Handbook of Clinical Neurology, Vol. 148 (3rd series)
Neurogenetics, Part II
D.H. Geschwind, H.L. Paulson, and C. Klein, Editors
https://doi.org/10.1016/B978-0-444-64076-5.00036-3

Chapter 36

The genetics of congenital myopathies

HERNAN D. GONORAZKY[1], CARSTEN G. BÖNNEMANN[2], AND JAMES J. DOWLING[1]*
[1]*Division of Neurology and Program of Genetics and Genome Biology, Hospital for Sick Children, Toronto, ON, Canada*
[2]*Neuromuscular and Neurogenetic Disorders of Childhood Section, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD, United States*

Abstract

Congenital myopathies are a clinically and genetically heterogeneous group of conditions that most commonly present at or around the time of birth with hypotonia, muscle weakness, and (often) respiratory distress. Historically, this group of disorders has been subclassified based on muscle histopathologic characteristics. There has been an explosion of gene discovery, and there are now at least 32 different genetic causes of disease. With this increased understanding of the genetic basis of disease has come the knowledge that the mutations in congenital myopathy genes can present with a wide variety of clinical phenotypes and can result in a broad spectrum of histopathologic findings on muscle biopsy. In addition, mutations in several genes can share the same histopathologic features. The identification of new genes and interpretation of different pathomechanisms at a molecular level have helped us to understand the clinical and histopathologic similarities that this group of disorders share. In this review, we highlight the genetic understanding for each subtype, its pathogenesis, and the future key issues in congenital myopathies.

INTRODUCTION

Congenital myopathies are a clinically and genetically heterogeneous group of conditions that most commonly present at or around the time of birth with hypotonia, muscle weakness, and respiratory distress (Bertini et al., 2011; Nance et al., 2012). They are associated with significant chronic care requirements, including continuous breathing and feeding support in some cases, and may result in mortality in the first years of life (Bertini et al., 2011; Nance et al., 2012). Historically, congenital myopathies have been described and enumerated based on findings seen on muscle biopsy. Based on biopsy features, congenital myopathies are typically subdivided into four categories (Fig. 36.1): nemaline myopathy (NM), core myopathy, centronuclear myopathy (CNM), and congenital fiber-type disproportion (CFTD) (Darras et al., 2014). The overall prevalence of congenital myopathies has not been precisely determined, though it is likely it occurs in at least 1:20,000 children (Hughes et al., 1996; Darin and Tulinius, 2000; Amburgey et al., 2011). In terms of subtypes, core myopathy appears to be the most common, followed by NM and CNM (Maggi et al., 2013). An accurate assessment of subtype-relative prevalence, however, has yet to be performed.

Knowledge of the genetics underlying congenital myopathies is rapidly changing the understanding of these conditions as well as the overall view of their categorization. To date, mutations in 32 different genes have been associated with a definitive clinical and histopathologic diagnosis of congenital myopathy (Table 36.1; Fig. 36.2) (Kaplan and Hamroun, 2014). These account for approximately 60% of cases of congenital myopathy (based on clinical gene panel assessment; Das, personal communication), meaning that an additional 40% of the genetic burden of disease remains to be solved (Maggi et al., 2013). Identification of these genetic causes has created an emerging picture of the pathogenic

*Correspondence to: James J. Dowling, MD, PhD, 15-09703 PGCRL, 686 Bay Street, Toronto, Ontario M5G 0A4, Canada. Tel: +1-416-813-7654, E-mail: james.dowling@sickkids.ca

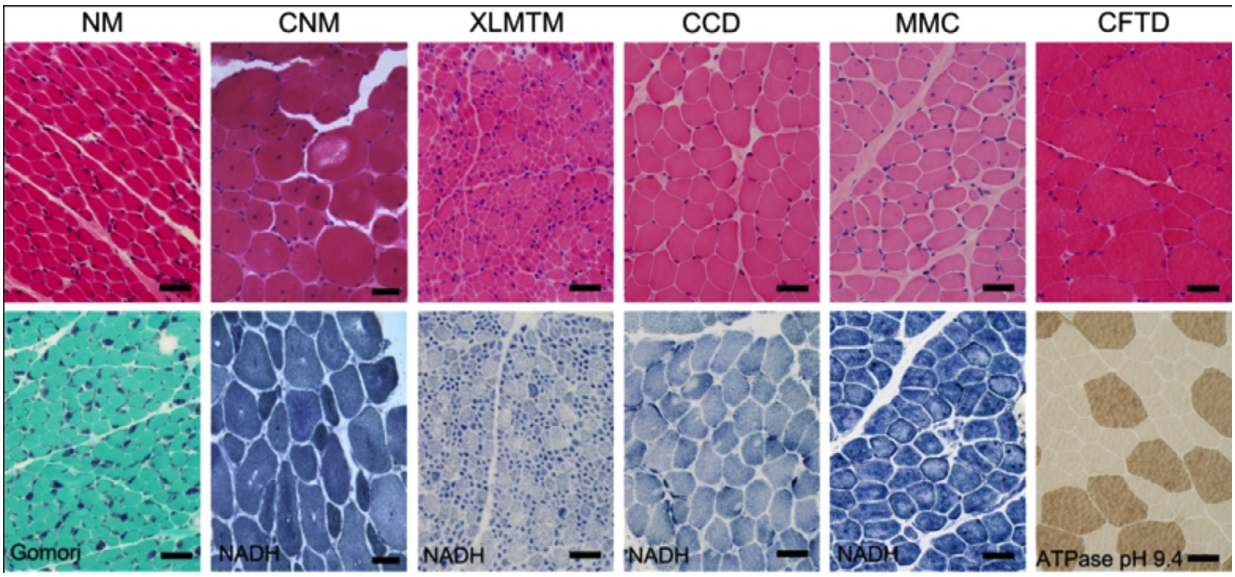

Fig. 36.1. *CCD*, central core disease; *CFTD*, congenital fiber type disproportion; *CNM*, central nuclear myopathy; *MMC*, multiminicore; *NM*, nemaline myopathy; *XLMTM*, X-linked *MTM*.

mechanisms likely responsible for causing disease in the subtypes of congenital myopathy. It has also enabled more sophisticated genotype–phenotype correlations to develop, and has deepened the understanding of clinical features of specific genetic subtypes. However, as yet this knowledge has not been translated into new therapies for these devastating disorders, though the development of animal models based on the genetics has resulted in the identification of several promising candidate therapies (Dowling et al., 2017). Lastly, the availability of congenital myopathy gene panels and whole-exome sequencing has shifted the diagnostic landscape, bringing into discussion the relationship between genetics and other diagnostic studies, as well as creating interesting questions regarding disease nomenclature and challenging conundrums related to variants of unknown significance (VUS).

Here we review the state of genetic understanding for each subtype of congenital myopathy, discuss how this understanding has generated a deepened appreciation of disease pathogenesis, and investigate several of the key issues created by the "genetic revolution" in congenital myopathies.

NEMALINE MYOPATHY

Clinical overview

NM is defined by the presence of nemaline rods or nemaline bodies on muscle biopsy. Rods, which are thought to be myofibrillar material that emerge/expand from the z band, are best appreciated on modified Gömori trichrome stain, or else visualized by electron microscopy (Dubowitz et al., 2013). Clinically, NM is a diverse disease, with presentations ranging from birth to adulthood. Patients may be loosely separated into clinical groupings based on age and severity of presentation: a severe infantile form, a "classic" congenital form, and late congenital form, and a childhood and adolescent form (Ryan et al., 2001). Between these groupings, a relatively consistent clinical feature is the presence of lower facial and bulbar weakness while the involvement of extraocular muscles is rare, with the result being that many children with NM, regardless of overall severity, require ongoing feeding and speech assistance (Wallgren-Pettersson et al., 2011).

Genetics overview and genotype–phenotype correlations

There are 12 known genetic causes of NM: *ACTA1, NEB, TPM2, TPM3, TNNT1, CFL2, KBTBD13, KLHL40, KLHL41, LMOD3, MYO18B,* and *MYPN*. *ACTA1* mutations are the most common dominant/de novo mutations, and *NEB* mutations are the most common recessive mutations (Tosch et al., 2006; Feng and Marston, 2009; Wallgren-Pettersson et al., 2011; Lehtokari et al., 2014). Mutations in *ACTA1* are generally considered to act in a dominant negative fashion, altering *ACTA1* polymerization into thin filaments (Ravenscroft et al., 2011). *TPM2* and *TPM3* mutations are generally also considered to act dominantly, interfering with tropomyosin polymer formation or function (Marttila et al., 2014) There are a small number of autosomal-recessive cases of *ACTA1*, *TPM2*, and *TPM3* as well (Feng and Marston, 2009; Marttila et al., 2014).

