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The congenital myopathies – inherited disorders of excitation-contraction coupling and muscle contraction

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ABSTRACT

The congenital myopathies (CMs) are a group of early-onset, non-dystrophic neuromuscular conditions with characteristic muscle biopsy findings, variable severity and a stable or slowly progressive course. Muscle weakness pronounced in axial and proximal muscle groups is common, whereas the degree of extraocular muscle weakness, cardiorespiratory and distal muscle involvement may implicate specific genes. Based on the predominant muscle biopsy finding, Central Core Disease (CCD), Multi-minicore Disease (MmD), Centronuclear Myopathy (CNM) and Nemaline Myopathy (NM) were the main CMs to be originally reported and still represent the major diagnostic categories. Mutations in more than 20 genes have been identified to date, encoding proteins implicated in skeletal muscle calcium homeostasis, excitation-contraction coupling (ECC), thin/thick filament assembly and interactions, and other mechanisms. Mutations in the skeletal muscle ryanodine receptor *(RYR1)* gene are the most frequent genetic cause, and CCD and MmD (the "core myopathies") are the most common subgroups. Widespread introduction of next generation sequencing (NGS) has vastly improved mutation detection in large genes such as *RYR1*, nebulin *(NEB)* and titin (*TTN)*, and identified novel genetic backgrounds. There is an increasing recognition that the originally described entities represent a partially skewed selection of a much wider phenotypical spectrum, as many patients may show only subtle, non-specific or multiple histopathological features, evading easy categorization. Most of the principal mechanisms have been largely resolved, but the etiology of the pathognomic histopathological features and secondary effects on muscle growth and atrophy pathways remain only poorly understood. Whilst so far management has been mainly supportive, therapy development is reaching the clinical trial stage in some conditions.

INTRODUCTION

The congenital myopathies (CMs) are a genetically heterogeneous group of early-onset muscle conditions characterized by variable degrees of muscle weakness and characteristic structural abnormalities on muscle biopsy. They are almost invariably disorders of disturbed ECC, the process whereby an electrical neuronal impulse is translated into muscle contraction through controlled Ca^{++} release leading to sarcomeric protein activation, or of proteins primarily involved in sarcomeric filament assembly and interaction. Nevertheless, recent findings suggest other less common pathogenic mechanisms. The concept of the CMs was established in the 1950s and 1960s, when the application of histochemical and ultrastructural techniques to diseased muscle identified histopathological features considered to be pathognomonic at the time. Recognition of these features – central cores, multi-minicores, central nuclei, nemaline rods – resulted in the designation of novel disease entities – Central Core Disease (CCD) [1], Multi-minicore Disease (MmD) [2], Centronuclear Myopathy (CNM) [3] and Nemaline Myopathy (NM) [4] – that still represent the major diagnostic categories.

Considerable progress has been made concerning the understanding of the phenotypical spectrum, diagnosis and management of the CMs. In addition to primary myopathic features, non-neuromuscular manifestations are observed in several forms, pointing to a role of the defective proteins in non-skeletal muscle tissues [5]. Muscle imaging, in particular muscle magnetic resonance imaging (MRI), has emerged as a powerful tool for deep phenotyping [6]. Presentations late in adulthood have now been recognized [7,8], and due 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 *(RYR1)* gene as the cause of Malignant Hyperthermia (MH) in 1991 and CCD in 1993 [9,10], mutations in more than 20 genes have been identified. Introduction of next generation sequencing (NGS) techniques into routine clinical diagnosis [11] has resulted in an improved detection rate of mutations in genes such as *RYR1*, nebulin *(NEB)* or titin *(TTN)*, due to their sheer size previously only studied with Sanger sequencing in few patients. These novel techniques have led to the recognition that different mutations in the same gene may give rise to variable histopathological phenotypes, whilst mutations in different genes may cause the same histopathological feature, often due to functional association of the defective proteins. Moreover, it has also become increasingly clear that in many CMs, non-specific or a combination of pathological abnormalities rather than a "pure" muscle pathology picture may be found. A classification based on predominant histopathological and associated clinical features is still practically useful; however, it is also helpful to consider these conditions according to the main underlying disease mechanisms.

In the present review, we will summarize genetic, clinical and pathological features of the major CMs. 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 CMs are limited, and mostly focused on the originally described major pathological variants, CCD, MmD, CNM and NM; the key characteristics of these entities are detailed below and illustrated in Figure 1.

CCD, initially described in the 1950s [1], and MmD [2] (also often referred to as the "core myopathies" [12]) derive their name from the histochemical appearance of focally reduced

oxidative enzyme activity, corresponding to myofibrillar changes on ultrastructural examination. Centrally located, well-demarcated cores running along a significant extent of the fibre axis on longitudinal sections are characteristic of CCD, whilst multiple cores of less well-defined appearance and of longitudinally more limited extent define MmD.

The hallmark of CNM is fibres with centralized nuclei, which vary in number and in terms of associated features between muscles and genetic backgrounds. NM (for review, [13]) is characterized by the presence of numerous nemaline rods that stain red on the Gomori trichrome and are confirmed on EM.

The overall prevalence of these CM variants has been estimated at 1 in 26000 [14]. While originally NM was considered the most frequent form, emerging data suggest that CMs with cores (CCD, MmD) are the most common subgroup. Marked genetic heterogeneity is now acknowledged and detailed in the sections on the major diagnostic categories below. In essence, recent data indicate that *RYR1* is the gene most frequently involved in the CMs, in particular CCD and MmD. Recessive *NEB* mutations and (*de novo*) dominant mutations in *ACTA1* encoding skeletal muscle alpha-actin are the most common known causes of NM, whereas X-linked recessive mutations in *MTM1* encoding myotubularin are believed to be the most common cause of CNM. Mutations in *TTN* are increasingly being recognized and may be implicated in a substantial proportion of currently unresolved CMs, as well as other neuromuscular disorders, including muscular dystrophies [15]. The genes implicated in the CMs are listed in Table 1 and the key clinico-pathological features associated with the most common genetic backgrounds are summarized in Table 2. Characteristic histopathological features are illustrated in Figure 1.

