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Therapeutic approaches in different congenital myopathies

Charlotte Gineste and Jocelyn Laporte

Abstract

Congenital myopathies are rare and severe genetic diseases affecting the skeletal muscle function in children and adults. They present a variable spectrum of phenotypes and a genetic heterogeneity. Subgroups are defined according to the clinical and histopathological features and encompass core myopathy, centronuclear myopathy, nemaline myopathy and other rare congenital myopathies. No approved treatment exists to date for any congenital myopathies. To tackle this important unmet need, an increased number of proof-of-concept studies recently assessed the therapeutic potential of various strategies, either pharmacological or genetic-based, aiming at counteracting muscle weakness or/and cure the pathology. Here, we list the implicated genes and cellular pathways, and review the therapeutic approaches preclinically tested and the ongoing/completed clinical trials for the different types of congenital myopathies.

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Introduction

Congenital myopathies refer to a group of rare genetically heterogeneous non-dystrophic skeletal muscle disorders. The prevalence was estimated to be approximately 1.50 per 100,000 in the all-age population, and 2.73/100,000 in the child population [1]. The different forms of the disease are distinguished based on clinical defects and histological anomalies. To date there is no approved therapy for any congenital myopathies. This review aims to describe the current status of the

therapeutic proof-of-concept studies and ongoing or completed clinical trials for congenital myopathies.

General phenotypes

The main clinical features of congenital myopathies are muscle weakness and hypotonia often present at birth. The clinical spectrum ranges from mild form (mainly childhood-onset) to severe forms (mainly neonatal). Patients typically show muscle atrophy and type 1 fiber predominance. Respiratory muscles are often affected, correlating with respiratory insufficiency. Patients may present other symptoms such as facial weakness, scoliosis, and eye or cardiac muscles involvement.

Subclasses and main differences

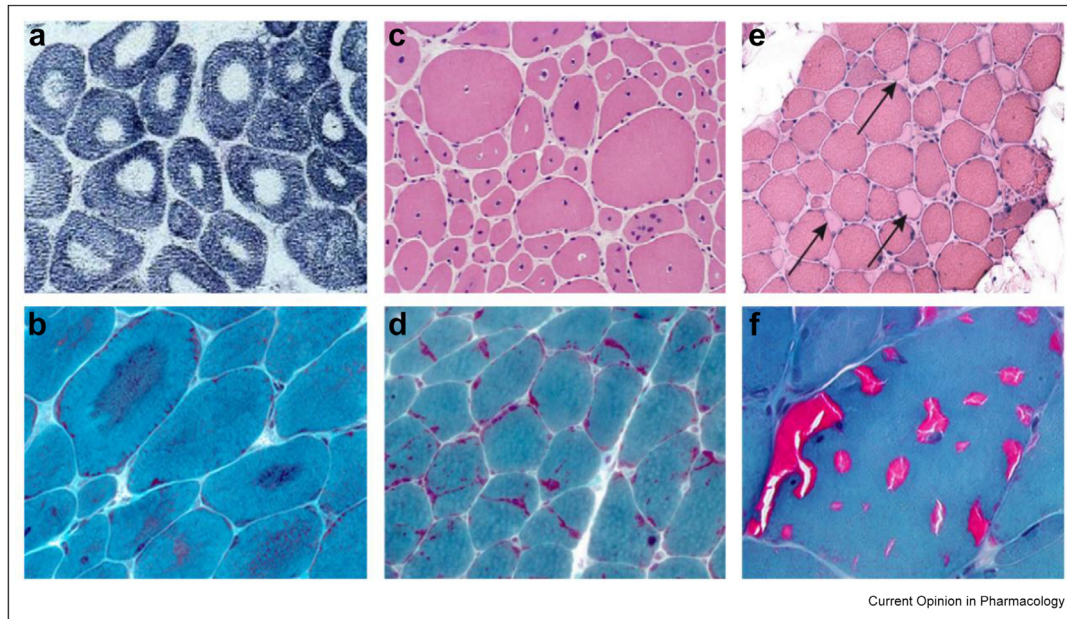
To date, the classification of congenital myopathies is mainly based on specific histological features found on muscle biopsies (Figure 1). Congenital myopathies can be divided into 4 main subgroups:

- Core myopathies
- Nemaline myopathy (NM)
- Centronuclear myopathies (CNM)
- Rare congenital myopathies

Core myopathies are characterized by the presence of areas with markedly reduced oxidative activity named “cores.” The localization, shape, size and number of cores define the different subtypes of core myopathies [2]. Individual cores centrally localized are found in central core disease (CCD). Numerous scattered small cores are usually found in multi-mini core disease (MmD). More recently, dusty cores have been identified in dusty core disease (DuCD) and described as irregular areas of myofibrillar disorganization with uneven oxidative stain and devoid of ATPase activity [3].

