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## Accepted Manuscript

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**Current therapeutic approaches to the congenital myopathies 1****Current and future therapeutic approaches to the congenital myopathies**

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**Current therapeutic approaches to the congenital myopathies 2****Abstract**

The congenital myopathies – including Central Core Disease (CCD), Multi-minicore Disease (MmD), Centronuclear Myopathy (CNM), Nemaline Myopathy (NM) and Congenital Fibre Type Disproportion (CFTD) – are a genetically heterogeneous group of early-onset neuromuscular conditions characterized by distinct histopathological features, and associated with a substantial individual and societal disease burden. Appropriate supportive management has substantially improved patient morbidity and mortality but there is currently no cure.

Recent years have seen an exponential increase in the genetic and molecular understanding of these conditions, leading to the identification of underlying defects in proteins involved in calcium homeostasis and excitation-contraction coupling, thick/thin filament assembly and function, redox regulation, membrane trafficking and/or autophagic pathways. Based on these findings, specific therapies are currently being developed, or are already approaching the clinical trial stage. Despite undeniable progress, therapy development faces considerable challenges, considering the rarity and diversity of specific conditions, and the size and complexity of some of the genes and proteins involved.

The present review will summarize the key genetic, histopathological and clinical features of specific congenital myopathies, and outline therapies already available or currently being developed in the context of known pathogenic mechanisms. The relevance of newly discovered molecular mechanisms and novel gene editing strategies for future therapy development will be discussed.

**Key words:**

CCD = Central Core Disease; CFTD = Congenital Fibre Type Disproportion; CNM = Centronuclear Myopathy; MmD = Multi-minicore Disease; NM = Nemaline Myopathy

## 1. Introduction

The congenital myopathies are a group of clinically heterogeneous, early-onset neuromuscular disorders characterized by normal CK and defined features on muscle biopsy (for review, [1]). Central Core Disease (CCD) and Multi-minicore Disease (MmD) (the “core myopathies), Nemaline Myopathy (NM), Centronuclear Myopathy (CNM) and Congenital Fibre Type Disproportion (CFTD) are the major entities. There are additional rarer forms, often limited to few families or patients.

The last decade has seen an exponential increase in the genetic resolution of the congenital myopathies. Mutations in almost 20 genes have been identified (Table 1), indicating both genetic diversity and substantial overlap between conditions previously considered to be distinct. Different mutations in the same gene may cause distinct congenital myopathies depending on the protein domain affected, whilst mutations in different genes may cause overlapping histopathological appearances due to functional association of the defective gene products. Mutations in the skeletal muscle ryanodine receptor (*RYR1*) gene, encoding the principal sarcoplasmic reticulum (SR) calcium release channel with a crucial role in excitation-contraction coupling, have emerged as the most common genetic cause implicated in most of the major forms [2, 3]. Proteins affected in the congenital myopathies are involved in calcium homeostasis and excitation-contraction coupling, thick/thin filament assembly and interactions, redox regulation, vesicular trafficking and/or autophagic pathways (Figure 1). In contrast to the congenital muscular dystrophies, sarcolemmal integrity is preserved, reflected in typically normal CK levels.

There is currently no cure for any of the congenital myopathies and management is largely supportive, aimed at preserving mobility and addressing orthopaedic, cardiorespiratory and other potential comorbidities such as an increased malignant hyperthermia (MH) risk (outlined in detail in [4]).

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Therapy development in the congenital myopathies faces a number of clinical and molecular challenges, some in common with other early-onset neuromuscular disorders and others more specific. On the clinical level, overall rarity of specific conditions and the marked clinical diversity associated with specific genetic defects makes recruitment for meaningful clinical trials a daunting task. On the molecular level, the sheer size and complexity of some of the proteins involved precludes viral transfer of the functional gene or exon skipping strategies as viable therapeutic options in many of these conditions. In addition, some of the underlying mechanisms are currently only partially understood.

The present review will summarize the key features of specific congenital myopathies, relevant animal models, and underlying pathogenic mechanisms (where known) as a basis for therapy development. We will outline currently available therapeutic approaches (Table 1) and the rationale behind potential future treatment strategies.

### 2. Core myopathies

The core myopathies (for detailed review, see [5]) are characterized by variable histopathological abnormalities on oxidative enzyme stains, well-defined and running along the longitudinal fibre axis in Central Core Disease (CCD) [6], and of more limited extent and patchier appearance in Multi-minicore Disease (MmD) [7]. As a group, core myopathies represent the most common subset of congenital myopathies, as recently confirmed in two independent studies from the USA and the United Kingdom [2, 3]. Core myopathies are most commonly linked to dominant and recessive mutations in the *RYR1* gene, or recessive mutations in the *SEPN1* gene encoding selenoprotein N. The histopathological appearance of cores on muscle biopsy is, however, not specific, and other genetic backgrounds, for example mutations in *TTN* [8] or *MYH7* [9] have to be considered, in particular if cardiac involvement is a feature and/or minicores are present.

