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Congenital myopathies: pathophysiological mechanisms and promising therapies

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Abstract

Congenital myopathies (CMs) are a kind of non-progressive or slow-progressive muscle diseases caused by genetic mutations, which are currently defined and categorized mainly according to their clinicopathological features. CMs exhibit pleiotropy and genetic heterogeneity. Currently, supportive treatment and pharmacological remission are the mainstay of treatment, with no cure available. Some adeno-associated viruses show promising prospects in the treatment of MTM1 and BIN1-associated myopathies; however, such gene-level therapeutic interventions target only specific mutation types and are not generalizable. Thus, it is particularly crucial to identify the specific causative genes. Here, we outline the pathogenic mechanisms based on the classification of causative genes: excitation-contraction coupling and triadic assembly (RYR1, MTM1, DNM2, BIN1), actin-myosin interaction and production of myofibril forces (NEB, ACTA1, TNNT1, TPM2, TPM3), as well as other biological processes. Furthermore, we provide a comprehensive overview of recent therapeutic advancements and potential treatment modalities of CMs. Despite ongoing research endeavors, targeted strategies and collaboration are imperative to address diagnostic uncertainties and explore potential treatments.

Keywords Congenital myopathies, Pathophysiological mechanisms, Therapy

Introduction

Congenital myopathies (CMs) result from genetic abnormalities affecting muscle development and typically manifest at birth or during early infancy. Unlike myotonic dystrophy and metabolic myopathies, CMs have a slow-progressive or non-progressive course, with rarely seen degeneration, necrosis, and regeneration of muscle fibers in the muscle. The distinctive features of CMs include pleiotropy and genetic heterogeneity [1]. More than 40 types of CMs have been reported, involving more than 30 causative genes [2]. There are various CMs with similar clinical manifestations, making it difficult to clinically diagnose patients. The manifestations include muscle weakness, hypotonia, respiratory distress, scoliosis, medulla oblongata involvement, and deformed feet [3]. Most patients have normal intelligence, the central nervous system is usually unaffected, serum creatine

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kinase levels are slightly elevated or normal, and electromyography mostly shows myogenic changes or normal. It is difficult to identify the specific type of CM according to the clinical presentation alone. Skeletal muscle biopsy is valuable in the diagnosis of CMs, and histopathologic or ultrastructural features within the muscle can be an important basis for differential diagnosis. For example, central core disease (CCD) is characterized by a lack of oxidase in the core of muscle fibers, centronuclear myopathy (CNM) is characterized by the presence of a central nucleus in muscle fibers, and nemaline myopathy (NM) is characterized by electron dense rods within muscle fibers [4]. In addition, abnormal structures such as aggregated columnar helical structures, cap-like structures, actin aggregates, and zebra-striated bodies can be observed within muscle fibers of patients with other types of CMs. However, skeletal muscle biopsy also has limitations. Due to the limitations of biopsy sampling, only focal pathological changes can be observed, that is, maybe typical pathological changes cannot be detected in a single muscle biopsy, and minor or nonspecific pathological changes do not assist in making a definitive diagnosis. In addition, structural changes of muscles in patients with CMs may change over time, further reducing the specificity of the patient's pathologic diagnosis. Therefore, repeated muscle biopsies may be required to make a definitive diagnosis.

The four types of CMs, including CCD, multimincore disease (MmD), CNM, and NM, have clearer histopathologic and clinical features, and there are more clinical data and samples than the other types of CMs. In addition, there are many other types of CMs, such as congenital fiber-type disproportion (CFTD), cylindrical spirals myopathy, and cap myopathy. Among them, mutations in ryanodine receptor type 1 (RYR1) seem to be the most common cause of CMs, especially CCD and MmD, while nebulin (NEB) recessive mutations and alpha-actin 1 (ACTA1) dominant mutations are closely related to NM, and X-linked recessive mutations of Myotubularin 1 (MTM1) are the most common cause of CNM [5]. The earlier classification of CMs was based on histopathologic features, and with the understanding of the genetic basis of the disease, it may be more scientific to classify CMs for myopathy through the causative gene. Grouping myopathies caused by mutations in the same causative gene helps to assess clinical symptoms and prognosis, which is more instructive for therapeutic measures and clinical trial design. However, this basis for categorization can blur the established diagnostic boundaries between subgroups of classical CMs. Given the genetic heterogeneity of CMs, different causative genes cause the same or similar pathologic phenotypes, and categorization by genes may put pressure on clinical diagnosis and treatment. Perhaps a classification criterion that integrates

multiple factors, such as pathologic features and causative genes, would be more appropriate.

Supportive therapy is mainly used for the current treatment of CMs, including maintaining normal body posture through corrective surgery, gastrostomy surgery for patients with dysphagia, regular testing of respiratory and cardiac functions, and timely non-invasive ventilation and coughing assistance for patients with respiratory muscle weakness. Other therapeutic measures include calcium agonists and other pharmacologic therapies and gene therapies [6]. Pharmacologic therapies are more categorized and more widely applicable, but mostly non-specific in improving complications of CMs. Gene therapy can address the underlying causative genes, but it needs to be recognized that many gene therapies are mutation-specific and only benefit a subset of patients suffering from a specific disease [7]. Given the wide variety of causative genes associated with CMs, further scientific research and development is necessary to fully cure more patients.

Pathogenesis

Excitation-contraction coupling and triadic assembly

Excitation-contraction coupling (ECC) converts electrical signals (plasma membrane depolarization) into chemical signals (cytoplasmic Ca^{2+} elevation) and relies on Triad to finely regulate sarcoplasmic Ca^{2+} levels during muscle contraction. Transverse tubules (T-tubules) are invaginations of the sarcolemma. In skeletal muscle cells, T-tubules are connected to the sarcoplasmic reticulum on both sides, forming tightly adherent membrane contacts called "Triad" [8–10]. The triad consists of many specialized structural components, such as voltage-sensing dihydropyridine receptors in the T tubule membrane and Ca^{2+} release channel, RYR1, in the sarcoplasmic reticulum. In ECC, depolarization of the T tubule membrane induces a conformational change in dihydropyridine receptors, which opens the Ca^{2+} channel RyR1 in the sarcoplasmic reticulum to release Ca^{2+} , thereby inducing muscle contraction (Fig. 1) [10]. MTM1, Dynamin 2 (DNM2), and Bridging Integrator 1 (BIN1) involved in the cell membrane remodeling process are closely related to triad biogenesis in skeletal muscle, and mutations in genes encoding related proteins cause varying degrees of ECC dysfunction, which is a very important pathogenic factor of CMs (Table 1).