CLINICO-PATHOLOGICAL AND GENETIC FEATURES

Congenital myopathies with cores – CCD, MmD and MH

In view of the pathological and genetic overlap, CCD, MmD (also referred to as the "core myopathies") and MH are discussed here in a single section.

CCD is most closely linked to dominant *RYR1* mutations, whereas MmD is genetically more heterogeneous, mainly due to recessive mutations in *RYR1* [16-18], *SEPN1* encoding selenoprotein N [19] and, less frequently, *MYH7* [20]. The histopathological appearance of MmD has also been described in some patients with recessive mutations in *MEGF10* encoding multiple epidermal growth factor 10 [21-24]. (Mini)cores on muscle biopsy may also be prominent in *TTN*-related myopathies [25], often in conjunction with other myopathic and dystrophic features, and may also occur in other neuromuscular disorders.

Clinically, CCD due to dominant *RYR1* mutations (for review, [12]) is usually a relatively mild condition, although early severe presentations, often associated with *de novo* inheritance, are on record [26]. Extraocular muscles are usually spared and facial, bulbar and respiratory involvement is typically mild. Congenital dislocation of the hips (CDH) and scoliosis are common. Most patients achieve independent ambulation and have a static or only slowly progressive course. Clinical features of predominantly recessively inherited MmD (for review [12]), are more variable. *SEPN1*-related myopathies [19,27] are characterized by marked weakness, early spinal rigidity, scoliosis and respiratory impairment. Recessively inherited *RYR1*-related core myopathies show a distribution of weakness and wasting similar to the *SEPN1*-related form but have additional extraocular muscle involvement and, with few exceptions, lack the severe

respiratory impairment [17,18]. Variable combinations of scoliosis, spinal rigidity, multiple, mainly distal contractures and an associated cardiomyopathy may occur in *TTN*- and *MYH7*-related forms [20,25]. *MEGF10*-related myopathies are associated with a very wide 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 on muscle biopsy[22]. Muscle MRI may 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 MH, a severe adverse reactions to volatile anaesthetics and muscle relaxants (for review, [30]), with some CCD-associated *RYR1* mutations also carrying an increased MHS risk. The association with recessive *RYR1*-related MmD is less well-established; however, MmD cases due to compound heterozygosity for dominant MHS *RYR1* mutations are on record [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 variable histopathological features [33]. Another recently recognized myopathy with similar clinico-pathological features is ***Native American myopathy (NAM)***, originally described in the Lumbee population of North Dakota and due to homozygosity for a founder mutation (p.W284S) in *STAC3* [34].

MHS-associated *RYR1* mutations have now also been identified as a common cause of ***exertional myalgia and rhabomyolysis (ERM)*** in otherwise healthy individuals with variable muscle biopsy findings [35]. Of note, exertional myalgia may be prominent in CCD [36], where mild to moderate CK elevations (up to 1000 IU/l), unusual in the context of other CMs, are also not uncommon. MHS-associated *RYR1* mutations may also give rise to a ***late-onset axial myopathy*** in previously healthy (or even particularly athletic) individuals [37,38].

Centronuclear Myopathy (CNM)

CNM (for review, [39]) is associated with X-linked recessive mutations in *MTM1* encoding myotubularin (X-linked myotubular myopathy, XLMTM) [40], autosomal-dominant mutations in *DNM2* encoding dynamin 2 [41] and *BIN1* encoding amphiphysin 2 [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] in one isolated pedigree. Heterozygous missense variants in *MTMR14* (or *hJUMPY*) identified in 2 patients with CNM may represent a genetic modifier of other genetic backgrounds [48].

In *MTM1*-related cases, the central nuclei are usually spaced down the long fibre axis, whereas in *DNM2*-related cases they may form chains; in the rare *BIN1*-related cases, clusters of central nuclei may occur. A typical feature of *MTM1*-related cases is central areas of enhanced oxidative enzyme activity and a pale peripheral halo. This finding and central nuclei are features shared with congenital myotonic dystrophy. Strictly centralized nuclei are more common than multiple internalized nuclei in the *MTM1*-, *DNM2*- and *BIN1*-related forms [40,41,44], whereas the opposite applies to *RYR1*-and *TTN*-related cases [43]. A radial distribution of sarcoplasmic strands with staining for NADH-TR and PAS is often seen in *DNM2*-related CNM [41]. "Necklace" fibres are often seen in milder *MTM1*-mutated cases or female carriers [49], and, occasionally, *DNM2*-related cases [50]. In most forms, ultrastructural triad abnormalities are observed [51].

Clinically, extraocular muscle involvement is the most consistent feature in all forms (for review [52]) except the *TTN*-, *SPEG*- and *CCDC78*-related form. The most severe form, XLMTM, typically presents in affected males with profound hypotonia, weakness and

contractures at birth, and associated bulbar and respiratory involvement almost always necessitating ventilation for survival. While the provision of constant respiratory support does improve life-expectancy in XLMTM there are recognized complications in some long-term survivors [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 are on record [55,56]. Additional characteristic features may include distal weakness, calf muscle hypertrophy, exertional myalgia/fatigue, peripheral or central nervous system involvement, and multisystem features such as neutropenia or cataracts. The peripheral axonal neuropathy CMTDIB is an allelic condition [57]. Recessive and, less frequently, dominantly inherited and milder, *BIN1*-related CNM have been reported in 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 myopathies (see above). Mutations in *TTN* are often associated with dysmorphic facial features, scoliosis, spinal rigidity and contractures [45], showing some overlap with the Emery-Dreifuss muscular dystrophy (EDMD) and the KDS spectrum. Cardiac involvement has only been reported in the *TTN*- and *SPEG*-related forms.

Nemaline Myopathy (NM)

NM has been associated with mutations in more than 10 genes to date, most commonly recessive mutations in the nebulin *(NEB)* gene [59,60], and (*de novo*) dominant mutations in the slow skeletal muscle α-actin *(ACTA1)* gene [61]. Dominant mutations in the alpha-tropomyosin *(TPM3)* [62], the beta-tropomyosin *(TPM2)* [63] and the *KBTBD13* [64] genes, as well as recessive mutations in *ACTA1* [65], in *TPM3* [66], TPM2 [67] the slow troponin T *(TNNT1)* [68], cofilin-2 *(CFL2)* [69], Kelch-repeat and BTB (POZ) domain containing 13 *(KBTBD 13)* [64] , *KLHL40* [70] ,

KLHL41 [71], leiomodin-3 *(LMOD3)* [72] , myopalladin *(MYPN)* [73,74] and myosin XVIIIB *(MYOB18)* [75]are less common or even limited to single families.