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Dominant mutations in *RYR1* are most commonly associated with the malignant hyperthermia susceptibility (MHS) trait [10], a pharmacogenetic reaction to halogenated anaesthetics and muscle relaxants, and CCD, a relatively mild, predominantly proximal congenital myopathy typically without significant extraocular or cardiorespiratory involvement. MHS-related *RYR1* mutations have also been associated with “induced” or adult-onset neuromuscular phenotypes, in particular exertional rhabdomyolysis [11] and late-onset axial myopathy [12]. Recessive mutations in *RYR1* mainly cause Multi-minicore Disease with external ophthalmoplegia [13], but also subgroups of Centronuclear Myopathy (CNM) [14] and Congenital Fibre Type Disproportion (CFTD) [15] (see below). Recessive mutations in *SEPNI* are the second most common cause of core myopathies [16], typically associated with the histopathological phenotype of MmD and clinical features of a predominantly axial myopathy with marked spinal rigidity, early-onset scoliosis and respiratory impairment almost invariably requiring respiratory support; congenital muscular dystrophy with spinal rigidity [17] and Mallory body myopathy [18] are allelic histopathological presentations .

Whereas the pathomechanisms underlying dominantly inherited *RYR1*-related MHS and CCD have been investigated in detail, recessive forms of *RYR1*-related myopathies remain only partially understood, with important implications for the development of therapeutic strategies. Whilst MHS and related phenotypes are generally attributed to a “hyperactive” RyR1 channel reflective of increased RyR1 agonist sensitivity and resulting disproportionate calcium release upon RyR1 stimulation, depletion of sarcoplasmic reticulum calcium stores with resulting increase in cytosolic calcium levels (“leaky channel” hypothesis) and disturbance of excitation-contraction coupling (E-C uncoupling hypothesis) have been proposed as explanations for RyR1 receptor malfunction in CCD and related myopathies (reviewed in [5]). Most functional studies concerning recessive *RYR1* mutations published to date indicate the marked reduction of the functional RyR1 protein levels as an important common denominator in this group, with

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impairment of calcium conductance rather than alterations of cytosolic or SR calcium levels as the most common downstream effect [19-21]. The precise function of selenoprotein N in muscle remains still uncertain but emerging evidence suggests a role in both antioxidant defense systems and (redox-regulated) calcium homeostasis: Selenoprotein N features a structural motif similar to those found in calcium-binding proteins [17] and, as demonstrated in normal zebrafish and the *sepn1* <sup>-/-</sup> morphant, may be functionally and spatially associated with RyR1 [22, 23], providing a possible molecular basis for the many clinico-pathological similarities between *RYR1*- and *SEPNI*-related core myopathies.

Because of the rapid recognition of *RYR1* mutations as one of the most common causes of neuromuscular disorders in humans, therapy development for these conditions is a major goal within the congenital myopathy field. Possible therapeutic strategies currently considered or already tried include modification of abnormal RyR1 function, correction of associated oxidative abnormalities, use of pharmacological compounds enhancing muscle contractility and/or neuromuscular transmission, and correction of the specific genetic defect.

Historically, the first pharmacological compound used to modify the abnormal Ca<sup>++</sup> amount released during an MH crisis was *dantrolene*, an agent that was shown in early studies to reduce the maximal rate of SR Ca<sup>++</sup> release [24] and inhibit caffeine induced ryanodine binding. In addition to its well-established role in MH management, dantrolene has also been used in the treatment of patients with *RYR1*-related (exertional) rhabdomyolysis [25], and anecdotal reports suggest that some patients with *RYR1*-related CCD may also benefit from dantrolene use [26, 27]. However, explorative use of dantrolene in *RYR1*-related myopathies ought probably to be limited to those carrying “leaky channel” mutations compared to those where calcium conductance is already reduced, considering that a RyR1 antagonist such as dantrolene may potentially worsen the consequences of the molecular defect in the latter group.



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RyR1 channels are situated within a highly complex macromolecular environment and undergo a number of dynamic modifications. One class of proteins involved in these complex interactions that have emerged as a possible pharmacological target for the treatment of *RYR*-related cardiac and skeletal myopathies are the calstabins, including calstabin 1 (FK506 binding protein 12 or FKBP12) mainly expressed in skeletal muscle and calstabin 2 (FKBP12.6) mainly expressed in cardiac muscle (for review, [28, 29]). Calstabins act as RyR1 channel-stabilizing proteins that increase the channel probability of being closed at the resting state, but their dissociation from the RyR receptor under conditions of stress and with increased phosphorylation and nitrosylation results in “leaky” channels and, ultimately, in reduction of the available SR calcium stores. Two pharmacological compounds that have been demonstrated to reduce SR calcium leak by enhancing RyR1-calstabin interactions are the 1,4-benzothiazepine derivatives JTV519 and S107 (also known as *Rycals*), suggesting them as potential treatments also for *RYR1*-related myopathies associated with excessive calcium release such as MH or ERM, or with increased SR calcium leak such as subgroups of CCD [30]. Concerns remain regarding non-skeletal muscle effects of these compounds originally developed for the treatment of cardiac disease, and their effects in recessive *RYR1*-related myopathies where calcium conductance may already be impaired (and increasing RyR1 channel closed probability may potentially have a detrimental rather than a beneficial effect).