Table 36.1

Classification of congenital myopathies by genes

Gene	Subtype	Inheritance pattern	Protein	Primary subcellular involvement	Possible pathogenesis
ACTA 1	• Nemaline myopathy (NM) • Cap disease (NM variant) • Zebra body myopathy (NM variant) • Congenital fiber type disproportion	AD, AR AD AD AD	Actin, alpha1, skeletal muscle	Thin filament involvement	Abnormal thin filament structure
TPM3	• Nemaline myopathy (NM variant) • Cap disease (NM variant) • Congenital fiber type disproportion	AD, AR AD AD	Tropomyosin 3		
TPM2	• Nemalin myopathy (NM) • Cap disease (NM variant)	AD AD	Tropomyosin 2 (beta		
TNNT1	• Nemalin myopathy (NM)	AR	Troponin T type 1 (skeletal, slow)		
NEB	• Nemaline myopathy (NM) • Core–rod myopathy	AR	Nebulin		Thin filament remodeling ± stability
LMOD3	• Nemalin myopathy (NM)	AR	Leiomodin 3		
KBTBD13	• Nemalin myopathy (NM)	AD	Kelch repeat and BTB (POZ) domain containing protein 13		
CFL2	• Nemalin myopathy (NM)	AR	Cofilin 2 (muscle)		
KLHL40	• Nemalin myopathy (NM)	AR	Kelch-like family member 40		
KLHL41	• Nemalin myopathy (NM)	AR	Kelch-like family member 41		
MYO18B	• Nemalin myopathy	AR	myosin 18B	Unknown	Unknown
RYR1	• Central core myopathy • Multiminicore myopathy • Core–rod myopathy • Nemalin myopathy • Congenital fiber type disproportion • Centronuclear myopathy • Congenital neuromuscular disease with uniform type 1 fiber	AD, AR AR AD, AR AR AR AR AD	Ryanodine receptor I	Triad involvement	Abnormal EC coupling
CACNAS1	• Congenital fiber type disproportion	AR	DHPR		
STAC3	• Native American myopathy	AR	SH3 and cysteine-rich domain containing protein3		
ORAI1	• Tubular aggregate myopathy	AD	Transmembrane protein 142A		Abnormal SOCE

Continued

Table 36.1

Continued

Gene	Subtype	Inheritance pattern	Protein	Primary subcellular involvement	Possible pathogenesis
STIM1	• Tubular aggregate myopathy	AD	Stromal interaction molecule 1		
SEPN1	• Multiminicore myopathy • Congenital fiber type disproportion	AR AR	Selenoprotein N1		Oxidative defects
CCDC78	• Centronuclear myopathy	AD	Coiled coil domain containing protein 78		Abnormal EC coupling?
BIN 1	• Centronuclear myopathy	AR,AD	Amphiphysin		Membrane remodeling ± stability
DNM2	• Centronuclear myopathy	AD	Dynamin 2		
MTM1	• Myotubular myopathy	XR	Myotubularin 1		
MTMR14[a]	• Centronuclear myopathy	*	Myotubularin-related protein 14		
SPEG	• Centronuclear myopathy with dilated cardiomyopathy	AR	*SPEG* complex locus		
PTPLA (=HCDA1)	• Congenital myopathy related to *PTPLA*	AR	Protein tyrosine phosphatase-like (3-hydroxyacyl-CoA dehydratase)		
TTN	• Centronuclear myopathy • Congenital myopathy with fatal cardiomyopathy	AR AR	Titin		
MYH7	• Myosin storage myopathy • Myosin storage myopathy with cardiomyopathy • Congenital fiber type disproportion	AD AR AD	Myosin, heavy chain 7, cardiac muscle, b		Abnormal ATPase and actin-binding properties Structural abnormalities
MYH2	• Myosin IIa myopathy	AD, AR	Myosin, heavy-chain 2, skeletal muscle	Heavy-chain neuromuscular junction (NJ)	Abnormal ATPase and actin-binding properties
CNTN1	• Compton-North Congenital myopathy	AR	Contactin-1		Structural abnormalities Aberrant NJ adhesion?
MEGF10	• Early-onset myopathy, areflexia respiratory distress and dysphagia • Minicores	AR AR	Multiple EGF-like domains 10	Satellite cells	Abnormal regulation of satellite cells
ZAK	• Congenital fiber type disproportion	AR	Sterile alpha motif and leucine zipper containing kinase AZK	Unknown	Mitogen-activated protein kinase (MAPK) signaling pathway

Adapted from Kaplan JC, Hamroun D (2015) The 2016 version of the gene table of monogenic neuromuscular disorders (nuclear genome). Neuromuscul Disord 25: 991–1020.

[a]Until now *MTMR14* has been proven to produce a myopathy only in animal models.

AD, autosomal-dominant; AR, autosomal-recessive; EC, excitation–contraction; SOCE, store-operated calcium entry.

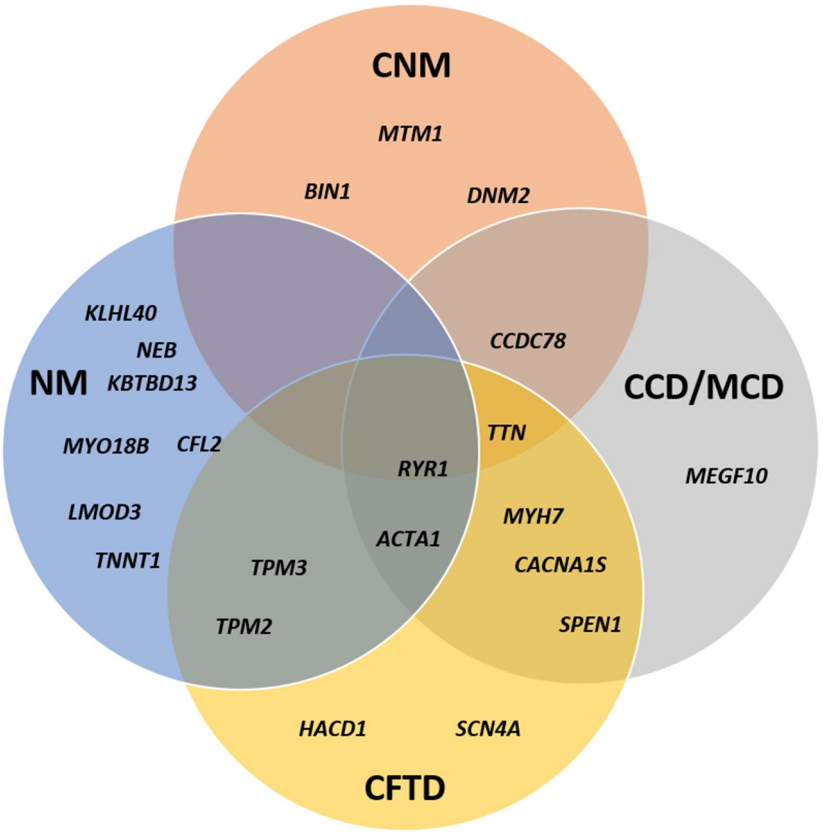

Fig. 36.2. Relationship in congenital myopathies between histopathology and genetics. The genetic causes of congenital myopathies are presented categorized based on the histopathologic subytpes with which they are associated. Note that several different genetic causes are associated with each histopathologic subtype, and that mutations in some genes can cause several different biopsy findings. *CCD/MCD*, central core disease/minicore disease; *CFTD*, congenital fiber type disproportion; *CNM*, central nuclear myopathy; *NM*, nemaline myopathy.

NEB, KLHL40, KLHL41, and *LMOD3* mutations classically cause disease through recessive loss of function, with mutations resulting in reduced expression and/or function (Pappas et al., 2010; Gupta et al., 2013; Garg et al., 2014; Yuen et al., 2014). Patients with *CFL2* (recessive), *TNNT1* (recessive, causing Amish NM), and *KBTBD13* (dominant) are very rarely encountered (Johnston et al., 2000; Agrawal et al., 2007; Sambuughin et al., 2010). The relative comparative frequency is imprecisely delineated outside the above broad generalizations; this is in part due to the recent identification of *LMOD3*, *KLHL40,* and *KLHL41* mutations. There are likely also regional and ethnic differences, both in terms of genetic subtype frequency and overall disease prevalence. For example, there is a common deletion (exon 55) found in *NEB* in individuals of Ashkenazi Jewish extraction (carrier frequency as high as 1:40) (Lehtokari et al., 2014).

In terms of general clinical features, *ACTA1*, *KLHL40*, and *LMOD3* mutations are most likely to be associated with severe infantile NM, often with death in the first year of life (Yuen et al., 2014; Colombo et al., 2015). In addition, a rare homozygous *CFL2* mutation has also recently been described in a family with fatal NM (Ong et al., 2014). The noninfantile *CFL2* phenotype is compatible with the classic form on NM, except for the presence of late foot drop and the lack of prominent facial weakness (Agrawal et al., 2007; Ockeloen et al., 2012). *NEB* mutations are most typically seen with classic congenital NM: i.e., onset in infancy with diffuse weakness, improvement with age to the point that ambulation is achieved, potentially persistent bulbar involvement (Pelin et al., 1999; Marttila et al., 2014).

The clinical phenotype related to *ACTA1* mutations can be highly variable, ranging from severe neonatal weakness to individuals having mild disease with minimal clinical involvement. While *ACTA1* patients typically share features such as bulbar weakness, muscle hypotrophy, and diffuse limb involvement, one *ACTA1* mutation is associated with a novel phenotype: hypertonia, muscle stiffness, and muscular hypertrophy (Feng and Marston, 2009; Jain et al., 2012). Although the complete loss or alteration of skeletal muscle α-actin would be hypothesized to be incompatible with fetal progression, even

patients with the severe lethal form of *ACTA1* mutation (i.e., fetal akinesia sequence) usually survive until birth. This is thought to be related to the compensatory expression of cardiac α-actin, the predominant isoform expressed in early human development until the end of the second trimester of gestation. As mentioned, there is also significant clinical variability in *ACTA1* clinical presentations, a fact that may be related to the levels of postnatal expression of cardiac actin (Nowak et al., 2007).

