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Therapeutic Aspects in Congenital Myopathies

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The congenital myopathies are a genetically heterogeneous and diverse group of early-onset, nondystrophic neuromuscular disorders. While the originally reported "classical" entities within this group – Central Core Disease, Multiminicore Disease, Nemaline Myopathy, and Centronuclear Myopathy – were defined by the predominant finding on muscle biopsy, "novel" forms with multiple, subtle, and unusual histopathologic 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 pharmacologic 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.
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Introduction

The congenital myopathies (CMs) are a group of genetically heterogeneous, nondystrophic conditions with distinct histopathologic and highly variable clinical features (for review, see ref.[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. While 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 orthopedic manifestations, management has been mainly based on a multidisciplinary approach involving various medical specialties and allied health professionals.[2] While 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, see ref.[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-pathologic features and

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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 CMs is currently in flux, with important implications for diagnosis, management but also therapy development (for review, see ref.[1]): Originally described in the 1950s and 1960s, the major disorders within this group – Central Core Disease (CCD), Multiminicore Disease (MmD), Nemaline Myopathy (NM) and Centronuclear Myopathy (CNM) – were classified based on the predominant histopathologic 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 (eg, the gene encoding the skeletal muscle ryanodine receptor, *RYR1*) may give rise to a wide range of different, histopathologically defined entities, whereas mutations in different genes (eg, those implicated in NM) may give rise to the same CM. While the CMs at the point of their original description were considered "pure" histopathologic entities, it has now also become apparent that those with multiple histopathologic 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 while 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 histopathologic 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–focused 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

CCD and *MmD*, often summarily referred to as the "core myopathies,"[4] are the most common CMs.[5] The principal histologic 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 genetically 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: While 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, see ref.[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 noninvasive 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 magnetic resonance imaging may help to differentiate genetically distinct core myopathies.[11]

CNM (for review, see ref.[12]) is characterized by the abundance of internalized and centralized nuclei on muscle biopsy, with variable additional histopathologic 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 supportive 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 Charcot-Marie-Tooth Disease (CMT), as well as other nonmuscular manifestations that may also occur in long-term survivors with XLMTM.[19]

NM, characterized by thread-like structures ("nemaline rods") on muscle biopsy that appear dark with the Gomori

trichrome stain (for review, see ref.[20] [Dubowitz 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 histopathologic 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 (eg, see ref.[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 magnetic resonance imaging may aid distinction between different genetic forms of NM.[34]

"Novel" CMs

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

CMs with nonspecific, multiple or rare histopathologic features: A substantial number of patients have been reported with mutations in known CM-associated genes but only nonspecific histopathologic features rather than the previously reported, more distinct structural abnormalities (for review, see ref.[1]); these nonspecific features include type 1 predominance or uniformity and congenital fiber disproportion (CFTD), that is, often predominant type 1 fibers that are significantly and consistently smaller than type 2 fibers. 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, and central nuclei − rather than the "pure" histopathologic presentations as summarized above. Some very rare histopathologic 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 histopathologic 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, see ref.[38]) are multisystem disorders associated with both dominant and recessive inheritance; neuromuscular manifestations comprise tubular aggregate myopathy but also myopathic manifestations with considerable overlap with the "classical" CMs. Recessively inherited, *PYROXD1-related CM*[39] features, histopathologic features of increased internalized nuclei, and myofibrillar disorganization and clinical symptoms of a moderately severe CM.

Hereditary myosin myopathies ("myosinopathies"; for review, see ref.[40]) comprise a wide range of neuromuscular phenotypes. Among 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 histopathologic 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 in *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.

CMs and the Malignant Hyperthermia 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 anesthetics, and depolarizing muscle relaxants (for review, see ref.[45]). While 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,*[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 *King-Denborough syndrome* and a high MH risk is *Native American myopathy*, 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 (for review, see ref.[1]) concern intracellular processes ensuring normal muscle maintenance and function, in particular ECC, the process whereby 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 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 among the most common causes of the CMs (Figure 1): 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, see refs.[50,51]). While the mechanisms underlying the more recently described recessive *RYR1*-related myopathies — MmD, CNM, and 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 CM 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, see ref.[38]) affect 2 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 with *SEPN1*[53,54] and the CNM-associated genes *DNM2* and *BIN1*.

