REVIEW ARTICLE

X-Linked Myotubular and Centronuclear Myopathies

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Abstract

Recent work has significantly enhanced our understanding of the centronuclear myopathies and, in particular, myotubular myopathy. These myopathies share similar morphologic appearances with other diseases, namely the presence of hypotrophic myofibers with prominent internalized or centrally placed nuclei. Early workers suggested that this alteration represented an arrest in myofiber maturation, while other hypotheses implicated either failure in myofiber maturation or neurogenic causes. Despite similarities in morphology, distinct patterns of inheritance and some differences in clinical features have been recognized among cases. A severe form, known as X-linked myotubular myopathy (XLMTM), presents at or near birth. Affected males have profound global hypotonia and weakness, accompanied by respiratory difficulties that often require ventilation. Most of these patients die in infancy or early childhood, but some survive into later childhood or even adulthood. The responsible gene (*MTM1*) has been cloned; it encodes a phosphoinositide lipid phosphatase known as myotubularin that appears to be important in muscle maintenance. In autosomal recessive centronuclear myopathy (AR CNM), the onset of weakness typically occurs in infancy or early childhood. Some investigators have divided AR CNM into 3 subgroups: 1) an early-onset form with ophthalmoparesis, 2) an early-onset form without ophthalmoparesis, and 3) a late-onset form without ophthalmoparesis. Clinically, autosomal dominant CNM (AD CNM) is relatively mild and usually presents in adults with a diffuse weakness that is slowly progressive and may be accompanied by muscle hypertrophy. Overall, the autosomal disorders are not as clinically uniform as XLMTM, which has made their genetic characterization more difficult. Currently the responsible gene(s) remain unknown. This review will explore the historical evolution in understanding of these myopathies and give an update on their histopathologic features, genetics and pathogenesis.

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INTRODUCTION

The centronuclear myopathies (CNMs) are a group of pathologically defined disorders that characteristically have a high proportion of small myofibers with centrally placed nuclei. Internalization of the nucleus in mature muscle fibers may be encountered in a wide range of muscle diseases, including some primary myopathies (e.g. myotonic dystrophy); however, additional clinical, genetic, and pathologic factors typically help distinguish these myopathies from the CNMs. This review focuses on the classic forms of CNMs and an important well-defined subgroup, X-linked myotubular myopathy (XLMTM).

Spiro and colleagues first coined the term "myotubular myopathy" in 1966 (1). They described a myopathy in a 12-year-old boy who had generalized muscle weakness since birth, ophthalmoplegia, and ptosis. Motor milestones were delayed and he became areflexic, with progressive weakness that lead to difficulty in walking and rising from a recumbent position. Muscle biopsy was characterized by numerous small myofibers with centrally placed nuclei that were surrounded by a clear zone on hematoxylin and eosin (H&E)-stained sections. The clear perinuclear zones were found to contain mitochondria and glycogen. The authors thought that the centrally nucleated myofibers were reminiscent of the myotube stage of muscle development and suggested that this morphologic alteration represented a developmental arrest (1).

One year later, Sher et al introduced the term familial "centronuclear myopathy" to describe 2 sisters with slowly progressive myopathy characterized by weakness and muscle wasting that began shortly after the first year of life (2). The weakness was generalized, but more pronounced, in the proximal and girdle muscle groups. Only one patient had facial muscle weakness, but ptosis was evident in both sisters. Histopathologically, the muscle was similar to the report of Spiro et al with small myofibers harboring centrally placed nuclei and clear perinuclear regions. Sher et al, however, thought the myofibers in their case differed significantly from fetal myotubes, and thereby favored the use of the more descriptive term "centronuclear myopathy" (2). Interestingly, the clinically asymptomatic mother of the patients they described underwent a muscle biopsy that revealed morphologic findings qualitatively similar, but less severe, than those of her daughters. The

authors postulated that the disease gene was inherited in an autosomal recessive manner.

The range of potential inheritance pattern was broadened by McLeod et al who described a family in which 16 members over 5 generations were afflicted with a myopathy that had a predominately limb-girdle distribution with relatively late onset of weakness, but no facial weakness, ptosis, or external ophthalmoplegia, and an autosomal dominant mode of inheritance (3). Muscle biopsy findings were similar to those previously described (1, 2).