The histopathological hallmark of nemaline myopathy is the presence of rod-like structures within the muscle fibers also called nemaline bodies. Rods are aggregates of proteins derived from the Z-disks [4]. Rods are mainly cytoplasmic but can sometimes have a nuclear localization. The number and distribution of rods can vary between muscles and between fibers. There is no correlation between the number of rods and the clinical severity.

Figure 1



Histopathological features of different subgroups of congenital myopathy. Main type of congenital myopathies: core myopathies with (a) central core disease and (b) dusty core disease, (c) centronuclear myopathy and (d) nemaline myopathy. Rare congenital myopathies: (e) myosin storage myopathy and (f) tubular aggregate myopathy. (a) The cores are unstained (white) by NADH-TR oxidative reaction that stains the intermyofibrillar network (blue). This patient has an autosomal dominant *RYR1*-related central core disease. (b) Dusty cores are irregular areas of reddish-purple granular material deposition on Gömöri trichrome stain. (c) Muscle fibres showing centrally located nuclei on hematoxylin and eosin stain, in a patient with autosomal dominant centronuclear myopathy caused by a *DNM2* mutation. (d) Nemaline bodies (rods) are stained red on a modified Gömöri trichrome staining and are present in the sarcoplasm and/or beneath the sarcolemma. This patient carries a mutation in *ACTA1* causing autosomal dominant nemaline myopathy. (e) Numerous muscle fibers show subsarcolemmal accumulation of material (arrows) that stains faintly red with hematoxylin–eosin. (f) Tubular aggregates are membrane tubules containing sarcoplasmic reticulum proteins that appear as red aggregates on Gömöri trichrome staining in this patient with tubular aggregate myopathy caused by the H109N mutation in *STIM1*. Images (a), (c) and (d) are adapted from [79] with permission from John Wiley and Sons. Image (b) is licensed under the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>) and is adapted from Garibaldi et al. [3]. Image (e) is licensed under the terms of the Creative Commons Attribution 2.0 International License (<https://creativecommons.org/licenses/by/2.0/>) and is adapted from [80]. Image (f) is adapted from [81] with permission.

Mislocalized nuclei in muscle cells, most often abnormally positioned in the center of the fiber without any excessive muscle regeneration, is commonly observed on biopsies from patients with centronuclear and myotubular myopathies [5].

Rare congenital myopathies refer to congenital myopathies presenting non-specific or rare histopathological characteristics such as the presence of tubular aggregates, myosin anomalies or accumulation, atrophy of fast fiber, or cap structures located at the periphery of the fibers. A combination of distinct ultrastructural features can also be found on some biopsies.

Genetic basis and main implicated cellular functions

Congenital myopathies are genetically heterogeneous with mutations found so far in more than 30 genes [6]. Some genes are associated solely with one type of congenital myopathy, while others are linked to several subclasses, for e.g. *RYR1*.

Nemaline myopathy is associated with mutations in at least 13 genes: *ACTA1* (actin alpha 1), *NEB* (nebulin), *TPM2* (β -tropomyosin), *TPM3* (α -tropomyosin), *KBTBD13* (Kelch repeat and BTB Domain containing 13), *KLHL40* (kelch-like family member 40), *KLHL41* (kelch-like family member 41), *LMOD3* (leiomodlin-3), *CFL2* (cofilin-2), *MYPN* (myopalladin), *MYO18B* (myosin XVIIIIB), *TNNT1* (slow Troponin T), *TNNT3* (fast Troponin T) and *RYR3* (ryanodine receptor 3) [4]. Recently, *CAP2* (encoding adenylyl cyclase-associated protein 2) was identified as a potential new gene for nemaline myopathy [7]. The most frequent forms are caused by recessive mutations in *NEB* and dominant mutations in *ACTA1*. In addition, mutations in *ACTA1* are responsible for the most severe cases.

Centronuclear myopathies are caused by mutations in at least 7 different genes and encoded proteins: *MTM1* (myotubularin), *DNM2* (dynamin 2), *BINI* (amphiphysin 2), *RYR1* (ryanodine receptor 1), *TTN* (titin), *SPEG* (striated muscle preferentially expressed protein kinase),