RYR1

Mutations in RYR1 are the most common cause in CMs. The RYR1 gene encodes the ryanodine receptor 1, which is a calcium release channel connecting the terminal cisternae and T-tubules at the end of the sarcoplasmic reticulum in skeletal muscle. This receptor is a key component of the ECC mechanism [53, 54]. Mutations

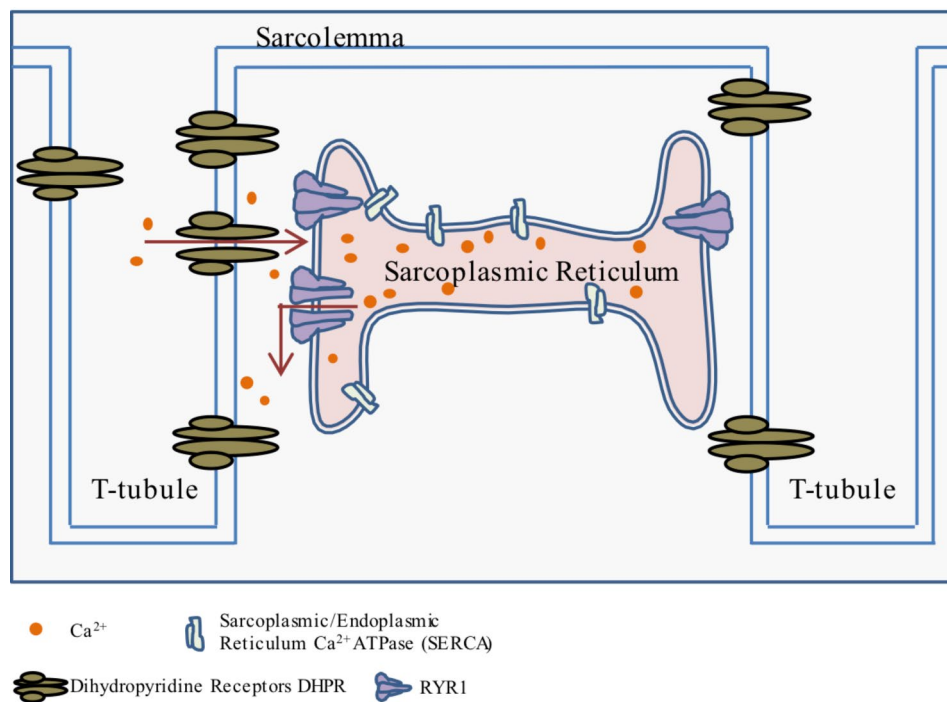


Fig. 1 Schematic diagram of the structure of the triad. The sarcoplasmic reticulum is a specialized smooth endoplasmic reticulum in the myofibrils, which is enlarged at both ends to form a flat sac known as the terminal cisterna. The sarcolemma, located at the site of the terminal cisterna, forms funnel-like invaginations, called T-tubules. The T-tubules in skeletal muscle cells are connected to the sarcoplasmic reticulum on both sides, forming a tightly adherent membrane contact called the "triad"

in RYR1 impair the normal process of calcium release, thereby limiting/reducing muscle contraction induced by neural excitation. In addition, mutations in RYR1 diminish the stability of RYR1, and Ca^{2+} , caffeine and volatile anesthetics predispose patients to malignant hyperthermia, which is characterized by fatal muscle tonus and spasms, hyperthermia and rhabdomyolysis [11]. Skeletal muscle biopsies of patients with RYR1 mutation-associated myopathies indicate pathological sarcoplasmic reticulum calcium leakage and increased calcium-activated protease activity [12]. Increased calcium-activated protease activity and mitochondrial Ca^{2+} overload led to pathological reactive oxygen species (ROS) production, thereby triggering muscle dysfunction. Both the recessive RYR1 mutant zebrafish model and RYR1 patients show high levels of oxidative stress. Treatment with antioxidants can correct this in zebrafish and improve their muscle function, suggesting a potential therapeutic option for this disease [55, 56].

Mutations in RYR1 are associated with several types of CMs, such as CCD, MmD and CNM. Mutations in RYR1 are closely associated with CCD. In patients with CCD, there is a lack of mitochondria in skeletal muscle, a reduction in local oxidase activity, and a well-defined core that extends for a considerable distance along the center of the longitudinal axis of myofibrils [5]. Depending on the specific type of mutation, CCD can be inherited in an

autosomal dominant or recessive manner, and it is generally accepted that functional alterations are the dominant form, whereas the reduction of RyR1 channels is the recessive form [57]. Clinically, CCD caused by dominant RyR1 mutations is usually less symptomatic. To date, the most mutations in RYR1 associated with CCD and MH are dominant missense mutations.

MTM1

MTM1 is a lipid phosphatase that regulates PI(3)P homeostasis in endosomes, which are crucial for cellular signaling and membrane transport [58]. MTM1 triggers endoplasmic reticulum remodeling by hydrolyzing PI(3)P, reducing membrane contacts between peripheral endoplasmic reticulum tubules and early endosomes in response to fluctuating nutrient conditions, such as starvation [58, 59]. This remodeling process delays mitochondrial fission, promotes the formation of a hyperfused mitochondrial network, and facilitates the transfer of fatty acids to mitochondria for β -oxidation. Consequently, it enhances the production of mitochondrial adenosine triphosphate (ATP) to maintain cellular energetic homeostasis. Furthermore, MTM1 initiates exocytosis by hydrolyzing PI(3)P in early endosomes and participates in regulating cargo protein delivery through mediating endosomal exocytosis [13, 14]. Overall, MTM1 is involved in the delivery of cargo proteins by regulating

