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Therapeutic aspects in congenital myopathies

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Abstract (150 words)

The congenital myopathies are a genetically heterogeneous and diverse group of early-onset, non-dystrophic neuromuscular disorders. Whilst the originally reported "classical" entities within this group - Central Core Disease (CCD), Multi-minicore Disease (MmD), Nemaline Myopathy (NM) and Centronuclear Myopathy (CNM) – were defined by the predominant finding on muscle biopsy, "novel" forms with multiple, subtle and unusual histopathological features have been described more recently, reflective of an expanding phenotypical spectrum. The main disease mechanisms concern excitation-contraction coupling, intracellular calcium homeostasis and thin/thick filament interactions.

Management to date has been mainly supportive. Therapeutic strategies currently at various stages of exploration include genetic interventions aimed at direct correction of the underlying genetic defect, enzyme replacement therapy and pharmacological approaches, either specifically targeting the principal effect of the underlying gene mutation, or addressing its downstream consequences more generally. Clinical trial development is accelerating but will require more robust natural history data and tailored outcome measures.

Introduction

The congenital myopathies (CMs) are a group of genetically heterogeneous, non-dystrophic conditions with distinct histopathological and highly variable clinical features (for review,[1]). Although onset is typically from birth or early infancy, an extremely wide spectrum of severity has been recognized, ranging from profoundly severe presentations within the fetal akinesia spectrum to milder forms with onset in adolescence or even in adulthood. Whilst most of the CMs are rare, as a group they are not uncommon and associated with a substantial individual and societal disease burden.

Reflective of the complex disease associations often involving cardiac, respiratory and orthopaedic manifestations, management has been mainly based on a multidisciplinary approach involving various medical specialties and allied health professionals [2]. Whilst such an approach has been highly effective in improving life expectancy and quality of life, there is currently no cure. However, in line with other neuromuscular disorders, therapy development aimed at correcting or ameliorating the underlying genetic defects is accelerating rapidly, and approaching the clinical trial stage. Speed of therapy development in the CMs is influenced by clinical urgency, but also the complexity, structure and function of the defective proteins, often affecting their suitability for different therapeutic strategies (for review, [3]). Corresponding to challenges in other early-onset conditions, clinical trial development is hampered by the rarity and clinical heterogeneity of individual disorders, and the resulting lack of robust natural history data and feasible outcome measures.

The following review will give an overview of the major CMs, their main clinico-pathological features and the most relevant underlying defects, with an emphasis on

their suitability for therapeutic modification. Key management principles will be briefly outlined and the most relevant therapeutic strategies summarized. Challenges of clinical trial design and areas for future research will be highlighted.

The congenital myopathies

The previously well-established concept of the congenital myopathies is currently in flux, with important implications diagnosis, management but also therapy development (for review [1]): Originally described in the 1950s and 1960s, the major disorders within this group – Central Core Disease (CCD), Multi-minicore Disease (MmD), Nemaline Myopathy (NM) and Centronuclear Myopathy (NM) – were classified based on the predominant histopathological feature on muscle biopsy and initially considered distinct and mutually exclusive entities. However, mainly prompted by the massively accelerated gene discovery over the last decade, this concept has been challenged, and more fluid boundaries, both between "specific" CMs but also between the CMs and other neuromuscular disorders, have been recognized: With mutations in more than 30 genes identified to date, it has become evident that different mutations in the same gene (for example, the gene encoding the skeletal muscle ryanodine receptor, *RYR1*) may give rise to a wide range of different, histopathologically defined entities (Figure 1), whereas mutations in different genes (for example, those implicated in NM) may give rise to the same CM. Whilst the CMs at the point of their original description were considered "pure" histopathological entities, it has now also become apparent that those with multiple histopathological features in the same muscle biopsy – cores, nemaline rods and central nuclei – are at least as, if not more common. Moreover, recent studies based on unbiased next generation approaches suggest a hitherto

unexpected overlap with other neuromuscular conditions, in particular those due to mutations in sodium and calcium channel genes associated with periodic paralysis and/or myotonia. Taken together, these observations suggest that whilst the "classical" entities may be relatively rare, the CMs in a wider sense may be much more common than previously estimated.

The rapid genetic resolution and the changing concepts concerning the CMs are of immediate relevance for management and therapy development: For example, as certain complications such as an associated cardiomyopathy are more dependent on the genetic background than on the histopathological diagnosis, identification of the specific gene defect will inform an appropriate management plan and anticipatory health surveillance more reliably than the specific muscle biopsy features. Disease mechanisms may vary considerably, not only between different genes but also for different mutations in the same gene, a notion that must be taken into account with regards to the development of therapeutic strategies. Moreover, considering that mutations in the same gene may give rise to distinct CMs, gene-focussed therapy approaches may be of potential benefit for more than one entity. Finally, the recently observed overlap with certain forms of myotonia and periodic paralysis suggests that already well-established treatments for these conditions may also be effective in the CMs, and that, vice versa, novel CM treatments may be of benefit in other neuromuscular disorders.

The "classical" congenital myopathies

Central Core Disease (CCD) and ***Multi-minicore Disease (MmD)***, often summarily referred to as the "core myopathies" [4], are the most common CMs [5]. The principal histological abnormality, focal reduction of oxidative stain ("cores"), is the same in CCD and MmD, but there are important differences both with regards to the number of cores on transverse sections and there longitudinal extent. CCD, one of the first CMs to be resolved in the early 1990s [6], has been mainly attributed to dominant mutations in the skeletal muscle ryanodine receptor *(RYR1)* gene. MmD, on the other hand, is due to recessive mutations in *RYR1* [7], *SEPN1* encoding selenoprotein N [8] and, less frequently, *MYH7* encoding beta myosin heavy chain 7 [9] and *TTN* encoding titin [10]. Depending on genetic background and mode of inheritance, different clinical manifestations have to be anticipated in the management of these conditions: Whilst generally a relatively mild condition without significant bulbar, cardiac or respiratory involvement, congenital dislocation of the hips, scoliosis and tendon Achilles contractures are frequently seen in dominantly inherited CCD (for review, [4]). *SEPN1*-related MmD [8], on the other hand, is characterized by a rapidly progressive scoliosis and severe respiratory impairment, often necessitating spinal fusion and non-invasive ventilation by early adolescence. A primary cardiomyopathy has only been observed in MmD related to recessive mutations in *TTN* and *MYH7* [9,10]. Extraocular muscle involvement is mainly a feature in *RYR1*-related MmD. Muscle MRI may help to differentiate genetically distinct core myopathies [11].

Centronuclear myopathy (CNM) (for review, [12]) is characterized by the abundance of internalized and centralized nuclei on muscle biopsy, with variable additional histopathological features depending on the genetic subgroup. With X-linked

myotubular myopathy (XLMTM) due to X-linked recessive mutations in myotubularin [13], the CNMs include one of the most severe CMs, with marked hypotonia and bulbar involvement, profound respiratory impairment often necessitating ventilation and associated with high mortality. Other relatively common CNM-related genetic backgrounds - autosomal-dominant mutations in *DNM2* encoding dynamin 2 [14] and *BIN1* encoding amphiphysin 2 [15], and autosomal-recessive mutations in *RYR1* [16], *BIN1* [17] and *TTN* [18] – usually give rise to milder presentations but may mimic the XLMTM phenotype in exceptional cases. *TTN*-related CNM is the only major form that may feature a primary cardiomyopathy, but unlike all other major subgroups does not have any extraocular muscle involvement. The *DNM2*-related form may show some overlap with an allelic form of CMT, as well as other non-muscular manifestations that may also occur in longterm survivors with XLMTM [19].