In terms of other specific clinical features, it should be noted that NM is not typically associated with extraocular muscle weakness. An exception to this is a subset of individuals with *LMOD3* and *KLHL40* mutations who have ophthalmoparesis, making these genetic diagnoses most likely in individuals with this clinical feature and NM pathology on biopsy (Ravenscroft et al., 2013b; Yuen et al., 2014). Interestingly, in addition to sharing clinical symptomatology (early onset, severe presentation with hypokinesia, arthrogryposis, respiratory and bulbar insufficiency, and early death), the *LMOD3* and *KLHL40* gene products have been documented to directly interact, suggesting a direct pathogenic connection (Garg et al., 2014).

Arthrogryposis is seen in several NM subtypes, but is quite prominent with *TPM2* mutations. In fact, mutations in *TPM2* have been found as a cause of distal arthrogryposis type II and type VII; patients with these conditions do not always have overt muscle weakness or features of NM on muscle biopsy (Krakowiak et al., 1997; Davidson et al., 2013). Of note, unlike what is predicted for *TPM2* mutations associated with muscle weakness, some mutations in *TPM2* that cause arthrogryposis are hypothesized to cause sarcomere hypercontractility (Mokbel et al., 2013). In contrast, *TPM3* mutations are not associated with congenital contractures, likely because *TPM2* is preferentially expressed during fetal development (Marttila et al., 2014).

Nonmuscle-related symptomatology is uncommon in any NM subtype. Patients are cognitively normal. They may have orthopedic complications and feeding and respiratory failure, all secondary complications of muscle weakness (Colombo et al., 2015). Heart involvement is rare; exceptions are some patients with mutations in *ACTA1* or *MYPN* that can manifest with cardiomyopathy (D'Amico et al., 2006; Miyatake et al., 2017).

In general, histopathology does not offer much to distinguish the different genetic forms of NM. Exceptions include the presence of nuclear rods, which are seen primarily with *ACTA1* mutations, the observation of many very small rods, seen with *KLHL40* and *CFL2* mutations, and the presence of unusual "barbell"-shaped rods, seen on electron microscopy with *LMOD3* mutations (Ockeloen et al., 2012; Ravenscroft et al., 2013b; Bonnemann et al., 2014; Nworu et al., 2015). Cap myopathy is considered a histopathologic variant of NM, and is associated with mutations in *ACTA1, TPM2, TPM3,* and *MYPN* (Tajsharghi et al., 2007; De Paula et al., 2009; Hung et al., 2010; Lornage et al., 2017). *ACTA1* mutations are also seen in zebra body myopathy (Nowak et al., 2007). Lastly, at times both cores and rods can be seen in a single biopsy. This is often referred to as "core-rod" myopathy. Known genetic causes of this phenomenon include mutations in *ACTA1*, *KBTBD13*, *TPM2*, *NEB*, and *RYR1* (North et al., 2014).

Disease pathomechanism(s)

Based on the known genetic causes of NM, a relatively consistent picture of the pathogenic mechanism(s) underlying the disease is presented. Given that *ACTA1*, *NEB*, *TPM2*, *TPM3*, *TNNT1*, and *LMOD3* are all components or direct modifiers of the actin thin filament, NM is essentially a disease of thin filament dysfunction. In other words, the actin filament is either not formed properly, or else its dynamic interaction with the myosin thick filaments is disturbed, with the end result being altered muscle contractile function (Ravenscroft et al., 2015).

Interestingly, several of the most recently identified NM genes are not direct components of the thin filament machinery. These include *KLHL40*, *KLHL41*, and *KBTBD13*, all of which are Kelch domain-containing proteins (Martilla et al., 2014). Evidence extrapolated from protein structural domains and from other similar Kelch proteins suggests that these proteins participate in the regulation of ubiquitination and protein turnover (Gupta and Beggs, 2014). Therefore one hypothesis as to their role in thin filament biology and in NM is that they participate specifically in the regulation of thin filament protein breakdown (Gupta and Beggs, 2014). However, it was recently shown that *KLHL40* can directly bind *LMOD3* and nebulin and can promote the stability of *LMOD3* by blocking its degradation (Garg et al., 2014). It is tempting to speculate that *KLHL41* and *KBTBD13* also may function in a similar fashion to regulate the levels of thin filament proteins.

Therapeutic considerations

At present, there are no specific therapies for NM. L-tyrosine has been shown in a limited case series to improve bulbar function, and in a preclinical model of *ACTA1* mutation to improve strength (Ryan et al., 2008; Nguyen et al., 2011). The mechanisms underlying its potential efficacy are unclear, and further study is necessary to demonstrate its true clinical effectiveness.

There is also a relative paucity of target therapeutics. One leading candidate therapeutic strategy is troponin activation. This treatment improves muscle contractile properties, and has been shown in cells from NM patients

to augment muscle force generation (Lee et al., 2013). It would thus be potentially applicable for the majority of NM patients (the clear exceptions would be those individuals with mutations causing myofibrillar hypersensibility to Ca^{2+}. Troponin modulators are currently in clinical trial for other neuromuscular disorders, and results from these studies may inform on the suitability of such drugs for patients with NM (de Winter et al., 2013).

Another strategy, specific for *ACTA1* mutations, is cardiac a-actin overexpression therapy. Ravenscroft et al. (2013a) showed that the severity of phenotype of *ACTA1* mutations is, in part, correlated with levels of the mutant ACTA1 protein, and that overexpression of ACTA in a mouse model of dominant *ACTA1* mutation reduces the relative proportion of mutant *ACTA1* and prevents many of the pathologic features of the mouse mutant (Ravenscroft et al., 2013a).

CENTRONUCLEAR MYOPATHY

Clinical overview

CNMs are a clinical and genetically heterogeneous myopathy subtype unified by the observation on muscle biopsy of central nuclei in >25% of muscle fibers. Additional histopathologic features include myofiber hypotrophy and distinctive patterns of disorganization of oxidative enzymes (Romero, 2010). Clinically, there is a broad range of symptom involvement, from several neonatal presentations to more mild adult disease (Bevilacqua et al., 2009; Bohm et al., 2012, 2014a). Ophthalmoparesis is quite commonly encountered, making it a useful clinical distinction from other myopathies in many cases (North et al., 2014).

Genetics overview and genotype–phenotype correlations

There are mutations in eight genes described as causes of CNM: *MTM1, DNM2, BIN1, RYR1, TTN, MTMR14, SPEG,* and *CCDC78* (Laporte et al., 1996; Bitoun et al., 2005; Tosch et al., 2006; Nicot et al., 2007; Wilmshurst et al., 2010; Majczenko et al., 2012; Ceyhan-Birsoy et al., 2013; Agrawal et al., 2014). Mutations in *MTM1* are associated with a specific subtype of CNM called myotubular myopathy (also referred to as X-linked CNM or X-linked MTM). As it is an X-linked gene, and mutations are typically loss-of-function alleles, the condition manifests primarily in boys, though there are occasional manifesting female carriers, usually with milder and later-onset manifestations (Savarese et al., 2016). The most frequent presentation of MTM is one of severe neonatal weakness, with involvement of the facial and extraocular muscles, including ptosis. Respiratory failure with requirement of mechanical ventilation is the most common situation. While the exact percentages are not known, death in infancy is common in MTM (Das et al., 2011). Those that survive the first year of life usually have extensive technology requirements, including wheelchair and ventilator dependence, and the mortality rate during childhood is 10% per year (Amburgey et al., 2017).

DNM2 mutations are the most common cause of autosomal-dominant CNM. The disease has essentially two distinct presentations, with some individuals (typically with de novo mutations in the PH domain) presenting in infancy and early childhood and others (with middle-domain mutations) presenting in late childhood or early adulthood. Ophthalmoparesis is seen regardless (Bohm et al., 2012). *RYR1* mutations are the most common autosomal-recessive cause of CNM. The typical mutation pattern is compound heterozygosity for one missense and one nonsense mutation. The clinical picture can resemble that of severe *DNM2* patients or can be similar to MTM (Wilmshurst et al., 2010).

BIN1 mutations are rare, though the spectrum of disease is expanding; typically this is a recessive subtype, though several families with very mild symptoms and dominant mutations have recently been described (Bohm et al., 2014a). Mutations in *SPEG*, *CCDC78,* and *MTMR14* are rare (Tosch et al., 2006; Majczenko et al., 2012; Agrawal et al., 2014); whether *MTMR14* mutations are truly causative or instead merely disease modifiers is a source of ongoing debate. The burden of *TTN* mutations in CNM is uncertain as the first patients were only recently identified (Ceyhan-Birsoy et al., 2013), and core-like lesions appear to be a more common histopathologic feature with *TTN* mutations.

