiber type disproportion. *Hum. Mutat.* **31**, E1544–E1550 (2010).
91. Laing, N. G. et al. Actin mutations are one cause of congenital fibre type disproportion. *Ann. Neurol.* **56**, 689–694 (2004).
92. Clarke, N. F. et al. *SEPN1*: associated with congenital fiber-type disproportion and insulin resistance. *Ann. Neurol.* **59**, 546–552 (2006).
93. Lamont, P. J. et al. Novel mutations widen the phenotypic spectrum of slow skeletal/β-cardiac myosin (*MYH7*) distal myopathy. *Hum. Mutat.* **35**, 868–879 (2014).
94. Vallat, J. M. et al. Coexistence of minicores, cores, and rods in the same muscle biopsy. A new example of mixed congenital myopathy. *Acta Neuropathol.* **58**, 229–232 (1982).
95. Schartner, V. et al. Dihydropyridine receptor (DHPR, *CACNA1S*) congenital myopathy. *Acta Neuropathol.* **133**, 517–533 (2017).
96. Monnier, N. et al. Presence of two different genetic traits in malignant hyperthermia families: implication for genetic analysis, diagnosis, and incidence of malignant hyperthermia susceptibility. *Anesthesiology* **97**, 1067–1074 (2002).
97. Jurkat-Rott, K. et al. A calcium channel mutation causing hypokalemic periodic paralysis. *Hum. Mol. Genet.* **3**, 1415–1419 (1994).
98. O'Grady, G. L. et al. Variants in the oxidoreductase PYROXD1 cause early-onset myopathy with internalized nuclei and myofibrillar disorganization. *Am. J. Hum. Genet.* **99**, 1086–1105 (2016).
99. Tajsharghi, H. & Oldfors, A. Myosinopathies: pathology and mechanisms. *Acta Neuropathol.* **125**, 3–18 (2013).
100. Tajsharghi, H. et al. Human disease caused by loss of fast IIa myosin heavy chain due to recessive *MYH2* mutations. *Brain* **133**, 1451–1459 (2010).
101. Martinsson, T. et al. Autosomal dominant myopathy: missense mutation (Glu-706 → Lys) in the myosin heavy chain IIa gene. *Proc. Natl Acad. Sci. USA* **97**, 14614–14619 (2000).
102. Willis, T. et al. A novel *MYH2* mutation in family members presenting with congenital myopathy, ophthalmoplegia and facial weakness. *J. Neurol.* **263**, 1427–1433 (2016).
103. Tsabari, R., Daum, H., Kerem, E., Fellig, Y. & Dor, T. Congenital myopathy due to myosin heavy chain 2 mutation presenting as chronic aspiration pneumonia in infancy. *Neuromuscul. Disord.* **27**, 947–950 (2017).
104. McMillin, M. J. et al. Mutations in *ECEL1* cause distal arthrogryposis type 5D. *Am. J. Hum. Genet.* **92**, 150–156 (2013).
105. Dieterich, K. et al. The neuronal endopeptidase ECEL1 is associated with a distinct form of recessive distal arthrogryposis. *Hum. Mol. Genet.* **22**, 1483–1492 (2013).
106. Shaaban, S. et al. Expanding the phenotypic spectrum of *ECEL1*-related congenital contracture syndromes. *Clin. Genet.* **85**, 562–567 (2014).
107. Todd, E. J. et al. Next generation sequencing in a large cohort of patients presenting with neuromuscular disease before or at birth. *Orphanet J. Rare Dis.* **10**, 148 (2015).
108. Bayram, Y. et al. Molecular etiology of arthrogryposis in multiple families of mostly Turkish origin. *J. Clin. Invest.* **126**, 762–778 (2016).
109. Coste, B. et al. Gain-of-function mutations in the mechanically activated ion channel PIEZO2 cause a subtype of distal arthrogryposis. *Proc. Natl Acad. Sci. USA* **110**, 4667–4672 (2013).
110. Zaharieva, I. T. et al. Loss-of-function mutations in *SCN4A* cause severe foetal hypokinesia or 'classical' congenital myopathy. *Brain* **139**, 674–691 (2016).
