Myopathology of Congenital Myopathies: Bridging the Old and the New

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Congenital myopathies (CMs) are a genetically heterogeneous group of neuromuscular disorders most commonly presenting with neonatal/childhood-onset hypotonia and muscle weakness, a relatively static or slowly progressive disease course, and originally classified into subcategories based on characteristic histopathologic findings in muscle biopsies. This enduring concept of disease definition and classification based on the clinicopathologic phenotype was pioneered in the premolecular era. Advances in molecular genetics have brought into focus the increased blurring of the original seemingly “watertight” categories through broadening of the clinical phenotypes in existing genes, and continuous identification of novel genetic backgrounds. This review summarizes the histopathologic landscape of the 4 “classical” subtypes of CM—nemaline myopathies, core myopathies, centronuclear myopathies, and congenital fiber type disproportion and some of the emerging and novel genetic diseases with a CM presentation.

Introduction

The concept of congenital myopathies (CMs) as a group of neuromuscular diseases based on a shared clinical picture of early-onset hypotonia and weakness, a relatively static or slowly progressive course and a classification based on distinctive histopathologic features into 4 subtypes—nemaline myopathies, core myopathies, centronuclear myopathies (CNMs), and congenital fiber type disproportion (CFTD) was pioneered in the premolecular era.[1-5] Advances in molecular genetics and the widespread use of next-generation-sequencing (NGS) have unraveled a far more complex gene-clinical phenotype-pathology relationship in this group of diseases. Increasingly, a switch to gene-based disease classification, that is *ACTA1*-related myopathies, *RYR1*-related myopathies, and so on, has revealed a broadening clinical spectrum ranging from severe and lethal fetal akinesia to milder, even adult presentations of disease.[6] A complex relationship between the genetic defect and associated pathology has emerged–one gene linked to multiple morphologic abnormalities, and multiple genes sharing the same morphologic abnormality.[7] It is also increasingly evident that a particular gene may be associated with a histopathologic spectrum that includes morphologic signatures straddling across previously well-delineated boundaries. Shared pathomechanisms across different genetic backgrounds are now well-recognized, and new mechanistic aspects in CM are being recognized through new gene discovery.[8,9] Notwithstanding these challenges, a pathology-based disease classification that is flexible and accommodative of the increasing gene-phenotype heterogeneity is still relevant, as along with the clinical phenotype and other investigative modalities, such as muscle MRI and neurophysiology, it can help in narrowing down the differential diagnosis, in prioritizing genetic testing, and in the interpretation of sequencing results to determine the pathogenicity of genetic variants.

This review begins with a brief summary of the clinical features and key pathomechanisms in CM. For the sake of simplicity and brevity, the pathology is reviewed under 2 main categories—“classical CM” including nemaline myopathies, core myopathies, CNMs and CFTD, and “emerging and novel CM” reflecting an ever increasing list of new genes linked with a CM presentation, with nonspecific or overlapping histopathologic signatures. In line with current thinking, cap disease, zebra body myopathy, actin accumulation, and rod-core or core-rod myopathy are regarded as variants of nemaline myopathy. Reducing body myopathy and sporadic late-onset nemaline myopathy are excluded from this classification, as their clinical presentations do not conform to a true CM.[7] Spheroid body myopathy is now assigned to myofibrillar myopathies (MFM).[10] It is not clear whether structural defects such as fingerprint bodies, cylindrical spirals, and cytoplasmic bodies are truly associated with distinct genetic entities. The diagnostic pitfalls and differential diagnoses are discussed. Defects in a number of genes (*MYH2, MYH3, MYH8, MYBPC1, ECEL1, TPM2, TPM3, TNNT2, ACTA1, and NEB*) are associated with distal arthrogryposis (DA), with an overlap in many with CM.[11] These genes mainly associated with DA are not discussed separately.

Clinical Features

Most CMs present at birth or in early infancy; a wide variation in clinical severity within each subtype is recognized, ranging from onset in utero with fetal akinesia, neonates with profound generalized weakness to milder

childhood onset cases with delayed motor milestones, and even adult presentations. The overall course is static or slowly progressive. Weakness is often generalized, or more prominent in limb-girdle and proximal limb muscles. There is often prominent facial weakness with or without ptosis, generalized hypotonia with hyporeflexia (frog-leg posturing), and respiratory and bulbar weakness. Rare hypertonic presentations are recognized.[12] Additional features such as prominent axial and/or respiratory weakness, weakness of ankle dorsiflexion/foot drop with pes cavus, ophthalmoplegia, cataracts, craniofacial dysmorphism, orthopedic complications such as early fixed kyphoscoliosis, spinal rigidity, congenital hip dislocation, DA multiplex and club feet, epsiodes of malignant hyperthermia (MH), and/or exertional myalgia with/without rhabdomyolysis can inform specific genotype-phenotype correlations that may help in prioritizing candidate gene testing.[13] Cardiomyopathy is uncommon, and when present independent of respiratory insufficiency, should prompt consideration of defects in *TTN, MYH7,* and rarely *ACTA1, MYPN, MYO18B, and SPEG*.[13-16] Serum CK is usually normal or mildly elevated. Electromyography can be normal or myopathic, and may occasionally show changes that appear even neurogenic in distal muscles or in severely affected neonates.[6] Nerve conduction studies are normal, but helpful in excluding neuropathies/neuronopathies. Specific tests including repetitive nerve stimulation and single fiber electromyography can be important in excluding congenital myasthenic syndromes (CMS), although, secondary abnormalities of the neuromuscular junction are now recognized in some CMs.[17] In neonates and infants, there can be a significant clinical overlap with other neuromuscular disorders, and the differential diagnosis is wide, including the congenital muscular dystrophies (CMD), CMS, metabolic/mitochondrial myopathies, spinal muscular atrophy, and Prader-Willi syndrome, all of whom can have a “floppy infant” presentation.[6]

Pathomechanisms

Cumulative evidence from studies in cell lines, patient tissues, and mutant animal models suggest shared pathomechanisms in CMs with areas of distinction within single gene disorders. These include defects in (1) sarcolemmal and intracellular membrane remodeling and excitation-contraction coupling (ECC), (2) mitochondrial distribution and function, (3) myofibrillar force generation, (4) atrophy and (5) autophagy.[18] The skeletal muscle triad, an essential muscle substructure has a key role in coordinating EC coupling. MH susceptibility mutations in *RYR1* and *CACNA1S* result in triad hypersensitivity to triggering agents and excessive calcium release from RyR1.[19,20] Mutations in *RYR1*-related myopathies result in aberrant EC coupling and diminished muscle force generation, either from reduced RyR1 expression (mostly recessive mutations) or altered SR-calcium release channel function (missense mutations).[21] Triad abnormalities are also implicated in the pathogenesis of CNMs. Structural changes in triads and/or altered EC coupling have been observed in zebrafish and mouse models of myotubular myopathy and in biopsies from patients with *MTM1* mutations. BIN1 is a regulator of T-tubule biogenesis and interacts directly with dynamin-2, which is also involved in membrane trafficking. Mutations in *MTM1, DNM2, BIN1*, and *RYR1*-related myopathies cause structural triad defects and aberrant EC coupling. The precise mechanisms are not understood, but may involve aberrant tubulogenesis and/or membrane trafficking.[22-24] Loss of STAC3, a putative linker or chaperone for DHPR-RyR1 interaction in *STAC3*-mutant mice or zebrafish disrupts EC coupling.[25,26] STIM1 and ORAI1, components of the store-operated calcium release-activated calcium channel and involved in store-operated Ca^{2+} entry, are present at the triad, but distinct from the DHPR-RYR1 interface.[27] Dominant mutations in *STIM1* and *ORAI1*-related tubular aggregate myopathies result in enhanced store-operated Ca^{2+} entry through constitutive activation of STIM1/ORAI1[28]; this mechanism is also implicated in MH pathogenesis.[29] Impaired sarcomeric function and myofibrillar force generation is a key effect of mutations in genes encoding thin filaments and their interacting proteins. Some of the underlying mechanisms include impaired force-generating capacity (nebulin-deficient mice, zebrafish and *NEB*-mutant patient muscle),[30-32] decreased Ca^{2+} sensitivity due to impaired actin-actin interactions (p.Asp286Gly *ACTA1*-mutant transgenic mice; cardiomyocytes expressing p.Met9Arg *TPM3* mutation),[33,34] and changes in cross-bridge cycling kinetics (muscle biopsies from patients with dominant or recessive *TPM3* mutations).