Nemaline myopathy (NM), characterized by thread-like structures ("nemaline rods") on muscle biopsy that appear dark with the Gomoeri trichrome stain (for review, [20] (Dubowitz, Sewry et al. 2013)), is the most genetically diverse CM and has been associated with mutations in more than 10 genes. The most common form is due to recessive mutations in *NEB* encoding nebulin [21], and characterized by onset in infancy with hypotonia and often pronounced feeding difficulties, with distal lower limb involvement, scoliosis and respiratory involvement developing in childhood or adolescence [22]. *(De novo)* dominant mutations in *ACTA1* [23], encoding skeletal muscle alpha actin and the second most common cause, give rise to severe neonatal presentations comparable to XLMTM, but milder presentations with later onset have also been reported. Other genetic backgrounds – including dominant and recessive mutations in the alpha-tropomyosin *(TPM3)* [24], the beta-tropomyosin *(TPM2)* [25] and the *KBTBD13* [26] genes, as well as recessive mutations in slow troponin T *(TNNT1)* [27],

cofilin-2 *(CFL2)* [28], and myopalladin *(MYPN)* [29] are less frequent, often limited to few families or distinct ethnic backgrounds.   Recent next generation studies into antenatally lethal neuromuscular disorders suggest NM due to recessive mutations in *KLHL40* [30]*, KLHL41* [31] and *LMOD3* [32] also as a relatively common histopathological association of the fetal akinesia sequence. Progressive proximal and neck weakness, gait abnormalities, poor exercise tolerance and a peculiar slowness of movements are the hallmark of *KBTBD13*-gene related NM [26], which is quite distinct from other forms. Many of the NM-associated genes have also been implicated in distal arthrogryposis (DA) syndromes (for example, [33]), a reflection of the distal involvement which is common throughout genetically distinct forms of NM. Extraocular muscle involvement is not a typical feature, except for some cases within the fetal akinesia spectrum. As in the core myopathies, muscle MRI may aid distinction between different genetic forms of NM [34].

"Novel" congenital myopathies

Reflective of the increasingly unbiased approach to the genetic investigation of patients with unresolved congenital myopathies through large-scale next generation sequencing, an expanding number of entities has been described with suggestive clinical but not always the histopathological features previously considered to be typical (for review, [1]). Within this group of "novel" congenital myopathies, there is also considerable overlap with other neuromuscular disorders, in particular the periodic paralyses, myotonias, myofibrillar and myosin storage myopathies.

Congenital myopathies with non-specific, multiple or rare histopathological features: A substantial number of patients have been reported with mutations in

known CM-associated genes but only non-specific histopathological features rather than the previously reported, more distinct structural abnormalities (for review, [1]); these non-specific features include type 1 predominance or uniformity and congenital fibre disproportion (CFTD), i.e. often predominant type 1 fibres that are significantly and consistently smaller than type 2 fibres. Some CM-associated genes, for example *RYR1, DNM2* and *TTN*, may mimic a congenital muscular dystrophy. Certain genetic backgrounds may also give rise to combinations of structural abnormalities – cores, rods, central nuclei – rather than the "pure" histopathological presentations as summarized above. Some very rare histopathological features, caps and zebra bodies, have also been attributed to mutations in NM-associated genes.

Mutations in *CACNA1S*, previously associated with dominantly inherited periodic paralysis and, less frequently, malignant hyperthermia (MH) [35,36], have recently also been implicated in CM phenotypes. **CACNA1S-related myopathy** [37] exists in both recessive and dominant forms, and histopathological and clinical features show considerable overlap with (recessive) *RYR1*-related myopathies. Indeed, the recognition of CMs as a manifestation of *CACNA1S* mutations and of periodic paralysis as a manifestation of *RYR1* mutations, respectively, indicates a consistent phenotypical spectrum of mutations in genes encoding essential components of the excitation-contraction coupling (ECC) machinery (see below). **STIM1- and ORAI1-related CMs** (for review, [38]) are multisystem disorders associated with both dominant and recessive inheritance whose neuromuscular manifestations comprise tubular aggregate myopathy (TAM) but also considerable overlap with the "classical" CMs. Recessively inherited, **PYROXD1-related CM** [39] features histopathological features of increased internalized nuclei and myofibrillar disorganization and clinical symptoms of a moderately severe CM.

Hereditary myosin myopathies ("myosinopathies") (for review, [40]) comprise a wide range of neuromuscular phenotypes. Amongst those, both dominantly inherited *MYH7-* and recessively inherited *MYH2*-related myopathies may feature cores on muscle biopsy and mimic MmD. There is also substantial overlap between hereditary myosinopathies and the DA spectrum. Two other recently described entities with histopathological features resembling core myopathies and clinical features in between the CM and the DA spectrum include recessively inherited ***ECEL1-related CM*** [41,42] and dominantly inherited ***PIEZO2-related CM*** [43] (also classified as DA5). Extraocular muscle involvement is a feature in the latter as well as *MYH2*-related myopathies and may cause diagnostic confusion with the recessive *RYR1*-related spectrum. Corresponding to the recent expansion of the *CACNA1S*-related spectrum, early-onset, severe ***SCN4A-related myopathies*** [44] typically due to loss-of-function mutations are another example of the expanding phenotypical spectrum associated with a gene previously mainly implicated in relatively mild, dominantly gain-of-function inherited forms of periodic paralysis and myotonia.

Congenital myopathies and the malignant hyperthermia (MHS) trait

A number of genes – *RYR1*, and, less frequently, *STAC3* and *CACNA1S* – that have been implicated in the CMs have also been linked with the ***Malignant Hyperthermia Susceptibility (MHS)*** trait, a profoundly severe, pharmacogenetic reaction to volatile anaesthetics and depolarizing muscle relaxants (for review, [45]). Whilst the link with MHS is well-established for dominant *RYR1* mutations associated with exertional rhabdomyolysis/myalgia (ERM) and some mutations giving rise to CCD, the association is less clear for recessively inherited *RYR1*-related CMs such as MmD, CNM and CFTD.

However, there is a subset of (often severe, early-onset) *RYR1*-related myopathies due to compound heterozygosity for *RYR1* mutations that appear to behave as dominants with regards to the MHS trait but as recessive with regards to the CM phenotypes [46]. Other *RYR1*-related myopathies with close links to the MHS trait include the **King-Denborough syndrome (KDS)** [47], a dysmorphic syndrome with short stature and scoliosis, and a **late-onset axial myopathy** [48] in previously healthy or even particularly athletic individuals compound heterozygous for MHS mutations.

Another recently recognized myopathy with marked similarities to *RYR1*-related KDS and a high MH risk is **Native American myopathy (NAM)**, originally described in native American Indians, the Lumbee population of North Carolina, and due to homozygosity for a founder mutation (p.W284S) in *STAC3* [49].

Pathogenesis

In contrast to the (congenital) muscular dystrophies, the integrity of the muscle membrane is usually preserved in the CMs, reflected in typically normal or only moderately elevated CK levels. Common pathogenic mechanisms (Table 1) [for review, [1]] concern intracellular processes ensuring normal muscle maintenance and function, in particular excitation-contraction coupling (ECC), the process thereby an electrical neuronal impulse is translated into muscle contraction through controlled calcium release from the sarcoplasmic reticulum (SR). During ECC, voltage-induced conformational changes of the dihydropyridine (DHPR) receptor localized on the transverse tubules indirectly lead to opening of the skeletal muscle ryanodine (RyR1) receptor, the principal sarcoplasmic reticulum (SR) calcium release channel, and, ultimately, muscle contraction through ordered interactions between thin and thick filaments. The process

is then terminated through calcium reuptake into the SR by specialized ATPases, the SERCAs.