As additional patients and families were reported, it became clear that the cases classified under this rubric differed in age of onset, clinical manifestations, and other features (4). Differences in inheritance pattern among affected families were an early clue that multiple disease etiologies, sharing a similar histopathology, likely underlie the myopathy in these subjects. A distinct X-linked recessive pattern of inheritance was observed in a subset of these cases (5, 6) and this led to a classification scheme that distinguished X-linked from autosomal cases (7).

Myonuclei are typically situated adjacent to the sarcolemma, but occasional, scattered myofibers (<5%) with centrally or eccentrically placed nuclei are considered normal on biopsy. A number of diverse conditions are known to alter myonuclear position; therefore central nucleation is a nonspecific finding. Myonuclear position is known to change with developmental stage. In fetal muscle the nuclei are situated in a centrally placed chain, however by birth the majority is found at the periphery near the sarcolemma. Hypertrophic and regenerating myofibers also tend to have a significant percentage of scattered, centrally or eccentrically placed nuclei; the same can be true in the early stage of muscular dystrophies. Chronic neuropathies may also show increased myofiber central nucleation. In some primary myopathies, such as CNM, XLMTM, and myotonic dystrophy, an increased proportion of myofibers with central nuclei is the rule. The mechanisms governing nuclear migration during development and in other aspects of muscle biology are not known. The disparate nature of conditions associated with internal myonuclei led early investigators to postulate a wide range of causes, including an arrest in muscle development (1), neurogenic causes (8–12), or impaired myofiber maturation (13, 14) to account for the morphologic findings in XLMTM and CNM. However, these early studies could not fully resolve these issues of etiology and pathogenesis. The advent of molecular genetic techniques has provided new insight into these issues, especially in the case of XLMTM.

X-LINKED MYOTUBULAR MYOPATHY

Clinical Findings

XLMTM is an X-linked disorder of congenital onset that is caused by *MTM1* mutations (15). In comparison to other congenital myopathies, patients with XLMTM have a rather homogeneous clinical presentation. Males are born with severe generalized hypotonia and weakness with respiratory insufficiency. Affected infants are areflexic and floppy with little or no anti-gravity movements at birth. A high percentage of

these patients require mechanical ventilation to maintain gas exchange. Ventilatory support is almost always required at birth and often for the duration of life, although spontaneous respirations can develop in a subset of patients, while others may require ventilation only during sleep. Many patients, require tube feeding due to impaired suck/swallow mechanisms. Ophthalmoplegia and facial weakness are common (7) and contractures of the hips and knees may be present, although these are generally less severe than those associated with congenital muscular dystrophy. Affected males typically have a large head size, are long in length, and weigh less than expected for the gestational age. Serum creatine kinase levels are within the normal range or are mildly elevated. Electromyography is often within normal limits but may show small polyphasic motor unit potentials and fibrillation potentials in some patients. In utero XLMTM is associated with weak or absent fetal movements and polyhydramnios. The patient's mother may have a history of miscarriages or premature births. Respiratory insufficiency often complicates the clinical course and is a frequent cause of death in these patients and the median survival is 29 months (16).

In a study of patients who survived one year, 50% required 24-hour ventilatory support, while 27% needed such support only at night (16). In another study of XLMTM subjects who survived at least one year, 74% (26 of 35) were living (the age range was 1–27 years). Eighty percent of these patients were completely or partially ventilator-dependent (17). Clinical management of XLMTM patients may be complicated by the inability to predict whether an individual patient will be able to achieve independent respiration. Cognitive development is normal unless the central nervous system is compromised by severe hypoxic episodes. The myopathy appears to be nonprogressive, but long-term survivors are apt to encounter myopathy-related complications including scoliosis, jaw malocclusion, and myopia. A variety of associated diagnoses have been reported in some long-term survivors, including premature adrenarche, pyloric stenosis, spherocytosis, gallstones, nephrocalcinosis and renal stones, bleeding diatheses responsive to vitamin K, and biochemical evidence of hepatic dysfunction. Hepatic peliosis, which is rare in children, has been reported in some XLMTM patients and may be associated with fatal hemorrhage. The involvement of tissues other than skeletal muscle in XLMTM survivors suggests that proper myotubularin function is important in these tissues and that survivors should be clinically monitored for these potential complications (17).