Nonmuscle symptoms are frequently encountered in patients with MTM. These can include rare conditions such as hepatic peliosis, unusual facial and extremity dysmorphisms, and bleeding diathesis, along with more common conditions such as scoliosis, cryptorchidism, and hip dislocations (Das et al., 2011). Cardiac involvement is rarely seen in any CNM; exceptions include patients with *SPEG* and *TTN* mutations (Agrawal et al., 2014; Chauveau et al., 2014b). Of note, TTN patients do not typically have ophthalmoparesis.

While all individuals with CNM have increased central nuclei, there are some histopathologic features that are more strongly associated with specific gene mutations, although these correlations are not without exceptions. *DNM2*-CNM fibers show typical intermyofibillar sarcomembranous network, described as "wheel pattern," with strands dispersing from the center to the periphery. *BIN1*-CNM has numerous small rounded type 1 fibers, some of them with clusters of centrally placed nuclei. MTM has the typical central nuclei resembling

myotubes on hematoxylin and eosin stain. In NADH-TR staining the fibers have a dark central region with a paler peripheral halo. Another feature is the necklace fibers that are basophilic rings with the nuclei aligned following the form of the cell (Romero, 2010). This last finding has been described in late-onset *MTM1*-CNM and in manifesting female carriers (Bevilacqua et al., 2009). *TTN*-CNM resembles *RYR1*-CNM with a high percentage of central and multiple internalized nuclei but, unlike the previously described genes, both can be associated with core-like areas (Ceyhan-Birsoy et al., 2013). Some individuals with *RYR1* mutations can have a histopathologic pattern that resembles MTM. *CCDC78* mutations are also associated with core-like areas and aggregates in addition to central nuclei and may be more appropriately recategorized as a core myopathy (Majczenko et al., 2012).

Disease pathomechanism(s)

Myotubularin (MTM1), dynamin-2 (DNM2), hJUMPY (MTMR14), striated muscle preferentially expressed protein kinase (SPEG), and amphyphisin-2 (BIN1) are proteins involved in the regulation of membrane traffic and remodeling. While the specific role of membrane trafficking in muscle formation is not completely understood, it is clear that the process is involved with the formation and maintenance of the excitation–contraction (EC) coupling apparatus. Most of the data has supported a role in T-tubule formation, though it is likely that these proteins influence terminal sarcoplasmic reticulum modeling as well. It follows that mutations in these genes disturb the structure of the T-tubule and the terminal sarcoplasmic reticulum and result in impairments in the EC coupling process (Nicot et al., 2007; Dowling et al., 2009; Al-Qusairi L et al., 2009; Gibbs et al., 2013, 2014; Agrawal et al., 2014).

Mutations in *MTM1* have also been shown in preclinical models to impair neuromuscular junction structure and function (Dowling et al., 2012b). Since neuromuscular junctions are also membrane specializations affected by the common pathophysiologic concept outlined above (i.e., membrane trafficking), this may be a more common property of CNMs, as similar (though less robust) evidence exists for DNM2 (Gibbs et al., 2013). Furthermore, patients with CNM of several genotypes have been reported to favorably respond to pyridostigmine, a drug that improves neuromuscular junction signaling (Robb et al., 2011; Gibbs et al., 2013).

RyR1 is a calcium channel located on the terminal sarcoplasmic reticulum. It is a core component of the EC coupling machinery, and mutations in the gene that result in CNM are thought to impair calcium release during EC coupling and thus limit/reduce muscle contraction stimulated by nerve excitation (Wilmshurst et al., 2010). Thus a clear pathomechanistic link between *RYR1* mutations and those seen in *MTM1*, *DNM2,* and *BIN1* is disturbance of the EC coupling process (Dowling et al., 2014).

TTN encodes for the giant sarcomeric ruler protein titin. The mutation in this gene can produce a wide range of disorders, including dilated cardiomyopathy, early-onset myopathy with fatal cardiomyopathy, limb girdle muscular dystrophy type 2J, and hereditary myopathy with early respiratory failure (Udd et al., 1998; Hackman et al., 2003; Carmignac et al., 2007; Hedberg et al., 2014). The *TTN*-related CNM presentation is seen with compound heterozygous mutations that typically involve at least one splice site or stop mutation. How alterations in *TTN*'s many functions (including myofiber elasticity and establishing passive muscle force) relate to the pathogenic themes of CNM (disturbed membrane traffic and altered EC coupling) is not clear at present. One possibility is that mutations interfere with titin–obscurin interactions (Ackermann et al., 2011; Randazzo et al., 2013). Obscurin is a linker protein required for sarcoplasmic reticulum organization; an obscurin knockout mouse model produces the disarrangement of the longitudinal sarcoplasmic reticulum, therefore the triad, and centralization of the nuclei resembling the CNMs (Lange et al., 2009).

Despite the advances in understanding of CNM pathomechanisms, it is still not certain why mutations in the known CNM genes result in the formation of central nuclei. One recent study identified BIN1 as a factor that promotes nuclear positioning through an N-WASP-dependent mechanism and showed that *BIN1* mutations disrupt this interaction and impair proper nuclear localization (Falcone et al., 2014).

Therapeutic considerations

There is considerable excitement in the CNM field related to the preclinical evaluation of gene therapy for MTM. In both murine and dog models, *MTM1* gene therapy has been shown to not only prevent disease development but also to arrest/reverse the MTM disease process after it has developed (Childers et al., 2014; Mack et al., 2017). Interestingly, enzyme replacement therapy with recombinant myotubularin (*MTM1*) has also been efficacious in a mouse model of the disease (Lawlor et al., 2013).

As mentioned above, alterations in the neuromuscular junction have been described in *MTM1* disease models. Perhaps not surprisingly, there are several case reports of patients with MTM benefiting from pyridostigmine, an acetylcholinesterase inhibitor that improves neuromuscular junction signaling (Robb et al., 2011). Further,

there are also case reports of patients with *RYR1* mutations and *DNM2* patients deriving similar benefits (Robb et al., 2011; Gibbs et al., 2013). Thus there seems to be a subtype-wide response (albeit modest) to neuromuscular junction augmentation therapy, although this still needs to be studied systematically across CNM subtypes.

Two new additional strategies for MTM that have shown promise in preclinical models relate to reduction/inhibition of genes that modify MTM1 function. One is inhibition of the lipid kinase PIK3C2B, an enzyme that synthesizes the phospholipid that is dephosphorylated by MTM1(Sabha et al., 2016). The other target is DNM2, reduction of which ameliorates pathology and improves muscle strength in MTM1 knockout mice (Cowling et al., 2014; Tasfaout et al., 2017).

CORE MYOPATHY

Clinical overview

Core myopathies are unified by the observation on muscle biopsy of areas lacking reactivity to the oxidative stains NADH, succinic dehydrogenase, as well as cytochrome c oxidase. These absent staining areas typically correlate with areas lacking mitochondria but containing disorganized myofibrils (as seen on electron microscopy) and come in two variants. Central cores represent areas of myofibrillar disorganization with absent mitochondria that span the longitudinal length of the myofiber, while minicores are small areas of disorganization typically in a more transverse orientation with little longitudinal extension (Dubowitz et al., 2013). Structured central cores refer to regions of absent mitochondria in which the myofibrillar apparatus is still preserved. As discussed below, core myopathies are largely caused by mutations in two genes, *RYR1* and *SEPN1* (Jungbluth et al., 2011).

The clinical features associated with this myopathy subtype are largely divided based on the underlying gene mutation. *SEPN1* mutations are most typically seen with minicore myopathy but are also associated with rigid spine muscular dystrophy, Mallory body myopathy, desmin-related myopathy, and CFTD (Ferreiro et al., 2004; Schara et al., 2008; Ardissone et al., 2016). Most patients with *SEPN1* mutations exhibit a consistent clinical phenotype. The most important clinical features are cervicoaxial weakness with a prominent lack of head control early in life along with later spinal rigidity and scoliosis, though patients often remain ambulant till adulthood (Scoto et al., 2011). Usually there is an early and progressive respiratory insufficiency with need for ventilation assistance in the first two decades of life. Around 8–9 years of age, affected children develop a thoracic scoliosis or a lumbar lordoscoliosis with cervical spine stiffness along with milder joint contractures in elbows, ankles, wrists, and sometimes in the temporomandibular joint (Scoto et al., 2011; Bonnemann et al., 2014). Of note, *SEPN1* mutations are all recessive, and likely result in loss of protein expression and function.

RYR1 mutations, which can be associated both with central cores and minicores, present with a very broad range of clinical signs and symptoms. Patients with central core disease (CCD) typically have mild, diffuse nonprogressive extremity weakness related to dominant mutations in *RYR1*. However, a subset of patients with CCD have a severe infantile presentation that results in significant disability, including respiratory failure and impaired ambulation. Such individuals typically have de novo dominant mutations, some of which can present with either severe or mild presentations (Bharucha-Goebel et al., 2013). Patients with *RYR1*-related minicore myopathy usually have a more severe clinical picture and are associated with recessive *RYR1* mutations. Weakness is often most prominent axially, though extremity involvement is seen as well, and musculoskeletal complications are frequently observed (scoliosis, hip dysplasias, chest wall deformities, and joint contractures) (Zhou et al., 2007; Amburgey et al., 2013). As in the centronuclear variant of recessive RYR1 mutations, ophthalmoparesis is quite common, and serves as a distinguishing clinical feature with SEPN1-related minicore myopathy (North et al., 2014). Of note, and as mentioned in previous sections, *RYR1* mutations are seen with essentially every histopathologic subtype of congenital myopathy, with core myopathy being the most prevalent presentation.