Defects in the genes encoding the key players of ECC - *RYR1* and *CACNA1S* - are amongst the most common causes of the CMs: Dominant *RYR1* mutations implicated in MHS, ERM and subgroups of CCD have been demonstrated to result in a hyperexcitable RyR1 receptor and excessive calcium release, whereas in others muscle weakness is attributed to constant calcium loss from the SR ("leaky channel hypothesis") and/or uncoupling of DHPR/RyR1 interactions [for review, [50,51]. Whilst the mechanisms underlying the more recently described recessive *RYR1*-related myopathies – MmD, CNM, CFTD – are currently less certain, those appear to involve a reduction of the functional RyR1 protein rather than malfunctioning of the individual RyR1 receptor. Dominant and recessive mutations in *CACNA1S* [37], encoding the CaV1.1 subunit of the DHPR receptor directly interacting with RyR1 in skeletal muscle and recently implicated in congenital myopathy phenotypes, have also been associated with disturbances of ECC, in particular decreased voltage-induced calcium release and reduction of the functional CaV1.1 protein. Mutations in genes encoding accessory proteins such as *STAC3* [49] probably cause weakness through their detrimental effect on the regular positioning and functioning of the ECC machinery [52], whereas mutations in *STIM1* and *ORAI1* (for review, [38]) affect two alternative pathways of intracellular calcium entry indirectly relevant for ECC, Store-Operated Calcium Entry (SOCE) and Excitation Contraction Coupled Calcium Entry (ECCE). In addition to these primary defects, secondary abnormalities of ECC and intracellular calcium homeostasis have also been reported in association in *SEPN1* [53,54] and the CNM-associated genes *DNM2* and *BIN1*. Calcium-induced thin and thick filament interactions, the molecular basis of all muscle contraction downstream of SR calcium release, are affected by mutations in the major

genes – *NEB*, *ACTA1* and the tropomyosins – implicated in NM, through structural alterations of thin and thick filaments prohibiting their regular assembly or function, and/or altering their calcium sensitivity [55-58]. Aggregation of abnormal protein is an additional factor in the myosinopathies [40], in particular those due to mutations in *MYH7*, and may play a role in other CMs primarily affecting the thin and thick filaments.

A number of additional pathomechanisms have been described in recent years, both in relation to genes implicated in the "classical" CMs but also the more recently described "novel" forms. These pathomechanisms include disturbances of myogenesis [59], alterations of redox regulation [39,60,61], and abnormalities of intracellular (membrane) trafficking and muscle (protein) quality control processes. Many of the more detailed mechanisms of how specific genetic backgrounds cause muscle weakness and wasting in the CMs, and the precise pathogenic mechanisms underlying the more recently identified gene mutations remain currently unresolved.

Management and therapy

Corresponding to other neuromuscular disorders, supportive management provided by a multidisciplinary team remains an essential aspect of the approach to the CMs and has substantially improved both quality of life and life expectancy. Whilst therapy development concerning the neuromuscular field so far has focused on the more common and severe conditions (for example, spinal muscular atrophy and Duchenne muscular dystrophy), therapies with the potential to improve or even cure the CMs are currently being developed. One of the most severe conditions within the congenital myopathy spectrum, X-linked myotubular myopathy (XLMTM) has been a particular focus of therapy development, and outcomes of ongoing clinical trials are already

expected in 2018. Therapies designed for the CMs (Table 2) (for review [3]) can be grossly divided in i) genetic therapies aimed at directly correcting the underlying genetic defect, ii) enzyme replacement therapy and iii) pharmacological therapies, including therapies that either very specifically target the principal effect of the underlying gene mutation, or address its downstream consequences in a more general way, thus being potentially applicable to a wider range of CMs rather than one specific entity only. Whilst some of these therapies as outlined below already hold considerable therapeutic promise, others are at a more conceptual stage and their eventual clinical applicability is currently far from certain. Considering that any of those is unlikely to fully correct the disease phenotype on its own and that the downstream consequences of some genetic defects are manifold, it is important to bear in mind that combined therapeutic approaches will be more likely required rather than focusing on a single approach alone. Lastly, as novel therapies will improve life expectancy in affected individuals, long-term manifestations of specific CMs potentially expanding the phenotypical spectrum will become more obvious, and are likely to require flexible adaptations of therapeutic strategies.

<u>Supportive management</u> of the CMs is not the major focus of the present paper and only the key principles are summarized below; more detailed information is available from a comprehensive, recently published review of the topic [2]. Considering often multiple comorbidities, an effectively co-ordinated multidisciplinary approach involving various medical and allied health professionals is essential for the effective management of the congenital myopathies. Supportive management principles are the same throughout different forms, with variable emphasis depending on the phenotypical manifestations of specific conditions: Joint contractures and scoliosis should be prevented and managed through regular physiotherapy input, appropriate seating and orthotic support, particularly in conditions where those features are prominent, such as

TTN- and *SEPN1*-related myopathies. With a view to potentially beneficial surgical interventions, orthopaedic input should be sought early, ideally at a tertiary neuromuscular centre experienced with the management of these conditions, including respiratory, cardiac and orthopaedic care. Dysarthria, feeding difficulties and poor weight gain are more common at the most severe end of the spectrum and in NM, and will benefit from regular speech language therapy input, dietary supplementation and gastrostomy insertion where needed. With the notable exception of dominantly inherited CCD, respiratory involvement is common throughout different forms and should prompt regular respiratory function monitoring (including sleep studies), as well as timely institution of (non-invasive) ventilation and, where beneficial, cough assist techniques. In contrast to other neuromuscular conditions, respiratory impairment does not evolve in parallel to the limb girdle weakness, and may be profound even in ambulant patients, particularly in *SEPN1-* and *NEB*-related myopathies. Although cardiac involvement is highly variable, regular cardiac monitoring should be performed at baseline in all genetically unresolved CMs, and in genetically resolved conditions where cardiomyopathies are a prominent recognized feature, particularly in *TTN-* and *MYH7*-related forms; in these forms, extension of cardiac assessment to relatives may also be warranted. A specific consideration is the potential association of CMs due to mutations in *RYR1*, and, less frequently, *STAC3* and *CACNA1S* with the MHS trait (see above) that must be anticipated for pre-operative planning in these patients.

Genetic therapies: Many of the genes mutated in the CMs such as *TTN*, *NEB* or *RYR1* are amongst the largest in humans, precluding *viral-based gene transfer* in most of these conditions, considering that AAV gene therapy approaches are unsuitable for genes with an mRNA over 4.5kb. Such an approach, delivery of the relatively smaller *MTM1* gene utilizing an AAV8 vector, has however been successfully demonstrated in two animal

models of XLMTM, the Mtm1-deficient mouse [62] and the naturally occurring Labrador retriever model of X-linked myotubular myopathy [63,64]. In both instances, viral-based gene transfer was well-tolerated, and resulted in improvement of clinico-pathological features in the murine and improved muscle strength, respiratory function and survival in the canine model. Considering that the Labrador retriever model shares many similarities with human XLMTM, these findings are encouraging, and corresponding experimental trials in children with XLMTM have recently started (NCT03199469).

CM forms due to heterozygous dominant negative or gain-of-function mutations – including (*de novo*) dominant forms of *RYR1*-, and, less frequently, *DNM2*-, *BIN1*- and *MYH7*-related myopathies - may benefit from new *gene editing strategies* [65] once those become clinically available. Based on the observation that carriers of *RYR1* null mutations are typically asymptomatic [66,67], selective silencing of the mutant allele may also become a feasible therapeutic strategy in dominant *RYR1*-related myopathies, particularly in neonatally severe forms due to *de novo* dominant mutations.