The majority of heterozygous female carriers of a *MTM1* mutation are asymptomatic; however, rare affected females have been reported. Typically they present as adults with slowly progressive muscle weakness and normal or mildly elevated serum creatine kinase levels (18–21). Recent reports describe heterozygous females who presented at a few months of age with clinical features suggestive of XLMTM and muscle biopsy findings consistent with XLMTM (22, 23). Generally, the disease is much less severe in females compared to the affected males in their family. Clinical manifestations may be seen in the setting of skewed (18, 24) or random X-inactivation (20, 21). Among mutation carrying females the disease phenotype seems to be more severe in those with skewed X-inactivation than in those with random X inactivation (25).

XLMTM Pathology

H&E-stained cross sections of XLMTM muscle show increased variability in fiber size, but this is generally not as extreme as that seen in dystrophic processes and the myofibers typically appear small and have a rounded profile. The cardinal histopathologic feature of XLMTM is the presence hypotrophic myofibers with relatively large centrally placed nuclei (Fig. 1A). The percentage of myofibers with centrally placed nuclei varies widely among cases, with a reported range of 2% to 60% (9, 26, 27). The percentage of myofibers with central nuclei can also vary from high power field to high power field in a given case. In longitudinal sections, regularly spaced nuclei may be observed running the length of a myofiber. Scattered unremarkable myofibers with nuclei in the expected peripheral location are nearly always seen and generally make up at least 10% of the total myofibers in a given biopsy. There is no consensus regarding the minimum number of central nuclei required to establish the diagnosis and the biopsy should be interpreted in the context of the clinical picture, and if possible, genetic findings. The proportion of centrally nucleated myofibers does not correlate with disease severity (26). Intrafusal fibers of the muscle spindle are uninvolved and in fact, may even have a larger diameter than the small, extrafusal muscle fibers. A clear peripheral zone characteristically surrounds the centrally placed nuclei. Fibrosis, necrosis, inflammation, degenerative and regenerative changes are typically not seen, and even when present, are not prominent. Some *MTM1* carriers who have had muscle biopsies have shown mild nonspecific changes that include the presence of small, centrally nucleated myofibers (5, 6, 28) or pathologic alterations that may only be evident ultrastructurally (18). More often the biopsy is morphologically normal (6, 29, 30).

On ATPase histochemistry the central perinuclear areas show no reaction, in keeping with a lack of perinuclear myofibrillar components in this region (Fig. 1B). A normal distribution of fiber types can be seen, but type 1 predominance with or without type 1 fiber hypotrophy is more common. Autopsy studies reveal that the degree of type 1 hypotrophy can vary among different muscles; that is, the tibialis anterior may have smaller fibers compared to muscles of the trunk and diaphragm (28, 31, 32). Central nucleation may be limited to type 1 fibers (33, 34) or type 2 fibers (35). Hypotrophy of type 2A fibers or deficiency of type 2B fibers may be encountered (12). An increased proportion of type 2C fibers, the least differentiated histochemical fiber type, has been reported (12). However, increased type 2C fibers and depletion of type 2B fibers are not specific and have been described in a variety of myopathies. Histochemical stains show increased PAS staining (Fig. 1C) and NADH-TR (Fig. 1D) in the center of affected myofibers, representing the accumulation of glycogen and mitochondria, respectively.

Vimentin and desmin are developmentally regulated intermediate filament proteins whose expression and distribution may be altered in XLMTM. In mature muscle, desmin is expressed at Z-lines, where it laterally links adjacent sarcomeres, and at the neuromuscular junction. It has been suggested that the immunohistochemical detection of vimentin and desmin may be an aid to diagnosis as the expression of these proteins is persistently high in XLMTM (Fig. 1E, F), whereas they are downregulated to low levels in mature neonatal muscle (13, 36). This has been used to support the idea of impaired maturation in XLMTM. Increased desmin staining may be either diffusely present or localized to the center of the myofiber (13, 36). Aberrant vimentin and desmin expression is not a universal feature of XLMTM and it has also been reported in adult onset cases of autosomal CNM, so it is also not specific (37). Interpreting these immunohistochemical stains can be challenging due to staining heterogeneity among myofibers in a given biopsy, and the fact that staining is not necessarily consistent among patient specimens (38). Perturbations in NCAM, utrophin and laminin alpha 5-chain expression have also been described (26). Fetal myosin is often expressed in many myofibers from affected neonates, but when older children are biopsied mature myosin isoforms can be identified (32).