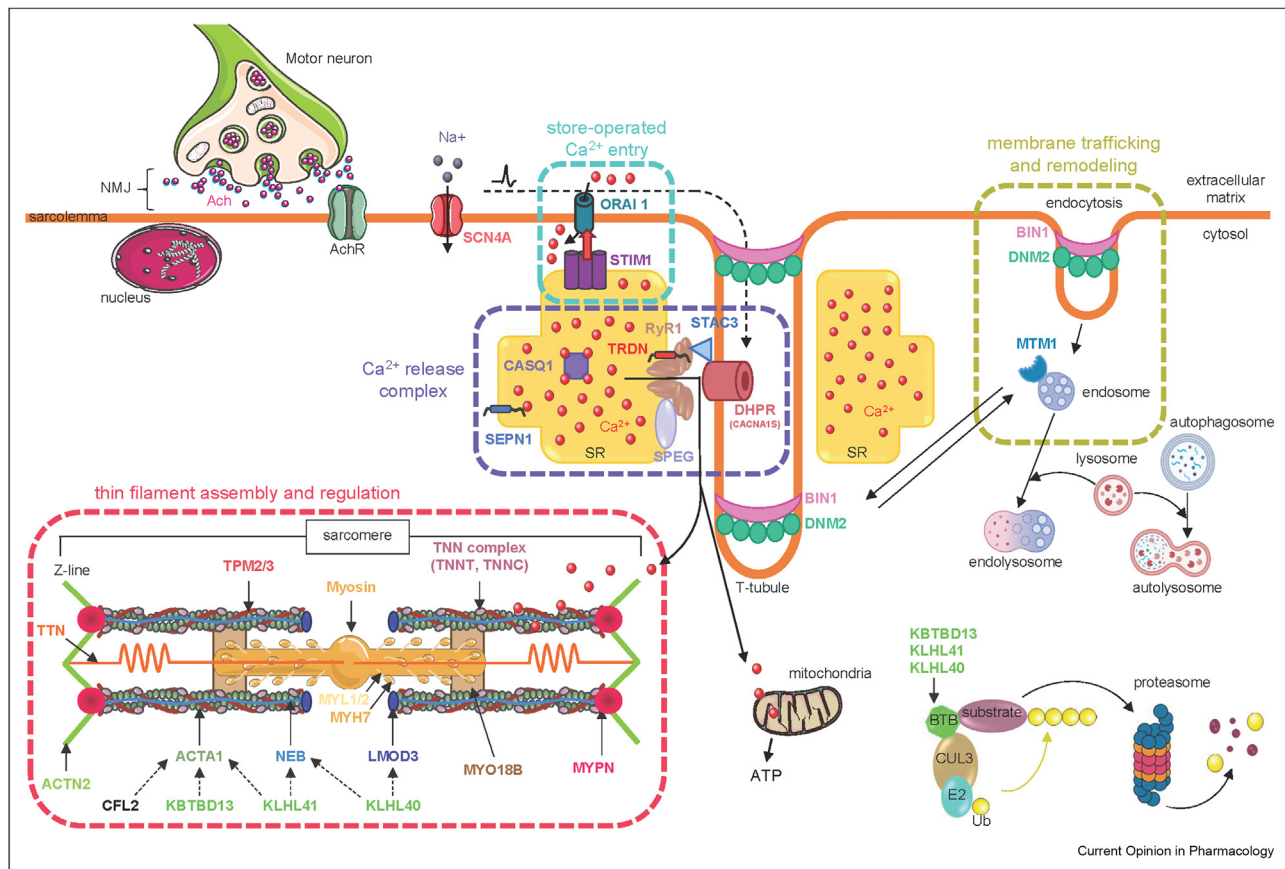
and *ZAK* (mitogen-activated protein kinase 20). CNM are divided in 3 main forms depending on the mutation mode of inheritance. The X-linked form of the disease is called myotubular myopathy (i.e. XLMTM) and is associated with mutations in *MTM1*. Mutations in *BIN1* (inherited as either autosomal recessive or autosomal dominant conditions), *DNM2* (main dominant form) and *RYR1* (recessively inherited) are the most frequent [5].

The causative genes for core myopathies are *RYR1*, *SEPN1* (encoding selenoprotein N, SEP1N), *MYH7* (myosin-7), *TTN*, *UNC45B* (protein unc-45 homolog B), *MEGF10* (multiple epidermal growth factor-like domains protein 10), *ACTN2* (actinin alpha 2), *CACNA1S* (dihydropyridine receptor alpha 1 subunit, DHPR),

FXR1 (RNA-binding protein), *TRIP4* (activating signal cointegrator 1), *ASCC1* (activating signal cointegrator 1 complex subunit 1), *ACTA1*, with *RYR1* and *SEPN1* being the main genes associated to CCD and MmD respectively [8].

There are other genes linked to rare congenital myopathies: *ORAI1* (calcium release-activated calcium channel 1), *STIM1* (stromal interaction molecule 1), *CASQ1* (calsequestrin 1), *MYL1* (myosin light chain 1), *CNTN1* (contactin-1), *TNNC2* (troponin C), *SCN4A* (sodium voltage-gated channel alpha subunit 4), *TRDN* (triadin), *PAX7* (paired box protein Pax-7), *KY* (kyphoscoliosis peptidase), *STAC3* (SH3 and cysteine rich domain 3), *PYROXD1* (pyridine nucleotide-