The number and distribution of nemaline rods varies between muscles and patients. Rods are believed to be derived from Z-lines, and may show continuity with Z-lines; they are mainly cytoplasmic but may also be nuclear, in particular in *ACTA1*-related NM [76] where there may be additional actin accumulation and compensatory expression of cardiac actin. Nemaline rods are usually seen in both fibre types except in patients with *TPM3* mutations, where they are limited to type 1 fibres. Numerous small rectangular rods in fibres with very few myofibrils are a feature of *KLHL40*-related NM [70].

Clinically, NM is highly variable and conventionally classified by age of onset and severity: Profoundly 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 of NM 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*-gene related NM is an unusual form characterized by progressive proximal and neck weakness, gait abnormalities, poor exercise tolerance and a peculiar slowness of movements [81]. Extraocular muscle involvement is only seen in a proportion of cases with *KLHL40*, *KLHL41* and *LMOD3* mutations. An associated cardiomyopathy may be seen in *MYPN*- and *MYO18B*-associated NM [74,75]. Many forms of NM may show marked distal involvement, and many of the causative genes have also been implicated in distinct distal arthrogryposis (DA) syndromes (for example, [82]). Muscle MRI may help to distinguish different genetic forms of NM [83].

Other congenital myopathies

Recent years have seen an expansion of the phenotypical spectrum of already known CM-associated genes, as well as the description of novel conditions that share some of the clinical and muscle biopsy findings with the better characterized entities without reaching a comparable level of histopathological "purity". These CMs with non-specific, multiple (structural) and unusual/other features are summarized in the following paragraph.

CMs with non-specific features. Marked ***type 1 predominance or uniformity*** is common in all CMs and may be the sole presenting feature [84]. Marked type 1 predominance and atrophy has also been reported in one consanguineous family with clinical features of a congenital myopathy and recessive mutations in *HACD1* encoding 3-hydroxyacyl-CoA dehydratase 1[85]; although recessive mutations in the corresponding canine gene PTPLA cause a form of CNM in dogs [86,87], increased central nuclei were not a feature in *HACD1*-mutated humans. ***Congenital fibre type disproportion (CFTD)***, the marked smallness of type 1 fibres compared to type 2 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.

CMs with multiple (structural) abnormalities, already recognized in the pre-molecular era [94], have now been largely genetically resolved and are often attributed to already previously identified genetic backgrounds: The common occurrence of cores and rods ("core-rod myopathy") has been attributed 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. There is also a rapidly increasing number of novel entities that do not readily fit into the conventional classification based on a single predominant histopathological abnormality: ***CACNA1S-related myopathy*** [95] is characterized by marked neonatal hypotonia, generalized weakness pronounced axially and variable extraocular, bulbar and respiratory involvement. *CACNA1S*-related myopathy is due to recessive and dominant mutations in the gene encoding the pore-forming subunit of DHPR in skeletal muscle (Cav1.1), previously associated with dominantly inherited forms of periodic paralysis (and, rarely, MHS phenotypes) [96,97]. Characteristic histopathological features include SR dilatation, increased internal nuclei and myofibrillar disorganization resembling minicores. Recessively inherited, ***PYROXD1-related CM*** [98] is an early-onset myopathy of moderate severity characterized by slowly progressive generalized weakness, facial and bulbar involvement, and increased internalized nuclei and myofibrillar disorganization on muscle biopsy. ***Hereditary myosin myopathies*** ("myosinopathies") (for review, [99]) comprise distinct distal arthrogryposis (DA) syndromes due to dominant mutations in *MYH3* and *MYH8* encoding two developmental myosin heavy chain (MyHC) isoforms, as well as CMs of variable onset and severity due to dominant and recessive mutations in *MYH2* and *MYH7,* the latter also implicated in Laing distal myopathy and myosin storage myopathy (MSM). In addition to the variable presence of cores on muscle biopsy, (recessive) *MYH2*-related myopathies [100-102] show marked reduction (or absence) of type 2A fibres [99,103], whereas accumulation of slow myosin ("hyaline bodies") may be seen in some *MYH7*-related cases. Both *MYH7*- and *MYH2*-related myopathies may also show increased connective tissue, internal nuclei, rimmed vacuoles, ring and lobulated fibres [20,93,99,103]. In the context of overlapping histopathological features, the presence of extraocular muscle involvement may cause diagnostic confusion with recessive *RYR1*-related MmD. Two other conditions combining ocular involvement, contractures within the DA spectrum and features of a CM are recessively inherited ***ECEL1-related CM*** [104-108] and

dominantly inherited ***PIEZO2-related CM*** [109] (also classified as DA5)**,** both associated with cores and increased internal nuclei on muscle biopsy. A recessive ***SCN4A-related CM*** due to homozygous or compound heterozygous mutations in *SCN4A* [110], encoding the α-subunit of the skeletal muscle voltage-gated sodium channel (Nav1.4) and previously associated with dominantly inherited myotonia and periodic paralysis has been recently described associated with a wide spectrum, from severe *in utero* (often early lethal) presentations to neonatal-onset conditions of variable severity. The phenotype is mainly characterised by hypotonia, facial and neck weakness, respiratory and swallowing difficulties and early-onset spinal deformities but interestingly, no clinical or electrophysiological evidence of myotonia. Mutations in the same gene have also been associated with a presentation featuring severe neonatal laryngospasm [111]. Histopathological features are characterized by a combination of increased fibre size variability and variable increases in fatty tissue, but do usually lack more distinct structural abnormalities[110]. Many of the genetic backgrounds implicated in the CMs – in particular *RYR1*, *TTN* and *DNM2* – may show marked increases in fat and connective tissue, ***features mimicking a congenital muscular dystrophy*** [112,113].