111. Singh, R. R. et al. Mutations in *SCN4A*: a rare but treatable cause of recurrent life-threatening laryngospasm. *Pediatrics* **134**, e1447–e1450 (2014).
112. Bharucha-Goebel, D. X. et al. Severe congenital *RYR1*-associated myopathy: the expanding clinicopathologic and genetic spectrum. *Neurology* **80**, 1584–1589 (2013).
113. Schessl, J. et al. MRI in *DNM2*-related centronuclear myopathy: evidence for highly selective muscle involvement. *Neuromuscul. Disord.* **17**, 28–32 (2007).
114. Clarke, N. F. et al. Cap disease due to mutation of the beta-tropomyosin gene (TPM2). *Neuromuscul. Disord.* **19**, 348–351 (2009).
115. Lehtokari, V. L. et al. Cap disease caused by heterozygous deletion of the β-tropomyosin gene *TPM2*. *Neuromuscul. Disord.* **17**, 433–442 (2007).
116. Sewry, C. A., Holton, J. L., Dick, D. J., Muntoni, F. & Hanna, M. G. Zebra body myopathy is caused by a mutation in the skeletal muscle actin gene (*ACTA1*). *Neuromuscul. Disord.* **25**, 388–391 (2015).
117. Lacruz, R. S. & Feske, S. Diseases caused by mutations in *ORAI1* and *STIM1*. *Ann. NY Acad. Sci.* **1356**, 45–79 (2015).
118. Gordon, C. P. & Litz, S. Multicore myopathy in a patient with anhidrotic ectodermal dysplasia. *Can. J. Anaesth.* **39**, 966–968 (1992).
119. Engel, A. G., Redhage, K. R., Tester, D. J., Ackerman, M. J. & Selcen, D. Congenital myopathy associated with the triadin knockout syndrome. *Neurology* **88**, 1153–1156 (2017).
120. Altmann, H. M. et al. Homozygous/compound heterozygous triadin mutations associated with autosomal-recessive long-QT syndrome and pediatric sudden cardiac arrest: elucidation of the triadin knockout syndrome. *Circulation* **131**, 2051–2060 (2015).
121. Olive, M. et al. New cardiac and skeletal protein aggregate myopathy associated with combined MuRF1 and MuRF3 mutations. *Hum. Mol. Genet.* **24**, 6264 (2015).
122. Zhang, L., Kelley, J., Schmeisser, G., Kobayashi, Y. M. & Jones, L. R. Complex formation between junctin, triadin, calsequestrin, and the ryanodine receptor. Proteins of the cardiac junctional sarcoplasmic reticulum membrane. *J. Biol. Chem.* **272**, 23389–23397 (1997).
123. Park, H. et al. Comparing skeletal and cardiac calsequestrin structures and their calcium binding: a proposed mechanism for coupled calcium binding and protein polymerization. *J. Biol. Chem.* **279**, 18026–18033 (2004).
124. Costello, B. et al. Characterization of the junctional face membrane from terminal cisternae of sarcoplasmic reticulum. *J. Cell Biol.* **103**, 741–753 (1986).
125. Treves, S. et al. Minor sarcoplasmic reticulum membrane components that modulate excitation–contraction coupling in striated muscles. *J. Physiol.* **587**, 3071–3079 (2009).
126. Rios, E. & Gyorke, S. Calsequestrin, triadin and more: the molecules that modulate calcium release in cardiac and skeletal muscle. *J. Physiol.* **587**, 3069–3070 (2009).
127. Guo, W. & Campbell, K. P. Association of triadin with the ryanodine receptor and calsequestrin in the lumen of the sarcoplasmic reticulum. *J. Biol. Chem.* **270**, 9027–9030 (1995).
128. Wium, E., Dulhunty, A. F. & Beard, N. A. Three residues in the luminal domain of triadin impact on Trisk 95 activation of skeletal muscle ryanodine receptors. *Pflugers Arch.* **468**, 1985–1994 (2016).
129. Caswell, A. H., Motoike, H. K., Fan, H. & Brandt, N. R. Location of ryanodine receptor binding site on skeletal muscle triadin. *Biochemistry* **38**, 90–97 (1999).