Genetics overview and genotype–phenotype correlations

RYR1 and *SEPN1* mutations combined are overwhelmingly the most common causes of core myopathy (Jungbluth et al., 2011). In fact, *RYR1* mutations are the most common cause of congenital myopathy in general and may well represent the most frequently encountered childhood muscle disease outside of Duchenne muscular dystrophy (Darras et al., 2014). The other gene associated with CCD is *MYH7*, which likely accounts for 10% of CCD cases. *MYH7* are most typically associated with Laing distal myopathy, and the phenotype in *MYH7*-CCD usually resembles features of this condition (slowly progressive, distal predominant weakness) (Naddaf and Waclawik, 2015). That said, *MYH7* mutations are increasingly identified in a broadening range of clinical situations, including axial myopathy resembling some *SEPN1/RYR1* patients, as well as hyaline body myopathy (Bohlega et al., 2004; Cullup et al., 2012). Mutations in other genes, particularly *ACTA1, KBTBD13, CCDC78,* and *TTN*, can result in cores, though in these cases the

"dominant" pathologic findings are often another subtype (nemaline rods for *ACTA1*, for example) (Laing et al., 2009; Chauveau et al., 2014a).

The spectrum of clinical syndromes and histopathologic subtypes for *RYR1* mutations is extremely broad (Bharucha-Goebel et al., 2013). Some data concerning genotype–phenotype correlations exist, though additional study is needed to make sense of this expanding field. It has been well documented that hyperactivating mutations in the N-terminus of the gene product are associated with malignant hyperthermia susceptibility (MHS), a pharmacogenetic condition of hypermetabolic reaction to volatile anesthetic exposure (Robinson et al., 2006). There are additional dynamic syndromes associated with *RYR1* mutations, including exertional rhabdomyolysis and heat illness/heat stroke (Capacchione and Muldoon, 2009; Dalmini et al., 2013). No obvious genotypic correlation exists for these conditions as of yet, and the extent of overlap with MHS-associated mutations is not clear. Mutations in the C-terminus are enriched in patients with CCD (Treves et al., 2008). These also can be associated with MHS. Recessive mutations are found throughout the extent of the gene. The specific histopathologic pattern caused by a given recessive mutation is hard to predict. Minicore myopathy cases are enriched for two missense mutations, while CNM cases tend to have one missense and one nonsense mutation. Of note, reduced levels of RyR1 protein, either documented by Western blot or inferred by mutation composition, are associated with a more severe clinical phenotype (Amburgey et al., 2013).

Disease pathomechanism(s)

SEPN1 encodes a member of the selenoprotein family called selenoprotein N1. Its function appears to be associated with regulating oxidative stress. Myotubes cultured from *SEPN1* patients have high levels of basal oxidative activity and sensitivity to oxidant exposure (Arbogast et al., 2009). In skeletal muscle, *SEPN1* is expressed at the sarcoplasmic reticulum, and some data support a role for it modulating EC coupling, either by a modulation of the Ca^{2+} reuptake through SERCA channels (Marino et al., 2015) or through a secondary alteration in Ryr1 (Arbogast and Ferreiro, 2010). Interestingly, *SEPN1* is expressed primarily in developing muscle (Castets et al., 2009); it therefore remains somewhat of a mystery how it exerts its effect on mature muscle and why it causes muscle disease outside of the neonatal period.

As already mentioned, RyR1 is a calcium channel located in the sarcoplasmic reticulum that is responsible for calcium release during the process of EC coupling. The primary pathomechanism related to *RYR1* mutation is alteration of regulated calcium release (Treves et al., 2008). In mutations associated with malignant hyperthermia, there is a hyperactive calcium release response (Robinson et al., 2006). In mutations associated with muscle weakness, be it in core myopathy or in other histopathologic settings, the overarching concept is one of reduced calcium release during EC coupling (Zhou et al., 2013). This can be the result of impaired RyR1–DHPR interactions, impaired RyR1 expression and stability, impaired RyR1 calcium release due to mutations in the channel pore, as well as other mechanisms (Zhou et al., 2013). Some mutations also affect calcium homeostasis on a more chronic level, producing a "leaky" RyR1 that has impaired opening and closing properties that chronically depletes sarcoplasmic reticulum calcium and diminishes its release (Avila and Dirksen, 2001). A relatively unexplored area related to *RYR1* mutations is potential effect on pathways other than EC coupling. Loss of RyR1 function has been associated with aberrant oxidative stress, suggesting that RyR1 participates in its regulation, perhaps through influencing calcium homeostasis (Dowling et al., 2012a).

Therapeutic considerations

Currently, there are no specific treatments for core myopathies and there has been a lack of rigorous controlled clinical trials for the few small molecules reported to have some benefit in these mutations. Dantrolene, a muscle relaxant and the only specific available effective drug to treat malignant hyperthermia, has been anecdotally reported in an individual case of CCD to improve endurance and muscle strength (Jungbluth et al., 2012). However, there are also descriptions that its administration produces an increase in muscle weakness. Therefore its true clinical value is still elusive (Dowling et al., 2014). Salbutamol has been studied in a small case series of patients with *RYR1* mutations, and shown to have potential benefit (Messina et al., 2004). The mechanism of action in this setting is unclear, and additional systematic study is clearly required to establish efficacy.

As mentioned above, there is evidence of increased oxidative cellular stress in models of both *SEPN1* and *RYR1* myopathies. In these models, the administration of *N*-acetylcysteine improves elements of the phenotype by rebalancing the redox state (Dowling et al., 2012a; Moulin and Ferreiro, 2017). Based on these data, *N*-acetylcysteine is now being considered as a potential therapeutic in both conditions. In fact, clinical trials are under way in France (for *SEPN1* myopathies) and in the United States (for RYR1 myopathies) to test its potential efficacy(Moulin and Ferreiro, 2017).

As the most obvious problem in core myopathies is impaired EC coupling, drugs that improve this process would be of great clinical interest. One class of drugs that may address this are called Rycals. Rycals stabilize the interaction of FKBP12 with RyR1 and augment/enhance its ability to release calcium. Rycals are being tested in heart failure (where secondary RyR2 dysfunction has been implicated) (Andersson and Marks, 2010; Marks, 2013) but have yet to be evaluated in RYR1 myopathy patients or models.

Lastly, gene-directed therapies may represent a way to address some of the *RYR1*-related myopathies. One example of this, provided by Rendu et al. (2013), was with cells from an individual with compound heterozygous mutation in *RYR1*. The researchers applied an exon-skipping strategy to remove a pseudo-exon formed by one of the mutations, with the result being restoration of RyR1 expression and functional calcium release (Rendu et al., 2013). This strategy is especially useful in dominant inherited disorders, and thus applicable to RYR1-related CCD and DNM2-related CNM. Loy et al. (2012) used this technique in two malignant hyperthermia and CCD mouse models with dominant *RYR1* mutations to selectively knock down the mutant allele, achieving a partial rescue in both.

OTHER CONSIDERATIONS

Additional myopathy subtypes

CFTD is often considered the fourth major histopathologic subtype of congenital myopathy. It is defined by the presence on muscle biopsy of type I fibers that are 50% smaller than type II fibers, usually in the setting of type I fiber predominance (Clarke, 2011). It is not clear if CFTD is truly its only entity, or instead an early general pathologic feature that precedes the development of more specific features such as rods, cores, or central nuclei. At present, the major known genetic causes of CFTD (*SEPN1*, *RYR1*, *TPM3*) are more commonly associated with other histologic pathologies.

Of note, a new congenital myopathy with nonspecific features was recently described associated with recessive mutations in *SCN4A*. Clinically, most patients present with elongated/myopathic facies, high arched palate, and generalized extremity weakness. Muscle biopsy findings are nonspecific and primarily show fiber size variation, and muscle magnetic resonance imaging (MRI) shows a characteristic pattern of muscle involvement (Zaharieva et al., 2016). Clinical severity ranges from severe (infantile onset with prominent morbidities) to mild. Based on the early reports and on our anecdotal experience, *SCN4A*-related myopathy is likely to be a relatively commonly encountered myopathy subtype. Of note, dominant mutations in *SCN4A* are well described to cause phenotypes such as myotonia congenita and periodic paralysis. Such mutations are associated with gain of function of the $Na_v1.4$ sodium channel, producing a sustained muscle contraction or a prolonged refractory state (Simkin and Bendahhou, 2011). The recessive mutations seen in *SCN4A* myopathy patients are thought to result in loss of protein function (Zhararieva et al., 2016; Gonorazky et al., 2017).

Another emerging subtype of congenital myopathy is tubular aggregate myopathy. This rare myopathy has primarily been observed in adults as a slowly progressive myopathy with prominent muscle cramps. Dominant mutations have been identified in *STIM1* and *ORAI1*, two components of the store-operated calcium machinery. Childhood cases with more severe weakness have now been identified, suggesting this disorder is likely to have a broader disease spectrum than first suspected (Bohm et al., 2014b; Endo et al., 2015). *STIM1* recessive mutations are associated with immune deficiency. Since none of the patients with tubular aggregate myopathy had evidence of immune dysfunction, it is considered that these mutations have a different tissue-specific impact (Bohm et al., 2014b).