Figure 2



Proteins involved and main affected pathways in congenital myopathies. Affected genes in NM mostly encode for proteins constitutive of the thin filaments or associated with their regulation and/or degradation by the ubiquitin-proteasome system. The genes related to CNM encode for proteins regulating membrane trafficking and remodeling and proteins of the SR Ca^{2+} release system. The Ca^{2+} release complex is often altered in core myopathies. Gene encoding for proteins of the store-operated Ca^{2+} entry (SOCE) are mutated in tubular aggregates myopathy. Proteins in bold are mutated in congenital myopathies. Myotubularin (*MTM1*), amphiphysin 2 (*BIN1*), dynamin 2 (*DNM2*), ryanodine receptor (*RyR1*), calsequestrin 1 (*CASQ1*), dihydropyridine receptor (*DHPR*), calcium release-activated calcium channel 1 (*ORAI1*), stromal interaction molecule 1 (*STIM1*), sodium voltage-gated channel alpha subunit 4 (*SCN4A*), triadin (*TRDN*), kelch repeat and BTB domain containing 13 (*KBTBD13*), kelch like family member 40/41 (*KLHL40/41*), leiomodlin 3 (*LMOD3*), myopalladin (*MYPN*), myosin XVIIIIB (*MYO18B*), actinin alpha 2 (*ACTN2*), titin (*TTN*), nebulin (*NEB*), tropomyosin 2/3 (*TPM2/3*), myosin light chain 1/2 (*MYL1/2*), myosin heavy chain 7 (*MYH7*), cofilin 2 (*CLF2*), troponin (TNN), actin alpha 1 (*ACTA1*), selenoprotein N (*SEPN1*), SH3 and cysteine rich domain 3 (*STAC3*). The figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license and partly created with [BioRender.com](https://www.biorender.com).

disulfide oxidoreductase domain-containing protein 1), *HACD1* (very-long-chain (3R)-3-hydroxyacyl-CoA dehydratase 1), *MYL2* (myosin light chain 2).

The genes associated to congenital myopathies encode proteins playing a critical role in muscle structure and function (Figure 2). The main pathomechanisms underlying muscle weakness in congenital myopathies are related to excitation-contraction coupling (ECC) and store-operated calcium entry (SOCE) (*RYR1*, *CACNA1S*, *SCN4A*, *SPEG*, *SEPNI*, *TRDN*, *STAC3*, *CASQ1*, *ORAI1*, *STIM1*), thin-thick filaments interactions and stability/turnover of thin filament proteins (*ACTA1*, *NEB*, *TPM2*, *TPM3*, *KBTBD13*, *KLHL40*, *KLHL41*, *LMOD3*, *CFL2*, *MYPN*, *MYO18B*, *TNNT1*, *TNNT3*, *MYH7*, *ACTN2*, *UNC45B*, *TTN*, *MYL1*, *MYL2*), and membrane trafficking and remodeling (*MTM1*, *DNM2*, *BIN1*). Myogenesis (*PAX7*, *MEGF10*) and regulation of oxidative stress (*PYROXD1*, *SEPNI*) are other mechanisms also altered in congenital myopathies.

Therapeutic strategies

Several strategies to treat congenital myopathies are currently explored. These consist in correcting or improving the underlying genetic defects mainly using genetic-based approaches, and normalizing the disease pathway or treating the general muscle imbalance mostly with pharmacological compounds (Table 1 and Figure 3).

Nemaline myopathy

The genes that are mutated in NM encode for proteins constitutive of thin filaments of the sarcomere or proteins related to thin filaments turnover, which mainly results in decreased Ca^{2+} sensitivity.

Preclinical trials mainly concern drug testing targeting the myofibrillar contraction, the neuromuscular junction (NMJ) or muscle atrophy, whereas gene-based therapy has been studied at a small scale.

A preclinical study showed that overexpression of ACTC (i.e. cardiac α -actin), a fetal and cardiac α -actin isoform, was therapeutic in the mouse model carrying the *ACTA1* D286G mutation but not in the model with the H40Y mutation [9]. This indicates that homolog overexpression may be of therapeutic interest for NM patients with specific mutations in *ACTA1*. In a similar way, expression of *MYL4* (i.e. myosin light chain 4), a myosin light chain isoform, restored thin/thick filament interface and improved muscle force in a *ACTA1* model [10].

Activation of troponin is the most studied therapeutic strategy for NM patients since calcium (Ca^{2+}) sensitizers have been developed. Positive effects on muscle force generation have been reported in murine models

of severe *ACTA1* form and in muscle fibers from either mouse models or NM patients with various mutations [11-17]. Altogether, this suggests that most NM patients may benefit from this therapeutic strategy. Similarly, activation of myosin to facilitate actomyosin interaction has been proven efficient to counteract force deficit in NM [18]. A therapeutic trial with pyridostigmine, an acetylcholine esterase inhibitor (AChEI), resulted in long-term benefits on the marked muscle weakness in a NM patient with a *KLHL40* mutation [19]. Inhibition of myostatin demonstrated therapeutic benefits in the *ACTA1* form for muscle mass and muscle strength, whereas no positive effect was observed in one murine model for *NEB*-related NM [20-22]. Therefore, myostatin is a target of interest for some forms of NM. L-tyrosine supplementation demonstrated beneficial effects in NM patients and in a mouse model of *ACTA1*^{H40Y} mutation [23,24]. Nevertheless, several preclinical studies in murine models for *NEB* and *ACTA1* forms showed no improvement of the muscle phenotype [25,26]. Further investigations are required to assess the real clinical effectiveness of amino acid supplementation to treat NM patients.