130. Groh, S. et al. Functional interaction of the cytoplasmic domain of triadin with the skeletal ryanodine receptor. *J. Biol. Chem.* **274**, 12278–12283 (1999).
131. Goonasekera, S. A. et al. Triadin binding to the C-terminal luminal loop of the ryanodine receptor is important for skeletal muscle excitation contraction coupling. *J. Gen. Physiol.* **130**, 365–378 (2007).
132. Gordon, A. M., Homsher, E. & Regnier, M. Regulation of contraction in striated muscle. *Physiol. Rev.* **80**, 853–924 (2000).
133. Abu-Abed, M., Mal, T. K., Kainosho, M., MacLennan, D. H. & Ikura, M. Characterization of the ATP-binding domain of the sarco(endo)plasmic reticulum Ca^{2+}-ATPase: probing nucleotide binding by multidimensional NMR. *Biochemistry* **41**, 1156–1164 (2002).
134. MacLennan, D. H., Asahi, M. & Tupling, A. R. The regulation of SERCA-type pumps by phospholamban and sarcolipin. *Ann. NY Acad. Sci.* **986**, 472–480 (2003).
135. MacLennan, D. H. & Kranias, E. G. Phospholamban: a crucial regulator of cardiac contractility. *Nat. Rev. Mol. Cell. Biol.* **4**, 566–577 (2003).
136. Asahi, M. et al. Sarcolipin regulates sarco(endo) plasmic reticulum Ca^{2+}-ATPase (SERCA) by binding to transmembrane helices alone or in association with phospholamban. *Proc. Natl Acad. Sci. USA* **100**, 5040–5045 (2003).
137. Kurebayashi, N. & Ogawa, Y. Depletion of Ca^{2+} in the sarcoplasmic reticulum stimulates Ca^{2+} entry into mouse skeletal muscle fibres. *J. Physiol.* **533**, 185–199 (2001).
138. Cherednichenko, G. et al. Conformational activation of Ca^{2+} entry by depolarization of skeletal myotubes. *Proc. Natl Acad. Sci. USA* **101**, 15793–15798 (2004).
139. Launikonis, B. S. & Rios, E. Store-operated Ca^{2+} entry during intracellular Ca^{2+} release in mammalian skeletal muscle. *J. Physiol.* **583**, 81–97 (2007).
140. Stiber, J. et al. STIM1 signalling controls store-operated calcium entry required for development and contractile function in skeletal muscle. *Nat. Cell Biol.* **10**, 688–697 (2008).
141. Peinelt, C. et al. Amplification of CRAC current by STIM1 and CRACM1 (Orai1). *Nat. Cell Biol.* **8**, 771–773 (2006).
142. Treves, S., Jungbluth, H., Muntoni, F. & Zorzato, F. Congenital muscle disorders with cores: the ryanodine receptor calcium channel paradigm. *Curr. Opin. Pharmacol.* **8**, 319–326 (2008).
143. Hwang, J. H., Zorzato, F., Clarke, N. F. & Treves, S. Mapping domains and mutations on the skeletal muscle ryanodine receptor channel. *Trends Mol. Med.* **18**, 644–657 (2012).
144. Maclennan, D. H. & Zvaritch, E. Mechanistic models for muscle diseases and disorders originating in the sarcoplasmic reticulum. *Biochim. Biophys. Acta* **1813**, 948–964 (2011).
145. Hirata, H. et al. Zebrafish relatively relaxed mutants have a ryanodine receptor defect, show slow swimming and provide a model of multi-minicore disease. *Development* **134**, 2771–2781 (2007).
146. Zhou, H. et al. RyR1 deficiency in congenital myopathies disrupts excitation–contraction coupling. *Hum. Mutat.* **34**, 986–996 (2013).
147. Zhou, H. et al. Epigenetic allele silencing unveils recessive *RYR1* mutations in core myopathies. *Am. J. Hum. Genet.* **79**, 859–868 (2006).
148. Ducreux, S. et al. Functional properties of ryanodine receptors carrying three amino acid substitutions identified in patients affected by multi-minicore disease and central core disease, expressed in immortalized lymphocytes. *Biochem. J.* **395**, 259–266 (2006).