Some additional genetic causes of congenital myopathy are not linked to a known histopathologic subtype. For example, *STAC3* mutations have been uncovered in a rare congenital myopathy called Native American myopathy (NAM). NAM is characterized by mild muscle weakness, an unusual gait, facial dysmorphisms, and susceptibility to malignant hyperthermia. A published *STAC3* mutation in NAM so far has only been described in Lumbee Native Americans (Horstick et al., 2013). However, it is likely that non-Lumbee will also be found with *STAC3* mutation.

Another example is mutation in *MEGF10*. Mutations in *MEGF10* have been described in an early-onset myopathy with respiratory failure, scoliosis, and joint contractures. The histopathology is largely nonspecific, with both myopathic and dystrophic changes, though minicores have been seen in some instances (Logan et al., 2011).

Finally, recessive missense mutations in *PYROXD1* have been described to be causative of an early-onset myopathy, slowly progressive with facial involvement, nasal speech, and swallowing difficulties. The muscle biopsies have features from multiple histopathologic subtypes: cores, central nuclei, rods, and myofibrillar disarrangement (O'Grady et al., 2016).

Variants of unknown significance

One of the emerging challenges in the diagnostic evaluation of patients with congenital myopathies is the frequent detection of VUS. This is particularly common

in large genes such as *TTN*, *RYR1*, and *NEB* (North et al., 2014). In some cases the clinical and histopathologic context provides sufficient ancillary data to support causality for a VUS. An example would be an *RYR1* VUS found in a patient with axial weakness, ophthalmoparesis, and cores on biopsy and a second pathogenic allele. Segregation analysis of VUS in families is of great importance. However, many times the situation is not straightforward, and supportive proof is lacking. Additional studies can provide some help. Muscle imaging such as by MRI is a useful diagnostic adjunct for congenital myopathies (Quijano-Roy et al., 2011). Given that distinct and reproducible patterns of MRI change can be observed associated with certain gene mutations, MRI can help support the pathogenicity of a rare variant. Other options for further analysis include RNA and protein studies on biopsy material (to assess for transcript or expression changes) and/or similar investigations using patient-derived cell lines. Unfortunately, in many cases the association between a variant and disease cannot be resolved. New assessment techniques (both computational and experimental) are clearly needed to help solve this dilemma.

New gene discovery

The "genomics" revolution has obviously impacted the genetic understanding of congenital myopathies. More than 32 genetic causes have now been identified, and these likely represent about two-thirds of the total genetic burden of disease for these disorders (Kaplan and Hamroun, 2015). The development of comprehensive, "next-gen"-based gene panels has revolutionized clinical diagnostics by providing rapid, affordable, and widely available testing. These panels have largely replaced conventional single-gene analysis by Sanger sequencing. The panels appear to have a hit rate of >50% when used as primary diagnostic tool, corroborating the assertion that the majority of genetic causes have been uncovered. However, they also reflect that mutations remain to be identified in many individuals. Mutations in the exomic sequence of additional (likely rare) genes are likely to account for some percentage of these cases. Such causes will be optimally uncovered by combining individual cohorts of unsolved cases; this strategy was used effectively to identify mutations in *LMOD3* and *KLHL41* in NM (Gupta et al., 2013; Yuen et al., 2014). In addition, a significant proportion of the remaining causes are likely to represent nonexomic mutations, be they regulatory, splicing, or deep intronic variants (Cummings et al., 2017). Successful identification and interpretation of these variants represent some of the next great challenges in gene discovery.

Disease nomenclature: histopathology versus gene mutation

Historically, congenital myopathies have been defined and distinguished based on histopathology (Dubowitz et al., 2013). This construction is increasingly being challenged as the genetic basis of disease is understood in the majority of cases. This is best demonstrated by considering the cohort of individuals with *RYR1* mutations. *RYR1* mutations have now been described in all histopathologic subtypes and in a broad range of distinctive clinical symptomatology. Therefore, it is likely more accurate (and more instructive) to consider patients with *RYR1* mutations together in a categorization of *RYR1*-related myopathies. Such a reclassification is particularly important in this setting, as it informs about clinical symptomatology and prognosis, and aids in rationale of therapy development and clinical trial design.

Gene-based classification makes sense for several congenital myopathy subtypes in addition to *RYR1*. These include *SEPN1*, where a range of histopathologic changes belies a consistent and unique clinical pattern, *MYH7* (where there is a range of both clinical presentations and histopathology), and *TTN* (largely because of the lack of cohesive histopathology). However, it is likely too soon to consider shifting entirely to a gene-based classification system. This is best understood when considering NM. At present, this grouping still provides valuable clinical information, as patients with NM share many features regardless of specific genetic cause, and may additionally benefit from a shared set of therapeutic strategies (given the shared pathomechanism of impaired thin filament function). This may change as more cases are identified with each individual genetic subtype of NM. In all, a system that incorporates genetics, histopathology, and clinical phenotypes, with an emphasis on one or more of these items depending on the context, is likely to be the most parsimonious and clinically useful.

Therapy development

There is obviously a great need for the identification and testing of new therapeutic strategies for congenital myopathies. In total, while case study data support potential modest efficacy for a few drug targets (such as salbutamol and pyridostigmine), these disorders currently have no rigorously validated treatments. There are clear barriers to therapy development for these disorders. Perhaps the biggest is a lack of subtype-specific natural history data. Several efforts are under way to rectify this, with multinational collaborative efforts representing an important advance for overcoming the relative rarity of congenital myopathies. Another barrier is the lack of

preclinical models for many of the important congenital myopathy subtypes. This is also being addressed, as not only mouse models but also novel animal and cell-based modeling strategies are being developed.

CONCLUDING REMARKS

The genetic basis for congenital myopathies is rapidly being solved, with mutations identified in the majority of cases. This has led to increased understanding of disease pathogenesis and has broadened genotype–phenotype understanding and improved clinical care. Unfortunately, this knowledge has yet to lead to therapies for these rare but devastating disorders. It has also led to new complexities, such as how to evaluate and interpret VUS and how best to classify and characterize these disorders. Future studies will be aimed at additional gene discovery, more sophisticated genotype–phenotype correlation, and, most importantly, development of new therapeutic strategies.

References

Ackermann MA, Ziman AP, Strong J et al. (2011). Integrity of the network sarcoplasmic reticulum in skeletal muscle requires small ankyrin 1. J Cell Sci 124: 3619–3630.

Agrawal PB, Greenleaf RS, Tomczak KK et al. (2007). Nemaline myopathy with minicores caused by mutation of the CFL2 gene encoding the skeletal muscle actin-binding protein, cofilin-2. Am J Hum Genet 80: 162–167.

Agrawal PB, Pierson CR, Joshi M et al. (2014). SPEG interacts with myotubularin, and its deficiency causes centronuclear myopathy with dilated cardiomyopathy. Am J Hum Genet 95: 218–226.

Al-Qusairi L, Weiss N, Toussaint A et al. (2009). T-tubule disorganization and defective excitation-contraction coupling in muscle fibers lacking myotubularin lipid phosphatase. Proc Natl Acad Sci U S A 106: 18763–18768.

Amburgey K, McNamara N, Bennett LR et al. (2011). Prevalence of congenital myopathies in a representative pediatric United States population. Ann Neurol 70: 662–665.

Amburgey K, Bailey A, Hwang JH et al. (2013). Genotype-phenotype correlations in recessive RYR1-related myopathies. Orphanet J Rare Dis 8: 117.

Amburgey K, Tsuchiya E, de Chastonay S et al. (2017). A natural history study of X-linked myotubular myopathy. Neurology 89: 1355–1364.

Andersson DC, Marks AR (2010). Fixing ryanodine receptor Ca leak – a novel therapeutic strategy for contractile failure in heart and skeletal muscle. Drug Discov Today Dis Mech 7: e151–e157.

Arbogast S, Ferreiro A (2010). Selenoproteins and protection against oxidative stress: selenoprotein N as a novel player at the crossroads of redox signaling and calcium homeostasis. Antioxid Redox Signal 12: 893–904.

Arbogast S, Beuvin M, Fraysse B et al. (2009). Oxidative stress in SEPN1-related myopathy: from pathophysiology to treatment. Ann Neurol 65: 677–686.

Ardissone A, Bragato C, Blasevich F et al. (2016). SEPN1-related myopathy in three patients: novel mutations and diagnostic clues. Eur J Pediatr 175: 1113–1118.

Avila G, Dirksen RT (2001). Functional effects of central core disease mutations in the cytoplasmic region of the skeletal muscle ryanodine receptor. J Gen Physiol 118: 277–290.

Bertini E, D'Amico A, Gualandi F et al. (2011). Congenital muscular dystrophies: a brief review. Semin Pediatr Neurol 18: 277–288.

Bevilacqua JA, Bitoun M, Biancalana V et al. (2009). "Necklace" fibers, a new histological marker of late-onset MTM1-related centronuclear myopathy. Acta Neuropathol 117: 283–291.