Another compound that has been suggested to stabilize RyR1 function is 5-aminoimidazole-4-carboximide ribonucleoside (or *AICAR*), an activator of the AMP-activated protein kinase (AMPK), a kinase and intracellular energy sensor upstream of the autophagy pathway. AICAR, a recognized enhancer of muscle performance [31], has recently been demonstrated to directly reduce RyR1 calcium leak and RNS and ROS production, an important aspect of the vicious cycle leading to fatal rhabdomyolysis in a mouse model of the human MH- and ERM- associated *RYR1* mutation Y522S [32]. These findings suggest AICAR as a potential

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treatment for *RYR1*-related myopathies associated with excessive calcium release or increased spontaneous calcium leak, but there are currently no data regarding its longterm use in human myopathies.

Another altogether different treatment approach is based on the observation of increased oxidative stress in patient cells and animal models. Recent studies have indicated that oxidative stress may be one of the causes underlying MH, and that oxidative stress is elevated in muscle of patients with MH and *RYR1*- and *SEPNI*-related myopathies [33-35]. A study on patient muscle cells and the zebrafish “relatively relaxed” mutant, a model of human recessive *RYR1*-related myopathies lacking the skeletal muscle *ryr1b* isoform, demonstrated elevation of oxidative stress markers and excessive production of reactive oxygen species of probable mitochondrial origin. Treatment with 200  $\mu$ M *N-acetylcysteine* (*NAC*) reduced oxidative stress markers, decreased muscle damage and improved swimming abilities in the zebrafish mutant [35]. The use of antioxidants (and more specifically, *NAC*) is also supported by the fact that the RyR1 protein contains more than 100 cysteine residues, and that some of these (namely Cys residues at amino acid positions 1040, 1303, 2436, 2656, 2606, 2611 and 3635) are hyper-reactive and act as redox sensors which modulate the channel activity [36, 37]. Taken together, these observations have provided the rationale for the use of *NAC* as a potential treatment for *RYR1*- and *SEPNI*-related myopathies, and have prompted the first clinical trials in humans currently underway. However, there are presently no data regarding its longterm use in these conditions, and genetic determinants of altered oxidative stress susceptibility (such Glucose 6-phosphate dehydrogenase (*G6PD*) deficiency, one of the most common metabolic conditions worldwide) will have to be taken into account for the interpretation of clinical trials.

Prompted by its previous use in other neuromuscular disorders, the  $\beta$ -agonist *salbutamol* has also been explored for the treatment of core myopathies with encouraging results in case reports and a small pilot study [26, 38, 39]. The precise mechanisms underlying the benefits of

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salbutamol in core myopathies are uncertain, but may include an anabolic effect as seen in other  $\beta$ -mimetics, increases in relaxation speed and contractility as described in cardiac muscle [40], and/or enhancement of neuromuscular transmission.

The feasibility of *genetic strategies* to treat *RYR1*-related myopathies has been demonstrated in one recessive case where exon skipping was successfully applied *in vitro* to remove an additional, paternally inherited exon associated with an unstable transcript and reduced RyR1 protein expression [41], resulting in increased expression of a functional RyR1 protein, and improved myotube morphology. Although these results are highly encouraging, a similar approach may only be applicable to the small number of pseudo-exon creating mutations in *RYR1*-related myopathies (estimated at 1-2%), as, in contrast to large structural proteins such as dystrophin, the RyR1 protein is unlikely to tolerate removal of whole exons through exon skipping considering its highly complex structure with probably little redundancy within functional domains. However, considering that even marked reduction of the functional RyR1 protein in carriers of recessive *RYR1* mutations appears to be well tolerated [41, 42], targeted gene editing may become a feasible strategy to silence selected dominant *RYR1* mutations associated with CCD in the future. A general caveat regarding the feasibility of gene therapy approaches to *RYR1*-related myopathies concerns the relative scarcity of recurrent *RYR1* mutations, suggesting that many of these currently highly time consuming and costly targeted approaches may only benefit a small number of (or even isolated) families only.

Potential for *future therapy development* in *RYR1*-related myopathies is suggested by the recent observation of altered expression levels of microRNAs (miRs) and histone deacetylase (HDACs) in patients with recessive *RYR1*-related myopathies (for further details, see article by Treves et al in this issue) [43, 44]. MicroRNAs are endogenous small (around ~22 nucleotides), non-coding RNAs that control gene expression by repressing translation or enhancing RNA degradation and that have been implicated in several cardiac and skeletal muscle pathologies.

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HDACs are involved in gene transcription and sequester the muscle specific transcription factor *meF2* [45]. The overexpression of class II HDACs, in particular HDAC4 and 5, suggests those as potential pharmacological targets, however, the precise role of such overexpression has to be ascertained in the first instance before considering modulating them as a feasible therapeutic strategy.