Table 1 Congenital myopathies-related genes and underlying mechanisms

Gene	Encoded protein	Function	Congenital myopathy	References
RYR1	Ryanodine receptor 1	a major component required for skeletal muscle ECC	CCD, MmD and CNM	[11, 12]
MTM1	Myotubularin 1	regulated endoplasmic reticulum, metabolic organelles and endosomal exocytosis	CNM (XLCNM)	[13–15]
DNM2	Dynamin 2	regulated T-tubule formation, autophagy, lipid droplet dynamics, mitochondrial metabolism, and endocytic trafficking of GLUT4	CNM (ADCNM)	[16, 17]
BIN1	Bridging integrator 1	essential for T-tubule biogenesis	CNM (ARCNM)	[10, 18, 19]
NEB	Nebulin	regulated the length and hardness of thin filaments, promoted filament activation and cross-bridge recruitment	NM	[20–23]
ACTA1	Alpha-actin 1	molecular basis for force production	NM, CFTD	[24–27]
TNNT1	Troponin T1	modulated actin-myosin interactions	NM	[28–31]
TPM2	β subunit of Tropomyosin	modulated actin-myosin interactions	NM	[32–34]
TPM3	α subunit of Tropomyosin	modulated actin-myosin interactions	NM	[35–37]
MEGF10	Multiple EGF-like domains protein 10	essential for muscle development and repair by regulating MuSCs proliferation and differentiation	EMARDD	[38–40]
TRIM32	Tripartite motif-containing protein 32	regulated cytoskeleton composition	Sarcotubular myopathy	[41, 42]
CFL2	Cofilin 2	regulated actin aggregation, maintained sarcomere structure, and modulated the cell cycle	NM	[43, 44]
CNTN1	Contactin 1	cell adhesion molecule; neural adhesion and neuromuscular junction protein	Compton-North congenital myopathy	[45, 46]
HACD1	3-hydroxyacyl-CoA dehydratase enzyme	involved in the elongation of the very long chain fatty acids	CNM	[47, 48]
SCN4A	Sodium channel protein type 4 subunit alpha	essential for the generation and propagation of the muscle action potential	ion channel-related congenital myopathy	[49, 50]
PYROXD1	pyridine nucleotide-disulphide oxidoreductase domain 1	involved in redox regulation	CNM, core myopathy, myofibrillar myopathy	[51, 52]

CCD, central core disease; CNM, centronuclear myopathy; NM, nemaline myopathy; MmD, multimincore disease; XLCNM, X-linked centronuclear myopathy; ADCNM, autosomal dominant centronuclear myopathy; ARCNM, autosomal recessive centronuclear myopathy; EMARDD, early-onset myopathy, areflexia, respiratory distress, and dysphagia

PI(3)P and thereby affecting the endoplasmic reticulum and early endosomes, regulating metabolic organelles to perform metabolic recombination in cells under limited nutrient supply, and regulating endosomal exocytosis. Loss-of-function mutation in MTM1 results in a defective shape of the endoplasmic reticulum, aberrant mitochondrial morphology, depletion of cellular ATP, and accumulation of endosomal β 1-integrin.

X-linked recessive mutations in MTM1 (XLCNM or XLMTM: X-linked myotubular myopathy; OMIM 310400) are the most common cause of CNM, and XLMTM is also the most common and severe form of CNM. Muscle biopsies of patients with CNM show severe alterations in the size of the patient's fibers, with the presence of very small round fibers that have large, aggregated nuclei in the center as well as abnormal central aggregates of mitochondria and other organelles [15]. These small round fibers are associated with myofibrillar undergrowth (i.e., dystrophy), and in addition, there is a link between small round fibers and fiber type [60]. Most of the small, rounded fibers are slow myofibers.

Fast myofibers are larger in cross-section, and even fiber hypertrophy is present. Mislocalization of mitochondria, myotubular elements and other organelles is another feature of XLMTM. Abnormal or absent triad structures and impaired ECC processes exist in XLMTM models and patient biopsies, which may be associated with MTM1 loss of function. In addition, another study found severe liver abnormalities in a zebrafish model with MTM1 loss of function [61]. This suggests that MTM1 mutations may be closely related to liver abnormalities, explaining the concomitant hepatobiliary diseases in some XLMTM patients.

The most common type of mutations in the MTM1 gene is the single nucleotide mutation, and other less common forms of mutations are deletions (small and large deletions, which can encompass the entire gene) and more complex rearrangements [15]. The majority of pathogenic MTM1 mutations lead to complete deletion of MTM protein, which is usually associated with severe phenotypes; however, there are also missense mutations that theoretically only partially (not completely) disrupt

protein function or RNA processing and/or protein stability, which are associated with less severe phenotypes.

DNM2

DNM encodes dynamins, which consist of five structural domains and play a key role in shaping and remodeling cellular membranes [62–64]. Three classical dynamin proteins are identified as follows: DNM1 exhibits predominant expression in the brain, while DNM3 is expressed in the brain, lung, and testis. In contrast, DNM2 demonstrates ubiquitous expression. Studies in *Drosophila*, zebrafish, and mice have suggested a potential role of DNM2 in the formation or maintenance of T-tubules, autophagy, lipid droplets, and mitochondrial metabolism. DNM2 is required for the release of autophagy precursors from recycling endosomes and the release process is mediated by DNM2-dependent scission of tubules, which is realized by the binding of DNM2 to LC3 [16, 17]. In addition, DNM2 regulates the clathrin-dependent and non-dependent endocytosis of glucose transporter-4 (GLUT4), which is crucial for insulin signaling pathways to achieve glucose homeostasis [65]. It is involved in cytoskeletal regulation, particularly actin formation and organization, controlling intracellular transport of GLUT4-containing vesicles by regulating actin polymerization.

Mutations in DNM2 lead to autosomal dominant centronuclear myopathy (ADCNM, OMIM 160150), and mutations affecting the central middle domain are usually associated with relatively mild clinical phenotypes, while mutations in the PH and GED structural domains tend to have more severe clinical phenotypes [16]. The self-assembly and lipid-binding capacity of DNM2 is required for effective membrane fission, but mutations in DNM2 in the central middle domain or PH structural domains lead to the formation of abnormally stable polymers with enhanced lipid-binding affinity and resistance to BIN1-mediated GTPase inhibition [8, 66, 67]. Unregulated membrane fission activity results in structural abnormalities in T-tubules and dysregulation of Ca^{2+} homeostasis. In cells with DNM2^{R465W} mutations, increased binding of DNM2^{R465W} to another chaperone protein, intersectin 1 (ITSN1), in the plasma membrane (ITSN1 serves as a scaffold for dynamin and other endocytosis proteins, and its recruitment to endocytosis sites can ensure its role in clathrin-mediated endocytosis) impedes DNM2 recruitment to the sites of autophagosome biogenesis (reduced binding of DNM2 to LC3 proteins), thereby impairing autophagy and leading to the accumulation of immature autophagosomes [16, 17, 68]. Impaired autophagic flux prevents the timely clearance of abnormal mitochondria and easily aggregated unfolded proteins that thereby accumulate in myocytes, which may be related to pathological phenotypes such as

vacuolization and centronucleus of myofibrils in CNM patients. In addition, DNM2 mutations in the ADCNM mouse model disorganize actin filaments, thereby affecting the translocation of GLUT4 to the plasma membrane, which explains the aberrant perinuclear accumulation of GLUT4 observed in muscle biopsies from CNM patients.

Targeted reduction of CNM2 expression has been reported to effectively prevent and reverse CNM associated with MTM1, BIN1 and DNM2 mutations in mouse models [65, 69–74]. It has been suggested that different splicing modalities lead to the formation of different DNM2 isoforms, and universal rather than muscle-specific phenotype is the main cause of CNM [75]. This suggests that subsequent therapies against CNM should not target muscle-specific DNM2 subtypes but should be designed or screened for AAV serotypes or oligonucleotides that target muscle.