Bharucha-Goebel DX, Santi M, Medne L et al. (2013). Severe congenital RYR1-associated myopathy: the expanding clinicopathologic and genetic spectrum. Neurology 80: 1584–1589.

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

Bohlega S, Abu-Amero SN, Wakil SM et al. (2004). Mutation of the slow myosin heavy chain rod domain underlies hyaline body myopathy. Neurology 62: 1518–1521.

Bohm J, Biancalana V, Dechene ET et al. (2012). Mutation spectrum in the large GTPase dynamin 2, and genotype-phenotype correlation in autosomal dominant centronuclear myopathy. Hum Mutat 33: 949–959.

Bohm J, Biancalana V, Malfatti E et al. (2014a). Adult-onset autosomal dominant centronuclear myopathy due to BIN1 mutations. Brain 137: 3160–3170.

Bohm J, Chevessier F, Koch C et al. (2014b). Clinical, histological and genetic characterisation of patients with tubular aggregate myopathy caused by mutations in STIM1. J Med Genet 51: 824–833.

Bonnemann CG, Wang CH, Quijano-Roy S et al. (2014). Diagnostic approach to the congenital muscular dystrophies. Neuromuscul Disord 24: 289–311.

Capacchione JF, Muldoon SM (2009). The relationship between exertional heat illness, exertional rhabdomyolysis, and malignant hyperthermia. Anesth Analg 109: 1065–1069.

Carmignac V, Salih MA, Quijano-Roy S et al. (2007). C-terminal titin deletions cause a novel early-onset myopathy with fatal cardiomyopathy. Ann Neurol 61: 340–351.

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

Ceyhan-Birsoy O, Agrawal PB, Hidalgo C et al. (2013). Recessive truncating titin gene, TTN, mutations presenting as centronuclear myopathy. Neurology 81: 1205–1214.

Chauveau C, Bonnemann CG, Julien C et al. (2014a). Recessive TTN truncating mutations define novel forms of core myopathy with heart disease. Hum Mol Genet 23: 980–991.

Chauveau C, Rowell J, Ferreiro A (2014b). A rising titan: TTN review and mutation update. Hum Mutat 35: 1046–1059.

Childers MK, Joubert R, Poulard K et al. (2014). Gene therapy prolongs survival and restores function in murine and canine models of myotubular myopathy. Sci Transl Med 6: 220ra210.

Clarke NF (2011). Congenital fiber-type disproportion. Semin Pediatr Neurol 18: 264–271.

Colombo I, Scoto M, Manzur AY et al. (2015). Congenital myopathies: natural history of a large pediatric cohort. Neurology 84: 28–35.

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

Cullup T, Lamont PJ, Cirak S et al. (2012). Mutations in MYH7 cause multi-minicore disease (MmD) with variable cardiac involvement. Neuromuscul Disord 22: 1096–1104.

Cummings BB, Marshall JL, Tukiainen T et al. (2017). Improving genetic diagnosis in Mendelian disease with transcriptome sequencing. Sci Transl Med 9.

D'Amico A, Graziano C, Pacileo G et al. (2006). Fatal hypertrophic cardiomyopathy and nemaline myopathy associated with ACTA1 K336E mutation. Neuromuscul Disord 16: 548–552.

Dalmini N, Voermans NC, Lillis S et al. (2013). Mutations in RYR1 are a common cause of exertional myalgia and rhabdomyolysis. Neuromuscular disorders: NMD 23: 540–548.

Darin N, Tulinius M (2000). Neuromuscular disorders in childhood: a descriptive epidemiological study from western Sweden. Neuromuscul Disord 10: 1–9.

Darras BT, Royden Jones JJ, Ryan MM et al. (2014). Neuromuscular disorders of infancy, childhood, and adolescence: a clinician's approach, Elsevier Science, London.

Das S, Dowling J, Pierson CR (2011). X-linked centronuclear myopathy. In: RA Pagon, MP Adam, HH Ardinger et al. (Eds.), GeneReviews. University of Washington, Seattle, WA.

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

De Paula AM, Franques J, Fernandez C et al. (2009). A TPM3 mutation causing cap myopathy. Neuromuscul Disord 19: 685–688.

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

Dowling JJ, Vreede AP, Low SE et al. (2009). Loss of myotubularin function results in T-tubule disorganization in zebrafish and human myotubular myopathy. PLoS Genet 5: e1000372.

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

Dowling JJ, Joubert R, Low SE et al. (2012b). Myotubular myopathy and the neuromuscular junction: a novel therapeutic approach from mouse models. Dis Model Mech 5: 852–859.

Dowling JJ, Lawlor MW, Dirksen RT (2014). Triadopathies: an emerging class of skeletal muscle diseases. Neurotherapeutics 11: 773–785.

Dowling JJ, H DG, Cohn RD et al. (2017). Treating pediatric neuromuscular disorders: the future is now. Am J Med Genet A.

Dubowitz V, Sewry CA, Oldfors A et al. (2013). Muscle biopsy: a practical approach, expert consult; online and print, 4: muscle biopsy: a practical approach. Philadelphia: Elsevier - Health Sciences Division.

Endo Y, Noguchi S, Hara Y et al. (2015). Dominant mutations in ORAI1 cause tubular aggregate myopathy with hypocalcemia via constitutive activation of store-operated Ca2+ channels. Hum Mol Genet 24: 637–648.

Falcone S, Roman W, Hnia K et al. (2014). N-WASP is required for Amphiphysin-2/BIN1-dependent nuclear positioning and triad organization in skeletal muscle and is involved in the pathophysiology of centronuclear myopathy. EMBO Mol Med 6: 1455–1475.

Feng JJ, Marston S (2009). Genotype-phenotype correlations in ACTA1 mutations that cause congenital myopathies. Neuromuscul Disord 19: 6–16.

Ferreiro A, Ceuterick-de Groote C, Marks JJ et al. (2004). Desmin-related myopathy with Mallory body-like inclusions is caused by mutations of the selenoprotein N gene. Ann Neurol 55: 676–686.

Garg A, O'Rourke J, Long C et al. (2014). KLHL40 deficiency destabilizes thin filament proteins and promotes nemaline myopathy. J Clin Invest 124: 3529–3539.

Gibbs EM, Clarke NF, Rose K et al. (2013). Neuromuscular junction abnormalities in DNM2-related centronuclear myopathy. J Mol Med (Berl) 91: 727–737.

Gibbs EM, Davidson AE, Telfer WR et al. (2014). The myopathy-causing mutation DNM2-S619L leads to defective tubulation in vitro and in developing zebrafish. Dis Model Mech 7: 157–161.

Gonorazky HD, Marshall CR, Al-Murshed M et al. (2017). Congenital myopathy with "corona" fibres, selective muscle atrophy, and craniosynostosis associated with novel recessive mutations in SCN4A. Neuromuscul Disord 27: 574–580.

Gupta VA, Beggs AH (2014). Kelch proteins: emerging roles in skeletal muscle development and diseases. Skelet Muscle 4: 11.

Gupta VA, Ravenscroft G, Shaheen R et al. (2013). Identification of KLHL41 mutations implicates BTB-Kelch-mediated ubiquitination as an alternate pathway to myofibrillar disruption in nemaline myopathy. Am J Hum Genet 93: 1108–1117.

Hackman JP, Vihola AK, Udd AB (2003). The role of titin in muscular disorders. Ann Med 35: 434–441.

Hedberg C, Melberg A, Dahlbom K et al. (2014). Hereditary myopathy with early respiratory failure is caused by mutations in the titin FN3 119 domain. Brain 137: e270.

Horstick EJ, Linsley JW, Dowling JJ et al. (2013). Stac3 is a component of the excitation-contraction coupling machinery and mutated in Native American myopathy. Nat Commun 4: 1952.

Hughes MI, Hicks EM, Nevin NC et al. (1996). The prevalence of inherited neuromuscular disease in Northern Ireland. Neuromuscul Disord 6: 69–73.

Hung RM, Yoon G, Hawkins CE et al. (2010). Cap myopathy caused by a mutation of the skeletal alpha-actin gene ACTA1. Neuromuscul Disord 20: 238–240.

Jain RK, Jayawant S, Squier W et al. (2012). Nemaline myopathy with stiffness and hypertonia associated with an ACTA1 mutation. Neurology 78: 1100–1103.

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

Jungbluth H, Sewry CA, Muntoni F (2011). Core myopathies. Semin Pediatr Neurol 18: 239–249.

Jungbluth H, Dowling JJ, Ferreiro A et al. (2012). 182nd ENMC International Workshop: RYR1-related myopathies, 15-17th April 2011, Naarden, The Netherlands. Neuromuscul Disord 22: 453–462.

Kaplan JC, Hamroun D (2014). The 2015 version of the gene table of monogenic neuromuscular disorders (nuclear genome). Neuromuscul Disord 24: 1123–1153.

Kaplan JC, Hamroun D (2015). The 2016 version of the gene table of monogenic neuromuscular disorders (nuclear genome). Neuromuscul Disord 25: 991–1020.

Krakowiak PA, O'Quinn JR, Bohnsack JF et al. (1997). A variant of Freeman-Sheldon syndrome maps to 11p15.5-pter. Am J Hum Genet 60: 426–432.