The mechanistic effects of various CM gene defects in causing the characteristic structural histopathologic alterations are not completely understood. Knock-in *RYR1* p.Try522Ser mutant mice develop MH susceptibility, and histologically, step-wise development of contracted sarcomeres and core-like regions secondary to mitochondrial dislocation and triad disruption (structured cores) followed by sarcomeric disintegration (unstructured cores).[35] Muscle biopsies of patients with recessive *RYR1* mutations and nemaline myopathies (*KBTBD13, ACTA1*, and *NEB*) show decreased RyR1 protein, decreased muscle-specific miRNAs, increased DNA methylation, and increased Class II HDAC (histone deacetylases) expression indicating shared epigenetic changes may activate a common pathophysiological pathway in some forms of CM.[36]

General Pathologic Features

There is commonly increased variation in fiber size for the age. Type I/slow fibers are frequently smaller (hypotrophy or atrophy) and randomly dispersed among larger fibers giving rise to the appearance of a “two-fiber population” and fiber size disproportion best appreciated in the oxidative stains. There is frequently predominance or uniformity of type I/slow fibers. Active necrosis and regeneration are not seen. Perimysial fatty infiltration can be striking in some cases. Marked perimysial and/or endomysial fibrosis is usually not seen, but can rarely occur. Inflammation or MHC Class I upregulation is not common. Using an antibody to fetal myosin heavy chain can reveal a population of very small fibers less than 5 µm in diameter that is difficult to discern with haematoxylin and eosin (HE) or oxidative enzyme staining.[11] These small fibers tend to be evenly dispersed across fascicles, and tend to diminish with increasing age. Infrequently, they appear to variably also coexpress fetal, fast and slow myosin isoforms. In biopsies from preterm and neonatal patients, these fibers can be obscured by the widespread developmental presence of fetal myosin across fascicles. Although not specific to CMs, the presence of these fibers within an overall nonspecifically myopathic background described above, even in the absence of specific structural abnormalities, should lead to the consideration of CM in the differential diagnosis. Layered on this myopathic background, 4 major histopathologic categories of CM can be defined, and are described in the subsequent paragraphs. Figure 1 illustrates the pathology and Table summarizes the pathologic spectrum and protein abnormalities associated with specific genes.

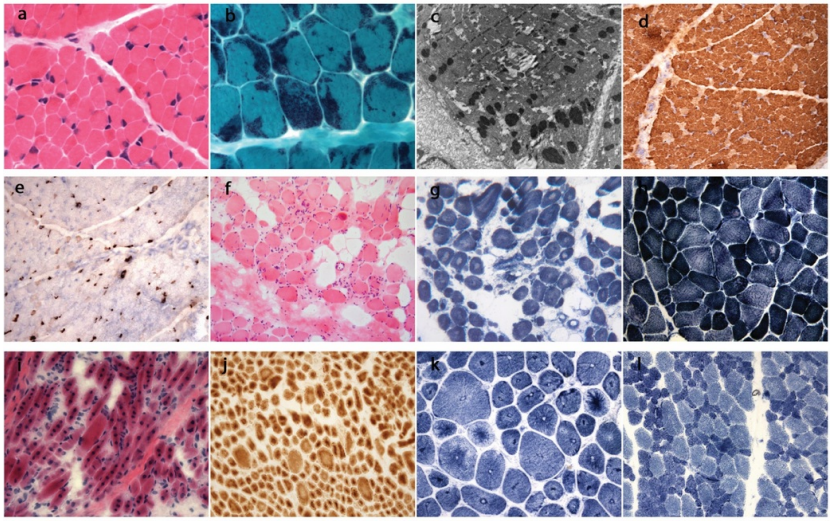

Figure 1 Pathologic features associated with congenital myopathy gene defects. Muscle biopsy from a patient with *ACTA1*-related CM. Sections stained with haematoxylin and eosin show refractile eosinophilic aggregates of nemaline rods (a) that appear as red staining inclusions in the Gomori Trichrome (b) in several fibers. Ultrastructurally the rods are seen as dense osmiophilic bodies, some of which appear to be inserted at the Z-line (c). Immunostaining with slow myosin heavy chain antibody shows marked slow fiber predominance (d), and there are several very small fibers staining with fetal myosin (e). Muscle biopsy from a patient with a dominant *RYR1* mutation. Marked myopathic size variation with increased internal nuclei and fat is seen in the haematoxylin and eosin stained section (f). Prominent central cores with uniformity of fiber typing is seen with NADH-TR staining (g). In contrast, the biopsy from a patient with recessive mutations in *SEPN1* shows preserved 2-fiber pattern and uneven staining/minicores in both fiber types with NADH-TR staining (h). Muscle biopsy from a patient with *MTM1*-related myotubular myopathy. Majority of the fibers show "myotubular" morphology with central nuclei that are spaced out along the length of the fiber with haematoxylin and eosin staining (i). There is central aggregation of oxidative staining and peripheral haloes, along with occasional "necklace" fibers with COX staining (j). Muscle biopsy from a patient with *DNM2*-related centronuclear myopathy. Fibers with characteristic radial sarcoplasmic strands are seen with NADH-TR staining (k). Muscle biopsy from a patient with *TPM3*-related congenital myopathy featuring fiber type disproportion, with small darkly stained type I fibers and larger lightly stained type II fibers with NADH-TR staining (l). (Color version of figure is available online.)