BIN1

BIN1 encodes bridging integrator 1 (also known as amphiphysin 2), which is an important membrane remodeling protein and plays an essential role in T-tubule biogenesis in skeletal muscle. BIN1 belongs to the BAR protein family and possesses a BAR structural domain associated with lipid binding. BIN1 has an amphipathic helix at the N-terminus and is usually considered to be a curvature-inducing module, but it has been suggested that it also senses curvature and other intramembrane stresses and is involved in membrane fission [63, 76]. Another structural domain shared between different BIN1 isoforms is the Src homology 3 (SH3) structural domain, which interacts with the PRD structural domains of dynamins to jointly regulate T-tubule biogenesis [77].

BIN1 mutations are associated with autosomal recessive centronuclear myopathy (ARCNM, OMIM 255200), and the majority of mutations occur in the BAR structural domain, the N-terminal amphipathic helix, and the C-terminal SH3 structural domain. CNM-associated mutations in the N-terminal amphipathic helix decrease the stability of the BIN1 scaffold. Mutations in the BAR structural domain are related to a decrease in its tubulation property [10, 18, 19]. The primary role of the SH3 structural domain of BIN1 is to bind dynamins, and mutations in the SH3 structural domain affect the interaction of BIN1 with dynamins. Muscle-specific isoforms of BIN1 have a distinctive PI motif, and this structural domain interacts with the specificity of phosphoinositides which is associated with phosphoinositides. Mutations in the PI motif are associated with severe forms of CNM, with the majority of patients dying within 2–4 years of onset [78]. This mutation occurs at the splicing acceptor site and results in the inability to form muscle-specific BIN1 isoforms, blocking the recruitment of phosphoinositides by BIN1 and thereby affecting

the recruitment of dynamins. BIN1 recruits DNM2 via PI(4,5)P2 to promote T-tubule biogenesis and inhibits the GTPase activity of DNM2 in a chemical-dependent manner to maintain T-tubule structure. Mutations in the SH3 structural domain of BIN1 render BIN1 unable to bind DNM2, and BIN1 fails to inhibit the GTPase activity of DNM2 [79–81]. The uncontrolled GTPase membrane fission activity results in the breakage and fragmentation of T-tubules. Overall, the biogenesis and maturation of T-tubules is realized by balancing the interaction of BIN1 with two proteins, MTM1 and DNM2 [82]. Low levels of DNM2 membrane fission activity promote T-tubule biogenesis, and high levels of MTM1 maintain T-tubule morphology, which are essential for T-tubule maturation.

Actin-myosin interaction and myofibril force production

Skeletal muscle is composed of cells that are extremely contractile. To achieve contractile function, each differentiated muscle cell contains myofibrils, which consist of repeating sarcomeres. The sarcomere is the smallest contractible unit of muscle, composed of overlapping myosin (thick filaments) and actin (thin filaments), with thin filaments in the opposite direction being anchored to the Z-wire at the end of the sarcomere and bipolar thick filaments occupying the center of the sarcomere [83, 84]. Muscle contraction is driven by actin-activated myosin [85, 86]. During each bridging cycle, the energy generated by ATP hydrolysis is converted into myosin head movement, which is a process regulated by calcium-mediated changes in protein conformation (Fig. 2). The length of the sarcomere determines the overlap between thin and

thick filaments, and thus the number of force-generating interactions between actin and myosin, which is closely related to the magnitude of the force produced; therefore, appropriate filament length is important for myofiber strength [87]. NEB- and ACTA1-specific mutations may affect the filament length, and shorter-than-normal filament lengths have been associated with lower myofiber force (Table 1). In addition, qualitative changes in actin-myosin interactions as well as myofiber disturbance are important factors in the production of lower muscle force. Troponin and tropomyosin act as protein switches that regulate actin-myosin interactions in response to changes in intracellular Ca^{2+} concentration in myocytes, and aberrant mutations in the genes encoding troponin (TNNT) and tropomyosin (TPM) at different loci differentially affect the force-producing process of myofibers, thereby leading to different myopathies.

NEB

NEB encodes nebulin protein, a macromolecular protein essential for skeletal muscle force production. Nebulin regulates the length and hardness of thin filaments, promotes filament activation and cross-bridge recruitment [20–22]. Its highly repetitive structure consists of 185 tandemly repeating structural domains associated with troponin and tropomyosin arrangement on the filaments [88, 89]. Nebulin stiffens the filaments by acting on the actin subunits on the filaments, finely regulates their helices, which is required for the movement of pro-tropomyosin/tropomyosin on the filaments. After activation of the skeletal muscle, nebulin promotes the aggregation

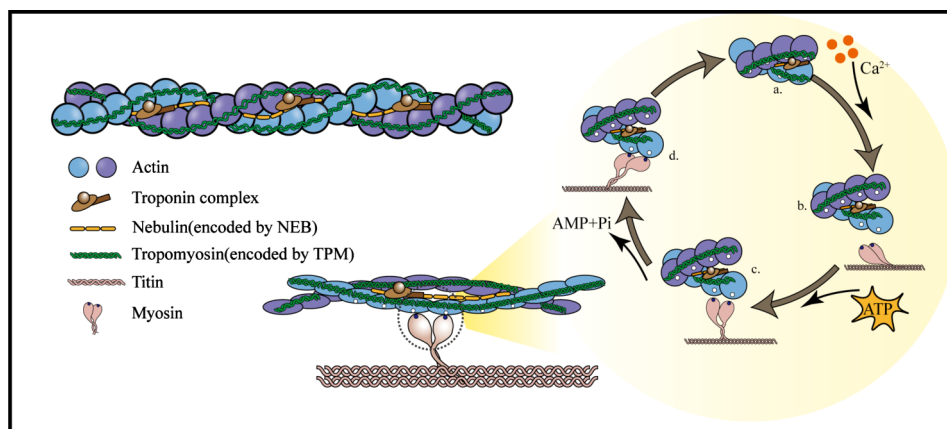


Fig. 2 Schematic diagram of fine myofilament composition and actin-myosin interactions. Troponin and tropomyosin act as protein switches that regulate actin-myosin interactions in response to changes in Ca^{2+} concentration within the myocyte. **(a and b)** When nerve impulses reach myofibers, Ca^{2+} is released from the sarcoplasmic reticulum and interacts with troponin complexes, Nebulin, and tropomyosin, leading to conformational changes in the thin filaments to expose myosin binding sites. **(c and d)** ATP is decomposed into ADP and inorganic phosphate, thereby releasing energy. The energy is utilized by the myosin head, causing the myosin head to bend and move forward. Myosin with the altered conformation binds to actin to form a muscle bridge. The forward movement of the myosin head shortens the distance between actin and myosin, resulting in actin slipping away from myosin and binding to new myosin to form a new muscle bridge. The above steps are repeated. The constant interaction between actin and myosin leads to muscle contraction, thereby generating force. Mutations in genes such as NEB, ACTA1, TNNT1, TPM2, and TPM3, which encode key proteins involved in the above process, result in aberrant protein function and cause different types of congenital myopathies