CMs with unusual or other features: Some of the genes associated with NM - *TPM2, TPM3, ACTA1, NEB* and *MYPN* – have also been implicated in rare myopathies with unusual histopathological features, Cap myopathy and Zebra body myopathy [73,114-116]. ***STIM1- and ORAI1-related CMs*** (for review, [117]) due to dominant gain-of-function mutations result in either tubular aggregate myopathy (TAM), a slowly progressive myopathy with variable extraocular muscle involvement, exertional myalgia and variable calf hypertrophy, or York platelet and Stormorken syndromes, related disorders with a CM, pupillary and platelet abnormalities, and variable multisystem involvement that form a clinical continuum. Recessive inheritance of loss-of-function mutations in *ORAI1* and *STIM1* lead to variable combinations of a severe combined immunodeficiency, ectodermal dysplasia and a CM, a combination reported already in the

pre-molecular era in association with minicores on muscle biopsy [118]. The ***"triadin knockout syndrome"*** due to compound heterozygosity for *TRDN* (null) mutations is a recessive cardiac arrhythmia syndrome with variable clinical and histopathological features of a CM, the latter characterized by focal dilation and degeneration of the lateral SR cisternae [119,120]; the highly variable penetrance of the myopathy associated with this entity remains however currently unaccounted for. Mutations in ***TRIM32***, ***TRIM54*** and ***TRIM63*** (encoding an ubiquitin E3 ligase and muscle-specific RING finger proteins MuRF3 and 1, respectively) have been associated with LGMD2H, sarcotubular myopathy (*TRIM32*), microtubular abnormalities and myosin-containing inclusions (*TRIM54* and *TRIM63*[121]), illustrating the increasingly fluid boundaries between the CMs and other neuromuscular disorders, in particular myofibrillar, protein aggregation and vacuolar myopathies.

PATHOGENESIS

The vast majority of the proteins implicated in the CMs have been associated with primary or secondary defects of muscle excitation-contraction coupling (ECC), intracellular calcium homeostasis and disturbed sarcomeric assembly and function (illustrated in Figure 2); other mechanisms are currently emerging.

ECC, muscle contraction and relaxation

Excitation-contraction coupling (ECC) is the process whereby an electrical signal generated by a neuronal action potential is converted into a chemical gradient, i.e. an increase in myoplasmic Ca^{++}, leading to muscle contraction. The two main players of skeletal muscle ECC are the ryanodine receptor sarcoplasmic reticulum (SR) Ca^{++} release channel (RyR1)

and the voltage sensing L-type Ca^{2+} channel dihydropyridine receptor (DHPR) (Figure 2). RyR1 is located on the SR junctional face membrane and the DHPR is located on the plasmalemma and transverse tubules, a plasmalemmal invagination running 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 a RyR1, and with every other RyR1 tetramer facing four DHPRs arranged in a characteristic checkerboard shape called a tetrad.

Apart from their principal regulation through direct interaction with DHPR, RyR1s are also regulated by Ca^{++} and Mg^{++}, and are additionally subjected to post-translational modifications (e.g. phosphorylation, sumoylation and nitrosylation) that affect the channel open probability. The junctional SR membrane contains the RyR1 as well as many other smaller proteins, including the structural proteins triadin and junctin, JP-45, the high capacity, low affinity Ca^{++} binding protein calsequestrin [122,123] in an area adjacent to RyR1, and others that play a role in the fine regulation of SR Ca^{++} release or in maintaining the structural integrity of the Ca^{++} release machinery [122,124-131].

Following Ca^{++} release from the SR, its binding to Troponin C, and direct thin-filament interaction, ***muscle contraction*** occurs in the sarcomere, a structure 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^{++} binding to troponin. The simplest model for sarcomeric Ca^{++} regulation is based on steric-blocking, where tropomyosin prevents myosin binding to the actin filament to generate force. Ca^{++} binding to troponin triggers a chain of reactions that result in azimuthal movements of tropomyosin around the filament to unmask binding sites on actin for myosin, the molecular motor and also major component of the thick filament, allowing force

production and motion [132] All these contractile proteins and related isoforms are differently expressed in slow and fast twitch muscles to fulfil different functional demands [132].

Termination of the contraction cycle and muscle relaxation is then achieved by RyR1 closure and by activation of the sarco/endoplasmic reticulum Ca^{++} ATPase (SERCA), the protein component responsible for pumping the Ca^{++} back into the SR [133]. SERCA activity can be modulated by two small regulatory proteins, sarcolipin and phospholamaban [134-136].

Although skeletal muscle ECC can occur in the presence of extracellular Ca^{++} in the nM range, there is a wide consensus that Ca^{++} ***entry from the extracellular space*** is essential to ensure prolonged muscle activity. Two main mechanisms of Ca^{++} entry have been identified in skeletal muscle, (i) excitation-coupled Ca^{++} entry (ECCE) via DHPR which is activated by a train of action potentials or prolonged membrane depolarisation, and (ii) store-operated Ca^{++} entry (SOCE) via STIM1 and Ora1 which is triggered by ER/SR store depletion [137-141].

ECC and Ca^{++} homeostasis abnormalities

Amongst the ***primary defects*** of ECC and Ca^{++} homeostasis, mutations in ***RYR1*** are the most common cause [7,18,142,143]: Based on functional studies utilizing cellular and animal models [for review [144]] [145], excessive Ca^{++} release and lower RyR1 activation thresholds are consequences of dominantly inherited MHS-associated *RYR1* mutations, whereas both SR calcium store depletion with resulting increased cytosolic calcium levels ("leaky channel" hypothesis) and disturbed EC coupling ("EC uncoupling hypothesis") have been proposed for dominantly inherited CCD [142]. Based on limited studies performed so far, quantitative reduction of RyR1 channels is a more likely mechanism than a qualitative RyR1 dysfunction in recessive *RYR1*-related myopathies [146-148]. (Secondary or primary) reduction of the Cav1.1 protein is seen in both recessive *RYR1*- and ***CACNA1S***-related CMs [95,146], the latter also showing disturbed ECC and, consequently, reduced

depolarization-induced SR Ca^{++} release in myotubes and mature muscle fibres. ***STAC3***, the gene homozygously mutated in NAM, targets Cav1.1 to the T-tubules and thus also participates in voltage-induced Ca^{++} release [149,150]. A similar mechanism is 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. Distinct alterations in store operated Ca^{++} influx have been described with dominant mutations in ***STIM1*** and ***ORAI1***, resulting in increased resting Ca^{++} levels due to constitutively active molecules mediating Ca^{++} influx independently of SR Ca^{++} levels [140,151]; the opposite effect, impaired Ca^{++} influx, is seen with recessive *ORAI1* mutations leading to reduced Orai1 expression [152].