### 3. Myotubular/Centronuclear myopathy (MTM/CNM)

Myotubular (or Centronuclear) Myopathies (MTM/CNM) are characterized by abundance of central nuclei on muscle biopsy (for review, [46]). CNM is due to X-linked recessive mutations in *MTM1* encoding myotubularin [“X-linked myotubular myopathy (XLMTM)”] [47], autosomal-dominant mutations in *DNM2* encoding dynamin2 [48] and the *BINI* gene encoding amphiphysin 2 [49], autosomal-recessive mutations in *BINI* [50], *RYR1* [14], and *TTN* encoding titin [51], and rarer backgrounds [52-54]. Whilst mutations in *MTM1* and *BINI* are typically associated with a “pure” CNM picture, histopathological appearance in particular in *DNM2*-, *RYR1*- and *TTN*-related forms is more variable and may also feature cores, CFTD and dystrophic features [14, 55, 56]. XLMTM due to X-linked recessive *MTM1* mutations is one of the most devastating neuromuscular disorders in humans with profound weakness, marked respiratory impairment and substantial mortality in affected males, whereas mutations in *DNM2*, *BINI*, *TTN* and *RYR1* are associated with more variable but on the whole milder phenotypes (for review, [46]). Extraocular involvement is prominent in all forms except *TTN*-related CNM. Non-skeletal muscle involvement – for example hepatic peliosis in XLMTM [57], cataracts in *DNM2*-related CNM [58] and dilated cardiomyopathy in association with *SPEG* mutations [54] - has been reported with different genetic backgrounds.

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A wide range of cellular and organismic models of the various genetic forms of CNM have been developed in recent years, as a basis for investigating underlying pathogenic mechanisms and as a prerequisite for therapy development (reviewed in [59]). The proteins initially implicated in CNM – myotubularin, dynamin 2 and amphiphysin 2 – are involved in intricately linked aspects of membrane trafficking and (re)modelling (reviewed in [60]). More specific pathogenic mechanisms (downstream of above “master mechanisms”) (reviewed in [59]) include defects of triads and calcium handling, autophagy, the neuromuscular junction, satellite cells, mitochondria, and the desmin cytoskeleton. *RYR1*-related CNM shares disturbed calcium homeostasis and excitation-contraction coupling as a common mechanism with other forms, but how mutations in *TTN* cause CNM is currently uncertain.

Therapy development efforts in the CNMs have been mainly focussed on XLMTM, considering the profound severity of the disorder. The enzymatic functions of the myotubularin protein suggest ***enzyme replacement therapy*** as a therapeutic option, and the relatively small size of the *MTM1* gene allows for therapies based on viral gene transfer-based ***gene therapy***. Delivery of functional myotubularin applying an AAV8-based vector has now been demonstrated to improve the clinical and histopathological phenotype in *Mtm1*-deficient mice, and to increase muscle strength, reduce respiratory impairment and prolong survival in a canine model of XLMTM [61], the Labrador retriever [62]. Similar improvements of contractile function and histopathological features have been observed following short term myotubularin enzyme replacement in *Mtm1d4* mice [63]. Results from these animal studies have been promising and have provided the basis for human enzyme replacement and gene therapy trials currently in preparation

Another potentially promising avenue for future therapy development is prompted by recent experimental evidence indicating that *dynamin 2 downregulation* may rescue the XLMTM phenotype in mice [64], emphasizing the close interaction of several CNM proteins in the same

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pathway and suggesting dynamin 2 downregulation as a possible therapeutic strategy for XLMTM.

As with for other conditions caused by heterozygous gain-of-function or dominant-negative mutations, dominant forms of CNM due to *DNM2* or *BINI* mutations may also be amenable to recently introduced gene editing strategies once those will approach the stage of clinical application [65].

Another therapeutic approach is based on the observation of neuromuscular junction (NMJ) abnormalities in patients and animal models of CNM [66], and the clinical overlap between the CNMs and certain forms of congenital myasthenic syndromes (CMS), suggesting *pharmacological compounds enhancing neuromuscular transmission* as a potential treatment modality [67, 68]. One such compound, pyridostigmine, has already been demonstrated to be of benefit in patients with genetically confirmed and unresolved forms of CNM [67, 68]. Salbutamol is another drug that has been used effectively in certain subgroups of CMS and may also be of benefit in other congenital myopathies as outlined above [38]. However, those observations based on small pilot studies and no large placebo-controlled double-blind studies concerning these compounds have been performed yet.

### 4. *TTN*-related myopathies

Although recessive *TTN* mutations have been linked with CNM [51], there is considerable overlap with other congenital myopathies, in particular the core myopathies [8, 56], and *TTN*-related myopathies are therefore covered here in a separate paragraph. The treatment approaches discussed below may also be potentially relevant to genes of similar size and mutation profiles such as *NEB*, and/or other proteins where abnormal aggregation may be an issue.

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Due to the large size of the *TTN* gene, its many exons, and a protein molecular weight of over 3 million Dalton, titin is highly susceptible to the stochastic impact of mutagenesis [69]. Premature stop-codons (caused by either mutations affecting correct splicing and leading to the inclusion of intronic sequences, or exonic nonsense mutations) as well as numerous missense variants abound in the *TTN* gene, with their functional impact and pathogenic relevance often difficult to ascertain. In CNM and other *TTN*-related Paediatric congenital myopathies reported to date, these variants are recessive [8, 51, 56, 70]. Although most *TTN* variants are private or very rare, some recessive missense variants have been linked to Autosomal-Recessive Multi-minicore Disease with Heart Disease (AR MmD-HD), with clear functional indication of structural disruption and complete loss of function of some key titin domains [8]. It appears that almost any combination of truncating and disruptive missense mutations in *TTN* can cause early-onset congenital myopathies with often vastly variable phenotypes, posing formidable challenges for accurate diagnostics but also for the development of therapeutic strategies that are realistically employable within the constraints of real clinical settings. Considering the main mutation mechanisms, several treatment strategies can be conceived and are pursued already in different contexts:

(I) In the presence of nonsense mutations, restoring the mRNA reading frame (either by exon-skipping or suppression of premature stop codons) could restore a functionally useful protein with largely intact mechanical and signalling functions and the potential to rescue or ameliorate the disease phenotype. This approach has been developed for the treatment of Duchenne muscular dystrophy (DMD), by exploiting initially the ability of certain aminoglycosides to enhance premature stop-codon read-through [71] and subsequent development of compounds with a similar mechanism of action but reduced toxicity such as PTC124 (Ataluren). Pharmacological suppression of premature stop codons is potentially applicable to a wide range of diseases, and pre-clinical and clinical studies are ongoing at various stages for DMD, cystic fibrosis, Werner

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Syndrome and other conditions [72-75], and should clarify the long-term clinical outcomes in comparison to established therapies. This approach should also be applicable to *TTN* truncating nonsense mutations, informed by prior studies in cellular and/or animal titinopathy models as a screening platform for the feasibility of stop-codon read-through therapy. It is not yet clear if the efficacy of ataluren is high enough to restore functional levels of dystrophin or titin to regain significant structural integrity and function, which is clearly easier with enzymes or ion channels where (partial) functional rescue can be achieved with much lower stoichiometry. An interesting point is whether the simultaneous intervention in other loss-of-function loci will be beneficial. MacArthur et al (2014) estimated that the human genome contains around 100 loss-of-function variants, with about 20 genes completely inactivated [76]. The impact of these variants can be hard to predict, leading to loss of mRNA or protein by specific degradative pathways [77]. This might pose not only a diagnostic problem (which truncating gene variants are truly pathogenic?), but could also be a challenge for global pharmacological read-through therapy that would lead to the re-expression of such loss-of-function loci.

(II) The use of protein-stabilising chaperone-like compounds (“chemical chaperones”) could be used to prevent the aggregation or degradation of misfolded titin domains carrying missense variants, or to promote their clearance by protein degradation pathways. This approach could alleviate the effects of proteotoxicity if a more normal function of the abnormal protein could be maintained. The recent use of the chemical chaperone 4-phenylbutyrate (4-PBA) demonstrated that this simple compound is capable of ameliorating protein aggregate formation and restoring basic mechanical functions in cellular models of plectinopathy [78], although it remains to be seen if these promising results can be reproduced at the organismic level. The chemical nature of 4-PBA leads to effects on multiple pathways, with a well-documented ability to inhibit histone deacetylases (HDAC) [79]. HDAC inhibition activates autophagy (for example, [80]), one of the two major ubiquitin-dependent protein degradation pathways, and it is possible that the stimulation



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of autolysosomal degradation of protein aggregates and thus their reduced proteotoxicity, rather than primary prevention of misfolding, might be mechanistically important in skeletal muscle. Clearly, developing better and more specific small-molecule inhibitors of protein aggregation could therefore add significantly to the arsenal of hereditary myopathy therapy but might require tailored approaches for certain classes or types of mutations. [81]

(III) In the case of haploinsufficiency, the absent protein would need to be restored if the above strategies are not effective. Current methods for gene delivery using chemical approaches or viral vectors are unsuited for gene or even protein delivery of a target with an mRNA of over 80 kb, making this last approach unlikely to be successful even in the medium future.

(IV) Compounds that directly target the biological activity of the mutant protein to disrupt that pathomechanism at its causative root. Such drug discovery is in its infancy for hereditary myopathies, but recent advances for small molecules targeting mutant beta-myosin and alleviating heart disease progression in mouse models [82] suggest that such approaches might be feasible in some cases. However, better understanding of the underlying biology would be required to devise robust screening protocols.

**5. Nemaline Myopathy (NM)**

Nemaline myopathy (NM) is one of the more common congenital myopathies [2, 83, 84] characterized by numerous nemaline rods on muscle biopsy. To date, 10 genes have been linked to this disorder, including *ACTA1*, *NEB*, *TPM3*, *TPM2*, *CFL2*, *TNNT1*, *LMOD3*, *KBTBD13*, *KLHL40*, and *KLHL41* [84-86] of which recessive mutations in *NEB* and (*de novo*) dominant mutations in *ACTA1* are the most common. NM is associated with highly variable degrees of muscle weakness [2, 83, 84, 87] ranging from profoundly affected infants within the fetal akinesia spectrum to only mildly affected adult-onset cases. Bulbar and respiratory impairment are

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common in many genetic forms but extraocular muscle involvement is not a typical feature, except in those due to mutations in the *KLHL* genes.

Seven of the 10 genes implicated in NM - i.e., skeletal alpha-actin (*ACTA1*), nebulin (*NEB*), tropomyosin (*TPM3* and *TPM2*), troponin T (*TNNT1*), cofilin (*CFL2*) and leiomodin-3 (*LMOD3*) - encode proteins constituting the sarcomeric thin filament [88, 89]. The sarcomeric thin filament is an essential component of normal muscle function, through conformational changes allowing myosin head binding to actin monomers and, ultimately, force production, prompted by  $\text{Ca}^{2+}$  binding to one of its components, troponin C. To understand how NM-related mutations disrupt normal thin filament function, experimental studies have used muscle samples from NM patients or from mouse models reproducing the human condition [86, 90-96]. These studies have revealed that the absence of certain proteins (e.g. nebulin or skeletal alpha-actin), or the presence of mutated amino acids in skeletal alpha-actin and tropomyosin, alter  $\text{Ca}^{2+}$  binding, thin filament conformation and/or the interaction between myosin and actin molecules [86, 95, 97-108], resulting in reduced force generation at the sarcomere and, ultimately, skeletal muscle weakness.