of myosin heads toward the thin filaments. Myosin exists in two different states of relaxation: “hyper-relaxed” and “disordered relaxed.” In the hyper-relaxed state, myosin heads interact with the backbone of thick filaments, limiting their interaction with actin; in the disordered relaxed state, myosin molecules are immobilized and can weakly bind actin in order to make a rapid transition to the active state when actin filaments open [90, 91]. The ATPase activity in the myosin head in the disordered relaxed state is 10 times higher than that in the hyper-relaxed state. It has been suggested that the ratio of myosin molecules in the hyper-relaxed state in muscle fibers of NM patients (which may be related to myosin-binding proteins involved in the isolation of the hyper-relaxed state) is disturbed, which affects the basal ATP consumption of the skeletal muscle and alters the energetic and metabolic state of NM muscle [22]. This may explain the phenomenon of glycogen deposition and aberrant mitochondria in the muscle of NM patients who are usually very thin.

NEB mutations lead to a wide spectrum of phenotypic variations in NM, ranging from severe fetal cases to adult-onset presentations [23]. Furthermore, identifying the causative mutation sites is impeded by the gene’s exceptionally long sequences [92]. Patients with an infantile onset develop diffuse weakness in infancy that improves with age and the patients gradually become able to walk [92, 93]. Patients with NM present with many characteristic nemaline rods or neuronal rods in the cytoplasm of the skeletal muscle. Studies in mouse models and NM patients have shown that nebulin deficiency reduces the sensitivity of skeletal muscle force production to Ca^{2+} . In addition, the helical structure of the thin filaments is disrupted in nebulin-deficient skeletal muscle, and the twisted filament helices alter the structure of the troponin complexes, impeding the movement of tropomyosin and resulting in reduced cross-bridge formation, and thus skeletal muscle can only produce low levels of force [94]. Fast skeletal muscle troponin activators can enhance force formation by retarding the rate of Ca^{2+} dissociation from troponin C (TnC) and promotes cross-bridge formation at a given Ca^{2+} concentration [95]. This suggests a therapeutic direction for NM.

ACTA1

The sarcomere is the smallest contractile unit of muscle, and the thin filament is a sarcomere microstructure essential for normal muscle contraction. The main structural and functional component of thin filaments is F-actin, formed by the polymerized helix of ACTA1-encoded α -actin. α -actin is a multifunctional protein that polymerizes and forms the helical chain of thin filaments, and the dynamic cross-bridges formed with the thick myosin filaments result in the sliding movement between

actin and myosin that shortens the contractile unit and generates the force that enables movement [24, 25]. Overall, actin interacts with myosin, tropomyosin, troponin, and nebulin as the molecular basis for force production in skeletal muscle.

ACTA1 mutations are associated with CMs, such as NM, CFTD, and zebra body myopathy [26, 27]. ACTA1-associated NM (NEM3, MIM: 617336) accounts for approximately 25% of all NM cases, and up to 50% of the severe cases of NM are associated with ACTA1 mutations, mostly heterozygous missense mutations that occur de novo and affect highly conserved amino acid sequences [25, 96]. Mouse models carrying missense ACTA1 mutations have shown that mutations in Asp-286Gly ACTA1 affect actin-actin interactions, impedes myosin cross-bridge binding, limits the number of strongly bound cross-bridges, and triggers a number of functional alterations in myofibers [97]. Heterozygous missense mutations (D292V, L221P, P332S) in the ACTA1 gene have been reported to be associated with CFTD [98]. CFTD is a rare CM, and its main pathological feature is that type I myofibers (slow myofibers) are generally smaller than type II myofibers (fast myofibers).

TNNT1

Tnnt1 encodes troponin T in slow skeletal muscle, a 30–35 kDa protein with ~220 to 300 amino acids. It plays a key role in regulating calcium for actin filament function. There are three homologous genes encoding different muscle fiber type-specific isoforms: Tnnt1 (slow skeletal muscle, sTnT), Tnnt3 (fast skeletal muscle, fTnT), and Tnnt2 (cardiac, cTnT). The N-terminal region of the three isoforms differs significantly, but they are highly conserved in the middle and C-terminal regions, which contain binding sites for TnC, TnI, and tropomyosin [99]. Of the 14 exons of TNNT1, exon 5, which encodes the N-terminal 11 amino acid fragments, is alternatively spliced, giving rise to high- and low-molecular weight sTnT variants [100]. Compared to high-molecular weight sTnT, low-molecular weight sTnT has a shorter N-terminal variable region, a change in molecular conformation, and an increased affinity for tropomyosin and TnI [28]. Muscle spindles are a special sensory organ in skeletal muscle. Under the activation of γ -motor neurons, the intrafusal fibers in muscle spindles contract to produce baseline tension, which is essential for sensing changes in muscle length [29]. Both sTnT and cTnT are expressed in spindle intrafusal fibers of adult mice, and low-molecular weight sTnT predominates in intrafusal fibers, suggesting that myofilaments are less sensitive to Ca^{2+} , which may be the key to maintaining proper tension in intrafusal nuclear bag fibers [101]. In a mouse model of ANM, sTnT-knockout mice exhibit impaired neuromuscular coordination, indicating abnormal muscle spindle

function [101, 102]. Further studies have shown that knockout of sTnT is compensated by an increase in cTnT in muscle spindles, and that this compensation produces abnormally high myofilament Ca^{2+} sensitivity, which generates hypertonicity in intrafusal nuclear bag fibers and increases the sensitivity of the spindle to changes in length and stretch [101, 102]. Compensatory upregulation of cTnT in intrafusal nuclear bag fibers may be a potential mechanism underlying abnormalities in neuromuscular reflex-related functions in patients with ANM, which may be related to patients' symptoms of tremors and clonus.