In those congenital myopathies where nonsense mutations are involved, *restoration of the mRNA reading frame* either through exon skipping (provided the reading frame is not disrupted) or through the *suppression of premature stop codons* could be considered to restore a functional protein. This latter approach has for example been applied in patients with Duchenne muscular dystrophy due to dystrophin nonsense mutation, utilizing the ability of the pharmacological compound PTC124 (or Ataluren) to increase premature stop-codon read-through [68], an ability shared with certain pharmacologically related but overall more toxic aminoglycosides. A similar approach could in theory also be applied to, for example, *TTN* and *NEB* nonsense mutations, however, it is currently uncertain if the efficacy of compounds such as Ataluren is high enough to restore the amount of functional protein sufficiently to achieve a clinical relevant improvement of the conditions

due to mutations in these genes. These considerations are particularly pertinent for proteins with a very short half- life, in contrast to stable proteins with longer half-life (such as dystrophin) that obviously provide better targets for such an approach. It is also currently uncertain how (and with what consequences) general enhancement of premature stop-codon read-through may affect the around 20 genes in the human genome that are constitutively inactivated due to loss-of-function mutations [69]. The remit of *exon skipping* in restoring the mRNA reading frame in the CMs is hampered by, in contrast to for example Duchenne Muscular Dystrophy, the relative rarity of specific genetic entities, the private nature of many of the causative mutations, and a relatively more complex structure of some of the proteins implicated that may not readily tolerate removal of whole constitutive exons. As proof-of-principle, exon skipping has, however, been successfully applied to remove a paternally inherited pseudo-exon associated with an unstable RyR1 transcript from the mRNA of a child with a recessive *RYR1*-related myopathy, resulting in increased functional RyR1 protein expression and improved myotube morphology *in vitro* [67]; unfortunately, pseudo-exon creating mutations are estimated at <2% of all *RYR1* mutations only and practical applicability of this potentially promising approach may thus be limited.

Down- or upregulation of genes acting in related pathways is a strategy currently considered for various forms of CNM and in NM secondary to recessive null mutations in *ACTA1*: The proteins encoded by 3 of the major genes implicated in CNM – *MTM1*, *DNM2* and *BIN1* – are intricately linked in the same intracellular trafficking pathways (for review, [15,70]), as demonstrated by the recent observation that *DNM2* downregulation ameliorates the XLMTM phenotype in mice [71]. Based on these observations, clinical trials aiming at *DNM2* downregulation in XLMTM and, possibly, *BIN1*-related forms of CNM, are currently at the planning stage. Targeting of class II

and III PI3 kinases acting upstream of myotubularin is another approach that has been demonstrated to improve the murine XLMTM phenotype [72] and may become a feasible therapeutic strategy in future. Patients with recessive *ACTA1* null mutations are rare and partly compensate the absence of skeletal muscle alpha-action by spontaneous upregulation of *ACTAC* encoded cardiac actin [73]. Based on these observations, cardiac alpha-actin upregulation has been investigated in 2 mouse models of *ACTA1*-related NM, the D286G and the H40Y line with however highly variable results [74] whose basis will have to be explored before this may be considered as a therapeutic option in affected humans.

Enzyme replacement therapy (ERT): *MTM1* is the only CM-implicated gene encoding a protein with predominantly enzymatic function, myotubularin, and XLMTM is thus the only CM currently considered for ERT. Mtm1d4 mice have shown improvement of histopathological features and contractile function following myotubularin ERT [75], providing the basis for human therapy trials currently in preparation.

Pharmacological therapies for the CMs are currently at highly variable stages of development and can be subdivided in those aimed at directly modifying altered protein function and those aimed at non-specifically ameliorating the often wide range of downstream effects of a specific genetic defect. Considering that some of the genes recently implicated in "novel" CMs have been previously associated with other neuromuscular disorders (for example, periodic paralyses and myotonias), it is conceivable that drugs already established for the treatment of these disorders may be repurposed for the treatment of the corresponding CMs.

Identification of pharmacological compounds aimed at effective *direct modification of altered protein function* is the ultimate aim of any therapy development but currently

at the early stages only for the CMs and other early-onset neuromuscular disorders.

Modification of RyR1 receptor calcium release has probably been most widely explored based on the longstanding experience with the RyR1 anatagonist Dantrolene in the treatment of acute MH crises, exploiting its ability to inhibit caffeine-induced ryanodine binding and to reduce the maximum rate of calcium release from the SR [76]. Case reports suggest that Dantrolene may also be effective in the treatment of *RYR1*-related CCD [77,78], *RYR1*-related rhabdomyolysis [79-81] and probably also the recently described *RYR1*-related bleeding disorder [82]. Other compounds that have attracted considerable interest are JTV519 and S107 (also known as Rycals), 1,4-benzothiazepine derivates with the ability to modify RyR1 function through their actions on the calstabins. Calstabins exist in 2 isoforms, calstabin 1 (or FK506 binding protein 12, FKBP12) mainly expressed in skeletal, and calstabin 2 (FKBP12.6) mainly expressed in cardiac muscle [83]. Calstabins exert their RyR stabilizing effect when associated with the RyR1 receptor through increasing its closed probability, and their dissociation from the receptor (for example, through metabolic modifications at times of stress) result in an increased open probability and a "leaky" channel. Rycals promote increased RyR-calstabin interactions both in skeletal muscle and the heart, and may thus be suitable for the treatment of *RYR1*-related myopathies associated with increased calcium release resulting in depleted SR stores [84]. Another compound to which a RyR1-stabilizing effect has been attributed is AICAR (5-aminoimidazole-4-carboximide ribonucleoside), a known activator of the AMP-activated protein kinase (AMPK), an energy sensor upstream of the autophagy pathway, and a recognized skeletal muscle performance enhancer [85]. In the RYR1 Y522S mouse, a murine model of *RYR1*-related MH and ERM, AICAR has been found to directly reduce two important contributors to the rhabdomyolysis, RyR1 calcium leak and the production of reactive

nitrogen and oxidatve species [86,87], and may thus be suitable for corresponding human *RYR1*-related phenotypes with similar pathomechanisms. Despite early promise in pre-clinical animal models or isolated cases, no data are currently available from larger clinical studies concerning Dantrolene, the Rycals and AICAR, and concerns remain regarding their longterm use and safety profiles in humans. Moreover, considering that their main mechanism is stabilization of a "leaky" RyR1 channel, efficacy of these compounds in *RYR1*-related myopathies associated with reduced rather than enhanced calcium conductance is currently uncertain. A number of pharmacological compounds *targeting thick and thin filament interactions* and thus promoting force generation [88,89] could potentially be of interest for the NMs but for various reasons have not reached the stage of clinical applicability yet: CK-2017357 (Cytokinetics Inc.) favours myosin activation and contraction by slowing the rate of calcium release from Troponin C [90] but preferentially affects type 2 fibres typically markedly reduced or absent in NM. CK-1827452 (or Omecamtiv Mecarbil, Cytokinetics Inc.) is another compound enhancing myosin activation in a fibre type-independent manner but also targets cardiac muscle, raising concerns about potential cardiac side-effects [91].

Amelioration of downstream effects is another approach that, although unlikely to cure specific conditions, may be of benefit for CMs with different genetic backgrounds that afffect similar mechanisms or pathways. Common downstream effects include muscle atrophy both macroscopically and on the single fibre level, increased oxidative stress, defective neuromuscular transmission and abnormal protein aggregation. Therapeutic strategies to address these common downstream manifestations have been devised with variable success, but if effective may also be of benefit in other neuromuscular disorders beyond the CMs.

Reduction of oxidative stress utilizing the antioxidant N-acetylcysteine (NAC) is a therapeutic approach based on the observation of increased oxidative stress markers in *SEPN1-* and *RYR1*-related myopathies, including MH [54,86,92], and improvement of both oxidative stress markers and clinical weakness in a zebrafish model of recessive *RYR1*-related myopathies, the relatively relaxed mutant [92]. The RyR1 receptor has redox-sensing abilities, mediated through a large number of cysteine residues that modulate channel activity [93], and a pharmacological effect of antioxidants such as NAC is therefore not unexpected. The first clinical studies with NAC in humans with *RYR1-* and *SEPN1*-related myopathies are currently underway [NCT02362425 and NCT02505087, respectively], with unresolved questions concerning potential risks of longterm use, in particular in patients with altered stress susceptibility such as G6PD deficiency, and decreased weight gain in animal models [94].

Structural and functional neuromuscular junction abnormalities have been observed with different genetic backgrounds and *enhancement of neuromuscular transmission* with acetylcholine esterase inhibitors has been utilized with some success in various forms of CNM, *RYR1*-related MmD and *KLHL40*-related NM [95-98], however, the number of cases studied so far is small. Some of the beneficial effects of Salbutamol – an established treatment modality in certain congenital myasthenic syndromes – observed in patients with *RYR1*-related myopathies [78,99,100] may also at least be partly due to its neuromuscular transmission-enhancing properties, although other pharmacological properties – for example an anabolic effect as seen in other beta-mimetics or enhancement of contractility as seen in cardiac muscle [101] – are also likely to contribute.