Despite improved understanding of the pathophysiological mechanisms underlying skeletal muscle weakness in NM, there is currently no cure and management is mainly symptomatic [109, 110], but a number of *pharmacological compounds* and *supplements* have been tested. Supported by preclinical data in a mouse model of *ACTA1*-related NM [90], a pilot study in 5 patients with NM suggested a beneficial effect of *L-Tyrosine*, with reduced fatigue and improvement of drooling [111]. A recent report of a single case with severe *KLHL40*-related NM suggested a sustained beneficial response to the acetylcholinesterase inhibitor pyridostigmine [112], indicating an associated neuromuscular transmission defect potentially amenable to treatment, corresponding to observations in other congenital myopathies, in particular the CNMs (see above).

Considering the key role of thin filament dysfunction in NM, *drugs targeting thin*

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*filaments and their interactions* directly have been recently developed [113, 114]. CK-2017357 (Cytokinetics Inc.) binds to the  $\text{Ca}^{2+}$  sensor, troponin C, thereby facilitating force production by slowing the rate of  $\text{Ca}^{2+}$  release from troponin C and consequently favouring myosin activation and impairing the relaxation process [115]. However, a possible clinical benefit in NM is uncertain as CK-2017357 preferentially affects fast-twitch muscle fibres which are typically lacking in NM. Another compound, *Omecamtiv Mecarbil* (formerly termed CK-1827452) (Cytokinetics Inc.) attaches to myosin molecules and could be potentially advantageous for weak slow-twitch muscle fibres [116]. However, Omecamtiv Mecarbil also binds to cardiac myosin molecules, raising the possibility of cardiac complications if given to patients with NM.

Considering the difficulties with directly targeting thin filament function, *other therapeutic strategies* are currently being developed. Muscle tissue from patients or animal models of NM contains a large number of atrophic fibres [2, 83, 84], suggesting increasing muscle fibre size and overall muscle mass by using *myostatin inhibitors* as a possible therapeutic strategy. Myostatin is a protein that is selectively expressed in skeletal muscle, cardiac muscle, and adipose tissue during late embryogenesis and adulthood and is an important negative regulator of muscle fibre size [117]. It binds to and signals through the activin type IIB receptor (ActRIIB) to down-regulate several key processes related to muscle fibre hypertrophy [117]. The potential for myostatin inhibition to promote muscle growth has led to development of a new class of myostatin and ActRIIB inhibitors as prospective therapeutic agents for myopathic, dystrophic and neurologic disorders [117], including NM but potentially also other congenital myopathies where fibre atrophy is a prominent feature such as XLMTM [118].

In the context of *ACTA1*-related NM, muscles from patients with rare null mutations partially compensate the absence of skeletal muscle  $\alpha$ -actin by expressing the *ACTC*-associated protein, cardiac  $\alpha$ -actin [119]. These findings have prompted investigation of *cardiac  $\alpha$ -actin upregulation* as a possible therapeutic avenue in various mouse models of *ACTA1*-related NM. In one

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transgenic line carrying the D286G mutation, muscle weakness and lethality were significantly reduced whilst in another model expressing H40Y, the up-regulation of *ACTC* did not have any noticeable positive or beneficial effect [120]. The mechanisms underlying such discrepancies remain to be determined.

### Congenital Fibre Type Disproportion

Congenital fibre type disproportion (CFTD) is most commonly defined by type 1 fibers being at least 25% smaller than type 2 fibres in the absence of other pathological features on muscle biopsy (for review[121]). There is marked genetic and clinico-pathological overlap with other congenital myopathies, in particular NM and *RYR1*-related myopathies, and although in many patients the histopathological appearance of “pure” CFTD persists, in others additional histopathological features such as nemaline rods, central nuclei or cores may evolve over time [15, 122-127]. CFTD is most commonly due to mutations in *TPM3* and *RYR1* and less frequently due to mutations in *ACTA1* and *SEPN1*. Although there are currently no therapeutic approaches specifically aimed at CFTD, these disorders are likely to benefit from therapy developments in other closely related congenital myopathies.

### Conclusions and outlook

Although there is currently no cure for any of the congenital myopathies, therapy development is rapidly progressing and informed by recent exponential advances concerning the genetic and molecular understanding of these conditions. Based on animal experimental evidence, gene therapy and enzyme replacement therapy trials are in preparation for X-linked myotubular myopathy (XLMTM), one of the most severe congenital myopathies. Clinical trials in humans

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with acetylcysteine in humans with *SEPNI*- and *RYR1*-related myopathies are currently in progress, based on the observation of marked oxidative abnormalities as an important pathogenic mechanism in these conditions. Off-label use of drugs such as dantrolene, pyridostigmine and salbutamol appears to be of benefit in small open-label pilot studies, but those findings will have to be confirmed in larger double-blind, placebo-controlled clinical trials. Unravelling novel disease mechanisms may lead to the development of therapies potentially applicable to several genetically distinct subgroups in subgroups intricately linked in the same molecular pathways. Novel gene editing strategies may provide new therapeutic options in particular for dominantly inherited conditions in future but do raise significant ethical questions associated with direct manipulation of the human genome.