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Table Spectrum of Pathologic Features and Protein Abnormalities Associated With Specific Congenital Myopathy Genes

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Gene	Protein	Inheritance	Pathologic Spectrum	Protein Abnormality IHC/IB†
Nemaline myopathies				
ACTA1	Filamentous α-actin	AD‡, AR^0	Rods in both fibre types; intranuclear rods; actin filament aggregates; caps/cap-like structures; zebra bodies; rods and cores	Rare homozygous null ACTA1 mutations show complete absence of filamentous α-actin and uniform increased expression cardiac actin
NEB	Nebulin	AR	Rods in both fiber types; rare cases show cap/cap-like lesions or rods and cores	Absent immunostaining for the nebulin SH3 domain-specific antibody in some severe neonatal cases
TPM2	β-tropomyosin	AD	Rods in both fiber types; caps	-
TPM3	α-tropomyosin (slow)	AD, AR	Rods restricted to slow fibers; caps	-
TNNT1	Troponin T (slow)	AR	Rods in both fiber types; severe fibrosis in some cases	Complete absence of the full length and mutant truncated slow skeletal Troponin T in Amish type nemaline myopathy due to homozygous nonsense truncating mutations
KBTBD13	Kelch repeat and BTB (POZ) domain	AD	Rods in both fiber types; rods and cores	-

	containing 13			
KLHL40	Kelch-like family member 40	AR	Numerous very small rods sometimes visible only ultrastructurally; few myofibrils in some fibers with many rods; square-shaped/rectangular rods	-
KLHL41	Kelch-like family member 41	AR	Rods in both fiber types	-
LMOD3	Leiomodin 3	AR	Rods in both fiber types; square-shaped/rectangular rods with a fringe	-
CFL2	Cofilin 2	AR	Rods in both fiber types; actin filament aggregates	-
MYPN	Myopallidin	AR	Slow fiber predominance; cytoplasmic and intranuclear rods	Decreased myopallidin levels detectable in sections immunostained with anti-MYPN antibody; undetectable full length MYPN on immunoblots of transdifferentiated myotubes from patients with recessive mutations
MYO18B	Myosin XVIIIB	AR	Small nemaline rods	Mutant truncated protein with loss of C-terminus detectable in immunoblots and in sections with antibody against C-terminal MYO18B protein in one case with homozygous recessive truncating mutations
Core myopathies				
RYR1	Ryanodine receptor 1	AD, AR	Central cores most closely associated with AD C-terminal mutations; minicores with indistinct fiber typing more frequently associated with recessive mutations, and more frequent noncore pathologies	Marked reduction of functional RyR1 protein in recessive cases; secondary reduction in other CM
SEPN1	Selenoprotein N	AR	minicores with cores in both fiber types; slow fiber predominance; nonspecific changes	-
MYH7	Slow/β-cardiac myosin heavy chain	AD	Both central cores and minicores in Laing distal myopathy; noncore pathologies	-
MYH2	Fast IIa myosin heavy chain	AD, AR	Minicores; slow fiber predominance; loss of fast IIa fibers; dystrophic-like changes with advanced disease	Marked reduction or absence of *MYH2* transcripts and protein
MEGF10	Multiple epidermal growth factor-like domains 10	AR	Dystrophic-like changes in cases with recessive null mutations; minicores in cases with recessive missense mutations	Total loss of detectable MEGF10 expression in cases with recessive null mutations
CFL2	Cofilin 2	AR	Minicores; slow fiber predominance; rods (see above)	Reduced sarcomeric immunostaining for cofilin-2
TTN	Titin	AR	Minicores in cases with recessive truncating mutations with or without cardiomyopathy or structural cardiac defects; noncore pathology	Loss of staining with C-terminal antibodies in sections and reduction or absence of C-terminal titin fragments on blots; secondary reduction or absence of calpain-3 in C-terminal titinopathies
ACTA1	Filamentous α-actin	AD	Rare “cores only” cases	-
Centronuclear myopathies				
MTM1	Myotubularin	X-linked	Central nuclei with characteristic “myotubular” pathology; identical morphology in cases of congenital myotonic dystrophy; slow fiber predominance in myotubular myopathy; carrier females, mild phenotype and older biopsies may show internal >> central nuclei, more frequent necklace fibers and “myotubular” features may be absent	Absence of endogenous protein detectable on immunoprecipitation/immunoblot analysis; absence of nuclear foci of muscle blind-like 1 in *MTM1*-related CM
DNM2	Dynamin 2	AD	Central nuclei, in chains in some fibers; fibers with radiating sarcoplasmic strands—may be absent in younger biopsies; atypical necklace fibers in some cases	-
BIN1	Bridging integrator 1	AR	Large clustered central nuclei, in chains in some fibers; perinuclear zone of increased oxidative staining	
MYMR14	Myotubularin-related protein 14	Possible association	Increased central nuclei	
RYR1	Ryanodine receptor	AR	Commonly associated with recessive cases; multiple internal nuclei >> strictly central nuclei	Reduced levels of RyR1 protein (see above)

	1			
TTN	Titin	AR	Commonly associated with recessive cases; multiple internal nuclei >> strictly central nuclei	Reduced or absent C-terminal titin (see above)
SPEG	Striated muscle preferentially expressed protein kinase	AR	Marked central nucleation; slow fiber predominance; a few necklace fibers,	Marked reduction or absence of SPEG protein on immunoblots and with immunofluorescence studies
CCDC78	Coiled-coil domain-containing protein 78	AD	Increased central nuclei, slow fiber predominance, core-like areas and sarcoplasmic aggregates	Sarcoplasmic aggregates immunoreactive for desmin, and actin; accumulation of CCDC78 and RyR1 within aggregates
Congenital fiber type disproportion (CFTD)				
RYR1	Ryanodine receptor 1	AR	CFTD may be the only pattern in some cases	-
SEPN1	Selenoprotein N	AR	CFTD may be the only pattern in some cases	-
ACTA1	Filamentous α-actin	AD	CFTD may be the only pattern in some cases	-
TPM2	β-tropomyosin	AD	CFTD may be the only or predominant feature	-
TPM3	α-tropomyosin (slow)	AD	CFTD alone is the most frequent pattern	-
MYH7	Slow/β-cardiac myosin heavy chain	AD	CFTD may be the only pattern in cases	-
Emerging or novel genes				
CACNA1S	Calcium channel, skeletal muscle dihydropyridine-sensitive, α-1S sub-unit	AD, AR	Slow fiber predominance; increased central/internal nuclei; core-like areas to more severe dystrophic-like features with mildly increased fibrosis; scattered regenerating /necrotic fibers	Decreased protein levels demonstrable in extracts of cultured myotubes on immunoblots and immunostaining in cells and frozen sections of skeletal muscle in recessive cases
SCN4A	Sodium channel, voltage-gated, type IV, α sub-unit	AR	Dystrophic-like fibrofatty infiltration with fast fiber predominance in fetal akinesia cases; slow fiber hypotrophy; mild slow predominance without specific structural alterations in the CM cases	-
STAC3	SH3 and cysteine-rich domains 3	AR	Mild myopathic features, mild slow fiber predominance and hypotrophy in infants; increased central nuclei in an adult	Reduced stability and expression of DHPRα seen in zebrafish STAC3 mutants
MYBPC3	Myosin-binding protein 3	AR	Single case with unexpected skeletal myopathy; slow fiber hypotrophy; focal myofibrillar disarray and partial loss of thick filaments on EM	Aberrant expression of cardiac MYBPC3 transcripts and protein, the latter restricted to small fibers expressing slow/β-cardiac myosin heavy chain
ZAK	Leucine zipper-and sterile α-motif containing kinase	AR	Increased central nuclei; peripheral mitochondrial accumulation, slow fiber predominance; atrophic fibers of both types; rimmed vacuoles in some cases	-
SRPK3	Protein kinase, serine/arginine-specific, 3	X-linked	*SRPK3*-null mice develop type II fiber specific centronuclear myopathy	-
PTPLA/HACD1	3-hydroxacyl-CoA dehydratase 1	AR	Centronuclear myopathy in French Labrador retrievers; slow fiber predominance and fiber size disproportion in biopsies from patients with mild CM	-

AD‡: autosomal dominant; AR^{0}: autosomal recessive; IHC/IB†: immunohistochemistry/immunoblot.