Laing NG, Dye DE, Wallgren-Pettersson C et al. (2009). Mutations and polymorphisms of the skeletal muscle alpha-actin gene (ACTA1). Hum Mutat 30: 1267–1277.

Lange S, Ouyang K, Meyer G et al. (2009). Obscurin determines the architecture of the longitudinal sarcoplasmic reticulum. J Cell Sci 122: 2640–2650.

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

Lawlor MW, Armstrong D, Viola MG et al. (2013). Enzyme replacement therapy rescues weakness and improves muscle pathology in mice with X-linked myotubular myopathy. Hum Mol Genet 22: 1525–1538.

Lee EJ, De Winter JM, Buck D et al. (2013). Fast skeletal muscle troponin activation increases force of mouse fast skeletal muscle and ameliorates weakness due to nebulin-deficiency. PLoS One 8: e55861.

Lehtokari VL, Kiiski K, Sandaradura SA et al. (2014). Mutation update: the spectra of nebulin variants and associated myopathies. Hum Mutat 35: 1418–1426.

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

Lornage X, Malfatti E, Cheraud C et al. (2017). Recessive MYPN mutations cause cap myopathy with occasional nemaline rods. Ann Neurol 81: 467–473.

Loy RE, Lueck JD, Mostajo-Radji MA et al. (2012). Allele-specific gene silencing in two mouse models of autosomal dominant skeletal myopathy. PLoS One 7: e49757.

Mack DL, Poulard K, Goddard MA et al. (2017). Systemic AAV8-mediated gene therapy drives whole-body correction of myotubular myopathy in dogs. Mol Ther 25: 839–854.

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

Majczenko K, Davidson AE, Camelo-Piragua S et al. (2012). Dominant mutation of CCDC78 in a unique congenital myopathy with prominent internal nuclei and atypical cores. Am J Hum Genet 91: 365–371.

Marino M, Stoilova T, Giorgi C et al. (2015). SEPN1, an endoplasmic reticulum-localized selenoprotein linked to skeletal muscle pathology, counteracts hyperoxidation by means of redox-regulating SERCA2 pump activity. Hum Mol Genet 24: 1843–1855.

Marks AR (2013). Calcium cycling proteins and heart failure: mechanisms and therapeutics. J Clin Invest 123: 46–52.

Marttila M, Hanif M, Lemola E et al. (2014). Nebulin interactions with actin and tropomyosin are altered by disease-causing mutations. Skelet Muscle 4: 15.

Marttila M, Lehtokari VL, Marston S et al. (2014). Mutation update and genotype-phenotype correlations of novel and previously described mutations in TPM2 and TPM3 causing congenital myopathies. Hum Mutat 35: 779–790.

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

Miyatake S, Mitsuhashi S, Hayashi YK et al. (2017). Biallelic mutations in MYPN, encoding myopalladin, are associated with childhood-onset, slowly progressive nemaline myopathy. Am J Hum Genet 100: 169–178.

Mokbel N, Ilkovski B, Kreissl M et al. (2013). K7del is a common TPM2 gene mutation associated with nemaline myopathy and raised myofibre calcium sensitivity. Brain 136: 494–507.

Moulin M, Ferreiro A (2017). Muscle redox disturbances and oxidative stress as pathomechanisms and therapeutic targets in early-onset myopathies. Semin Cell Dev Biol 64: 213–223.

Naddaf E, Waclawik AJ (2015). Two families with MYH7 distal myopathy associated with cardiomyopathy and core formations. J Clin Neuromuscul Dis 16: 164–169.

Nance JR, Dowling JJ, Gibbs EM et al. (2012). Congenital myopathies: an update. Curr Neurol Neurosci Rep 12: 165–174.

Nguyen MA, Joya JE, Kee AJ et al. (2011). Hypertrophy and dietary tyrosine ameliorate the phenotypes of a mouse model of severe nemaline myopathy. Brain 134: 3516–3529.

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

North KN, Wang CH, Clarke N et al. (2014). Approach to the diagnosis of congenital myopathies. Neuromuscul Disord 24: 97–116.

Nowak KJ, Sewry CA, Navarro C et al. (2007). Nemaline myopathy caused by absence of alpha-skeletal muscle actin. Ann Neurol 61: 175–184.

Nworu CU, Kraft R, Schnurr DC et al. (2015). Leiomodin 3 and tropomodulin 4 have overlapping functions during skeletal myofibrillogenesis. J Cell Sci 128: 239–250.

Ockeloen CW, Gilhuis HJ, Pfundt R et al. (2012). Congenital myopathy caused by a novel missense mutation in the CFL2 gene. Neuromuscul Disord 22: 632–639.

O'Grady GL, Best HA, Sztal TE et al. (2016). Variants in the oxidoreductase PYROXD1 cause early-onset myopathy with internalized nuclei and myofibrillar disorganization. Am J Hum Genet 99: 1086–1105.

Ong RW, AlSaman A, Selcen D et al. (2014). Novel cofilin-2 (CFL2) four base pair deletion causing nemaline myopathy. J Neurol Neurosurg Psychiatry 85: 1058–1060.

Pappas CT, Krieg PA, Gregorio CC (2010). Nebulin regulates actin filament lengths by a stabilization mechanism. J Cell Biol 189: 859–870.

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

Quijano-Roy S, Carlier RY, Fischer D (2011). Muscle imaging in congenital myopathies. Semin Pediatr Neurol 18: 221–229.

Randazzo D, Giacomello E, Lorenzini S et al. (2013). Obscurin is required for ankyrinB-dependent dystrophin localization and sarcolemma integrity. J Cell Biol 200: 523–536.

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

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

Ravenscroft G, Miyatake S, Lehtokari VL et al. (2013b). Mutations in KLHL40 are a frequent cause of severe autosomal-recessive nemaline myopathy. Am J Hum Genet 93: 6–18.

Ravenscroft G, Laing NG, Bonnemann CG (2015). Pathophysiological concepts in the congenital myopathies: blurring the boundaries, sharpening the focus. Brain 138: 246–268.

Rendu J, Brocard J, Denarier E et al. (2013). Exon skipping as a therapeutic strategy applied to an RYR1 mutation with pseudo-exon inclusion causing a severe core myopathy. Hum Gene Ther 24: 702–713.

Robb SA, Sewry CA, Dowling JJ et al. (2011). Impaired neuromuscular transmission and response to acetylcholinesterase inhibitors in centronuclear myopathies. Neuromuscul Disord 21: 379–386.

Robinson R, Carpenter D, Shaw MA et al. (2006). Mutations in RYR1 in malignant hyperthermia and central core disease. Hum Mutat 27: 977–989.

Romero NB (2010). Centronuclear myopathies: a widening concept. Neuromuscul Disord 20: 223–228.

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

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

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

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

Savarese M, Musumeci O, Giugliano T et al. (2016). Novel findings associated with MTM1 suggest a higher number of female symptomatic carriers. Neuromuscul Disord 26: 292–299.

Schara U, Kress W, Bonnemann CG et al. (2008). The phenotype and long-term follow-up in 11 patients with juvenile selenoprotein N1-related myopathy. Eur J Paediatr Neurol 12: 224–230.

Scoto M, Cirak S, Mein R et al. (2011). SEPN1-related myopathies: clinical course in a large cohort of patients. Neurology 76: 2073–2078.

Simkin D, Bendahhou S (2011). Skeletal muscle Na channel disorders. Front Pharmacol 2: 63.

Tajsharghi H, Ohlsson M, Lindberg C et al. (2007). Congenital myopathy with nemaline rods and cap structures caused by a mutation in the beta-tropomyosin gene (TPM2). Arch Neurol 64: 1334–1338.

Tasfaout H, Buono S, Guo S et al. (2017). Antisense oligonucleotide-mediated Dnm2 knockdown prevents and reverts myotubular myopathy in mice. Nat Commun 8: 15661.

Tosch V, Rohde HM, Tronchere H et al. (2006). A novel PtdIns3P and PtdIns(3,5)P2 phosphatase with an inactivating variant in centronuclear myopathy. Hum Mol Genet 15: 3098–3106.

Treves S, Jungbluth H, Muntoni F et al. (2008). Congenital muscle disorders with cores: the ryanodine receptor calcium channel paradigm. Curr Opin Pharmacol 8: 319–326.

Udd B, Haravuori H, Kalimo H et al. (1998). Tibial muscular dystrophy – from clinical description to linkage on chromosome 2q31. Neuromuscul Disord 8: 327–332.

Wallgren-Pettersson C, Sewry CA, Nowak KJ et al. (2011). Nemaline myopathies. Semin Pediatr Neurol 18: 230–238.

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

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

Zaharieva IT, Thor MG, Oates EC et al. (2016). Loss-of-function mutations in SCN4A cause severe foetal hypokinesia or 'classical' congenital myopathy. Brain 139: 674–691.

Zhou H, Jungbluth H, Sewry CA et al. (2007). Molecular mechanisms and phenotypic variation in RYR1-related congenital myopathies. Brain 130: 2024–2036.

Zhou H, Rokach O, Feng L et al. (2013). RyR1 deficiency in congenital myopathies disrupts excitation-contraction coupling. Hum Mutat 34: 986–996.