Secondary defects of ECC and Ca^{++} homeostasis have been demonstrated in ***SEPN1***-mutated myotubes and in the SEPN1 KO mouse model [153,154], probably due to RyR1 redox modifications. Many of the genes implicated in CNM – ***MTM1*** [40], ***DNM2*** [41], and ***BIN1*** [44] – code for proteins that have an important role in intricately linked intracellular membrane trafficking pathways and may thus indirectly affect muscle Ca^{++} handling and ECC, probably secondary to abnormalities of triad assembly and the ECC machinery (for review, [155]). Although such abnormalities have been demonstrated in mouse models of both *DNM2*- and *MTM1*-related myopathies [156], a recent study on *MTM1*-mutated human myoblasts failed to demonstrate any alterations in ECC and Ca^{++} release, indicating that those alterations may reflect long-term effects *in vivo* [157]. Lastly, pathogenicity of ***TTN*** mutations, although probably multifactorial, is also likely to include several mechanisms implicated in ECC, including calpain-3 mediated RyR1 recruitment to the triad, and obscurin-mediated interactions between the T-tubules, SR and the sarcomere.

Abnormalities of sarcomeric assembly and function

The majority of the genes implicated in NM to date – *NEB* [59], *ACTA1* [61], *TPM2* [158], *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 [reviewed in [159]]: Mediated through lowered Ca^{++} sensitivity, dominant *ACTA1* mutations exert a dominant negative effect on muscle function, whereas recessive *ACTA1* mutations abolish functional protein expression, with phenotype severity probably reliant on the expression of compensatory proteins such as ACTC [160,161]. Rarely, ACTA1 may result in enhanced muscle contractility[162,163]. *NEB* mutations affect the specific role of nebulin in thin filament regulation and force generation [164]. The specific effects of various NM-associated mutations on nebulin interactions with actin and tropomyosin [165], thin filament length and force generation [166] has been demonstrated in two recent studies *in vitro*. *MYO18B* recently found to be mutated in one family with a severe form of NM[75] encodes an unconventional myosin with a more general role in sarcomeric assembly and maintenance[167,168].

Many of the genes more recently implicated in NM - *KBTD13*, *KLHL40*, *KLHL41* and *LMOD3* – encode a group of Kelch- and associated proteins that are not primary thin filament components but that are involved in muscle quality control processes [169] and may thus affect myofibrillar assembly and function indirectly. Evidence for a direct interaction between KLHL40, nebulin and leiomodin 3, respectively, has been recently provided [170]. The myosinopathies [99], disorders of the thick filament, are likely to cause muscle disease by two principal mechanisms, disturbed thick filament interaction and function, and, in particular in *MYH7*-related CMs [99], aggregation of abnormal protein.

Other pathogenic mechanisms implicated in the CMs

Whilst some of the proteins implicated in the CMs are very specifically involved in ECC and calcium homeostasis, others have (putative) additional roles in and beyond muscle. Selenoprotein N encoded by *SEPN1* is a member of a protein family mediating the various biological effects of selenium and in muscle has been specifically implicated in ***myogenesis***, a role shared with MEGF10 mutated in a rarer form of MmD[23], and ***redox regulation*** [171,172]. The important role of normally functioning redox regulation for muscle health is also illustrated by the recent identification of recessive mutations in the oxireductase *PYROXD1* as a cause of early-onset congenital myopathies [98]. Reflective of 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 desmin cytoskeleton, satellite cell activation and the neuromuscular junction (for review, [155]). Abnormalities of muscle membrane systems have also been described in association with canine *HACD1/PTPLA*-related CNM[86,87], a naturally occurring animal model of a non-specific congenital myopathy recently described in humans[173]. The CNM-associated genes *MTM1* and *DNM2* have now also been implicated in pathways that may affect muscle protein turnover and/or ***muscle growth and atrophy pathways***: Disturbances of the autophagy pathway have been reported in zebrafish and mouse models of myotubularin deficiency, associated with atrogin upregulation and atrophy [174-176]. Abnormalities of autophagosome maturation and autophagic flux have also been described in a mouse model of *DNM2*-related CNM, associated with marked muscle atrophy and weakness [177]. Autophagy and other degradation pathways may be also be affected in *TTN*-related CNM, through abrogation of calpain-3 mediated protein turnover with C-terminal truncating *TTN* mutations or its links with the ubiquitin ligase myospryn [178], or through disruption of the link between the kinase domain and the autophagy cargo adaptors Nbr1 and SQSTM1 by M-band disrupting *TTN* mutations [25]. Intriguingly, the typical

histopathological appearance of CNM has now also been reported in primary disorders of autophagy[179,180], 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 recently reported in *RYR1*-related myopathies [181] but may also be relevant for other congenital myopathies [157]. How mutations in *ECEL1, PIEZO2* and *SCN4A* cause specific early-onset CMs is currently uncertain.

DIAGNOSTIC APPROACH

A structured diagnostic approach to the CMs is summarized in [182]. Whilst many features on ***clinical assessment*** – weakness and hypotonia pronounced axially – are consistent but non-specific, others, in particular the degree of distal, extraocular muscle, cardiac and respiratory involvement, may indicate specific genetic backgrounds. Useful ***laboratory investigations*** include serum CK levels, typically normal or slightly elevated, and acetylcholine receptor (AChR) antibodies, to exclude autoimmune myasthenic conditions [183]. ***Neurophysiological studies*** including electromyography (EMG) and nerve conduction studies (NCS) are mainly useful for excluding congenital neuropathies, myotonic disorders [111] or congenital myasthenic syndromes [184]. ***Muscle imaging*** (for review [6])**,** in particular muscle ultrasound (US) as a screening test and muscle magnetic resonance imaging (MRI) for a more detailed assessment, may reveal diagnostic patterns of selective muscle involvement. ***Muscle biopsy*** assessment with a standard panel of histological, histochemical and immunohistochemical stains (for review, [13]) will confirm the specific CM, and exclude distinct conditions with overlapping pathological features such as the congenital muscular dystrophies (CMDs) [185], myofibrillar myopathies (MFMs) [186] and autophagic vacuolar myopathies (AVMs) [187]. Electron

microscopy (EM) is very helpful to clarify the pathognomic structural abnormalities seen with light microscopy. Concomitant analysis of multiple CM-associated genes through NGS is rapidly becoming the preferred diagnostic approach. Functional studies will become increasingly relevant for pathogenicity assessment of variants in large genes such as *TTN*, *NEB* and *RYR1*, in which genetic variants of uncertain significance are not uncommon even in healthy control populations.