On semithin sections, centrally located nuclei with irregular contours are often seen (Fig. 1G). Some myofibers appear tubular in profile due to empty central areas that are devoid of myofibrils and nuclei. Mitochondria and glycogen may accumulate in the center of the myofiber (Fig. 1H). Focal myofibrillar abnormalities such as Z-disk streaming or complete sarcomeric loss may be detected using oil or phase contrast microscopy.

Electron microscopy reveals irregularly contoured central nuclei (Fig. 1I) that may have prominent nucleoli, and at times a generous amount of heterochromatin. Longitudinal sections are optimal for visualizing the mitochondria and glycogen that aggregate around the poles of the nucleus (Fig. 1J). Myofibrils can be seen either juxtaposed to nuclei or separated from nuclei by a swath of cytoplasm that is part of the perinuclear halo seen by light microscopy. Foci of myofibrillar degeneration or disorganization, membranous debris, myelin figures, and empty vesicle-like structures have been reported to occupy the perinuclear region (39, 40). A properly formed myofibrillar apparatus predominates, but focal Z-band streaming may be encountered. A reduction in the proportion of satellite cell nuclei has been reported in XLMTM muscle (41), but other studies have shown no significant alteration (9). Ultrastructurally, the sarcolemmal membrane, basal lamina, T-tubules, and triads are unremarkable. A variety of membrane bound structures have been described in some reports, but these do not appear to be consistent features (9).

XLMTM Pathogenesis

The X-linked nature of this disorder facilitated genetic analysis and, in 1990, linkage studies assigned the gene to Xq28 (42, 43). Three patients with fortuitous deletions helped narrow the candidate area (44, 45). The responsible gene was positionally cloned and named *MTM1* (15). *MTM1* spans approximately 100 Kb at the genomic level, contains 15 exons, and lies approximately 20 Kb proximal to a related gene, (*MTMR1*) (46). *MTM1* mRNA is ubiquitously expressed in all tissues and is detected as a 4.0-Kb transcript on Northern blots, while alternate polyadenylation sites generate a smaller, 2.4-Kb skeletal muscle- and testis-specific transcript. The physiologic significance of these different *MTM1* transcripts is unknown. The protein product is a phosphatidylinositol
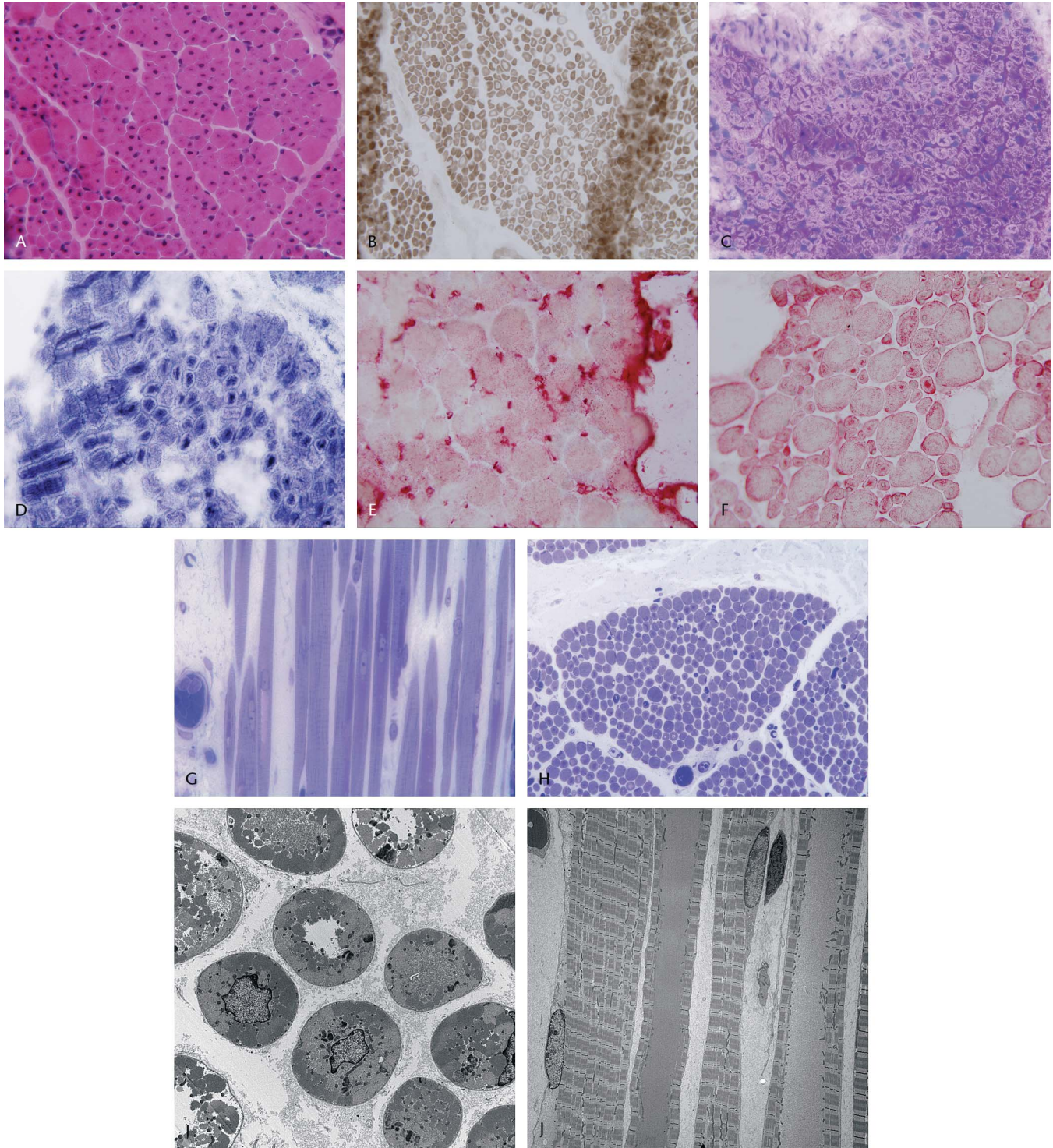
FIGURE 1. XLMTM quadriceps biopsy findings in a newborn male. **(A)** Frozen section stained with H&E reveals increased variation in fiber size and numerous small rounded fibers with centrally located nuclei. **(B)** ATPase pH 4.3 reveals type 1 predominance and numerous ''myotubes'' due to an absence of central myofibrils (original magnification: 400×). **(C)** PAS and **(D)** NADH-TR showing increased central staining corresponding to the accumulation of glycogen and mitochondria, respectively (original magnification: 600×). Immunohistochemistry for vimentin **(E)** and desmin **(F)** showing increased expression of these intermediate filaments and central location of desmin (original magnification: 600×). **(G, H)** Semithin sections stained with toluidine blue showing central nuclei and abundant accumulation of glycogen (original magnification: 600×). **(I, J)** Electron micrographs reveal irregular contours of central nuclei and adjacent cores of abundant glycogen (original magnifications: **[I]** = 3,500×; **[J]** = 1,800×).