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**Conflict of interest:**

HJ served as a member of the Advisory Board for Audentes Therapeutics, a pharmaceutical company developing genetic therapies for XLMTM.

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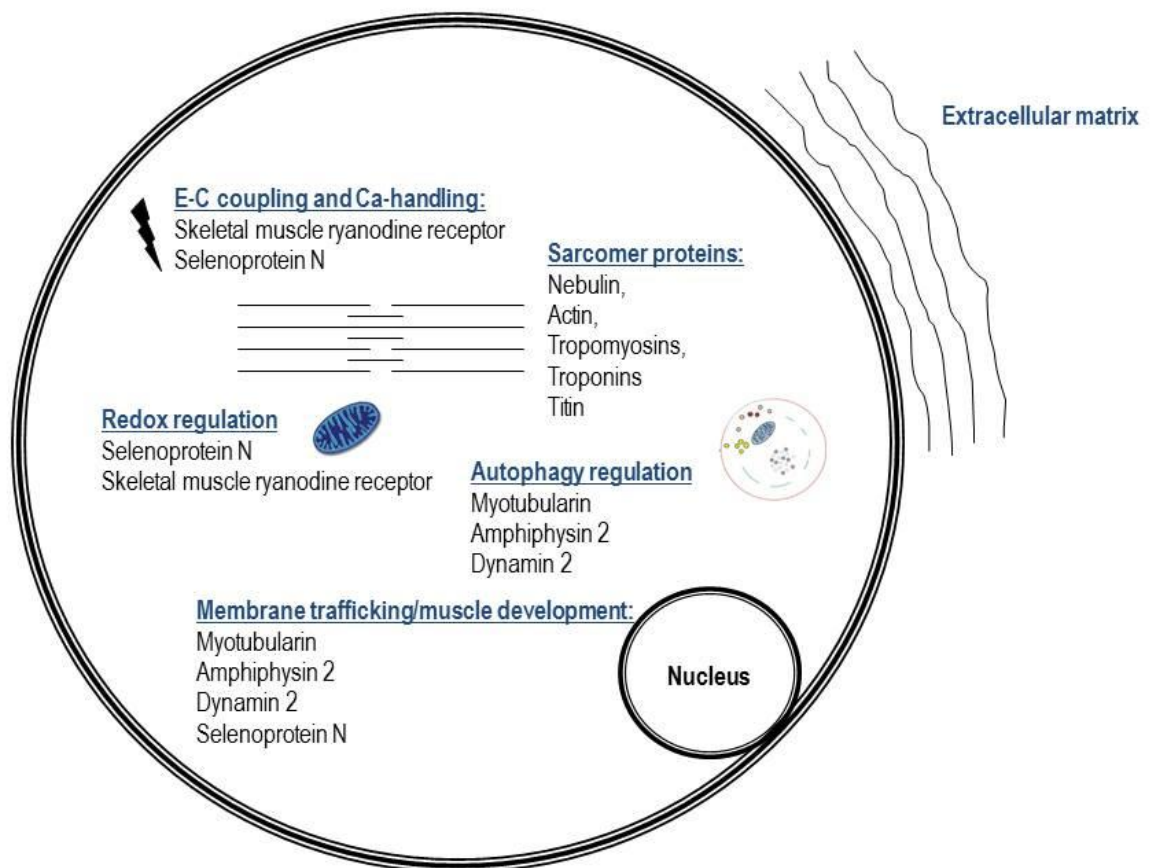
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**Figure and Table legends:****Figure 1**

**Schematic representation of pathogenic mechanisms implicated in the congenital myopathies (selection).** A significant proportion of proteins involved in the congenital myopathies play a role in various aspects of Excitation-Contraction (EC) coupling, the process by which a neuronal impulse is translated into muscle contraction, mediated by calcium release from the sarcoplasmic reticulum (SR) into the cytosol and resulting in contractile thin/thick filament interactions. Abnormalities of the triad, the ultrastructural correlate of the EC machinery, are also an important downstream effect of defective membrane formation and remodelling in various genetic forms of CNM due to mutations in *MTM1*, *DNM2* and *BIN1*.



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**Table 1: Genetics of the major congenital myopathies.** AD = autosomal dominant, AR = autosomal recessive, XLR = X-linked recessive inheritance. The most common genetic backgrounds within each group are indicated in bold. \*typically combination of central nuclei and cores; \*\*one isolated case with *RYR1*-related CNM and a *de novo* heterozygous missense mutations has also been reported. \*\*\* typically combination of (central) nuclei, (mini)cores and other myofibrillar abnormalities