A nonsense mutation in exon 11 of TNNT1 forms a stop codon that results in the loss of 83 amino acids from the C-terminal end of the sTnT protein, causing the loss of the primary site of interaction with TnI and TnC and the second site of interaction with tropomyosin [99]. sTnT with no tropomyosin binding site fails to integrate into myofilaments and is degraded in myocytes to avoid toxic effects [31, 103]. Deletion of sTnT results in severe NM in childhood, which is characterized by pathological changes in fiber size, atrophy of slow type I muscle fibers and hypertrophy of fast type II muscle fibers, and the presence of nemaline rods in muscle fibers [30, 31]. It is generally believed that hypertrophy of type II fibers is a compensatory response to atrophy of type I fibers.

TPM2 and TPM3

Tropomyosin is encoded by at least four distinct genes, TPM1-4, wherein TPM1 and TPM3 encode the α -subunit of the muscle Tropomyosin α - β dimer, while TPM2 encodes the β -subunit of the Tropomyosin α - β dimer. Selective splicing of TPM generates many tissue-specific isoforms [104]. TPM1 is mainly expressed in cardiac and type II fast muscle fibers, whereas TPM2 and TPM3 are mainly expressed in type I slow muscle fibers. Tropomyosin serves as a protein switch to turn on or off the actin/myosin interaction and plays a key regulatory role in muscle force generation. In the resting state of muscle, tropomyosin polymers line up along actin filaments, covering the myosin-binding site and inhibiting the activation of the contractile unit; during muscle contraction, the intracellular Ca^{2+} level increases, inducing a conformational change in the troponin/tropomyosin complex and exposing the myosin-binding site to trigger force generation [98].

In addition to being involved in sarcomere contraction, tropomyosin is also involved in the regulation of early skeletal muscle development. Skeletal muscle development relies on cytoskeletal dynamics to direct muscle precursor migration and myofiber morphogenesis, while TPM2 mutations are likely to adversely affect cytoskeletal dynamics, which may disrupt overall myogenesis [32–34]. Myogenesis begins with mononuclear muscle

precursor cells known as myoblasts, which differentiate and fuse with each other to form multinucleated myotubes. The Multinucleated myotube cells attach to tendon cells through a process of myotube guidance and then are assembled with sarcomeres to become mature into contractile myofibers [34]. Drosophila and zebrafish models of the TPM2 mutation exhibit abnormal muscle morphology, possibly because of improper regulation of actin dynamics during myoblast migration, fusion and myotube lengthening [105]. This suggests that TPM2 mutations may disrupt myoblast fusion and myotube guidance prior to sarcomere assembly, thereby hindering muscle morphogenesis, and thus muscular dysplasia may be an important etiological component of TPM2-associated myopathies. Bonnet et al. found that RNA-binding protein quaking (QKI) and TPM3 are both essential in early myofibril formation and QKI controls TPM3 transcript levels through physical interaction with its 3'UTR [35]. This also suggests that muscle defects in CMs associated with human TPM3 mutations may be due to defects in myofiber formation rather than impaired regulation of muscle contraction.

The TPM2 gene is located on chromosome 9 and is highly expressed in skeletal muscle, and CM-associated TPM2 mutations appear to be de novo and affect conserved amino acid sequences. TPM2 expression is higher in slow muscle fibers (65%) than in fast muscle fibers (29%) [106]. The TPM3 locus is located on chromosome 1 and encodes the muscle fiber-specific isoform of α -Tropomyosin in type I slow muscle. TPM3 missense mutation (M9R) is associated with a late-onset form of NM in childhood, which weakens the affinity of TPM3 with actin and leads to local structural instability of tropomyosin coiled-coil dimers, while homozygous nonsense mutation is associated with a severe infantile form of NM, and functional TPM3 may be absent from these patients [36, 37]. Mutations in TPM2 and TPM3 are associated with CMs, such as NM, cap myopathy, and CFTD. Cap myopathy is a rare CM, characterized by a well-demarcated cap-like structure underneath the myofibrillar membrane and abnormal accumulation of tropomyosin. Highly oxidized type I slow muscle fibers predominate in the skeletal muscle of patients with CFTD who have hypotonia.

Massive evidence suggests that the mechanism of muscle dysfunction in Tropomyosin-associated myopathies depends on the specific type of mutations. Tropomyosin p.K49del, p.E139del, and p.Q147P mutations have been reported to weaken the binding of tropomyosin to actin and disrupt the helical structure of tropomyosin, which may be associated with abnormalities in protein secondary structure [107]. Other mutations are associated with abnormal calcium sensitivity of myofilaments. p.R91G, p.K7del, Arg168His (R168H) cause an

increase in calcium sensitivity, while p.E41K and p.E117K decrease calcium sensitivity [108–111]. And beyond that, the p.K7del mutation perhaps alters the β -tropomyosin-troponin interaction and the tropomyosin-actin interaction [108]. An abnormal sensitivity of myofilaments to Ca^{2+} may account for hypertonia or hypotonia in certain patients, while chronic muscle overcontraction resulting from increased myofibril sensitivity to calcium could explain joint spasms in patients.

Other biological processes

MEGF10 and TRIM32

Multiple EGF-like domains protein 10 (MEGF10) is a transmembrane protein that contains multiple EGF-like repeats located on the plasma membrane. It serves as a regulatory factor for muscle development and is highly expressed in activated muscle satellite cells (MuSCs), regulating their proliferation, differentiation, and fusion into multinucleated muscle fibers [39]. In adult skeletal muscle, MuSCs are usually in a state of mitotic arrest but can be induced to enter the cell cycle under load or trauma, thus forming a population of precursor cells. After undergoing several rounds of division, they fuse with existing fibers or form new ones with multiple nuclei [112]. Additionally, MuSCs possess the capacity for self-renewal and replenishment of the quiescent MuSC pool following activation [113–115]. MEGF10 plays a key role in muscle development and repair by regulating the differentiation program of MuSCs and maintaining their self-renewal through activating the Notch signaling pathway [38]. When MEGF10 is silenced in mouse muscles, it results in downregulation of components of the Notch signaling pathway, severe depletion of self-renewing MuSC pools, and premature differentiation of MuSCs [38, 116]. Extracellular part of MEGF10 contains 17 EGF-like domains, and multiple tyrosine phosphorylation residues in the cytoplasmic domain mediate cell proliferation and differentiation signals [117]. Mutations in MEGF10 may affect the conformation of cysteine in the EGF-like domain, leading to defects in tyrosine phosphorylation of MEGF10 and impairment of intracellular signaling [40]. The proliferation and migration of MEGF10^{-/-} MuSCs are reduced, and the regenerative ability of MEGF10^{-/-} mouse skeletal muscles is impaired [118]. Mutations in MEGF10 cause early-onset myopathy, areflexia, respiratory distress, and dysphagia (EMARDD, OMIM 614399) [39, 40], which results in decreased muscle tone, respiratory failure, delayed reflexes, swallowing difficulties, and a marked reduction in the number of multinucleated muscle fibers in patients' muscles.