149. Nelson, B. R. et al. Skeletal muscle-specific T-tubule protein STAC3 mediates voltage-induced Ca^{2+} release and contractility. *Proc. Natl Acad. Sci. USA* **110**, 11881–11886 (2013).
150. Polster, A., Nelson, B. R., Olson, E. N. & Beam, K. G. Stac3 has a direct role in skeletal muscle-type excitation–contraction coupling that is disrupted by a myopathy-causing mutation. *Proc. Natl Acad. Sci. USA* **113**, 10986–10991 (2016).
151. Bohm, J. et al. Constitutive activation of the calcium sensor STIM1 causes tubular-aggregate myopathy. *Am. J. Hum. Genet.* **92**, 271–278 (2013).
152. Volkers, M. et al. Orai1 deficiency leads to heart failure and skeletal myopathy in zebrafish. *J. Cell Sci.* **125**, 287–294 (2012).
153. Jurynec, M. J. et al. Selenoprotein N is required for ryanodine receptor calcium release channel activity in human and zebrafish muscle. *Proc. Natl Acad. Sci. USA* **105**, 12485–12490 (2008).
154. Arbogast, S. et al. Oxidative stress in *SEPN1*-related myopathy: from pathophysiology to treatment. *Ann. Neurol.* **65**, 677–686 (2009).
155. Jungbluth, H. & Gautel, M. Pathogenic mechanisms in centronuclear myopathies. *Front. Aging Neurosci.* **6**, 339 (2014).
156. Cowling, B. S., Toussaint, A., Muller, J. & Laporte, J. Defective membrane remodeling in neuromuscular diseases: insights from animal models. *PLoS Genet.* **8**, e1002595 (2012).
157. Bachmann, C. et al. Cellular, biochemical and molecular changes in muscles from patients with X-linked myotubular myopathy due to MTM1 mutations. *Hum. Mol. Genet.* **26**, 320–332 (2017).
158. Wallgren-Pettersson, C., Sewry, C. A., Nowak, K. J. & Laing, N. G. Nemaline myopathies. *Sem. Pediatr. Neurol.* **18**, 230–238 (2011).
159. Ravenscroft, G. et al. Mouse models of dominant *ACTA1* disease recapitulate human disease and provide insight into therapies. *Brain* **134**, 1101–1115 (2011).
160. Ravenscroft, G. et al. Actin nemaline myopathy mouse reproduces disease, suggests other actin disease phenotypes and provides cautionary note on muscle transgene expression. *PLoS ONE* **6**, e28699 (2011).
161. Jain, R. K. et al. Nemaline myopathy with stiffness and hypertonia associated with an *ACTA1* mutation. *Neurology* **78**, 1100–1103 (2012).
162. Donkervoort, S. et al. *TPM3* deletions cause a hypercontractile congenital muscle stiffness phenotype. *Ann. Neurol.* **78**, 982–994 (2015).
163. Ochala, J. et al. Disrupted myosin cross-bridge cycling kinetics triggers muscle weakness in nebulin-related myopathy. *FASEB J.* **25**, 1903–1913 (2011).
164. Marttila, M. et al. Nebulin interactions with actin and tropomyosin are altered by disease-causing mutations. *Skelet. Muscle* **4**, 15 (2014).
165. de Winter, J. M. et al. Mutation-specific effects on thin filament length in thin filament myopathy. *Ann. Neurol.* **9**, 959–969 (2016).
166. Ajima, R. et al. Deficiency of *Myo18B* in mice results in embryonic lethality with cardiac myofibrillar aberrations. *Genes Cells* **13**, 987–999 (2008).
167. Gurung, R. et al. A zebrafish model for a human myopathy associated with mutation of the unconventional myosin MYO18B. *Genetics* **205**, 725–735 (2017).
168. Gupta, V. A. & Beggs, A. H. Kelch proteins: emerging roles in skeletal muscle development and diseases. *Skelet. Muscle* **4**, 11 (2014).
169. Garg, A. et al. KLHL40 deficiency destabilizes thin filament proteins and promotes nemaline myopathy. *J. Clin. Invest.* **124**, 3529–3539 (2014).