(PI)-lipid phosphatase of 603 amino acids known as myotubularin (15, 47).

PIs are quantitatively minor phospholipids that are essential to a wide variety of cellular signaling pathways governing diverse cellular functions such as growth, metabolism, apoptosis, differentiation, vesicle trafficking, and actin rearrangement (48, 49). PIs are precursors of second messengers such as I(1,4,5)P3 and diacylglycerol known to function downstream of specific extracellular signals. A number of kinases phosphorylate PIs at positions 3-, 4-, and 5- of the inositol group, while phosphatases remove phosphate groups from these positions to generate various PI species (48, 49). A variety of proteins possess lipid-binding domains such as pleckstrin homology (PH), Fab1p/YOTB/Vac1p/EEA1 (FYVE), and others that have variable specificity for the different PI classes (50). By directly binding to proteins in cell signal transduction pathways, PIs regulate the assembly of multiprotein complexes, or recruit proteins to targeted subcellular sites of function. By removing phosphate moieties from the inositol ring of these lipids, phosphatases counteract protein complex formation and targeting. Dysregulation in the orchestration of PI kinase and phosphatase activity lead to perturbations in the critical cellular functions mediated by these lipids and ultimately, pathology (48, 49).

Database searches yielded a number of closely related genes and myotubularin is considered the archetypical member of a new gene family. Currently 14 human members of the family have been identified, (including *MTM1*) and are known as the myotubular myopathy (*MTM*)-related genes (*MTMR1-13*). Eight of these are predicted to possess phosphatase activity while at least some of the remainder are catalytically inactive and may play regulatory roles by interacting with catalytically active forms (51–53). Like *MTM1*, most of the *MTMR* genes are ubiquitously expressed. Other members of this gene family have been linked to neuromuscular disease, with *MTMR2* and *MTMR13* mutations associated with autosomal recessive Charcot-Marie-Tooth neuropathy (54, 55). *MTM1* is highly conserved phylogenetically with at least 20 related genes present in a diverse spectrum of eukaryotic organisms from yeast to mammals (53).