Muscle atrophy both on the macroscopic and single fibre level is a common feature in many congenital myopathies and *stimulation of muscle growth pathways* is therefore

at least in principle a feasible approach. One class of drugs that have been considered in this context are inhibitors of myostatin, a negative regulator of muscle mass [102] expressed in adipocytes, skeletal and cardiac muscle that downregulates muscle growth pathways mediated by its binding to the activin type IIb (ActRIIB) receptor. Novel myostatin and ActRIIB inhibitors have been developed that – in addition to the CMs – may be of potential use in other myopathic, dystrophic and neurological disorders where muscle atrophy is a prominent feature. The recent observation of altered microRNA (miRs) and histone deacetylase (HDACs) expression (i.e. increase of the class II HDACs 4 and 5) in patients with recessive *RYR1*-related and other CMs [103] suggest involvement of additional pathways implicated in muscle growth that may be potentially pharmacologically targeted: MiRs are small non-coding RNAS with an indirect role in gene regulation, through their variable ability to repress translation and/or enhance RNA degradation, and have been implicated in a wide range of disease processes, including neuromuscular disorders. HDACs may affect muscle growth in different ways, through their actions upstream of the autophagy pathway, involvement in gene transcription and sequestration of the muscle specific transcription factor mef2 [104].

Aggregation of misfolded proteins is a mechanism that, in contrast to the myofibrillar myopathies, has not been extensively considered as a therapeutic target in the CMs but plays a recognized role in *MYH7*- [40] and in a subset of *TTN*-related CMs, and is likely to be implicated in the various forms of NM. *Prevention of protein aggregates* and reduction of proteotoxicity, either through primarily preventing their aggregation or secondarily promoting their clearance, is thus a feasible strategy in these and, possibly, other forms. The available range of chemical chaperones with protein-stabilizing qualities is currently still limited, but the recent demonstration that the

chemical chaperone 4-phenylbutyrate (4-PBA) effectively reduces protein aggregation and restores function in *PLEC*-related epidermolysis bullosa simplex with muscular dystrophy (EBS-MD) *in vitro* [105] confirms at least the principal usefulness of such compounds. 4-PBA acts through several pathways [106], including autophagy induction mediated by HDAC inhibition, and it is currently uncertain if the beneficial effect observed in *PLEC*-mutated cells is due to primary prevention of misfolding, increased (autophagy-mediated) clearance, or a combination of both. Interestingly, a beneficial effect of 4PBA has recently also been suggested in a mouse model of a *RYR1*-related myopathy [107]. Although the number of chemical chaperones is increasing, problems that still need addressing concern difficulties achieving pharmacologically relevant concentrations and a relative lack of target specifity.

Other pharmacological approaches include *L-Tyrosine supplementation*, so far only tried in a small cohort of patients with NM [108] based on promising preliminary results in a relevant animal model [109], that resulted in some improved secretion management but no other obvious functional benefit.

Challenges of therapy development and clinical trial design

Challenges of therapy development and clinical trial planning are the same in the CMs as in other early-onset neuromuscular disorders, and reflect the extreme rarity of most of these conditions, their genetic heterogeneity, the paucity of detailed natural history information and the lack of validated outcome measures.

Clinical trial design needs to consider the severity of the condition under study and the mechanism(s) of action of the chosen intervention. As in most of the CMs follow a relatively static course with less severe progressive replacement of muscle by connective

tissue and fat, maintaining stability is a less suitable endpoint compared to more severely progressive neuromuscular conditions such as the muscular dystrophies. On the other hand, treatments targeting defective EC coupling, one of the most commonly implicated disease mechanisms, could result in a discernable increase in muscle strength, an outcome measure that can be accurately measured in older individuals and thus provide early proof-of-concept of target engagement as well as a first indication of clinical efficacy.

There are only very few clinical trials currently ongoing in the CMs, illustrating some of the principles outlined above. An example of a clinical trial targeting a severely affected population is the AAV gene therapy trial in XLMTM: In addition to the obligatory safety measures as the main emphasis of this Phase 1 trial, outcome measures informed by an ongoing natural history study will include validated functional scales, measures of respiratory muscle strength, and other secondary measures of motor function. Survival and time off ventilator are ultimate endpoints that will also provide a clinical meaning to the outcome of this study. The prototype of a trial for a less severe condition in which the primary endpoint is to decrease muscle damage and to improve exercise tolerance is the N-acetylcysteine (NAC) trial in older individuals (above the age of 7 and older) with *RYR1*-related myopathies. This trial will run for a minimum of 12 months and will include as outcome measures both serum biomarkers of anti-oxidant effect (primary), and the 6 minute walk test as a measurement of improved endurance (secondary) at an interval following establishment of a baseline. These two different studies illustrate the challenges of identifying the most informative outcome measure in a profoundly severe condition where improvement is unexpected but may be dramatic, and in milder condition with less potential for rapid improvement and lack of baseline data for the selected outcome measure. The need to have robust information concerning baseline

and outcome measures will become increasingly important in CM-related clinical studies, in particular those targeting milder and more stable cohort of patients.

Conclusions and outlook

Therapy development in the CMs is currently still in its infancy but opens the realistic perspective of a gradual transition from a palliative to a curative approach to these conditions. Whilst some of the therapies being developed very specifically target distinct genetic entities, others, in particular those focusing on enhancing ECC or thin/thick filament interactions, may be of benefit for a wider range of different CMs, or even other neuromuscular disorders. Lack of comprehensive natural history data and robust outcome measures are the major bottlenecks for effective clinical trial planning. Further refinement of currently available genetic approaches, in particular viral-based gene transfer and gene editing techniques, are likely to change the therapeutic landscape even further in coming years.

Disclosure of interests:

HJ and FM have served on the Advisory Board of Audentes Therapeutics and Dynacure, two companies working on treatments for X-linked myotubular (XLMTM) and Centronuclear Myopathy. The authors have no commercial, proprietary, or financial interest in any products or companies described in this article.

Figure and Table legends

Figure 1

Clinical features of *RYR1*-related myopathies. a) Male infant with recessive *RYR1*-related CNM mimicking XLMTM; b) boy with dominantly inherited *RYR1*-related CCD demonstrating a positive Gowers'sign; c-d) male adolescent with a personal and family history of (exertional) rhabdomyolysis due to a dominant MH-associated *RYR1* mutation; and e) adult with late-onset axial myopathy due to a heterozygous *RYR1* mutation. The extremely wide phenotypical spectrum illustrates the difficulties obtaining informative natural history data, identifying coherent cohorts and reliable outcome measures as a basis for clinical trials.

Table 1

Main pathogenic mechanisms implicated in the congenital myopathies. Common genes and phenotypes are indicated in bold, genes highlighted in red indicate those also implicated in the Malignant Hyperthermia Susceptibility (MHS) trait. For each mechanism, only the most relevant genetic backgrounds and the most commonly associated phenotypes are indicated (for a more comprehensive review, also of rarer backgrounds and phenotypes, see). Most protein defects implicated in the congenital myopathies exert their pathogenic effects through multiple mechanisms. * = secondary ECC defects have been described in *MTM1*-, *DNM2*- and *BIN1*-related CNM (due to abnormalities of triadic assembly), and in *SEPN1*-related myopathies (due to abnormal redox modification of ryanodine receptors). ECC = Excitation-contraction coupling; CCD = Central Core Disease, MmD = Multi-minicore Disease (MmD), CNM = Centronuclear Myopathy, XLMTM = X-linked myotubular myopathy; NM = Nemaline

Myopathy, CM = congenital myopathy with multiple or non-specific features; TAM = Tubular Aggregate Myopathy; MSM = Myosin Storage Myopathy; DA = Distal Arthrogryposis; AD = autosomal-dominant; AR = autosomal-recessive

Table 2

Therapeutic approaches to the congenital myopathies. Amongst the therapeutic strategies summarized, enzyme replacement therapy (ERT) and viral-based gene transfer in X-linked myotubular myopathy (XLMTM), as well as antioxidant therapy in *RYR1*-and *SEPN1*-related myopathies have already (or are approaching) the clinical trial stage. * Salbutamol probably exerts its effects through additional mechanisms other than enhancement of neuromuscular transmission (see main text). CCD = Central Core Disease, CNM = Centronuclear Myopathy, NM = Nemaline Myopathy; AD = autosomal-dominant

REFERENCES

1.   Jungbluth H. The congenital myopathies – inherited disorders of excitation-contraction coupling and muscle contraction. *Nature Neurology Reviews* in press

2.   Wang CH, Dowling JJ, North K, et al. Consensus statement on standard of care for congenital myopathies. *Journal of child neurology.* 2012;27(3):363-382.