Nemaline Myopathies

Mutations in 12 genes (*ACTA1, NEB, TPM2, TPM3, TNNT1, KBTBD13, KLHL40, KLHL41, LMOD3, CFL2, MYPN*, and *MYO18B*) have been described in association with nemaline myopathies till date. These genes encode for

proteins that are components of sarcomeric thin filaments, interact with or modulate thin filament function. Recessive mutations in nebulin (*NEB*) and de novo dominant mutations in slow skeletal muscle alpha-actin (*ACTA1*) are most common.[37,38] Genetic confirmation of nemaline myopathies can be complicated due to the large number of genes involved, the large size of *NEB* and the fact that many pathologically confirmed cases do not link to any of the known loci, pointing to further genetic heterogeneity. The defining feature of nemaline myopathies is the presence of thread-like or rod-like inclusions in myofibers that stain red with the modified Gomori Trichrome (MGT) stain. It is easy to overlook the presence of nemaline rods in HE stained sections, but they can be identified as refractile eosinophilic structures. A careful screening of HE and MGT stained sections under high-power optics should be mandatory in any case of suspected CM. In the MGT stain, the distinction between rods and other red staining inclusions and structures such as cytoplasmic bodies and mitochondria can be difficult. Rods can also be difficult to identify in biopsies from preterm and neonatal patients due to small fiber size, and screening with fluorescent microscopy can be helpful, as they display autofluorescence enhanced with HE at different excitation wavelengths.[7] Routine screening of toluidine blue-stained semithin resin sections is another useful strategy for identifying rods and other inclusions. Labeling with fluorophore conjugated phalloidin (a skeletal α-actin binding toxin) can be helpful in confirming nemaline rods and aggregates of filamentous actin. Cardiac actin (developmental skeletal α-actin isoform) appears to label nemaline rods from diverse genetic backgrounds, but diffusely high expression in developmentally immature fibers can obscure the rod-specific fluorescent signal. The number and distribution of rods can be highly variable within the same muscle, and between patients, and in general there is no correlation between their number and clinical severity. There is no minimum number defined for making a pathologic diagnosis, but in general they should be a substantial feature in the biopsy. Patients with mutations in *NEB* and *ACTA1* have been described with no rods visible with light and electron microscopy[39,40] but sampling has to be considered. Rods can be seen as a normal feature adjacent to myotendinous insertions and extraocular muscles, ageing muscle, in paraspinal muscle in patients with adult-onset axial myopathy/camptocormia with Parkinsonism,[41] sporadic late-onset nemaline myopathy[42] and orthopedic spine disease, and as a minor feature in a diverse range of myopathies including some *RYR1*-related cases.[43] Rods are most frequently cytoplasmic, can be patchy or diffusely distributed across fascicles. Within myofibers, they can be peripherally clustered, under the sarcolemma or surrounding nuclei or appear as linear streaks. They are usually not seen within intrafusal fibers and vascular smooth muscle. Rods are thought to be Z-line derivatives. Ultrastructurally, they frequently appear as rod-shaped or ovoid osmiophilic structures often placed parallel to the long-axis of the sarcomere, seen to be inserted in the Z-line, and showing a similar lattice structure. Sometimes they appear as thickened Z-lines and display a fringe of thin filaments. Areas containing rods can display minimal architectural abnormality or show massive myofibrillar disarray, core-like lesions and poorly formed myofibrils. Rods appear enriched in many Z-line and thin filament proteins including actin, alpha-actinin, tropomyosin, nebulin, and myotilin.[11] Desmin decorates their periphery as with the Z-line. The lesional spectrum of *ACTA1*-related pathologies is broader, and includes actin filament aggregates, caps/cap-like structures, cores/core-like areas, and zebra bodies. Originally regarded as distinct entities, it is becoming increasingly apparent that a mix of lesions may occur in any one case, some of the features may develop with increasing age, and it is more appropriate to regard these as belonging to the *ACTA1*-related pathologic spectrum.[37] Actin thin filament aggregates appear as homogenous pale areas in HE and MGT stains. Cap/Cap-like lesions can be identified in HE, MGT, and NADH-TR stains as sharply demarcated peripheral zones and comprise accumulations of disorganized myofibrils containing mainly thin filaments, and haphazardly arranged, thickened Z-lines. Caps show reduced staining for myosin ATPase and poor immunoreactivity for myosin heavy chains, but can be immunoreactive for several proteins including actin, troponin T, tropomyosin, nebulin, and desmin. Cap/cap-like lesions have been associated so far with mutations in *ACTA1, TPM2, TPM3*, and *NEB*.[44-47] Rods and cores in the same biopsy have been reported in *ACTA1, NEB, KBTBD13, CFL2*, and *RYR1*-related cases-so-called rod-core myopathy.[7] Prominent clusters of rods may mimic cores in the oxidative stains, as these areas are often devoid of mitochondria, thereby leading to an over-diagnosis of rod-core myopathy. The diagnosis should be restricted to cases with presence of rods and cores as distinct lesions, either within the same fiber or in different fibers. Zebra bodies are now considered to be part of the *ACTA1*-related spectrum.[48]

Pathology is rarely predictive of the genotype in nemaline myopathies. Intranuclear rods have been reported in *ACTA1*–related cases and in 2 patients with recessive mutations in *MYPN*.[14,49] Ultrastructurally, presence of rectangular rods and rods with an attached fringe of myofibrils have been regarded as features of *KLHL40* and *LMOD3*-related cases, respectively, but these may not be entirely specific pathologic associations.[7] In rare cases with homozygous null ACTA1 mutations (with complete absence of filamentous α-actin, the adult skeletal muscle isoform), there is uniform increased expression in postnatal skeletal muscle of cardiac actin. Cardiac actin is normally downregulated by birth, but persists as the major isoform in the adult heart. Nemaline rods form in such homozygous cases, suggesting that the presence of mutant protein is not essential for rod formation.[37] Cases with higher cardiac actin expression appear to show a milder clinical phenotype, thereby making upregulation of another actin isoform an attractive therapeutic strategy in human actinopathies.[37,50] A complete absence of the full length and mutant truncated slow skeletal Troponin T with accompanying slow fiber hypotrophy and abundant nemaline rods was seen in patients with *TNNT1*-related Amish type nemaline myopathy due to homozygous nonsense truncating mutations.[51] Biopsies from patients with recessive *NEB* mutations usually do not show an absence of protein. Exceptions to this are seen in some severe neonatal cases where an absence is detectable with immunostaining for the nebulin SH3 domain-specific antibody.[52] Rods restricted to slow/type I fibers are seen in *TPM3*-related cases, as α-tropomyosin 3 is the predominant isoform in slow/type I skeletal muscle fibers.[53] Recessive, loss-of-function mutations in *MYPN,* encoding myopallidin, a sarcomeric protein exclusively localized to striated muscle, have been reported to cause a relatively mild, childhood- to adult-onset slowly progressive nemaline myopathy with cardiac involvement. Intranuclear rods were seen in 2 patients. Decreased myopallidin levels were detectable in sections immunostained with an antibody to myopallidin, and full-length myopallidin was undetectable on immunoblots of transdifferentiated myotubes from patients.[14] Recessive null mutations in *MYO18B* have been reported in 2 patients with Klippel-Feil anomaly, facial dysmorphism, and myopathy. Few rods were identified ultrastructurally.[54] Recessive truncating mutations have been reported in a third case with a severe nemaline myopathy and cardiomyopathy. The mutant truncated protein with loss of the C-terminus was detectable in immunoblots and in sections with antibody against C-terminal MYO18B protein.[15]