Myotubularin has significant homology with dual specificity protein tyrosine phosphatases (PTP) and contains the active site motif characteristic of this superfamily (15). Myotubularin also contains a GRAM domain that is structurally similar to pleckstrin homology (PH) domains that bind PIs (56). These domains are found in a number of proteins important in cell signaling, cytoskeletal organization, and vesicle trafficking. A SET interaction domain (SID) allows myotubularin and related proteins to interact with other proteins with SET domains. SET domains are known to occur in a number of proteins that modulate epigenetic mechanisms of gene regulation important to growth and differentiation. Myogenic differentiation is inhibited when C2C12 cells (mouse myoblasts) are transfected with Sbf1 (*MTMR5*), a SET-binding protein that is a catalytically inactive cognate of myotubularin, suggesting that the interaction of myotubularin-related proteins with SET domains may be important for proper myogenic differentiation (57). The function of the SID motif in myotubularin is unknown. Myotubularin and some related proteins have coiled coil and PDZ-binding motifs near the C-terminus that would permit protein-protein interactions (51). Several MTMR family members also have additional PI binding sites, such as FYVE and PH domains (51, 52). The functional implications of all these sequence motifs are largely unclear.

Despite conservation in amino acid sequence that indicated an active PTP motif, studies showed that myotubularin had poor PTP activity (57–59). The catalytic sites of Sac1p (a yeast PI phosphatase known to be important in vesicle trafficking) and myotubularin are similar and this finding led to experiments that showed myotubularin can dephosphorylate phosphatidylinositol 3-phosphate PI(3)P in vitro and in vivo (58, 59). This was not surprising as the tumor suppressor PTEN is also a member of the PTP superfamily and has PI phosphatase activity (60). A catalytic profile similar to that of myotubularin was noted for several MTMRs, suggesting unified substrate specificity to this protein family (61). Significantly, some missense mutations responsible for XLMTM abrogate myotubularin phosphatase activity (59).

If all catalytically active members of the ubiquitously expressed myotubularin family have a similar catalytic profile, then why is this seemingly large amount of functional redundancy required? Differences in spatiotemporal expression pattern and subcellular location of the myotubularin family members may account for this phenomenon by providing myotubularin family members with access to different intracellular PI pools upon which they perform their catalytic function (61, 62). *MTM1* and *MTMR2* transcripts are differentially regulated in C2C12 cells with increasing expression of the former and decreasing expression of the latter as myogenic differentiation proceeds (61). Tagging studies performed in COS1 cells that overexpress myotubularin or MTMR2 show relatively distinct subcellular localizations, with some overlap, as both show cytoplasmic localization. However, myotubularin overexpression induces the formation of membrane projections to which it was recruited; a change not seen in cells overexpressing *MTMR2* (58, 61). Additional studies have shown that Rac1 induced plasma membrane remodeling recruits myotubularin to membrane ruffles and this appears to be independent of phosphatase activity (62).

A substrate of myotubularin, PI(3)P, interacts with FYVE domain-containing proteins and is an important mediator of vesicular trafficking. Therefore a number of studies have examined myotubularin's role in vesicular trafficking. When human *MTM1* is expressed in yeast, PI(3)P levels decrease and a vacuolar phenotype reminiscent of the VPS34 (yeast PI 3-kinase) mutant manifests (58). However, this phenotype is not seen when human myotubularin is overexpressed in mammalian cells (62). Myotubularin (but not MTMR2) has been shown to metabolize endosomal PI(3)P pools in some reports (61), but not in others (62). Myotubularin dephosphorylates another constituent of the endosomal membrane, phosphatidylinositol (3,5)-bisphosphate (PI[3,5]P2), yielding phosphatidylinositol 5-phosphate (PI[5]P), which may induce myotubularin to form a heptameric ring (63). It has been suggested that the ring form is the active configuration and that dissociation is inactivating (63, 64). An XLMTM causing mutation (R69C), occurring in the GRAM domain, hampers the ability of myotubularin to respond to PI(5)P, suggesting

that this may function as a critical feedback loop (63). Other studies have shown that the GRAM domain of myotubularin interacts with PI(3,5)P2 and that this interaction is critical to myotubularin's function in endosomal trafficking and vacuolar morphology (65). In this study the R69C mutation was associated with decreased PI(3,5)P2 binding (65). Clearly further investigation remains to be performed in this aspect of myotubularin biology.