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. CCD, Central Core Disease; MH, malignant hyperthermia; XLMTM, X-linked myotubular myopathy.

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. The genes and major pathogenic mechanisms implicated in the CMs are summarized in Table 1.

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

Table 1 Main Pathogenic Mechanisms Implicated in the Congenital Myopathies

Mechanism	Gene	Protein	Phenotype(s) and Inheritance
ECC and calcium homeostasis	*CACNA1S*	Calcium channel, voltage-dependent, L-type, alpha 1S subunit	CM (AD,AR)
	RYR1	Ryanodine receptor 1 (skeletal)	**CCD (AD), MmD,** CNM (AR)
	STAC3	SH3 and cysteine-rich domain 3	CM(AR)
	ORAI1	Calcium release-activated calcium modulator 1	TAM (AD,AR)
	STIM1	Stromal interaction molecular 1	TAM (AD,AR)
Thick-thin filament assembly, myofibrillar force generation and protein turnover	***NEB***	Nebulin	**NM (AR)**
	ACTA1	Alpha actin, skeletal muscle	**NM (AD,**AR)
	TPM2	Tropomyosin 2 (beta)	NM (AD)
	TPM3	Tropomyosin 3	NM (AD)
	MYH2	Myosin, heavy polypeptide 2, skeletal muscle	CM (AD,AR)
	MYH7	Myosin, heavy polypeptide 7, cardiac muscle, beta	MmD, MSM (AD,AR)
	KBTBD13	Kelch repeat and BTB (POZ) domain containing 13	NM (AD)
	KLHL40	Kelch-like family member 40	**NM (AR)**
	KLHL41	Kelch-like family member 41	NM (AR)
	TTN	Titin	**CNM,** MmD (AR)
Other cellular processes			
-Myogenesis	***SEPN1****	Selenoprotein N1	**MmD (AR)**
	MEGF10	Multiple EGF-like-domains 10	CM (AR)
	MTM1*	Myotubularin	**XLMTM (X-linked)**
-Membrane and intracellular trafficking	*BIN1**	Amphiphysin	CNM (AR,AD)
	*DNM2**	Dynamin 2	CNM (AD)
-Redox regulation	*PYROXD*	Pyridine nucleotide-disulfide oxidoreductase domain-containing protein 1	CM (AR)
-Unknown and other	*ECEL1*	Endothelin converting enzyme-like protein 1	CM, DA (AR)
	PIEZO2	Piezo-type mechanosensitive ion channel component 2	CM,DA (AD)
	SCN4A	Sodium channel, voltage gated-type IV, alpha subunit	CM (AR)

AD, autosomal-dominant; AR, autosomal-recessive; CCD, Central Core Disease; CM, congenital myopathy with multiple or nonspecific features; CNM, Centronuclear Myopathy; DA, Distal Arthrogryposis; ECC, excitation-contraction coupling; MmD, Multiminicore Disease, MSM, myosin storage myopathy; NM, Nemaline Myopathy; TAM, tubular aggregate myopathy; XLMTM, X-linked myotubular myopathy.

Common genes and phenotypes are indicated in bold. 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 ref.1). 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).

substantially improved both quality of life and life expectancy. While therapy development concerning the neuromuscular field so far has focused on the more common and severe conditions (eg, 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 CM spectrum, XLMTM has been a particular focus of therapy development, and outcomes of ongoing clinical trials are already expected in 2019. Therapies designed for the CMs (summarized in Table 2; for review, see ref.[3]) can be grossly divided in (1) genetic therapies aimed at directly correcting the underlying genetic defect, (2) enzyme replacement therapy, and (3) pharmacologic 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. While 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.

Supportive management 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 excellent review of the topic.[2] Considering often multiple comorbidities, an effectively coordinated multidisciplinary approach involving various medical and allied health professionals is essential for the effective management of the CMs. 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, orthopedic input should be sought early, ideally at a tertiary neuromuscular center experienced with the management of these conditions, including respiratory, cardiac, and orthopedic 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