Core Myopathies

The term “core myopathies” encompasses the premolecular era designations of central core disease (CCD) and multi-minicore disease (MmD). This reflects the recognition of multiple genetic associations along an overlapping spectrum of clinical phenotypes and core pathology in muscle biopsies. Mutations in *RYR1, SEPN1, MYH7, MYH2, MEGF10, TTN, CFL2*, and *ACTA1* have been described in association with the presence of cores. The defining feature in biopsies is the presence of cores of varying morphologies. In the classical description of CCD, the cores appear as well-delineated amorphous areas in the HE and MGT stains that are devoid of oxidative enzyme staining. In longitudinal sections, they can be seen traversing down a considerable length of the fiber, and can be surrounded by a rim of enhanced oxidative and/or PAS staining. In transverse sections, the cores can be central or eccentric, and single or multiple within a fiber. Ultrastructurally, cores are devoid of mitochondria. The sarcoplasmic reticulum and T-tubule networks may also be reduced. The myofibrillar disruption can range from subtle misalignment of myofibrils and slight irregularity of the Z lines (structured cores) to marked myofibrillar disruption and disarray (unstructured cores) with Z-line streaming. Unstructured cores lack myosin ATPase staining. Immunohistochemically cores show accumulation of several myofibrillar proteins including desmin, myotilin, alpha-B-crystallin, filamin-C, and heat shock proteins.[11] In MmD, multiminicores comprise multiple small foci of myofibrillar disruption and loss of mitochondria that appear in oxidative stains as multiple small foci within a fiber devoid of oxidative enzyme staining, or uneven oxidative staining imparting a mottled or moth-eaten appearance, when more pronounced. Larger lesions have been described as multicores. Ultrastructurally, there is varying degree of focal disruption affecting a few sarcomeres, or subtle misalignment of myofibrils with loss of mitochondria. In both central cores and minicores, the loss of mitochondria can extend beyond the boundaries of the lesion.

The CCD clinicopathologic phenotype is most closely associated with dominant missense mutations in the C-terminal domain of *RYR1* with few exceptions.[55] Recessive *RYR1* mutations have been rarely reported in association with severe CCD presenting with fetal akinesia.[56] Biopsies in RYR1-related CCD usually show marked predominance or uniformity of slow fibers. The latter may be the only feature in biopsies from young individuals, and cores may develop with age.[57,58] CCD pathology in skeletal muscle has been described in association with missense mutations in *MYH7*-related hypertrophic cardiomyopathy in the absence of significant muscle weakness, and rarely even in the absence of cardiac hypertrophy.[59] The *MYH7* p.K1729del tail mutation is associated with an extended clinical spectrum of Laing distal myopathy and muscle biopsies from several patients showed CCD.[60] An allelic disease is *MYH7*-related myosin storage myopathy due to dominant mutations in the distal end of the tail of slow/β-cardiac myosin heavy chain. Onset varies from childhood to adulthood, with limb-girdle, scapuloperoneal or distal limb weakness. Muscle biopsies show sharply demarcated hyaline aggregates in slow fibers that stain with myosin ATPase and are immunoreactive for slow myosin but not desmin. Ultrastructurally they contain granular and partly filamentous material.[61] Recessive mutations in SEPN1 account for the majority of cases with the classic phenotype of MmD characterized by early spinal rigidity, scoliosis, and respiratory impairment. There is a considerable overlap with rigid spine muscular dystrophy (RSMD1), an allelic disease due to recessive mutations in *SEPN1*.[62] RYR1-related MmD with ophthalmoplegia is associated with recessive mutations in *RYR1*. The mutations are spread throughout the gene and frequently result in marked reduction of the functional RYR1 protein.[63-65] Multiple small lesions “minicores” are more commonly seen with *SEPN1*-related MmD, whereas multiple larger lesions “multicores” are more often associated with *RYR1*-related MmD.[62] Analysis of a large cohort of 106 patients with recessive *RYR1* mutations revealed nearly 50% of cases to be noncore myopathy related, and these cases were more likely to be associated with hypomorphic mutations.[66] *RYR1* mutations are associated with other phenotypes including malignant hyperthermia susceptibility (MHS), King-Denborough syndrome, exertional myalgia and rhabdomyolysis, and late-onset axial myopathy. The pathology in these cases may be nonspecific, and cores are not always present.[7] Cores are present in both slow and fast fibers in *SEPN1*-related MmD.[62] In *RYR1*-related MmD the cores are often associated with an indistinct fiber typing. Multiminicores have also been reported in *MYH7*-related distal myopathy with an extended phenotypic spectrum due to dominant missense and charge reversal mutations or deletions. The pathology in these cases is variable, and biopsies in addition to minicores often show CFTD, slow fiber predominance, and in some cases larger cores, increased central nuclei, Mallory-like inclusions, and occasional necrotic or regenerating fibers.[67] Dominant and recessive mutations in *MYH2* (encoding myosin heavy chain IIa isoform expressed in fast IIa skeletal muscle fibers) are associated with a congenital proximal myopathy with joint contractures and ophthalmoplegia. Biopsies show atrophy and recessive cases show loss of IIa fibers with structural changes including minicores, slow fiber predominance, and sometimes vacuoles. Recessive cases show marked reduction or absence of *MYH2* transcripts and protein. With disease progression, biopsies can show dystrophic features with fibrosis, fatty infiltration, and structural changes that are not restricted to IIa fibers.[61] Recessive missense mutations in *MEGF10* are associated with a CM with severe weakness, respiratory impairment, scoliosis, joint contractures, and minicores in biopsies.[68] Recessive null mutations with a total loss of detectable MEGF10 expression cause a more severe CM with diaphragmatic weakness, areflexia, and dysphagia (EMARDD), but lack minicores in biopsies.[69] Dystrophic-like changes with fibrofatty infiltration and scattered regenerating fibers are also seen. Multiminicores were frequently observed in biopsies from patients with homozygous M-line recessive truncating mutations in *TTN* causing early-onset myopathy with fatal cardiomyopathy, and recessive truncating *TTN* mutations causing CM with structural cardiac defects, Emery-Dreifuss muscular dystrophy or arthrogryposis.[70,71] Minicores were commonly associated with slow fiber predominance and increased internal/central nucleation in these cases. One of the molecular consequences of recessive truncating C-terminal mutations is primary truncation of titin without an impact on its sarcomeric incorporation. This is evidenced by normal sarcomeric labeling with titin antibodies to epitopes in the N-terminal and A-I junction, but loss of staining with antibodies to epitopes in the C-terminal.[72] Reduction or absence of C-terminal titin fragments can be demonstrated on Western Blots.[72,73] Ultrastructurally, dissolution of the M-line structure without overt Z-line abnormalities in some myofibrils has been reported. Another important consequence is the loss of protein interactions of C-terminal titin. Within the titin C-terminus, next to the M10 domain is the is7 region, encoded by the alternatively spliced second last exon 362 (Mex5). It contains the M-line binding site for the muscle specific protease calpain 3.[74] Loss of this M-line titin binding site due to truncation of titin results in a secondary reduction or absence of calpain 3 that is demonstrable on Western Blot, and is a common feature of C-terminal recessive titinopathies, all with primary or secondary titin truncations.[73,75] Exceptionally, 2 dominant mutations in *ACTA1* are reported to cause a