Centronuclear myopathy

BIN1, *DNM2* and *MTM1* are functionally interconnected due to their role in membrane trafficking and remodeling. In addition, *DNM2* seems to play a main role in pathogenesis and is increased in *BIN1*-, *DNM2*- and *MTM1*-related CNM.

CNM is the congenital myopathy for which preclinical therapeutic testing has been performed with a particular focus on gene-based therapy [27]. To restore *MTM1* expression and thus normalize the disease phenotype, gene therapy was successful in several preclinical models [28,29]. A clinical trial was started in *MTM1* patients with adeno-associated virus (AAV) delivery of the *MTM1* cDNA (complementary DeoxyriboNucleic Acid) but was stopped because of high hepatic toxicity linked to liver dysfunction in *MTM1* patients. Another related strategy to improve muscle phenotype consists in enzyme replacement by delivering a recombinant *MTM1* by intramuscular injection [30]. Also, positive outcomes have been reported after overexpression of the *MTM1* homolog, *MTMR2* (encoding myotubularin-related protein 2) [31,32]. Reduction of *DNM2* level by using various new technologies, i.e. antisense oligonucleotides (ASO), CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats), shRNA (i.e. short hairpin RiboNucleic Acid) [33-39] has improved muscle phenotypes in models for three main forms of CNM, suggesting *DNM2* reduction or normalization may be beneficial for CNM patients with different genetic forms. However, the first clinical trial consisting in ASO injection has been interrupted due to tolerability issues. A potential gene-therapy alternative is the

overexpression of *BINI*, a negative regulator of *DNM2*, which improved phenotype in murine models for *MTM1*- and *DNM2*-related CNM [40,41].

The therapeutic effects of several drugs have also been assessed for CNM. AchEI have been tested to improve signaling at the NMJ and resulted in favorable outcomes in preclinical models and in patients [42,43]. Myostatin inhibition to target muscle atrophy demonstrated some positive effects on muscle mass and muscle force in preclinical models [44,45]. However, its therapeutic efficiency in patients might be hampered as *MTM1* and *DNM2* patients have very low plasma level of myostatin [46,47]. Other drugs have been used in preclinical models in order to reduce the abnormal level of the PI3P (phosphatidylinositol 3-phosphate) lipid via inhibition of the lipid kinase PIK3C2B (phosphatidylinositol 4-phosphate 3-kinase C2 domain-containing subunit beta) [48,49], to correct epigenetic alteration through histone deacetylases inhibition [50], or to activate autophagy by modulating mTOR (mechanistic target of rapamycin) pathway [51]. All have demonstrated muscle phenotype improvement. Tamoxifen showed an amelioration of muscle phenotypes in a preclinical model for *MTM1* and its therapeutic efficiency in patients is currently clinically tested (Table 1). Although tamoxifen mechanism of action in muscle is still unknown, its repurposing may be the best option for rapid translation to patients as it is an already FDA-approved drug (i.e. approved by Food and Drug Administration).

Core myopathies

Many genes mutated in this subgroup of myopathies code for proteins of the Ca^{2+} release complex and proteins associated with the myofilaments (Figure 2). Interestingly, it was recently demonstrated in a *SEPN1* mouse model that *SEPN1* function as a redox-sensitive calcium sensor of the endoplasmic reticulum suggesting a role for *SEPN1* in excitation-contraction coupling [52,53].

So far, therapeutic proof-of concept studies mainly target RyR1 as it is the most frequently mutated gene in core myopathies. Mutations in *RYR1* can be dominant or recessive and result in distinct channel defects as leaky RyR1, excitation-contraction uncoupling, or loss of RyR1.

RYR1 is a too large to fit into an AAV so that the classic gene replacement therapy cannot be applied. Exon skipping strategy of an abnormal extra exon was applied to primary muscle cells from a patient with a *RYR1* mutation and resulted in increased RyR1 protein and restored Ca^{2+} release [54]. Further investigations are needed to determine the efficacy of such strategies in preclinical models. The other genetic modulation that promoted muscle improvement consisted in targeting

the mutant allele with siRNA (i.e. small interfering RNA) [55].