170. Castets, P. et al. Satellite cell loss and impaired muscle regeneration in selenoprotein N deficiency. *Hum. Mol. Genet.* **20**, 694–704 (2011).
171. Castets, P. et al. Selenoprotein N is dynamically expressed during mouse development and detected early in muscle precursors. *BMC Dev. Biol.* **9**, 46 (2009).
172. Beggs, A. H. et al. *MTM1* mutation associated with X-linked myotubular myopathy in Labrador Retrievers. *Proc. Natl Acad. Sci. USA* **107**, 14697–14702 (2010).
173. Dowling, J. J., Low, S. E., Busta, A. S. & Feldman, E. L. Zebrafish MTMR14 is required for excitation–contraction coupling, developmental motor function and the regulation of autophagy. *Hum. Mol. Genet.* **19**, 2668–2681 (2010).
174. Fetalvero, K. M. et al. Defective autophagy and mTORC1 signaling in myotubularin null mice. *Mol. Cell. Biol.* **33**, 98–110 (2013).
175. Al-Qusairi, L. et al. Lack of myotubularin (MTM1) leads to muscle hypotrophy through unbalanced regulation of the autophagy and ubiquitin–proteasome pathways. *FASEB J.* **27**, 3384–3394 (2013).
176. Durieux, A. C. et al. A centronuclear myopathy–dynamin 2 mutation impairs autophagy in mice. *Traffic* **13**, 869–879 (2012).
177. Sarparanta, J. et al. Interactions with M-band titin and calpain 3 link myospryn (CMYA5) to tibial and limb-girdle muscular dystrophies. *J. Biol. Chem.* **285**, 30304–30315 (2010).
178. McClelland, V. et al. Vici syndrome associated with sensorineural hearing loss and evidence of neuromuscular involvement on muscle biopsy. *Am. J. Med. Genet. A* **152A**, 741–747 (2010).
179. Byrne, S. et al. *EPG5*-related Vici syndrome: a paradigm of neurodevelopmental disorders with defective autophagy. *Brain* **139**, 765–781 (2016).
180. Rokach, O. et al. Epigenetic changes as a common trigger of muscle weakness in congenital myopathies. *Hum. Mol. Genet.* **24**, 4636–4647 (2015).
181. North, K. N. et al. Approach to the diagnosis of congenital myopathies. *Neuromuscul. Disord.* **24**, 97–116 (2014).
182. Hacohen, Y. et al. Fetal acetylcholine receptor inactivation syndrome: a myopathy due to maternal antibodies. *Neurol. Neuroimmunol. Neuroinflamm.* **2**, e57 (2015).
183. Kinali, M. et al. Congenital myasthenic syndromes in childhood: diagnostic and management challenges. *J. Neuroimmunol.* **201–202**, 6–12 (2008).
184. Bonnemann, C. G. et al. Diagnostic approach to the congenital muscular dystrophies. *Neuromuscul. Disord.* **24**, 289–311 (2014).
185. Selcen, D. Myofibrillar myopathies. *Neuromuscul. Disord.* **21**, 161–171 (2011).
186. Nishino, I. Autophagic vacuolar myopathy. *Semin. Pediatr. Neurol.* **13**, 90–95 (2006).
187. Wang, C. H. et al. Consensus statement on standard of care for congenital myopathies. *J. Child Neurol.* **27**, 363–382 (2012).
188. Jungbluth, H., Ochala, J., Treves, S. & Gautel, M. Current and future therapeutic approaches to the congenital myopathies. *Semin. Cell Dev. Biol.* **64**, 191–200 (2017).
189. Childers, M. K. et al. Gene therapy prolongs survival and restores function in murine and canine models of myotubular myopathy. *Sci. Transl Med.* **6**, 220ra10 (2014).
190. Rendu, J. et al. Exon skipping as a therapeutic strategy applied to an *RYR1* mutation with pseudo-exon inclusion causing a severe core myopathy. *Hum. Gene Ther.* **24**, 702–713 (2013).
191. Monnier, N. et al. A homozygous splicing mutation causing a depletion of skeletal muscle RYR1 is associated with multi-minicore disease congenital myopathy with ophthalmoplegia. *Hum. Mol. Genet.* **12**, 1171–1178 (2003).