“core only” myopathy with slow fiber predominance in the skeletal muscle of all affected individuals.[76]

Centronuclear Myopathies

Mutations in *MTM1, DNM2, BIN1, MTMR14, RYR1, TTN, SPEG*, and *CCDC78* have been associated with centronuclear myopathiesCNMs to date. The defining feature in muscle biopsies is the increased presence of fibers with centrally located nuclei in myofibers. These may be accompanied by increased numbers of internal nuclei, including multiple nuclei. The term “myotubular myopathy,” originally introduced due to the morphologic similarities in affected muscle with fetal myotubes, is now restricted to define X-linked CNM.[7,11] Central nuclei are exceedingly rare in pediatric biopsies from individuals in whom a primary neuromuscular disorder is excluded, and the presence of even a few central nuclei in the absence of overt regeneration should be regarded as abnormal. The majority of centronuclear genes encode for proteins that are implicated in various aspects of membrane trafficking and remodeling relevant to endocytosis, vesicle transport, autophagy, nuclear localization, cell signaling, triad function, and other essential cellular processes.

X-linked myotubular myopathy is caused by mutations in the *MTM1* gene, which encodes a 3′-phosphoinositides phosphatase called myotubularin 1. Affected neonates have the most severe phenotype of all CNM.[8] Muscle biopsies show distinctive features. A high proportion of myofibers show large centrally placed nuclei, which appear regularly spaced down the length of the fiber. The central areas are darkly stained with PAS and oxidative enzyme stains, with a characteristic pale peripheral halo that is devoid of mitochondria but retains myofibrils. The perinuclear zones are often devoid of organelles and may appear as unstained “holes.” Central nuclei are present in both slow and fast fiber types, as well as in fibers with and without developmental myosin heavy chain isoforms.[7] There is often accompanying slow fiber hypotrophy and/or predominance. Ultrastructurally, there is central accumulation of glycogen and mitochondria, myofibrillar disarray, displaced and/or prominent stacked triads, and collections of dense tubules. Immunoprecipitation/immunoblot analysis can show an absence of endogenous protein; this is rarely necessary in routine diagnostic practice. Severely affected female infants, and mild or overtly manifesting female carriers have been described, some related to skewed X-inactivation. Carrier females can show myopathic alterations in skeletal muscle with increased internal/central nucleation even in absence of skewed X-inactivation.[77] The number of internal nuclei can exceed the number of central nuclei, and the characteristic “myotubular” morphology can be absent in biopsies from adult males and carrier females with a milder phenotype. Characteristic “necklace fibers” containing a subsarcolemmal loop or rim of enhanced oxidative staining associated with internal nuclei were originally identified in these patients, but subsequently also identified in the severe neonatal form, with evidence suggesting an increase in their number over time.[78,79] In 2 children, the percentage of centrally nucleated fibers was greater in the biceps than the quadriceps, suggesting a site-specific influence on pathology.[79] A male child with a milder phenotype, with biopsies taken at 15 and 18 months of life, showed slow fiber predominance and hypotrophy with no central nuclei.[80] It appears that the pathology in *MTM1*-related myopathy can be influenced by several factors including clinical severity, age, and site of the biopsy. Autosomal dominant CNM is mostly due to mutations in *DNM2*. The disease usually presents as a relatively mild form of autosomal dominant late-childhood or early-adult onset distal myopathy, and de novo mutations with a severe early-onset phenotype have also been described.[8] A characteristic feature is the presence of fibers with sarcoplasmic strands radiating from the center like a spoke-wheel and best observed with PAS and NADH-TR stains, in addition to central nuclei, sometimes in chains, and slow fiber predominance. The presence of fibers with radiating sarcoplasmic strands may be age-related, and biopsies from younger individuals may lack this signature. “Atypical” necklace fibers lacking nuclei on the subsarcolemmal loop of oxidative staining may be a feature in some cases.[81] Recessive mutations in *BIN1* are a rare cause of CNM. A prominent feature in biopsies is clustered, multiple central nuclei that may form chains down the length of fibers, and a surrounding zone of enhanced oxidative staining. Autosomal recessive loss-of-function mutations in *SPEG*, encoding striated muscle preferentially expressed protein kinase, an MTM1-interacting protein, have recently been identified to cause a severe, congenital-onset CNM with dilated cardiomyopathy. Biopsy findings include marked central nucleation, slow fiber predominance, a few necklace fibers, and marked reduction or absence of SPEG protein by immunoblot and immunofluorescence studies.[16] Recessive missense and nonsense mutations in *RYR1* have been associated with a CNM, with a phenotypic severity in between the severe *MTM1*-related CNM and mild dominant cases of *DNM2*-related CNM. The number of centralized nuclei was lower than in other forms of CNM, and exclusive central nucleation or prominent core pathology was not a feature in 1 cohort. The presence of multiple internalized nuclei was a consistent finding. In some patients, follow-up biopsies showed well-formed cores, yet again pointing to an age-related phenomenon.[82] A similar pathologic signature with a high proportion of coexisting central and multiple internal nuclei is reported in association with congenital/early onset *TTN*-related CNM with or without dilated cardiomyopathy, or with structural cardiac defects, Emery Dreifuss muscular dystrophy or arthrogryposis due to recessive truncating mutations in *TTN*.[70,72,83] Many of the cases also showed prominent minicore pathology and were formally designated as a core myopathy, illustrating the difficulties in defining such cases due to presence of more than 1 major structural defect. Heterozygous missense variants in *MTMR14* (or *hJUMPY*) identified in 2 patients with CNM may represent a genetic modifier of other genetic backgrounds.[7] *CCDC78* encodes for coiled-coil domain-containing protein 78, a protein primarily expressed in skeletal muscle with sarcoplasmic, sub-sarcolemmal and perinuclear localization. A rare autosomal dominant mutation in *CCDC78* has been associated with a mild congenital CNM.[84] Muscle biopsies from affected individuals showed increased central nuclei, slow fiber predominance, core-like areas and sarcoplasmic aggregates immunoreactive for desmin and actin. These aggregates also accumulated CCDC78 and RyR1.

Congenital Fiber Type Disproportion

The term CFTD was coined by Brooke in the premolecular era based on the consistent observation of smaller type I fibers in fiber size histograms from biopsies of a group of children with congenital-onset hypotonia and nonprogressive muscle weakness of varying severity.[85] A similar picture was observed in other cases with congenital onset myopathy, including familial cases. As type I/slow fiber hypotrophy is relatively common in various forms of CMs and other myopathies, the accepted pathologic criteria for diagnosing CFTD include type I/slow fiber hypotrophy with type I fibers at least 12% smaller than type II fibers; subsequently revised to 25%, and in the absence of any

other pathologic features in biopsies in patients with a clinical presentation of CM.[5]

Although there is existing debate whether CFTD truly qualifies as a distinct clinical/nosologic entity, a pure CFTD histologic pattern has been associated with mutations in *RYR1, SEPN1, ACTA1, TPM2, TPM3* and *MYH7*.[13,86-89] Interestingly the *MYH7* mutation affected a multigenerational family causing an autosomal dominant myopathy. Biopsy from a younger individual showed a pure CFTD picture, whereas that from an older individual showed evidence of myosin storage.[89] The conventional diagnosis has been made with NADH-TR and myosin ATPase stains. With the increasing application of myosin heavy chain immunohistochemistry, it is apparent that even in cases with a "pure" histochemical pattern of CFTD, the demonstration of varying numbers of histochemically small type I and larger type II being hybrids expressing more than 1 myosin heavy chain isoform introduces further complexity in defining such cases. Furthermore it is possible that other structural lesions associated with the CFTD genes including cores, nemaline rods, caps and other lesions may develop over time.