Drugs tested for core myopathies target the functional alterations of RyR1, the increased oxidative stress, the protein aggregates and the altered DNA methylation. The chemical compound dantrolene, which blocks RyR1, was tested in patients with RyR1-related myopathies [56]. Similarly, the use of Rycals, a novel class of chemical molecules that restore the calstabin1 binding to the RyR1 channel, stabilized the closed state of RyR1 which results in reduced Ca^{2+} leak and thus enhanced Ca^{2+} release and ECC. Therefore, Rycals represent a potential valid treatment for *RYR1*-related myopathies with a leaky RyR1 [57]. Antioxidant treatment demonstrated benefit in different *RYR1*-models [58,59] but failed to achieve its primary endpoint in *RYR1* patients [60]. The clinical effectiveness of antioxidant was also tested in *SEPN1* patients after positive effects in cells from patients carrying *SEPN1*-null mutations were observed [61] and final results are expected. Chemical chaperones are potential drugs to be repurposed for myopathies related to *RYR1*. Benefits of 4-phenylbutyrate (4-PBA) administration were demonstrated in a mouse model carrying the I4895T mutation in *Ryr1* leading to endoplasmic reticulum stress originating from the uncoupled receptor [62]. The combination of two drugs targeting DNA and class II histone enzymes improved the muscle phenotype of a *RYR1* mouse model of multi-mini core disease [63]. Recently, p38, a mitogen-activated protein kinase, was identified as a potential modifier of RyR1 signaling [64]. The potential therapeutic effect for *RYR1*-related disease or diseases with Ca^{2+} handling alterations remains to be confirmed. Finally, salbutamol, a β_2 adrenergic receptor agonist, was well-tolerated and beneficial in patients with central core disease or multi-mini core disease.

Others/rare myopathies

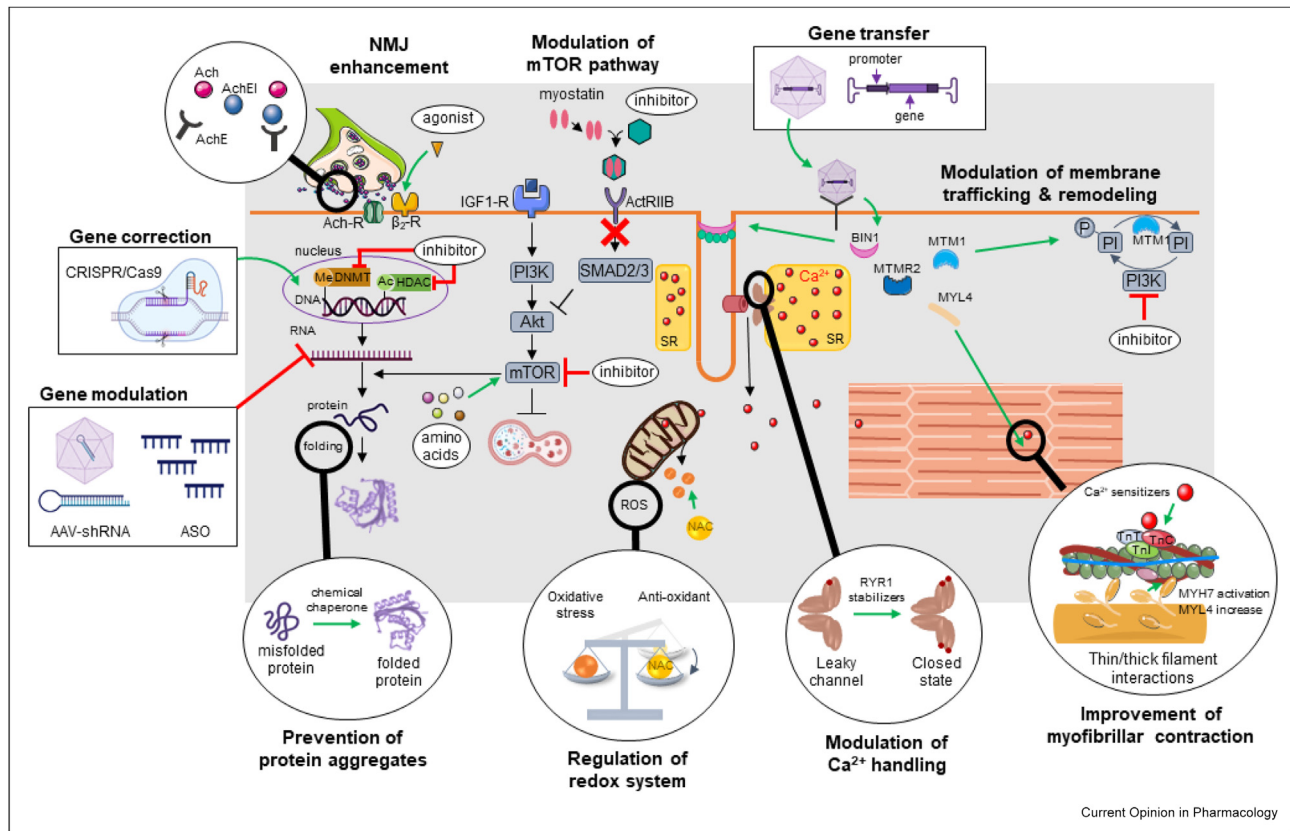
Few therapeutics approaches were reported for rare congenital myopathies, most consisting in testing strategies already proven efficient for other congenital myopathies. Upon exposure to troponin activator, contractility of patient's myofiber carrying mutation in *TNNC2* was improved [65]. This provides evidence that Ca^{2+} sensitizers might be of interest for patients with troponin mutations independently of the troponin subunit. Also, a clinical trial is ongoing with the β_2 adrenoceptor agonist salbutamol for patients with different types of congenital myopathy. The only genetic-based approach for a rare congenital myopathy was recently published and consisted in *Orai1* silencing to prevent the development of tubular aggregate myopathy in mice [66].