192. Barton-Davis, E. R., Cordier, L., Shoturma, D. I., Leland, S. E. & Sweeney, H. L. Aminoglycoside antibiotics restore dystrophin function to skeletal muscles of *mdx* mice. *J. Clin. Invest.* **104**, 375–381 (1999).
193. MacArthur, D. G. & Lek, M. The uncertain road towards genomic medicine. *Trends Genet.* **28**, 303–305 (2012).
194. Cowling, B. S. et al. Reducing dynamin 2 expression rescues X-linked centronuclear myopathy. *J. Clin. Invest.* **124**, 1350–1363 (2014).
195. Sabha, N. et al. PIK3C2B inhibition improves function and prolongs survival in myotubular myopathy animal models. *J. Clin. Invest.* **126**, 3613–3625 (2016).
196. Ravenscroft, G. et al. Cardiac α-actin over-expression therapy in dominant *ACTA1* disease. *Hum. Mol. Genet.* **22**, 3987–3997 (2013).
197. Nowak, K. J. et al. Nemaline myopathy caused by absence of α-skeletal muscle actin. *Ann. Neurol.* **61**, 175–184 (2007).
198. Lawlor, M. W. et al. Enzyme replacement therapy rescues weakness and improves muscle pathology in mice with X-linked myotubular myopathy. *Hum. Mol. Genet.* **22**, 1525–1538 (2013).
199. Fruen, B. R., Mickelson, J. R. & Louis, C. F. Dantrolene inhibition of sarcoplasmic reticulum Ca^{2+} release by direct and specific action at skeletal muscle ryanodine receptors. *J. Biol. Chem.* **272**, 26965–26971 (1997).
200. Timmins, M. A. et al. Malignant hyperthermia testing in probands without adverse anesthetic reaction. *Anesthesiology* **123**, 548–556 (2015).
201. Michalek-Sauberer, A. & Gilly, H. Prophylactic use of dantrolene in a patient with central core disease. *Anesth. Analg.* **86**, 915–916 (1998).
202. Jungbluth, H., Dowling, J. J., Ferreiro, A. & Muntoni, F. 217th ENMC International Workshop: *RYR1*-related myopathies, Naarden, The Netherlands, 29–31 January 2016. *Neuromuscul. Disord.* **26**, 624–633 (2016).
203. Andersson, D. C. & Marks, A. R. Fixing ryanodine receptor Ca leak — a novel therapeutic strategy for contractile failure in heart and skeletal muscle. *Drug Discov. Today Dis. Mech.* **7**, e151–e157 (2010).
204. Marks, A. R. Calcium cycling proteins and heart failure: mechanisms and therapeutics. *J. Clin. Invest.* **123**, 46–52 (2013).
205. Pold, R. et al. Long-term AICAR administration and exercise prevents diabetes in ZDF rats. *Diabetes* **54**, 928–934 (2005).
206. Lanner, J. T. et al. AICAR prevents heat-induced sudden death in RyR1 mutant mice independent of AMPK activation. *Nat. Med.* **18**, 244–251 (2012).
207. de Winter, J. M. et al. Troponin activator augments muscle force in nemaline myopathy patients with nebulin mutations. *J. Med. Genet.* **50**, 383–392 (2013).
208. de Winter, J. M. et al. Effect of levosimendan on the contractility of muscle fibers from nemaline myopathy patients with mutations in the nebulin gene. *Skelet. Muscle* **5**, 12 (2015).
209. Amthor, H. & Hoogaars, W. M. Interference with myostatin/ActRIIB signaling as a therapeutic strategy for Duchenne muscular dystrophy. *Curr. Gene Ther.* **12**, 245–259 (2012).
210. Durham, W. J. et al. RyR1 S-nitrosylation underlies environmental heat stroke and sudden death in Y522S RyR1 knockin mice. *Cell* **133**, 53–65 (2008).
211. Dowling, J. J. et al. Oxidative stress and successful antioxidant treatment in models of RYR1-related myopathy. *Brain* **135**, 1115–1127 (2012).