MANAGEMENT AND THERAPY DEVELOPMENT

Supportive management (outlined in detail in [188]) is based on a multidisciplinary approach: Regular physiotherapy and provision of orthotic support is beneficial to prevent contracture development and to maintain mobility. Dysarthria and feeding difficulties will benefit from regular speech language therapy input; in some cases bulbar involvement and poor weight gain may require gastrostomy insertion. Regular respiratory function monitoring (including sleep studies) and proactive respiratory management (including timely non-invasive ventilation and cough assistance techniques) are mandatory particularly in forms where substantial respiratory involvement, often out of proportion to the degree of limb girdle weakness, is recognized. Regular cardiac monitoring is crucial in CMs with consistently associated cardiomyopathies (in particular *TTN*- and *MYH7*- related forms), but also in individuals where the genetic defect is uncertain. Considering often complex comorbidities, orthopaedic (in particular scoliosis) surgery should be undertaken at a tertiary neuromuscular centre. MHS has to be anticipated in the anaesthetic management of *RYR1*- and *STAC3*-mutated patients and those with unresolved genetic backgrounds.

Already available or currently developed therapies for the CMs detailed below are reviewed in [189].

Genetic therapies: Due to their enormous size, *viral-based gene transfer* is unsuitable for most genes commonly implicated in the CMs. However, delivery of *MTM1* through an AAV8-based vector has been demonstrated to improve the clinico-pathological phenotype in Mtm1-deficient mice and a canine model of XLMTM [173,190]. Restoring the mRNA reading frame is in theory applicable to various CMs where nonsense mutations are implicated. *Exon skipping* has been successfully applied *in vitro* to remove the incorporation of a pseudo-exon in the mRNA of a child with a recessive *RYR1*-related myopathy [191]. Considering that carriers of truncating *RYR1* mutations are asymptomatic [191,192], mutant gene selective silencing may also become feasible therapeutic strategy for dominant *RYR1*-related myopathies in future. *Pharmacological suppression of stop codons* [193] with compounds such as PTC124 (Ataluren) is a potential approach in CMs where nonsense mutations are involved, although it is currently uncertain if such an approach will increase normal protein levels sufficiently to restore structural integrity and function, and what the effects on the many loss-of-function variants in the human genome [194] will be. *Down- or upregulation of genes acting in related pathways* may become particular relevant for different forms of CNM: Recent studies demonstrate that *dynamin 2 downregulation* [195], or targeting of class II and III PI3 kinases in muscle [196] can rescue the phenotype in XLMTM animal models, suggesting pharmacological modification of intricately linked pathways a potential treatment modality for XLMTM and, possibly, other forms of CNM. Upregulation of cardiac actin may be a therapeutic approach for patients with *ACTA1* null mutations [197,198].

Enzyme replacement therapy is currently only relevant to XLMTM due to loss of myotubularin function, where in *Mtm1 KO* mice improvements of contractile function and histopathological features have been observed following short term myotubularin enzyme replacement [199].

Pharmacological therapies potentially applicable to the CMs can be grossly divided into 3

principal approaches: i) direct modification of altered protein function (for example modification of RyR1 release in *RYR1*-related myopathies) or ii) enhancement of thin-thick filament interactions (for example, in some NMs), and iii) those aimed at non-specifically ameliorating downstream effects of the specific gene mutation. *Modification of RyR1 Ca^{++} release* through the specific RyR1 antagonist Dantrolene [200] is the established emergency treatment for MH but has also been effectively used in few patients with *RYR1*-related ERM [35,201] and CCD [202,203]. Other compounds with the potential to treat excessive SR Ca^{++} release and/or increased SR Ca^{++} leak are the calstabin-stabilizing 1,4-benzothiazepine derivatives JTV519 and S107 ("Rycals") (for review, [204,205]) and the AMPK activator AICAR (or 5-aminoimidazole-4-carboximide ribonucleoside) [206,207], however, safety profiles of these compounds in humans and their roles in *RYR1*-related myopathies associated with reduced rather than enhanced calcium conductance are currently uncertain. *Enhancement of filament interactions* and promotion of force production [208,209] (either by slowing the rate of calcium release from troponin C or directly targeting myosin molecules) are potentially valuable for some NMs, however, concerns remain concerning fibre type specificity and/or potential cardiac side-effects of the molecules utilized. *Modification of downstream effect of specific gene mutations* comprises various approaches: Inhibition of myostatin, an important negative regulator of muscle fibre size [210], may be applicable to CMs where fibre atrophy is prominent. Based on the observation of increased oxidative stress and a favourable response to these compounds in animal models [154,211,212], antioxidants such as *N-acetylcysteine (NAC)* are currently being investigated in clinical trials concerning *RYR1*- and *SEPN1*-related myopathies. Based on neuromuscular junction/transmission abnormalities in CNM, *RYR1*-related MmD and *KLHL40*-related NM [213-216], acetylcholinesterase inhibitors have been used with some benefit in a small number of patients. Two other compounds where an apparent benefit was demonstrated in two small open label pilot studies are salbutamol in core