An *Mtm1* knockout mouse reproduces many features of the human disease (66). However unlike human XLMTM, the mouse model starts with an asymptomatic phase that is followed by a generalized progressive myopathy that ensues at about 4 weeks of age. The animals become progressively weaker and paralysis develops in the later stages; their life span is dramatically shortened to an average of 59 days due to respiratory insufficiency. Morphologically, the muscle shows variation in myofiber size and hypotrophy, which is most pronounced in type 1 myofibers. The percentage of myofibers with central nuclei increases with progression of the myopathy.

In the *Mtm1* knockout mouse, muscle differentiation proceeds normally in the first few asymptomatic weeks of life, nuclei are in the expected subsarcolemmal location, and the proper complement of developmentally regulated myosin heavy chain isomers is expressed (66). Central nucleation of myofibers occurs over time and is accompanied by ultrastructural changes such as myofibrillar disarray, Z-line streaming, and mitochondrial aggregation, among others. This suggests that myotubularin may be critical in maintaining the internal structural organization of myofibers and that other lipid phosphatases cannot compensate for a loss of myotubularin expression in skeletal muscle (66).

The initial report that *MTM1* mutations caused XLMTM detailed seven mutations (15). Now that an efficient methodology for mutation detection has been devised (47), mutation testing is available as a clinical service (www.genetests. org) and nearly 200 different mutations, occurring in over 300 families, have been reported in the literature (19, 67–72). Mutations have been described throughout the entire coding sequence of *MTM1*, however, over two-thirds of reported mutations occur in exons 4, 5, 8, 9, 11, and 12 (19). Biancalana et al reported that for 328 described *MTM1* mutations, approximately 30% were missense mutations, 20% were nonsense mutations, 20% were small insertions/deletions, 20% were splice site alterations, and 7% are large deletions (71). Five frequently mutated sites accounted for 20% of all *MTM1* mutations and they include IVS11-10A>G (results in an insertion of 3 amino acids between the PTP and SID domains due to the generation of a new splice site), R241C, c.141-144 delAGAA (frameshift at amino acid 48), R37X, and R421Q. Truncating mutations were nearly always associated with severe myopathy and early demise (16, 71), however, some patients with distal C-terminal truncations had a mild course (73). A number of nontruncating mutations give rise to a severe phenotype and these alterations are not restricted to a particular functional domain of myotubularin (16, 71). Missense mutations affecting the PTP or SID domain tend to be associated with severe disease, illustrating the critical nature of these domains to myotubularin function (73). Certain missense mutations are seen in some patients with a mild clinical course and are

compatible with survival to adult age (68, 71, 74). The mild phenotype can probably be attributed to some residual myotubularin function in these patients. Generally, myotubularin is absent in XLMTM patient muscle and those patients who do have detectable myotubularin levels tend to harbor a missense mutation or a small insertion or deletion (75).

Due to poor genotype-phenotype correlation, *MTM1* mutation analysis is of limited use in predicting prognosis, but it is critical in order to confirm the diagnosis and document carrier status since over 80% of probands' mothers are mutation carriers (71, 73, 76). Even though the incidence of germline mosaicism is unknown, prenatal testing should still be offered to all women who have borne an affected boy, because a number of cases with maternal and grand-paternal germline mosaicism have been described (73, 77, 78).

AUTOSOMAL CENTRONUCLEAR MYOPATHIES

Clinical

Patients with autosomal CNMs have been loosely divided into groups depending on the age of onset: an early-onset subgroup with a mode of inheritance that is mostly recessive or sporadic and a late-onset subgroup often with dominant mode of inheritance (7). The dominant cases tend to present in adulthood or adolescence and recessive cases present earlier, but the boundaries are blurred. In contrast to XLMTM the autosomal forms are rarely fatal in childhood. On examination, patients tend to have diffuse weakness that preferentially involves either proximal or distal muscles. Electromyographic findings may be myopathic or normal and serum creatine kinase levels are often within the normal range (7). A recent examination of a series of 29 patients from 12 families supported the validity of separating cases by age of onset and further subdivided recessive and dominant cases using additional clinical parameters (79).