Diagnostic Pitfalls and Differential Diagnoses

In neonates and infants, there can be a significant clinical overlap with other neuromuscular disorders, and the differential diagnosis is wide, including CMD, CMS, metabolic/mitochondrial myopathies, spinal muscular atrophy, and secondary involvement of skeletal muscle due to a central cause or within a syndromic context, for example Prader-Willi syndrome, all of whom can have a "floppy infant" presentation. It is important to read a muscle biopsy with the knowledge of the clinical presentation and the investigations at hand, including creatine kinase and lactate levels, and neurophysiology at the very least. Often biopsies may show mild, nonspecific features such as mild size variation, subtle architectural abnormalities, and persistence of immature myosin heavy chain isoforms beyond the appropriate developmental stage. Distinction between primary vs secondary myopathic involvement is virtually impossible in such cases. Type I/slow fiber predominance/uniformity is a common CM signature, and at times may be the only abnormal finding. However, a similar picture may emerge in biopsies from a chronically denervated muscle, and in absence of other indicators such as angular or grouped/fascicular atrophy, it is hard to distinguish neurogenic vs myopathic slow predominance, particularly in needle biopsies. To further complicate the picture, core lesions ranging from CCD-type cores to minicores may develop in chronically denervated muscle. Accompanying slow fiber hypertrophy is more suggestive of chronic denervation and reinnervation, but may also occur in *RYR1*-related CM. Fast predominance is uncommon in CM, but has been reported in some cases associated with *MYH7* mutations.[67] Biopsies from individuals with exertional myalgia and/or rhabdomyolysis of diverse metabolic causes and mutations in *CAV3* may show pathology identical to MmD.[90] A minor degree of disruption of the internal architecture can be seen in virtually every myopathic muscle of diverse genetic and acquired etiologies. Focal areas of loss of oxidative staining due to loss of mitochondria and loss/disarray of myofibrils are a common feature in MFM. Ultrastructurally, the appearances overlap with those of unstructured cores. Multiple myofibrillar proteins accumulate within cores and can be mistaken for abnormal protein aggregation in MFM. Focal areas of subsarcolemmal oxidative pallor adjacent to capillaries, and central poorly demarcated pallor, often more pronounced in type II fibers in biopsies of older children and adults are a normal microanatomical feature, not to be mistaken for cores. Mild central nucleation and minicores with preserved fiber typing especially in biopsies from older individuals in *MICU1*-related myopathy can mimic a core myopathy or a mild CNM.[91] The presence of clusters of regenerating and rarely necrotic fibers in addition is a distinctive feature of *MICU1*-related myopathy.[91] Abnormal positioning, size, and shape of mitochondria are integral structural abnormalities in CM; however canonical features of primary mitochondrial disease including ragged red fibers, mosaic, or diffuse COX-negative fibers and/or complex I/complex IV immunodeficient fibers are not seen except in adults as an age-related finding. CMS are a genetically heterogeneous group of disorders of neuromuscular transmission, often misdiagnosed as CMD or CM. Recognition of the presence of secondary neuromuscular transmission defects in several types of CM makes the diagnosis even more challenging, especially in the early stage of the disease. This overlap also extends to the muscle pathology, and biopsies from CMS patients often show myopathic features including type I/slow fiber predominance, slow fiber hypotrophy resembling fiber size disproportion, uneven oxidative staining and minicores.[92] Extensive fibrosis and fatty infiltration mimicking a CMD have been reported in *RYR1, SEPN1, MYH2, MYH7, TTN, TNNT1*, and *ACTA1*-related CM.[7,37,61,70] Severe fibrosis may be present at birth in some cases of *RYR1* and *TTN*-related CM. Even in these cases, overt necrosis, regeneration, and inflammation are usually not seen. The structural abnormalities in *MTM1*-related CM and congenital myotonic dystrophy (cDM1) due to expansion in *DMPK* are virtually identical,[11] and molecular exclusion of cDM1 in such cases is mandatory. Useful indicators in biopsies include absence of nuclear foci of muscle blind-like 1 in *MTM1*-related CM[93] and depletion of type I/slow fibers in cDM1. Figure 2 illustrates the pathology of some of these diagnostic confounders.

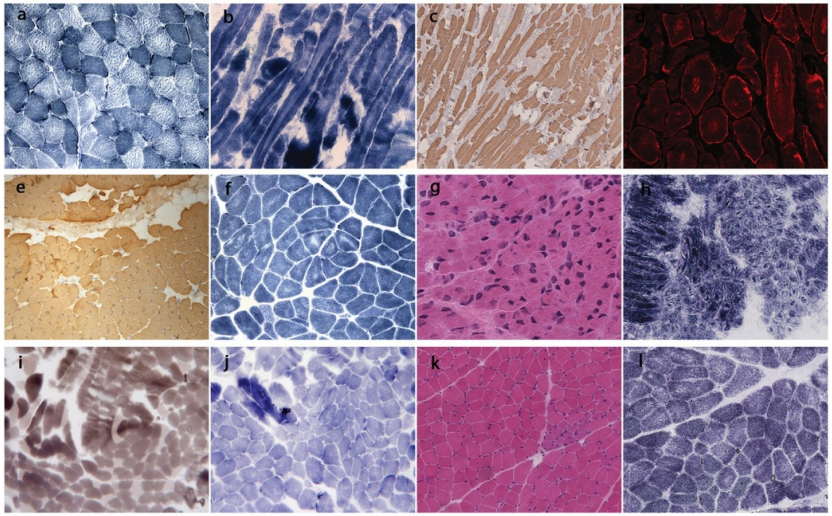

Figure 2 Pathologic mimics of congenital myopathies. Muscle biopsy from an adolescent with “minimal change” histology. There is central pallor in many fibers, especially prominent in type II fibers. Areas adjacent to endomysial capillaries also show pale staining with NADH-TR stain. These are normal developmental features mimicking minicores (a). Muscle biopsy from a patient with a demyelinating neuropathy and chronic denervation mimicking features of central core disease. There is marked type I/slow fiber predominance evident on staining with NADH-TR (b) and slow myosin (c). Striking cores running down a considerable length of the fibers are seen (b). There is desmin accumulation within several cores (d). Muscle biopsy from a patient with *DYNC1H1*-related distal spinal muscular atrophy. There is uniformity of slow fibers on staining with slow myosin (e) and staining with NADH-TR shows minicores in several fibers (f). Muscle biopsy from a patient with congenital myotonic dystrophy. Increased central nucleation is seen with haematoxylin and eosin staining (g), and characteristic “myotubular” morphology is seen with NADH-TR staining (h). Muscle biopsy from a patient with congenital myasthenia and mutations in *DOK7*. Type I fiber predominance is seen withy ATPase staining at pH 4.5 (i), and minicores are seen in many fibers with NADH-TR staining (j). Muscle biopsy from a patient with *MICU1*-related myopathy. Mild central nucleation (k) and minicores (l) are features that mimic a congenital myopathy. A differentiating feature is the presence of clusters of basophilic regenerating fibers (k).

alt-text: Figure 2

Pathology of Emerging and Novel Congenital Myopathies

The presence of a substantial number of molecularly unresolved cases with a clinicopathologic diagnosis of CM suggests further genetic heterogeneity within this group of neuromuscular disorders. The systematic application of NGS has started to bridge this gap by continuous discovery of new mutations in known CM genes, extending the phenotypic spectrum of known non-CM genes such as those associated with channelopathies to CM, and identification of new disease causing genes. The following paragraph summarizes the pathology associated with some of these emerging and novel CM genes. The full phenotypic spectrum of many of these conditions is not yet known. Figure 1 illustrates the pathology associated with some of these gene defects. (Please delete this entire last sentence in this paragraph.)