myopathies [217-219] and, also supported by pre-clinical data from a relevant animal model [220], L-Tyrosine in NM [221]. For those disease entities where misfolded proteins or domains play unequivocal primary roles in the disease process (e.g. titin in AR MmD-HD), the development of compounds acting as "chemical chaperones" might bear promise: A pharmacochaperone approach, using the small amphipathic compound 4-phenylbutyrate, was recently shown to alleviate some of the pathological features in a mouse model of *PLEC*-associated epidermolysis bullosa simplex with muscular dystrophy (EBS-MD) [222], although it is uncertain if the observed effect was due to stabilisation of misfolded mutant protein, or its clearance through autophagy induction by the "pleiotropic" drug 4-phenylbutyrate (4PBA) [223,224]. A beneficial effect of 4PBA has recently also been suggested in a mouse model of a *RYR1*-related myopathy [225]. The range of chemical chaperones is increasing rapidly [226], but their effective concentrations (IC_{50}) are often still very low [227], 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 clinico-pathological spectrum of the CMs, which – in addition to the "classical" entities CCD, MmD, CNM and NM – now encompass a wide range of early-onset non-dystrophic neuromuscular disorders with variable combinations of structural defects. CMs due to mutations in *RYR1*, the most common genetic cause, show a continuum with intermittent induced myopathies – MH and (exertional) rhabdomyolysis – in otherwise healthy individuals, whilst there is substantial overlap with the distal arthrogryposis and protein aggregation myopathy spectrum particularly in forms where sarcomeric proteins are implicated. Unravelling the underlying molecular mechanisms has not only advanced the understanding of the CMs but also our

knowledge of normal muscle physiology and homeostasis: Whilst the primary genetic defects and principal pathogenic mechanisms have been largely elucidated, downstream effects on muscle growth and atrophy pathways, the role of (genetic) modifiers and the molecular basis for the common histopathological features remain largely uncertain. Specific therapies utilizing multiple – including 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.

FIGURE AND TABLE LEGENDS

FIGURES

Figure 1

Muscle pathology in congenital myopathies, illustrating the key pathological features of the congenital myopathies, central cores, multiple minicores, central nuclei and nemaline rods. *RYR1*–related Central Core Disease (CCD) (a-c), *SEPN1*–related Multi-minicore Disease (MmD) (d-f), Centronuclear Myopathy (CNM) (g-i *MTM1*- and j-l *DNM2*-related), and *ACTA1*–related Nemaline Myopathy (NM) (m-o). Muscle biopsies stained with haematoxylin and eosin (H&E) (a,d,g,j,m), NADH-TR (b,e,h,k), modified Gomori Trichrome (n), slow myosin heavy chain (c,f,i,l), and myosin ATPase pH 4.6 (o). **a)** Child with dominant *RYR1*-related CCD shows myopathic fibre size variation and marked perimysial fatty infiltration. **b)** Most fibres contain a single central or eccentric 'core' with a well-delineated zone of diminished or absent oxidative staining; some of these also show a rim of enhanced oxidative staining surrounding the core lesion. **c)** There is uniformity of type I/slow fibres. **d)** Adolescent with recessive *SEPN1*-related MmD shows myopathic fibre size variation and perimysial fatty infiltration. **e,f)** Fibre typing is preserved with predominance of type 1/slow fibres, and both type I and type II fibres display foci areas of diminished or absent oxidative staining (multi-minicores) and occasionally larger lesions. **g)** Male neonate with severe X-

linked recessive myotubular myopathy (XLMTM) shows centrally placed nuclei in a large number of fibres. **h)** The majority of fibres display pale peripheral halos and **(i)** type I/slow fibres are predominant. **j)** Adult with *DNM2*-related CNM shows marked increase in central nucleation and perimysial fatty infiltration. **k)** Many fibres display 'radial strands' radiating from a centrally placed nucleus. **l)** There is type I/slow fibre predominance and hypotrophy creating fibre size disproportion; central nuclei are present in both fibre types. **m)** Severely affected neonate with *de novo* dominant *ACTA1*-related NM shows myopathic fibre size variation with an appearance of two fibre populations mostly of smaller type I and larger type II fibres (see o). **n)** Numerous thread-like inclusions are seen in both fibre sizes and appear red with the modified Gomori trichrome and eosinophilic with haematoxylin and eosin (m). **o)** Pale stained type I fibres are often more severely affected and atrophic/hypotrophic. Scale bar: (a-f, i-m, o = 100 µm; g,h,n = 10 µm)

Figure 2

Subcellular localization of the main proteins implicated in skeletal muscle excitation-contraction coupling (ECC), thin-thick filament interaction and assembly. Mutations in genes encoding components of the ECC machinery and thin-thick filaments of skeletal muscle are commonly mutated in the congenital myopathies. The transverse tubules are invaginations of the plasma membrane where the DHPR complex (containing STAC3) is located. This membrane compartment faces the sarcoplasmic reticulum (SR) junctional face membrane (JFM), containing the ryanodine receptor calcium release channel (RyR1) as well as JP-45 and the strucutural proteins triadin and junctin. Calsequestrin bound to calcium forms a mesh-like structure within the lumen of the SR terminal cisternae. JP-45 also interacts with calsequestrin via its lumenal carboxy-terminal domain. Calcium release into the cytosol results in sarcomeric shortening through specific interactions between thin-thick

filaments, in particular sliding of actin past myosin filaments. The ECC is terminated through SR calcium re-uptake through SERCA calcium pumps. SERCAs are present in the terminal cisternae as well as the longitudinal SR, and are regulated by phospholamban, myoregulin and sarcolipin. The calcium-bufferring protein sarcalumenin is also located in the longitudinal sarcoplasmic reticulum and terminal cisternae and is also involved in regulating SERCA activity. (Objects not to scale). Image kindly provided by Christoph Bachmann, Departments of Anesthesia and Biomedicine, Basel University Hospital, Basel, Switzerland.