3.   Jungbluth H, Ochala J, Treves S, Gautel M. Current and future therapeutic approaches to the congenital myopathies. *Semin Cell Dev Biol.* 2017;64:191-200.

4.   Jungbluth H, Sewry CA, Muntoni F. Core myopathies. *Seminars in pediatric neurology.* 2011;18(4):239-249.

5.   Maggi L, Scoto M, Cirak S, et al. Congenital myopathies--clinical features and frequency of individual subtypes diagnosed over a 5-year period in the United Kingdom. *Neuromuscular disorders : NMD.* 2013;23(3):195-205.

6.   Zhang Y, Chen HS, Khanna VK, et al. A mutation in the human ryanodine receptor gene associated with central core disease. *Nat Genet.* 1993;5(1):46-50.

7.   Jungbluth H, Zhou H, Hartley L, et al. Minicore myopathy with ophthalmoplegia caused by mutations in the ryanodine receptor type 1 gene. *Neurology.* 2005;65(12):1930-1935.

8.   Ferreiro A, Quijano-Roy S, Pichereau C, 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.* 2002;71(4):739-749.

9.   Cullup T, Lamont PJ, Cirak S, et al. Mutations in MYH7 cause Multi-minicore Disease (MmD) with variable cardiac involvement. *Neuromuscular disorders : NMD.* 2012;22(12):1096-1104.

10.   Chauveau C, Bonnemann CG, Julien C, et al. Recessive TTN truncating mutations define novel forms of core myopathy with heart disease. *Human molecular genetics.* 2014;23(4):980-991.

11.   Klein A, Jungbluth H, Clement E, et al. Muscle MRI in congenital myopathies due to Ryanodine receptor type 1 (RYR1) gene mutations

12.   Romero NB. Centronuclear myopathies: a widening concept. *Neuromuscular disorders : NMD.* 2010;20(4):223-228.

13.   Laporte J, Hu LJ, Kretz C, et al. A gene mutated in X-linked myotubular myopathy defines a new putative tyrosine phosphatase family conserved in yeast. *Nat Genet.* 1996;13(2):175-182.

14.   Bitoun M, Maugenre S, Jeannet PY, et al. Mutations in dynamin 2 cause dominant centronuclear myopathy. *Nat Genet.* 2005;37(11):1207-1209.

15.   Bohm J, Biancalana V, Malfatti E, et al. Adult-onset autosomal dominant centronuclear myopathy due to BIN1 mutations. *Brain : a journal of neurology.* 2014.

16.   Wilmshurst JM, Lillis S, Zhou H, et al. RYR1 mutations are a common cause of congenital myopathies with central nuclei. *Ann Neurol.* 2010;68(5):717-726.

17.   Nicot AS, Toussaint A, Tosch V, et al. Mutations in amphiphysin 2 (BIN1) disrupt interaction with dynamin 2 and cause autosomal recessive centronuclear myopathy. *Nat Genet.* 2007;39(9):1134-1139.

18.   Ceyhan-Birsoy O, Agrawal PB, Hidalgo C, et al. Recessive truncating titin gene, TTN, mutations presenting as centronuclear myopathy. *Neurology.* 2013.

19. Herman GE, Finegold M, Zhao W, de Gouyon B, Metzenberg A. Medical complications in long-term survivors with X-linked myotubular myopathy. *J Pediatr.* 1999;134(2):206-214.

20. Dubowitz V, Sewry CA, Oldfors A. *Muscle Biopsy: A Practical Approach.* 4th ed: Saunders; 2013.

21. Pelin K, Hilpela P, Donner K, et al. Mutations in the nebulin gene associated with autosomal recessive nemaline myopathy. *Proc Natl Acad Sci U S A.* 1999;96(5):2305-2310.

22. Ryan MM, Schnell C, Strickland CD, et al. Nemaline myopathy: a clinical study of 143 cases. *Ann Neurol.* 2001;50(3):312-320.

23. Nowak KJ, Wattanasirichaigoon D, Goebel HH, et al. Mutations in the skeletal muscle alpha-actin gene in patients with actin myopathy and nemaline myopathy. *Nature genetics.* 1999;23(2):208-212.

24. Laing NG, Wilton SD, Akkari PA, et al. A mutation in the alpha tropomyosin gene TPM3 associated with autosomal dominant nemaline myopathy. *Nat Genet.* 1995;9(1):75-79.

25. Donner K, Ollikainen M, Ridanpaa M, et al. Mutations in the beta-tropomyosin (TPM2) gene--a rare cause of nemaline myopathy. *Neuromuscul Disord.* 2002;12(2):151-158.

26. Sambuughin N, Yau KS, Olive M, et al. Dominant mutations in KBTBD13, a member of the BTB/Kelch family, cause nemaline myopathy with cores. *Am J Hum Genet.* 2010;87(6):842-847.

27. Johnston JJ, Kelley RI, Crawford TO, et al. A novel nemaline myopathy in the Amish caused by a mutation in troponin T1. *Am J Hum Genet.* 2000;67(4):814-821.

28. Agrawal PB, Greenleaf RS, Tomczak KK, et al. Nemaline myopathy with minicores caused by mutation of the CFL2 gene encoding the skeletal muscle actin-binding protein, cofilin-2. *American journal of human genetics.* 2007;80(1):162-167.

29. Miyatake S, Mitsuhashi S, Hayashi YK, et al. Biallelic Mutations in MYPN, Encoding Myopalladin, Are Associated with Childhood-Onset, Slowly Progressive Nemaline Myopathy. *Am J Hum Genet.* 2017;100(1):169-178.

30. Ravenscroft G, Miyatake S, Lehtokari VL, et al. Mutations in KLHL40 are a frequent cause of severe autosomal-recessive nemaline myopathy. *American journal of human genetics.* 2013;93(1):6-18.

31. Gupta VA, Ravenscroft G, Shaheen R, et al. Identification of KLHL41 Mutations Implicates BTB-Kelch-Mediated Ubiquitination as an Alternate Pathway to Myofibrillar Disruption in Nemaline Myopathy. *American journal of human genetics.* 2013;93(6):1108-1117.

32. Yuen M, Sandaradura SA, Dowling JJ, et al. Leiomodin-3 dysfunction results in thin filament disorganization and nemaline myopathy. *J Clin Invest.* 2014;124(11):4693-4708.

33. Davidson AE, Siddiqui FM, Lopez MA, et al. Novel deletion of lysine 7 expands the clinical, histopathological and genetic spectrum of TPM2-related myopathies. *Brain.* 2013;136(Pt 2):508-521.

34. Jungbluth H, Sewry CA, Counsell S, et al. Magnetic resonance imaging of muscle in nemaline myopathy. *Neuromuscul Disord.* 2004;14(12):779-784.

35. Jurkat-Rott K, Lehmann-Horn F, Elbaz A, et al. A calcium channel mutation causing hypokalemic periodic paralysis. *Hum Mol Genet.* 1994;3(8):1415-1419.