While muscle weakness and fatigue are limiting factors to physical activity in patients with congenital

Table 1					
Therapeutic approaches tested for congenital myopathies.					
Gene	Strategy	Target/purpose	Compound	References	Clinical trial/status
Nemaline myopathy					
ACTA1	Genetic				
	gene compensation – homolog overexpression	ACTC expression	genetic cross (TgActa1 x TgACTC)	[9]	Preclinical study
	gene upregulation	MYL4 overexpression	AAV6-MyI4	[10]	Preclinical study
	Pharmacology				
	drug to primary function				
	myofibrillar interaction increase	troponin activation	tirasemtiv (Ca ²⁺ sensitizer)	[69]	Preclinical study
	drug to general muscle function				
	amino acid supplementation	unknown target	L-tyrosine	[23,25]	Preclinical study
	TGFβ signaling pathway inhibition	myostatin inhibition	ActRIIB-mFc, mRK35	[20,22]	Preclinical study
NEB	Pharmacology				
	drug to primary function				
	myofibrillar interaction increase	troponin activation	Ca ²⁺ sensitizers (tirasemtiv, levosimodan, CK-2066260)	[11-15]	Preclinical study
	myofibrillar interaction increase	MYH7 activation	omecamtiv mecarbil	[18]	Preclinical study
	drug to general muscle function				
	TGFβ signaling pathway inhibition	myostatin inhibition	ActRIIB-mFc	[21]	Preclinical study
	amino acid supplementation	unknown target	L-tyrosine, L-carnitine, taurine, creatine	[26]	Preclinical study
TPM2/3	Pharmacology				
	drug to primary function				
	myofibrillar interaction increase	troponin activation	EMD 57033, CK-1909178 (Ca ²⁺ sensitizers)	[16,17]	Preclinical study
TPM3	Pharmacology				
	drug to general muscle function				
	amino acid supplementation	unknown target	L-carnitine	[70]	Preclinical study
	amino acid supplementation	unknown target	L-tyrosine	[24]	Clinical study
KLHL40	Pharmacology				
	drug to general muscle function				
	NMJ transmission enhancement	AchE inhibition	pyridostigmine	[19]	Clinical study
Centronuclear myopathy					
MTM1	Genetic				
	gene replacement	MTM1 expression	AAV8-MTM1	[28,29]	ASPIRO; NCT03199469
	enzyme replacement	MTM1 expression	3E10Fv-MTM1	[30]	Preclinical study
	gene compensation – homolog overexpression	MTMR2 expression	AAV9-MTMR2	[31,32]	Preclinical study
	gene downregulation	DNM2 level reduction	genetic cross (<i>Mtm1^{-y}</i> x <i>Dnm2^{+/-}</i>), RNAi <i>DNM2</i> (AAV2/9-shRNA, ASO)	[37-39]	DYN101 Unite-CNM; NCT04033159
	gene downregulation	PI3P level reduction	genetic cross (<i>Mtm1^{-y}</i> x <i>Pik3c2bKO</i>)	[48]	Preclinical study
	gene upregulation	BIN1 increase	AAV9-BIN1	[40]	Preclinical study
	gene modulation – TGFβ signaling pathway inhibition	myostatin inhibition	AAV-PropD76A	[46]	Preclinical study
	Pharmacology				
	drug to primary function				
		PI3K inhibition	PI3P level reduction	wortmannin	[48,49]
drug to general muscle function					
	NMJ transmission enhancement	AchE inhibition	pyridostigmine	[42,71]	Clinical and preclinical study