Tripartite motif-containing protein 32 (TRIM32) is a member of the tripartite motif (TRIM) family and consists of three interconnected motifs. It acts as an E3 ubiquitin ligase, participating in a series of post-translational

modifications by attaching ubiquitin moieties to target substrates [119]. TRIM32 binds to myosin head and neck regions and ubiquitinates actin, which may be involved in regulating cytoskeleton composition [41]. There are reports that expressing specific pathological mutations in *Drosophila thin* (the homologue of TRIM32) can lead to abnormal myofibrillar abnormalities in fruit flies [120, 121]. In addition, the lack of Trim32 in the mouse hippocampus is related to upregulation of Notch1, indicating that Trim32 may be related to the Notch signaling pathway; however, its exact relationship with skeletal muscle development and myopathy still needs further exploration [120, 122]. TRIM32 mutations are associated with sarcotubular myopathy (OMIM 268950), and most of the mutations occur in highly conserved NHL domains. Patients with sarcotubular myopathy exhibit mild to moderate symmetric proximal muscle weakness and emaciation and have difficulties with vigorous activity [42]. A muscle biopsy shows varying sizes of muscle fibers, an increased number of nuclei, and small vacuoles in some muscle fibers.

The Notch signaling pathway plays a key role in the regulation of skeletal muscle development and regeneration. Proteins encoded by MEGF10 and TRIM32 are associated with this pathway and involved in regulating muscle function; their mutations are associated with specific types of CMs [120]. Further exploration of the Notch signaling pathway in skeletal muscle development, homeostasis, and disease background holds great promise for understanding the convergence mechanism of muscular diseases and identifying new therapeutic targets.

Cofilin 2 (CFL2)

CFL2 encodes for the cofilin2 protein, which resides in the formed sarcomere and belongs to the skeletal muscle-specific actin depolymerizing factor (ADF)/cofilin protein family. CFL2 maintains the length of F-actin through its actin-serving activity and contributes to regulating actin filament dynamics by maintaining the structure of the sarcomere [43]. Reduction or functional deficiency of CFL2 affects the development and maintenance of sarcomere structure, leading to muscle dysfunction. Rosen et al. investigated the pathogenicity of the human CFL2 p.A35T mutation and concluded that this mutation may lead to protein misfolding and splicing defects in the body, resulting in reduced expression of CFL2 and myopathy [123]. The absence of *Drosophila* cofilin (DmCFL) leads to actin aggregation, muscle structure collapse, and weakness similar to NM patients [44]. In addition, CFL2 plays a crucial role in regulating cell cycle progression during myogenic differentiation of C2C12 cells by influencing cell proliferation and the expression of myogenic transcription factors. Knockout of the CFL2 gene promotes cell cycle transition from the G0/G1 phase to the

G2/M phase, stimulates cell proliferation, and markedly downregulates the expression of myogenic transcription factors such as MyoD, MyoG, and MEF2C, thereby impairing the differentiation of C2C12 myoblasts and the formation of myotubes [124]. Overall, CFL2 is involved in regulating actin aggregation and maintaining sarcomere structure, which may also involve regulation of the cell cycle, thereby affecting muscle regeneration after injury. CFL2 mutations have been found in some patients with NM, and the role of CFL2 in increasing sarcomere production suggests that muscle growth may promote the occurrence and development of NM, especially during periods of rapid muscle growth after birth and during adolescence [125–127]. This is consistent with clinical findings that symptoms worsen or develop further during adolescent growth spurts in some patients with NM.

Furthermore, several other genes associated with CMS include CNTN1, HACD1, SCN4A, and PYROXD1 (Table 1). CNTN1 functions as a neural adhesion and neuromuscular junction protein. HACD1 is involved in elongating very long chain fatty acids (VLCFA). SCN4A is pivotal in muscle action potential, while PYROXD1 functions as a nuclear-cytoplasmic oxidoreductase.

Treatments

The management of CMs has shown some improvement over the past three years (Table 2). The potential treatment methods for CMs can be roughly divided into the following aspects: treatment for potential genetic defects, calcium/myosin regulation targeting muscle contraction, and treatments targeting genes involved in the same way [128].

Treatments for potential genetic defects

Currently, there is no approved treatment for CMs in clinical practice. Therefore, the primary focus of patient care lies in managing accompanying symptoms, particularly respiratory dysfunction which can be latent and life-threatening even with mild clinical symptoms. This often necessitates mechanical support ranging from non-invasive ventilation during nighttime sleep to near-permanent mechanical ventilation via tracheostomy.

Most patients suffer from systemic muscle atrophy, and facial involvement varies. Muscle pathological features of the face include a high, arched palate and drooping upper eyelids, but generally no involvement of extraocular muscles [2, 144]. Many patients experience persistent

Table 2 Treatment strategies of congenital myopathies

Strategy	Gene targets	Myopathy model	Phase	References	
Gene therapy	MTM1 (AAV)	BIN1-related CNM; XLCNM	preclinical	[129, 130]	
	MTM1 (AAV)	XLCNM	preclinical	[131]	
	MTM1 (AAV)	XLCNM	clinical trial	[132]	
	BIN1 (AAV)	DNM2-related myopathy; XLCNM	preclinical	[133, 134]	
	DNM2 (AAV)	CNM	preclinical	[70]	
	DNM2(CRISPR/Cas9)	CNM	preclinical	[135]	
	DNM2 (antisense oligonucleotides ASOs)	CNM linked to BIN1; ADCNM	preclinical	[73, 74]	
	DNM2(ASOs)	XLCNM	I/II clinical trial	NCT04033159	
	Drug therapy	Rycal: a RyR stabilizing molecule	RYR1-related myopathies	provides a rationale for a clinical trial (NCT04141670)	[12]
		Tamoxifen	Bin1- and Dnm2-related CNM	preclinical	[136]
Acetazolamide		ion channel-related congenital myopathy	clinical trial	[137]	
tirasemtiv: a fast skeletal muscle troponin activator		NM	preclinical	[138]	
omecamtiv mecarbil (OM): a small molecule activator of Myh7(slow skeletal muscle troponin activator)		nebulin-based NM	preclinical	[139]	
L-carnitine		congenital myopathies linked to TPM3 mutations	preclinical	[140]	
TMP269 and 5-aza-2-deoxycytidine (5-Aza): HDAC and DNA methyltransferase inhibitors		congenital myopathies linked to recessive RYR1 mutations	preclinical	[141]	
valproic acid: HDAC inhibitor		XLCNM	preclinical	[142]	
metformin: autophagy-promoting compounds		BAG3 myofibrillar myopathy	preclinical	[143]	

CNM, centronuclear myopathy; NM, nemaline myopathy; MmD, multimincore disease; XLCNM, X-linked centronuclear myopathy; ADCNM, autosomal dominant centronuclear myopathy

involvement of the medulla oblongata, with difficulties in chewing and swallowing that require a gastrostomy to assist with feeding. For infants and young children who have a weak suction ability, tube feeding is necessary [27]. Patients with severe CMs and skeletal deformities such as clubfoot/clubfeet, pectus excavatum, scoliosis, and short ribs may require surgical correction or conservative treatment based on their condition. Contractures are a significant issue for patients, leading to various joint symptoms and disability. Some patients also experience excessive joint laxity, particularly in the shoulders, hands, and knees. [145]. Additionally, up to 17% of XLMTM patients may suffer from liver and gallbladder diseases, with a small number at risk of life-threatening hepatic peliosis [15, 146]. These issues necessitate attention and appropriate management.