36. Monnier N, Krivosic-Horber R, Payen JF, et al. Presence of two different genetic traits in malignant hyperthermia families: implication for genetic analysis, diagnosis, and incidence of malignant hyperthermia susceptibility. *Anesthesiology.* 2002;97(5):1067-1074.

37. Schartner V, Romero NB, Donkervoort S, et al. Dihydropyridine receptor (DHPR, CACNA1S) congenital myopathy. *Acta Neuropathol.* 2017;133(4):517-533.

38. Lacruz RS, Feske S. Diseases caused by mutations in ORAI1 and STIM1. *Ann N Y Acad Sci.* 2015;1356:45-79.

39. O'Grady GL, Best HA, Sztal TE, et al. Variants in the Oxidoreductase PYROXD1 Cause Early-Onset Myopathy with Internalized Nuclei and Myofibrillar Disorganization. *Am J Hum Genet.* 2016;99(5):1086-1105.

40. Tajsharghi H, Oldfors A. Myosinopathies: pathology and mechanisms. *Acta Neuropathol.* 2013;125(1):3-18.

41. Dieterich K, Quijano-Roy S, Monnier N, et al. The neuronal endopeptidase ECEL1 is associated with a distinct form of recessive distal arthrogryposis. *Human molecular genetics.* 2013;22(8):1483-1492.

42. McMillin MJ, Below JE, Shively KM, et al. Mutations in ECEL1 cause distal arthrogryposis type 5D. *American journal of human genetics.* 2013;92(1):150-156.

43. Coste B, Houge G, Murray MF, et al. Gain-of-function mutations in the mechanically activated ion channel PIEZO2 cause a subtype of Distal Arthrogryposis. *Proc Natl Acad Sci U S A.* 2013;110(12):4667-4672.

44.   Zaharieva IT, Thor MG, Oates EC, et al. Loss-of-function mutations in SCN4A cause severe foetal hypokinesia or 'classical' congenital myopathy. *Brain : a journal of neurology.* 2016;139(Pt 3):674-691.

45.   Rosenberg H, Davis M, James D, Pollock N, Stowell K. Malignant hyperthermia. *Orphanet J Rare Dis.* 2007;2:21.

46.   Kraeva N, Heytens L, Jungbluth H, et al. Compound RYR1 heterozygosity resulting in a complex phenotype of malignant hyperthermia susceptibility and a core myopathy. *Neuromuscular disorders : NMD.* 2015;25(7):567-576.

47.   Dowling JJ, Lillis S, Amburgey K, et al. King-Denborough syndrome with and without mutations in the skeletal muscle ryanodine receptor (RYR1) gene. *Neuromuscular disorders : NMD.* 2011.

48.   Loseth S, Voermans NC, Torbergsen T, et al. A novel late-onset axial myopathy associated with mutations in the skeletal muscle ryanodine receptor (RYR1) gene. *Journal of neurology.* 2013;260(6):1504-1510.

49.   Horstick EJ, Linsley JW, Dowling JJ, et al. Stac3 is a component of the excitation-contraction coupling machinery and mutated in Native American myopathy. *Nature communications.* 2013;4:1952.

50.   Treves S, Jungbluth H, Muntoni F, Zorzato F. Congenital muscle disorders with cores: the ryanodine receptor calcium channel paradigm. *Curr Opin Pharmacol.* 2008;8(3):319-326.

51.   Maclennan DH, Zvaritch E. Mechanistic models for muscle diseases and disorders originating in the sarcoplasmic reticulum. *Biochim Biophys Acta.* 2010.

52. Wong King Yuen SM, Campiglio M, Tung CC, Flucher BE, Van Petegem F. Structural insights into binding of STAC proteins to voltage-gated calcium channels. *Proc Natl Acad Sci U S A.* 2017;114(45):E9520-E9528.

53. Jurynec MJ, Xia R, Mackrill JJ, et al. Selenoprotein N is required for ryanodine receptor calcium release channel activity in human and zebrafish muscle. *Proc Natl Acad Sci U S A.* 2008;105(34):12485-12490.

54. Arbogast S, Beuvin M, Fraysse B, Zhou H, Muntoni F, Ferreiro A. Oxidative stress in SEPN1-related myopathy: from pathophysiology to treatment. *Ann Neurol.* 2009;65(6):677-686.

55. Ravenscroft G, Jackaman C, Bringans S, et al. Mouse models of dominant ACTA1 disease recapitulate human disease and provide insight into therapies. *Brain.* 2011;134(Pt 4):1101-1115.

56. Ochala J, Lehtokari VL, Iwamoto H, et al. Disrupted myosin cross-bridge cycling kinetics triggers muscle weakness in nebulin-related myopathy. *FASEB J.* 2011.

57. Donkervoort S, Papadaki M, de Winter JM, et al. TPM3 deletions cause a hypercontractile congenital muscle stiffness phenotype. *Ann Neurol.* 2015;78(6):982-994.

58. de Winter JM, Joureau B, Lee EJ, et al. Mutation-specific effects on thin filament length in thin filament myopathy. *Annals of neurology.* 2016.

59. Logan CV, Lucke B, Pottinger C, et al. Mutations in MEGF10, a regulator of satellite cell myogenesis, cause early onset myopathy, areflexia, respiratory distress and dysphagia (EMARDD). *Nat Genet.* 2011;43(12):1189-1192.

60. Castets P, Bertrand AT, Beuvin M, et al. Satellite cell loss and impaired muscle regeneration in selenoprotein N deficiency. *Hum Mol Genet.* 2011;20(4):694-704.

61. Castets P, Maugenre S, Gartioux C, et al. Selenoprotein N is dynamically expressed during mouse development and detected early in muscle precursors. *BMC Dev Biol.* 2009;9:46.

62. Buj-Bello A, Fougerousse F, Schwab Y, et al. AAV-mediated intramuscular delivery of myotubularin corrects the myotubular myopathy phenotype in targeted murine muscle and suggests a function in plasma membrane homeostasis. *Hum Mol Genet.* 2008;17(14):2132-2143.

63. Beggs AH, Bohm J, Snead E, et al. MTM1 mutation associated with X-linked myotubular myopathy in Labrador Retrievers. *Proc Natl Acad Sci U S A.* 2010;107(33):14697-14702.

64. Childers MK, Joubert R, Poulard K, et al. Gene therapy prolongs survival and restores function in murine and canine models of myotubular myopathy. *Science translational medicine.* 2014;6(220):220ra210.

65. Cathomen T, Ehl S. Translating the genomic revolution - targeted genome editing in primates. *The New England journal of medicine.* 2014;370(24):2342-2345.

66. Monnier N, Ferreiro A, Marty I, Labarre-Vila A, Mezin P, Lunardi J. A homozygous splicing mutation causing a depletion of skeletal muscle RYR1 is associated with multi-minicore disease congenital myopathy with ophthalmoplegia. *Hum Mol Genet.* 2003;12(10):1171-1178.

67.   Rendu J, Brocard J, Denarier E, et al. Exon skipping as a therapeutic strategy applied to an RYR1 mutation with pseudo-exon inclusion causing a severe core myopathy. *Human gene therapy.* 2013;24(7):702-713.

68.   McDonald CM, Campbell C, Torricelli RE, et al. Ataluren in patients with nonsense mutation Duchenne muscular dystrophy (ACT DMD): a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet.* 2017;390(10101):1489-1498.

69.   MacArthur DG, Lek M. The uncertain road towards genomic medicine. *Trends in genetics : TIG.* 2012;28(7):303-305.

70.   Jungbluth H, Gautel M. Pathogenic mechanisms in centronuclear myopathies. *Frontiers in aging neuroscience.* 2014;6:339.

71.   Cowling BS, Chevremont T, Prokic I, et al. Reducing dynamin 2 expression rescues X-linked centronuclear myopathy. *J Clin Invest.* 2014;124(3):1350-1363.