212. Natera-de Benito, D. et al. *KLHL40*-related nemaline myopathy with a sustained, positive response to treatment with acetylcholinesterase inhibitors. *J. Neurol.* **263**, 517–523 (2016).
213. Robb, S. A. et al. Impaired neuromuscular transmission and response to acetylcholinesterase inhibitors in centronuclear myopathies. *Neuromuscul. Disord.* **21**, 379–386 (2011).
214. Gibbs, E. M. et al. Neuromuscular junction abnormalities in DNM2-related centronuclear myopathy. *J. Mol. Med.* **91**, 727–737 (2013).
215. Dowling, J. J. et al. Myotubular myopathy and the neuromuscular junction: a novel therapeutic approach from mouse models. *Dis. Model. Mech.* **5**, 852–859 (2012).
216. Messina, S. et al. Pilot trial of salbutamol in central core and multi-minicore diseases. *Neuropediatrics* **35**, 262–266 (2004).
217. Schreuder, L. T. et al. Successful use of albuterol in a patient with central core disease and mitochondrial dysfunction. *J. Inherit. Metab. Dis.* **33**, S205–209 (2010).
218. Jungbluth, H., Dowling, J. J., Ferreiro, A. & Muntoni, F. 182nd ENMC International Workshop: *RYR1*-related myopathies, 15–17th April 2011, Naarden, The Netherlands. *Neuromuscul. Disord.* **22**, 453–462 (2012).
219. Nguyen, M. A. et al. Hypertrophy and dietary tyrosine ameliorate the phenotypes of a mouse model of severe nemaline myopathy. *Brain* **134**, 3516–3529 (2011).
220. Ryan, M. M. et al. Dietary L-tyrosine supplementation in nemaline myopathy. *J. Child Neurol.* **23**, 609–613 (2008).
221. Winter, L. et al. Chemical chaperone ameliorates pathological protein aggregation in plectin-deficient muscle. *J. Clin. Invest.* **124**, 1144–1157 (2014).
222. Kusaczuk, M., Bartoszewicz, M. & Cechowska-Pasko, M. Phenylbutyric acid: simple structure — multiple effects. *Curr. Pharm. Des.* **21**, 2147–2166 (2015).
223. Cuadrado-Tejedor, M., Ricobaraza, A. L., Torrijo, R., Franco, R. & Garcia-Osta, A. Phenylbutyrate is a multifaceted drug that exerts neuroprotective effects and reverses the Alzheimer's disease-like phenotype of a commonly used mouse model. *Curr. Pharm. Des.* **19**, 5076–5084 (2013).
224. Lee, C. S. et al. A chemical chaperone improves muscle function in mice with a RyR1 mutation. *Nat. Commun.* **8**, 14659 (2017).

225. Vega, H., Agellon, L. B. & Michalak, M. The rise of proteostasis promoters. *IUBMB Life* **68**, 943–954 (2016).
226. Yuste-Checa, P. et al. Pharmacological chaperoning: a potential treatment for PMM2-CDG. *Hum. Mutat.* **38**, 160–168 (2017).
227. Kim, E., et al. Characterization of human cardiac calsequestrin and its deleterious mutants. *J. Mol. Biol.* **373**, 1047–1057 (2007).
228. Tang, L., et al. Structural basis for Ca^{2+} selectivity of a voltage-gated calcium channel. *Nature* **505**, 56–61 (2014).
229. Zamoon, J., Mascioni, A., Thomas, D. D. & Veglia, G. NMR solution structure and topological orientation of monomeric phospholamban in dodecylphosphocholine micelles. *Biophys. J.* **85**, 2589–2598 (2003).
230. Efremov, R. G., Leitner, A., Aebersold, R. & Raunser, S. Architecture and conformational switch mechanism of the ryanodine receptor. *Nature* **517**, 39–43 (2015).
231. Toyoshima, C., Nakasako, M., Nomura, H. & Ogawa, H. Crystal structure of the calcium pump of sarcoplasmic reticulum at 2.6 Å resolution. *Nature* **405**, 647–655 (2000).

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H.J., S.T., F.Z., J.O., C.S., M.G. and F.M. researched data for the article. All authors made substantial contributions to discussion of the content, wrote the article and reviewed and/or edited the manuscript before submission.

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