DNM2	TGFβ signaling pathway inhibition	myostatin inhibition	ActRIIB-mFc	[44,45]	Preclinical study
	epigenetic repurposing	HDAC inhibition	valproic acid	[50]	Preclinical study
DNM2	autophagy activation	target unknown	tamoxifen	[72,73]	TAM4MTM; NCT04915846
	Genetic gene deletion	mTOR pathway inhibition	RAD001, AZD8055	[51]	Preclinical study
BIN1	gene downregulation	DNM2 level reduction	allele-specific CRISPR	[36]	Cell study
	gene upregulation	DNM2 level reduction	RNAi <i>DNM2</i> (AAV2/9-shRNA, ASO, allele-specific AAV1-shRNA)	[33-35]	DYN101 Unite-CNM; NCT04033159
BIN1	Pharmacology <i>drug to general muscle function</i>	BIN1 increase	AAV9- <i>BIN1</i>	[41]	Preclinical study
	NMJ transmission enhancement	AchE inhibition	pyridostigmine	[43]	Clinical and preclinical study
Core myopathy RYR1	gene modulation – downregulation	DNM2 reduction	RNAi <i>DNM2</i> (ASO)	[74]	Preclinical study
	Genetic gene correction – exon-skipping	RyR1 expression	allele-specific pseudo-exon skipping (U7-AON)	[54]	Cell study
RYR1	gene downregulation	RyR1 knockdown	allele-specific RNAi (siRNA)	[55]	Preclinical study
	Pharmacology <i>drug to primary function</i>	modification of RyR1 calcium release	dantrolene, FKBP12 stabilizers	[56,57]	ARM 210 RYR1-RM; NCT04141670
RYR1	RyR1 antagonist	oxidative stress reduction	N-acetylcysteine	[58-60]	NCT02362425
	repurposing – antioxidant <i>drug to general muscle function</i>	AchE inhibition	pyridostigmine	[75]	Preclinical study
RYR1	NMJ transmission enhancement	HDAC and DNMT inhibition	TMP269, 5-Aza	[63]	Preclinical study
	epigenetic – enzymes	endoplasmic reticulum stress and protein folding	4-PBA	[62]	Preclinical study
SEPN1	protein aggregates prevention	β2 adrenoreceptor agonist	salbutamol	[76]	Clinical study
	NMJ enhancement	p38MAPK inhibition	SB203580, SB202190	[64]	Cell study
SEPN1	repurposing	oxidative stress reduction	N-acetylcysteine	[61]	SeINac; NCT02505087
	Pharmacology <i>drug to primary function</i>	hypertrophy pathway	none	[67,68,77,78]	Clinical and preclinical study
Other/several myopathies various	repurposing – antioxidant				
	exercise				
TNNC2	Pharmacology <i>drug to general muscle function</i>	β2 adrenoreceptor agonist	salbutamol		COMPIS; NCT05099107
	NMJ enhancement				
STIM1	Pharmacology <i>drug to primary function</i>	troponin activation	tirasemtiv (Ca ²⁺ sensitizer)	[65]	Preclinical study
	myofilament interaction increase	<i>Orai1</i> knockdown	genetic cross (<i>Stim1</i> ^{R304W/+} x <i>Orai1</i> ^{+/-}), RNAi <i>Orai1</i> (AAV9-shRNA)	[66]	Preclinical study
STIM1	Genetic gene downregulation				

Figure 3



Main therapeutic strategies in congenital myopathies and main pathways targeted. Gene-base strategies include gene correction at DNA level in the nucleus by using CRISPR/cas9 technology (*DNM2* for CNM), gene modulation at the mRNA level by using antisense oligonucleotide (ASO) or exon-skipping (*DNM2* for CNM, *RYR1* for core myopathies, *MYH7* for NM), shRNA (*DNM2* for CNM and *ORAI1* for tubular aggregate myopathy), allele-specific shRNA (*DNM2* for CNM), and gene transfer by using AAV (*MTM1*, *MTMR2* and *BIN1* for CNM, *MYL4* for NM). Pharmacological compounds used in several types of congenital myopathies target the NMJ (AChE and agonist of β_2R) and mTOR pathways (myostatin inhibitor). Drugs tested specifically for CNM target membrane trafficking (inhibitor of PI3K), modulation of mTOR pathway (mTOR inhibitor) and of histone modification (HDAC inhibitor). For NM, pharmacological intervention aimed at modulating protein translation (amino acid supplementation) and actomyosin interactions (troponin activators, MYH7 activation and MYL4 increase). Oxidative stress regulation (antioxidant as NAC), RyR1 functional modulation (inhibitor of Ca^{2+} leak), protein folding improvement (chemical chaperone) are pharmacologically targeted in core myopathies. The figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license and partly created with BioRender.com.

myopathies, several studies demonstrated that training is safe and does not exacerbate the pathology. Physical training when possible might be an option at least to limit the development of muscle weakness and/or atrophy and thus improve quality of life [67,68].

Conclusion

Many preclinical studies have been performed and many targets have been identified so that several clinical trials could be initiated. Until now, results of completed clinical trials are not very conclusive, but ongoing clinical trials may be proven safe and efficient. Moreover, many therapeutic options have been only preclinically tested indicating that a large number of options remains to be evaluated in patients. Most preclinical studies focused on strategies to limit the development of the

disease. Nevertheless, it is important to keep considering approaches aiming at reversing the pathology for patients severely affected. Also, drug repurposing may benefit from a rapid clinical translation as compared to gene-based therapies that, despite being promising, still require long development stages. Finally, no combined therapies have been evaluated so far, indicating room for improvement towards effective therapies for patients with congenital myopathies.

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Declaration of competing Interest

JL is co-founder of Dynacure.

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