Recessive or dominant mutations in *CACNA1S* ($Ca_v1.1$), a gene previously associated with heterozygous dominant mutations causing hypokalemic periodic paralysis and MHS, encoding the pore-forming subunit of the dihydropyridine receptor (DHPR), a voltage-gated L-type Ca^{2+} channel that is located at the triad, on the T-tubule and effects ECC with its interaction with RYR1, the Ca^{2+} release channel on the sarcoplasmic reticulum, have been recently identified by whole exome sequencing in a cohort of patients presenting with CM, with generalized hypotonia, severe axial and generalized weakness and ophthalmoplegia in some cases.[94] Muscle pathology ranged from myopathic alterations with type I/slow fiber predominance or uniformity, increased central/internal nuclei and core-like areas to more severe dystrophic-like features with mildly increased fibrosis with scattered regenerating/degenerating fibers. The intermyofibrillar network appeared unusually prominent with “alveolar or reticular” staining patterns with NADH-TR. Ultrastructure demonstrated abnormal triads with dilated T-tubules and sarcoplasmic reticulum. In recessive cases, decreased protein levels were demonstrable in extracts of cultured myotubes on Western Blots and immunostaining in cells and frozen sections of skeletal muscle. *SCN4A* encodes the α-subunit of the adult skeletal muscle voltage-gated sodium channel $Na_v1.4$. Dominant gain-of-function mutations in SCN4A are a well-established cause of myotonia and periodic paralysis. In a cohort of individuals from 6 unrelated kindreds, whole exome sequencing identified homozygous or compound heterozygous loss-of-function mutations in SCN4A, with phenotypes ranging in severity from fetal akinesia to classic CM.[91] Biopsies from the fetal akinesia cohort showed marked dystrophic-like fibrofatty infiltration with fast fiber predominance, and mild myopathic features with slow fiber hypotrophy and mild slow predominance without specific structural alterations in the CM cohort. A homozygous missense mutation in *STAC3* (SH3 and cysteine-rich domain 3) was recently identified to cause Native American myopathy, characterized by congenital-onset muscle weakness, joint contractures, facial dysmorphism, and

MHS. A key role for STAC3 in ECC via regulation of Ca^{2+} channels has been identified.[26,95] Reduced stability and expression of DHPRα was seen in zebrafish STAC3 mutants. Muscle biopsies from infants are reported to show mild myopathic features with mild slow predominance, slow fiber hypotrophy, and increased central nuclei in an adult.[95] Recessive mutations in *CTCN1*, encoding contactin-1, a neural adhesion and neuromuscular junction protein are associated with a familial lethal CM with nonspecific myopathic changes and a secondary loss of sarcolemmal β-syntrophin and α-dystrobrevin in muscle biopsies.[96] Mutations in *MYBPC3* encoding the cardiac isoform of myosin binding protein C are a frequent cause of hypertrophic cardiomyopathy. An infant with a homozygous p.R943X mutation in *MYBPC3* with fatal cardiomyopathy also had an unexpected skeletal myopathy. Muscle biopsy showed myopathic changes with slow fiber hypotrophy, ultrastructural evidence of focal myofibrillar disarray and partial loss of thick filaments, and aberrant expression of cardiac ~~*MYBPC3*~~ *(MYBPC3)* transcripts and protein, the latter restricted to small fibers expressing slow/ β cardiac myosin heavy chain.[97] Homozygous mutations in *kinase ZAK* (mitogen activated protein triple kinase ZAK) have recently been identified in 3 consanguineous families with a slowly progressive CM. Muscle biopsies show a combination of myopathic size variation with increased central nucleation, prominent peripheral/subsarcolemmal mitochondrial accumulation, overall slow/type I predominance with atrophic fibers of both types, and rimmed vacuoles in some cases. *SRPK3*, a member of the serine arginine protein kinase family, and a novel *MEF2* (myocyte enhancer factor 2)—regulated gene is preferentially expressed in striated muscle expression from embryonic to adult stages in mice. *SRPK3*-null mice develop type II fiber specific CNM.[98] Till date, there are no reports of *SRPK3*-related human myopathy. Autosomal recessive loss-of-function mutations in *PTPLA/HACD1* (protein tyrosine phosphatase-like A) segregate with a CNM in French Labrador retrievers.[99] No confirmed cases of *PTPLA/HACD1*-related human myopathy are reported to date. HACD1 (3-hydroxyacyl-CoA dehydratase 1) is involved in catalyzing the dehydration step of very long chain fatty acid synthesis. Homozygous recessive mutations in *HACD1* are reported in individuals from a consanguineous family resulting in a mild CM with gradual improvement with age. Biopsies were taken from affected individuals at a young age and showed slow fiber predominance and fiber size disproportion without cores or central nuclei.[100]

Conclusions

The conventional clinicopathologic classification with a focus on structural/morphologic abnormalities in muscle biopsies of individuals with CM provides a robust framework in directing molecular genetic testing in most clinical settings. However, the increasing application of unbiased NGS to molecularly unresolved CM cohorts has brought into focus the ever-increasing heterogeneity between genotypes and clinical and pathologic phenotypes. Among the CMs, the widest pathologic spectrum is seen in association with defects in *ACTA1, RYR1, TTN*, and *MYH7*—the 4 “chameleon” genes. Overlapping morphologic signatures in muscle biopsies extend beyond the CM spectrum, and careful consideration must be given to the potentially broad differential diagnoses including neurogenic conditions, myasthenic syndromes, channelopathies, and muscular dystrophies. Pathomechanisms for several CM related genes are now resolved with proteins implicated in Ca^{2+} homeostasis, intracellular membrane remodeling, ECC, myofibrillar force generation, mitochondrial dysfunction, and autophagy. Yet, how exactly these perturbed pathways lead to distinctive morphologic abnormalities is not understood. Genes encoding muscle ion channels and their regulatory proteins are an emerging group of CM with yet incompletely understood shared mechanistic pathways that lead to a CM phenotype. In the not too distant future, genetic testing will precede the need for a muscle biopsy in most clinical scenarios where a congenital onset primary neuromuscular disorder is suspected. In this context of “reverse pathology” where biopsies will be performed to ascertain molecularly unresolved or uncertain cases, functional testing including protein expression studies will assume increasing importance. The challenge for pathologists will be of integrating these tests into the routine diagnostic pipeline.

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