72.   Sabha N, Volpatti JR, Gonorazky H, et al. PIK3C2B inhibition improves function and prolongs survival in myotubular myopathy animal models. *J Clin Invest.* 2016;126(9):3613-3625.

73.   Nowak KJ, Ravenscroft G, Jackaman C, et al. Rescue of skeletal muscle alpha-actin-null mice by cardiac (fetal) alpha-actin. *The Journal of cell biology.* 2009;185(5):903-915.

74.   Ravenscroft G, McNamara E, Griffiths LM, et al. Cardiac alpha-actin over-expression therapy in dominant ACTA1 disease. *Hum Mol Genet.* 2013;22(19):3987-3997.

75. Lawlor MW, Armstrong D, Viola MG, et al. Enzyme replacement therapy rescues weakness and improves muscle pathology in mice with X-linked myotubular myopathy. *Human molecular genetics.* 2013;22(8):1525-1538.

76. Fruen BR, Mickelson JR, Louis CF. Dantrolene inhibition of sarcoplasmic reticulum Ca2+ release by direct and specific action at skeletal muscle ryanodine receptors. *J Biol Chem.* 1997;272(43):26965-26971.

77. Michalek-Sauberer A, Gilly H. Prophylactic use of dantrolene in a patient with central core disease. *Anesthesia and analgesia.* 1998;86(4):915-916.

78. Jungbluth H, Dowling JJ, Ferreiro A, Muntoni F. 182nd ENMC International Workshop: RYR1-related myopathies, 15-17th April 2011, Naarden, The Netherlands. *Neuromuscular disorders : NMD.* 2012.

79. Dlamini N, Voermans NC, Lillis S, et al. Mutations in RYR1 are a common cause of exertional myalgia and rhabdomyolysis. *Neuromuscular disorders : NMD.* 2013;23(7):540-548.

80. Timmins MA, Rosenberg H, Larach MG, Sterling C, Kraeva N, Riazi S. Malignant hyperthermia testing in probands without adverse anesthetic reaction. *Anesthesiology.* 2015;123(3):548-556.

81. Scalco RS, Voermans NC, Piercy RJ, Jungbluth H, Quinlivan R. Dantrolene as a possible prophylactic treatment for RYR1-related rhabdomyolysis. *Eur J Neurol.* 2016;23(8):e56-57.

82. Lopez RJ, Byrne S, Vukcevic M, et al. An RYR1 mutation associated with malignant hyperthermia is also associated with bleeding abnormalities. *Sci Signal.* 2016;9(435):ra68.

83. Marks AR. Calcium cycling proteins and heart failure: mechanisms and therapeutics. *J Clin Invest.* 2013;123(1):46-52.

84.	Andersson DC, Marks AR. Fixing ryanodine receptor Ca leak - a novel therapeutic strategy for contractile failure in heart and skeletal muscle. *Drug discovery today. Disease mechanisms.* 2010;7(2):e151-e157.

85.	Pold R, Jensen LS, Jessen N, et al. Long-term AICAR administration and exercise prevents diabetes in ZDF rats. *Diabetes.* 2005;54(4):928-934.

86.	Durham WJ, Aracena-Parks P, Long C, et al. RyR1 S-nitrosylation underlies environmental heat stroke and sudden death in Y522S RyR1 knockin mice. *Cell.* 2008;133(1):53-65.

87.	Lanner JT, Georgiou DK, Dagnino-Acosta A, et al. AICAR prevents heat-induced sudden death in RyR1 mutant mice independent of AMPK activation. *Nature medicine.* 2012;18(2):244-251.

88.	de Winter JM, Buck D, Hidalgo C, et al. Troponin activator augments muscle force in nemaline myopathy patients with nebulin mutations. *J Med Genet.* 2013;50(6):383-392.

89.	de Winter JM, Joureau B, Sequeira V, et al. Effect of levosimendan on the contractility of muscle fibers from nemaline myopathy patients with mutations in the nebulin gene. *Skelet Muscle.* 2015;5:12.

90.	Russell AJ, Hartman JJ, Hinken AC, et al. Activation of fast skeletal muscle troponin as a potential therapeutic approach for treating neuromuscular diseases. *Nat Med.* 2012;18(3):452-455.

91.	Malik FI, Hartman JJ, Elias KA, et al. Cardiac myosin activation: a potential therapeutic approach for systolic heart failure. *Science.* 2011;331(6023):1439-1443.

92.   Dowling JJ, Arbogast S, Hur J, et al. Oxidative stress and successful antioxidant treatment in models of RYR1-related myopathy. *Brain : a journal of neurology.* 2012;135(Pt 4):1115-1127.

93.   Voss AA, Lango J, Ernst-Russell M, Morin D, Pessah IN. Identification of hyperreactive cysteines within ryanodine receptor type 1 by mass spectrometry. *J Biol Chem.* 2004;279(33):34514-34520.

94.   Pinniger GJ, Terrill JR, Assan EB, Grounds MD, Arthur PG. Pre-clinical evaluation of N-acetylcysteine reveals side effects in the mdx mouse model of Duchenne muscular dystrophy. *J Physiol.* 2017;595(23):7093-7107.

95.   Robb SA, Sewry CA, Dowling JJ, et al. Impaired neuromuscular transmission and response to acetylcholinesterase inhibitors in centronuclear myopathies. *Neuromuscular disorders : NMD.* 2011.

96.   Dowling JJ, Joubert R, Low SE, et al. Myotubular myopathy and the neuromuscular junction: a novel therapeutic approach from mouse models. *Disease models & mechanisms.* 2012;5(6):852-859.

97.   Gibbs EM, Clarke NF, Rose K, et al. Neuromuscular junction abnormalities in DNM2-related centronuclear myopathy. *Journal of molecular medicine.* 2013;91(6):727-737.

98.   Natera-de Benito D, Nascimento A, Abicht A, et al. KLHL40-related nemaline myopathy with a sustained, positive response to treatment with acetylcholinesterase inhibitors. *Journal of neurology.* 2016.

99.   Messina S, Hartley L, Main M, et al. Pilot trial of salbutamol in central core and multi-minicore diseases. *Neuropediatrics.* 2004;35(5):262-266.

100. Schreuder LT, Nijhuis-van der Sanden MW, de Hair A, et al. Successful use of albuterol in a patient with central core disease and mitochondrial dysfunction. *J Inherit Metab Dis.* 2010.

101. Bers DM. Cardiac excitation-contraction coupling. *Nature.* 2002;415(6868):198-205.

102. Amthor H, Hoogaars WM. Interference with myostatin/ActRIIB signaling as a therapeutic strategy for Duchenne muscular dystrophy. *Current gene therapy.* 2012;12(3):245-259.

103. Rokach O, Sekulic-Jablanovic M, Voermans N, et al. Epigenetic changes as a common trigger of muscle weakness in congenital myopathies. *Human molecular genetics.* 2015.

104. Miska EA, Karlsson C, Langley E, Nielsen SJ, Pines J, Kouzarides T. HDAC4 deacetylase associates with and represses the MEF2 transcription factor. *The EMBO journal.* 1999;18(18):5099-5107.

105. Winter L, Staszewska I, Mihailovska E, et al. Chemical chaperone ameliorates pathological protein aggregation in plectin-deficient muscle. *J Clin Invest.* 2014;124(3):1144-1157.

106. Cuadrado-Tejedor M, Ricobaraza AL, 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.* 2013;19(28):5076-5084.

107. Lee CS, Hanna AD, Wang H, et al. A chemical chaperone improves muscle function in mice with a RyR1 mutation. *Nat Commun.* 2017;8:14659.

108. Ryan MM, Sy C, Rudge S, et al. Dietary L-tyrosine supplementation in nemaline myopathy. *J Child Neurol.* 2008;23(6):609-613.

109. Nguyen MA, Joya JE, Kee AJ, et al. Hypertrophy and dietary tyrosine ameliorate the phenotypes of a mouse model of severe nemaline myopathy. *Brain : a journal of neurology.* 2011;134(Pt 12):3516-3529.