Table 2 Therapeutic Approaches to the Congenital Myopathies

Therapeutic Approach		Condition/Gene	Compound(s)	Reference(s)
Genetic	Viral-based gene transfer	XLMTM, others	AAV8 vector-based *MTM1* transfer	62,64
	Gene editing	Various	Various	65
	Suppression of premature stop codons	Various	Various	68
	Exon skipping	CCD, other AD CMs	Various	67
	Protein downregulation (DNM2)	CNM *(MTM1, BIN1)*	Various	71
	Targeting of class II and III PI3 kinases	CNM *(MTM1)*	Various	72
	Protein upregulation (ACTAC)	NM *(ACTA1)*	Various	74
ERT	Myotubularin replacement	XLMTM	Myotubularin	75
Pharmacologic	Modification of RyR1 receptor calcium release	*RYR1*-RM (AD)	Dantrolene, Rycals, AICAR	76-81; 84,87
	Targeting thin/thick filament interactions	NM, others	CK-2017357, CK-1827452	90,91
	Reduction of oxidative stress	*RYR1* + *SEPN1*-RM	*N*-acetylcysteine (NAC)	92
	Enhancement of neuromuscular transmission	CNMs, others	Pyridostigmine, Salbutamol*	95-99
	Stimulation of muscle growth pathways	Various	ActRIIB inhibitors, others	102
	Prevention of protein aggregates	*MYH7*-RM, others	4-phenylbutyrate (4-PBA)	105,107

AD, autosomal-dominant; CCD, Central Core Disease; CNM, Centronuclear Myopathy; NM, Nemaline Myopathy.

Among 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 reached (or are approaching) the clinical trial stage.

*Salbutamol probably exerts its effects through additional mechanisms other than enhancement of neuromuscular transmission (see main text).

(noninvasive) 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 preoperative planning in these patients.

Genetic therapies: Many of the genes mutated in the CMs such as *TTN, NEB,* or *RYR1* are among the largest in humans, precluding *viral-based gene transfer* in most of these conditions, considering that aden-associated virus (AAV) gene therapy approaches are unsuitable for genes with an mRNA over 4.5 kb. Such an approach, delivery of the relatively smaller *MTM1* gene utilizing an AAV8 vector, has, however, been successfully demonstrated in 2 animal models of XLMTM, the Mtm1-deficient mouse[62] and the naturally occurring Labrador retriever model of XLMTM.[63,64] In both instances, viral-based gene transfer was well-tolerated, and resulted in improvement of clinicopathologic 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 CMs 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 pharmacologic 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, see refs.[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 the 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]; the basis of this variability 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 histopathologic features and contractile function following myotubularin ERT,[75] providing the basis for human therapy trials currently in preparation.

Pharmacologic 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 nonspecifically 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 (eg, 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 pharmacologic 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 antagonist 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 derivatives with the ability to modify RyR 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 RyR receptor through increasing its closed probability, and their dissociation from the receptor (eg, 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 5-aminoimidazole-4-carboximide ribonucleoside (AICAR), a known activator of the AMP-activated protein kinase, 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 2 important contributors to the rhabdomyolysis, RyR1 calcium leak, and the production of reactive nitrogen and oxidative species,[86,87] and may thus be suitable for corresponding human *RYR1*-related phenotypes with similar pathomechanisms. Despite early promise in preclinical 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 long-term 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 pharmacologic 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.) favors myosin activation and contraction by slowing the rate of calcium release from Troponin C[90] but preferentially affects type 2 fibers typically markedly reduced or absent in NM. CK-1827452 (or Omecamtiv Mecarbil, Cytokinetics Inc.) is another compound enhancing myosin activation in a fiber-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 affect similar mechanisms or pathways. Common downstream effects include muscle atrophy both macroscopically and on the single fiber 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 pharmacologic 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 long−term 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 case 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 pharmacologic 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 fiber level is a common feature in many CMs 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 receptor. Novel myostatin and activin type IIb inhibitors have been developed that − in addition to a potential role in the CMs − may be of potential use in other myopathic, dystrophic, and neurologic disorders where muscle atrophy is a prominent feature. The recent observation of altered microRNA and histone deacetylase (HDACs) expression (ie, 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: microRNAs are small noncoding 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 its role for example in 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 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 4−PBA 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 specificity.

Other pharmacologic approaches include *L-Tyrosine supplementation*, an approach so far only tried in a small cohort of patients with NM[108] based on promising preliminary results in a relevant animal model,[109] resulting 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 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 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 antioxidant effect (primary), and the 6-minute walk test as a measurement of improved endurance (secondary) at an interval following establishment of a baseline. These 2 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 a 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. While 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.

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