Calcium/myosin regulation targeting muscle contraction

Mutations in certain genes can lead to a decrease in the sensitivity of skeletal muscle troponin to Ca^{2+} . By adjusting the sensitivity of muscle fibers to Ca^{2+} through exogenous activators, the patient's muscle strength can be improved. Tirasemtiv is a fast skeletal muscle troponin activator that selectively increases the sensitivity of fast-twitch muscle fibers to calcium and has shown good therapeutic effects in animal models. However, in a phase 3 clinical trial conducted among patients with amyotrophic lateral sclerosis, tirasemtiv did not meet the endpoint due to treatment-related side effects such as dizziness, fatigue, nausea, weight loss, and insomnia [147]. Reldesemtiv (formerly CK-2127107) is a second-generation fast skeletal muscle troponin activator that has fewer side effects compared with tirasemtiv in structure. However, no clinical trials have been conducted on it specifically for patients with CMs [148, 149].

Omecamtiv microbial (OM), a small-molecule cardiac myosin activator (MYH7 or β -MHC), is currently in phase 3 clinical trials for the treatment of heart failure [139]. Myh7, or β -MHC, is expressed both in the heart and slow-twitch skeletal muscle, indicating that OM may also be effective for slow-twitch muscles. The research results of nemaline myopathy in animal models confirm its therapeutic effect on skeletal muscle [139]. Unlike various existing calcium sensitizers, OM does not affect calcium transients, thereby limiting potential side effects and making it a promising therapeutic option. It should be noted that troponin activators are only suitable for specific mutation types, and some pathogenic gene mutations cause an increase in calcium sensitivity in patients with CMs, which is not suitable for such drugs. Rycals are a class of compounds derived from the 1,4-benzothiazepine backbone. They stabilize RyR1 channel complexes by restoring calstatain1 binding, alleviate intracellular Ca^{2+} overload, and improve skeletal muscle function. There is

evidence that drug therapy targeting epigenetic enzymes can improve muscle strength, RyR1 protein content, and muscle ultrastructure in a mouse model of CMs associated with recessive RYR1 mutations [141]. This study provides conceptual evidence for the development of drugs that modify the entire genome epigenetically when treating patients with CMs, representing a new direction in drug therapy.

Treatments targeting genes involved in the same way

The therapeutic approach targeting genes involved in the same pathway does not correct pathogenic genes but aims to compensate for specific protein deficiencies by enhancing the expression of related genes. For example, BIN1, MTM1, and DNMT2 interact to jointly regulate the biogenesis and stability of T-tubules. In DNMT2-related myopathy, treatment can be achieved by regulating the expression level of BIN1 to improve skeletal muscle condition [150]. Delivering therapeutic gene modifiers to skeletal muscles is a pressing challenge due to their dispersion throughout various parts of the body. While recombinant adeno-associated viruses (rAAVs) are commonly used for gene replacement therapy and gene editing, selective transduction of specific tissues remains a challenge after systemic administration. However, capsid variants of AAVs, such as those containing RGD motifs, have been identified to efficiently deliver to muscle tissues, providing a practical tool for treatment development and testing [151]. Another major limitation of virus-based gene transfer strategies is the limited packaging capacity of viruses, such as AAVs, which limits their ability (approximately 5 kb) to deliver large genes like NEB into cells. An alternative approach could be to introduce functional protein fragments or truncated proteins, for example, injecting AAV that encapsulates the nebulin protein fragment (containing the Z-disk region and super repeats of nebulin) in a NEB knockout mouse model [128]. However, despite this injection, marked improvements in the structure and function of mouse sarcomere have not been observed, so further experimental exploration is necessary. Silencing mutated mRNA without affecting normal alleles through allele-specific RNA interference is also a therapeutic strategy that is worthy of development. Due to the diverse types of pathogenic genes and complex mutation types in CMs, Dudhal et al. [152] developed multifunctional siRNAs that target two non-pathogenic single nucleotide polymorphisms (SNPs) with the aim of generating universal small interfering RNAs without considering mutation type. This innovative approach has significantly enriched the molecular toolbox, as this type of small interfering RNA can effectively target all DNMT2 mutations and possesses broad applicability, thus warranting further exploration and development.

Prospectives

CMs are a rare genetic disease caused by various types of mutations in genes involved in multiple physiological processes, including muscle ECC and triadic assembly, interactions between thick and thin muscle filaments, production of myofibrillar force, MuSCs activation and regeneration, and ion channel functions. The pleiotropy and genetic heterogeneity of this disease make clinical diagnosis very challenging, and there are numerous and diverse types of gene mutations that prevent effective treatment at the genetic level. As such, most current treatment strategies focus on specific mutation types only and lack universality across patients.

Currently, while drug therapy remains the primary treatment option for CMs in clinical practice, targeted therapeutic strategies and novel drugs are under development to better meet the needs of patients. Despite ongoing research efforts, there remain unclear areas in clinical diagnosis and identification, as well as unknown fields and potential treatment possibilities related to pathogenic molecular mechanisms and treatment strategies. To overcome this rare disease, it is crucial for researchers and medical professionals to continue collaborating and delving deeper into the field of CM research.

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Author contributions

Bin Dai, Meiyuan Li and Yuntian Shen contributed to the conception of the study; Han Zhang, Mengyuan Chang, Daiyue Chen, Jiawen Yang, Yijie Zhang, Jiacheng Sun and Xinlei Yao performed the data analyses; Han Zhang, Mengyuan Chang and Daiyue Chen drafted the manuscript; Bin Dai, Meiyuan Li, Yuntian Shen, Hualin Sun and Xiaosong Gu reviewed and modified the manuscript; All authors read and approved the final manuscript.

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

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Competing interests

The authors declare that they have no competing interests.

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