	GENE	LOCUS	INHERITANCE	PROTEIN
<b><u>Nemaline myopathy</u></b>				
	<i>ACTA1</i>	1q42	AD/AR	skeletal $\alpha$ - actin
	<i>CFL2</i>	14q12	AR	Cofilin-2
	<i>KBTBD13</i>	15q25	AD	Kelch 13
	<i>KLHL40</i>	3p21	AR	Kelch-like 40
	<i>KLHL41</i>	2q31	AR	Kelch-like 41
	<i>NEB</i>	2q.2	AR	Nebulin
	<i>TPM2</i>	9p13	AD/AR	$\beta$ -tropomyosin
	<i>TPM3</i>	1q2	AD/AR	$\alpha$ -tropomyosin
	<i>TNNT1</i>	19q13	AR	Troponin T
<b><u>Core myopathies</u></b>				
Central Core Disease	<b><i>RYR1</i></b>	<b>19q13</b>	AD/AR	<b>Ryanodine receptor 1</b>
Multiminicore Disease	<b><i>RYR1</i></b>	19q13	AD/AR	Ryanodine receptor 1
	<b><i>SEPN1</i></b>	1p36	AR	<b>Selenoprotein N1</b>
+/- cardiomyopathy	<i>TTN</i>	2q31	AR	Titin
	<i>MYH7</i>	14q11	AD	slow myosin heavy chain
<b><u>Centronuclear myopathy</u></b>				

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<u>Myotubular myopathy</u>	<i>MTM1</i>	Xq28	XLR	Myotubularin
<u>Centronuclear myopathy</u>	<i>BIN1</i>	2q14	AR	Amphiphysin
	<i>CCDC78</i>	16p13.3	AD*	coiled-coil domain-containing protein 78
	<i>DNM2</i>	19p13	AD	Dynamin-2
	<i>MYF6</i>	12q21.31	AD	Myogenic factor 6
	<i>RYR1</i>	19q13	AR**	Ryanodine receptor 1
	<i>SPEG</i>	2q35	AR	SPEG complex locus
	<i>TTN</i>	2q31	AR***	Titin
<b><u>Congenital Fibre Type Disproportion</u></b>				
	<i>ACTA1</i>	1q42	AD	skeletal $\alpha$ -actin
	<i>HACD1</i>	10p12	AR	3-hydroxyacyl-CoA dehydratase 1
	<i>MYH7</i>	<b>14q11</b>	AD	slow myosin heavy chain
	<i>RYR1</i>	<b>19q13</b>	AR	Ryanodine receptor 1
	<i>SEPN1</i>	<b>1p36</b>	AR	Selenoprotein N1
	<i>TPM2</i>	9q13	AD	$\beta$ -tropomyosin
	<i>TPM3</i>	1q2	AD	$\alpha$ -tropomyosin

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**Table 2: Therapeutic approaches to the congenital myopathies.** \* = first trials with N-acetylcysteine currently in progress.

<b>Therapeutic approach</b>	<b>CM</b>	<b>Gene</b>	<b>Type of study</b>	<b>Reference</b>
<i>Gene therapy</i>				
<b>Exon skipping</b>	<b>CCD</b>	<i><b>RYR1</b></i>	<b>Proof of Principle</b>	<b>41</b>
<b>Gene transfer</b>	<b>XLMTM</b>	<i><b>MTM1</b></i>	<b>Proof of Principle</b>	<b>61</b>
<i>Enzyme replacement</i>				
<b>Enzyme replacement therapy</b>	<b>XLMTM</b>	<i><b>MTM1</b></i>	<b>Proof of Principle</b>	<b>63</b>
<i>Modulation of SR calcium release</i>				
<b>Dantrolene</b>	<b>MH, ERM</b>	<i><b>RYR1</b></i>	<b>Case studies</b>	<b>26</b>
<b>Dantrolene</b>	<b>CCD</b>	<i><b>RYR1</b></i>	<b>Case studies</b>	<b>27</b>
<b>AICAR</b>	<b>MH, ERM</b>	<i><b>RYR1</b></i>	<b>Proof of Principle</b>	<b>32</b>
<b>Rycals</b>	<b>MH, CCD</b>	<i><b>RYR1</b></i>	<b>Proof of Principle</b>	<b>29</b>
<i>Modulation of neuromuscular transmission</i>				
<b>Pyridostigmine</b>	<b>CNM</b>	<i><b>MTM1</b></i>	<b>Open-label pilot study</b>	<b>68</b>
<b>Pyridostigmine</b>	<b>NM</b>	<i><b>KLHL40</b></i>	<b>Case study</b>	<b>112</b>
<b>Salbutamol</b>	<b>MmD, CCD</b>	<i><b>RYR1</b></i>	<b>Open-label pilot study</b>	<b>38</b>
<i>Modulation of filament interaction</i>				
<b>CK-2017357</b>	<b>NM</b>	<i><b>Various</b></i>	<b>Proof of Principle</b>	<b>115</b>
<b>CK-1827452</b>	<b>NM</b>	<i><b>Various</b></i>	<b>Proof of Principle</b>	<b>116</b>
<i>Other approaches</i>				
<b>Myostatin inhibition</b>	<b>CNM</b>	<i><b>MTM1</b></i>	<b>Proof of Principle</b>	<b>118</b>
<b>N-acetylcysteine</b>	<b>CCD</b>	<i><b>RYR1</b></i>	<b>Proof of Principle*</b>	<b>35</b>
<b>N-acetylcysteine</b>	<b>MmD</b>	<i><b>SEPNI</b></i>	<b>Proof of Principle*</b>	<b>35</b>