TABLES

Table 1

Genes implicated in the congenital myopathies and related conditions. Genes that are most commonly implicated in the "classical" structural congenital myopathies (and their most commonly associated histopathological features) are highlighted in bold. AD = autosomal-dominant; AR = autosomal-recessive; CM = Congenital myopathy (non-specific); CCD = Central Core Disease; MmD = Multi-minicore Disease; CNM = Centronuclear Myopathy (CNM); XLMTM = X-linked myotubular myopathy; NM = Nemaline Myopathy; CFTD = Congenital Fibre Type Disproportion; KDS = King-Denborough syndrome (KDS); NAM = North American Myopathy (NAM); TAM = Tubular Aggregate Myopathy (TAM); MSM = Myosin Storage Myopathy; DA = Distal Arthrogryposis; EOM = extraocular muscle involvement; CN = central nuclei;

Table 2

Genetic, clinical and pathological features associated with different genetic backgrounds commonly implicated in the congenital myopathies. *RYR1* = skeletal

muscle ryanodine receptor gene; *SEPN1* = selenoprotein N gene; *TTN* = titin gene; *MTM1* = myotubularin gene, *DNM2* = dynamin gene, *NEB* = Nebulin gene, *ACTA1* = skeletal muscle α-actin gene; *KLHL40* = kelch-like family member 40. - = not reported, + = infrequent, ++ = common and +++ = very common. a = right ventricular impairment secondary to respiratory involvement. b = includes both congential cardiac defects and acquired cardiomyopathies.

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Table 1

Gene symbol	Chromosome location	Protein	Condition	Inheritance
Proteins involved in SR calcium release, ECC and/or triadic assembly				
RYR1	19q13.1	Ryanodine receptor 1 (skeletal)	**CCD** **MmD** CNM CFTD KDS	AD, AR AD, AR AR AR AR, AD
STAC 3	12q13.3	SH3 and Cystein-rich domain 3	NAM	AR
ORAI1	12q24.31	Calcium release-activated calcium modulator 1	TAM	AD
STIM1	11p15.4	Stromal interactin molecular 1	TAM Stormorken syndrome	AD AD
MTM1	Xq28	Myotubularin	XLMTM	X-linked
BIN1	2q14	Amphiphysin	**CNM**	AR, AD
DNM2	19p13.2	Dynamin 2	*CNM*	AD
SPEG	2q35	SPEG complex locus	CM with CN and cardiomyopathy	AR
CCDC 78	16p13.3	Coiled-coil domain containing protein 78	CM with cores and CN	AD
CACNA1S	1q32	Calcium channel, voltage-dependent, L type, alpha 1S subunit	CM with EOM	AD, AR
SEPN 1	1p36.13	Selenoprotein N1	**MmD** CFTD	AR AR
Proteins involved in thick-thin filament assembly and interaction, myofibrillar force generation and protein turnover				
NEB	**2q22**	**Nebulin**	**NM**	**AR**
ACTA 1	1q42.1	Alpha actin, skeletal muscle	**NM** CFTD Cap myopathy	AD, AR AD, AR AD, AR AD, AR
TNNT 1	19q13.4	Slow troponin T	NM	AR
TPM2	9p13	Tropomyosin 2 (beta)	**NM** Cap myopathy DA1A DA2B Escobar syndrome	AD AD AD AD AR
TPM3	1q21.2	Tropomyosin 3	**NM** CFTD Cap myopathy	AD AD AD
MYH2	17p13.1	Myosin, heavy polypeptide 2, skeletal muscle	CM with EOM	AD, AR
MYH3	17p13.1	Myosin, heavy polypeptide 3, skeletal muscle, embryonic	DA 2A, 2B and 8	AD
MYH7	14q12	Myosin, heavy polypeptide 7, cardiac muscle, beta	CFTD MmD MSM	AD AR AR
MYH8	17p13.1	Myosin, heavy polypeptide 8, skeletal muscle, neonatal	Trismus, pseudocamptodactyly syndrome Carney complex	AD AD
KBTBD13	15q22.31	Kelch repeat and BTB (POZ) domain containing 13	NM with cores	AD

KLHL 40	2p22.1	Kelch-like family member 40	**NM**	AR
KLHL4 1	2q31.1	Kelch-like family member 41	NM	AR
LMOD 3	3p14.1	Leiomodin 3 (fetal)	NM	AR
MYBP C3	11p11.2	Cardiac myosin binding protein-C	CM with cardiomyopathy	AR
MYPN	10q21.3	Myopalladin	NM with cardiomyopathy	AR
TTN	2q31	Titin	**CNM** MmD	AR AR
Proteins involved in other cellular processes or with unknown functions				
CFL2	14q12	Cofilin 2 (muscle)	NM with cores	AR
CNTN 1	12q11-q12	Contactin-1	CM lethal	AR
ECEL 1	2q37.1	Endothelin converting enzyme-like protein 1	DA5	AR
PIEZO 2	18p11.21-22	Piezo-Type mechanosensitive ion channel component 2	Marden-Walker syndrome DA3 DA5 DA with impaired proprioception	AD AD AD AR
MEGF 10	5q23.2	Multiple EGF-like-domains 10	CM with minicores CM with areflexia, respiratory distress and dysphagia	AR AR
HACD 1	10p12.33	Protein tyrosine phosphatase-like (3-Hydroxyacyl-CoA dehydratase	CM	AR
SCN4 A	17q23.3	Sodium channel, voltage gated type IV, alpha subunit	CM	AR
TRIM3 2	9q33.2	Tripartite motif-containing 32	Sarcotubular myopathy	AR
PYRO XD1	12p12.1	Pyridine nucleotide-disulfide oxidoreductase domain-containing protein	CM	AR

Table 2

Gene	***RYR1***	***RYR1***	***SEPN1***		***MTM1***	***DNM2***	***NEB***	***ACTA1***	
Frequency	+++	+++	++	++	++	+	++	++	+

Onset									
- Infancy	++	+++	+		+++	+	+++	++	+++
- Childhood	+++	++	+++	+	+	+	+	++	+
- Adulthood	++	+	-	-	-	+++	-	-	-
Clinical									
- EOM	+	+++	-	-	+++	+++	-	-	++
- Bulbar	+	+++	++	++	+++	++	++	++	+++
- Distal	-	+	-	++	+	+++	++	+	+
- Respiratory	+	++	+++	++	+++	+	++	++	+++
- Cardiac	-	+	+[a]		-	-	-	+	
- Contractures	+	+	+		+++	++	++	++	+++
Histopathology									
- Cores	+++	+++	+++	++	-	+	+	+	
- Central nuclei	++	++	-		+++	+++	-	-	
- Nemaline rods	+	+	-	+	-	-	+++	+++	+++
- FTD	+	+++	+	+	+	-	-	+	
- Connective	++	++	++		-	+	-	-	