Patients with AD CNM often present with an insidious onset and may not come to medical attention until the third decade of life. Although the onset of weakness is usually predominantly proximal, some patients also have weakness of distal muscle groups. AD CNM has 2 clinical subgroups that are distinguished by the presence or absence of diffuse muscle hypertrophy. Both forms of AD CNM are relatively mild compared to AR CNM. The course is slowly progressive and if independent ambulation is lost it generally occurs well into adulthood. Ptosis is common but ophthalmoparesis is only occasionally seen with some limitation of upward gaze. AD CNM patients with hypertrophy present at a younger age and have a more rapid course than their counterparts with AD CNM and no hypertrophy (79). There is no increase in adipose or connective tissues on muscle biopsy in these cases of AD CNM whether hypertrophy is clinically evident or not, so the basis of the muscle hypertrophy in this subset of AD CNM cases is unknown. However, increased adipose tissue has been reported in a sporadic case (80) and calf pseudohypertrophy has been documented in some AD families (81, 82).

The presence or absence of ophthalmoparesis in early-onset cases has been suggested as a means to clinically classify AR CNM (79). Patients with ophthalmoparesis tend to be

more severely affected than those without this finding. Dysmorphic features characteristic of severe myopathies, such as elongated facies and high arched palate, may arise in these patients. Patients with late-onset AR CNM and no ophthalmoparesis have a clinical phenotype similar to patients with AD CNM (79); these children typically have some neuromuscular impairment as they grow and develop. A few cases with AR CNM have a severe phenotype that can mimic XLMTM (7).

Autosomal CNM Pathology

H&E-stained sections show excess variability in the size of myofibers which are generally less rounded and more polygonal compared to XLMTM myofibers. This may be due to the fact that these subjects tend to present at an older age and are therefore biopsied later in life than many XLMTM patients (Fig. 2A). Generally small fibers have centrally placed nuclei, but hypertrophic fibers and fibers of normal size may also have central nuclei (Fig. 2B). In autosomal CNM a myofiber generally has a single, centrally placed nucleus, but clusters of centrally and eccentrically placed nuclei can also be seen. The nuclei tend to be smaller and more hyperchromatic in comparison to XLMTM. A clear peripheral zone can be seen around the central nuclei of some fibers. In longitudinal sections the nuclei appear to form chains. As with XLMTM, there is no recognized threshold proportion of centrally nucleated myofibers needed to make the diagnosis and the proportion of centrally placed nuclei can vary widely from case to case. Fifty percent of AR CNM cases and approximately 40% of AD CNM cases show over 80% of myofibers with central nuclei

(79). The majority of all patients had over 20% of myofibers with central nuclei (79). A moderate increase in endomysial fibrosis has been described in association with severe AR CNM associated with progression to the loss of ambulation in adolescence and restrictive lung disease (79). Myofiber necrosis, phagocytosis, inflammation, degeneration, and regeneration are only rarely seen in autosomal CNM, and when present are not abundant. Myofiber depletion with replacement by adipose tissue may be seen in longstanding cases.

ATPase histochemistry reveals myofibers with centrally placed clear cores or perinuclear halos, the lack of reactivity being due to an absence of myofibrils. Type 1 hypotrophy and/or predominance is seen in most cases (79). The central nuclei are commonly seen in these small type 1 fibers. Type 2 hypertrophy or scattered type 2C fibers may be present and an absence of type 2B fibers has been described (83). PAS stains show glycogen accumulation at the poles of central nuclei. Oxidative enzyme stains reveal centrally placed deposits of reaction product in myofibers due to perinuclear mitochondrial aggregation. In adult cases, acid phosphatase staining may reveal central staining corresponding to lipofuscin. A distinctive radial arrangement of the intermyofibrillar network is seen with NADH-TR, appearing as spokes of a wheel radiating from a centrally placed nucleus in approximately 80% of AR CNM and AD CNM cases (Fig. 2C) (79).

Immunohistochemistry can show increased desmin expression in either the center of myofibers, where the staining surrounds nuclei, or it can be diffusely present throughout the myofiber (Fig. 2D) (84). Vimentin expression may or may not be increased in scattered myofibers (37, 84). As in

FIGURE 2. (A) Deltoid biopsy of a sporadic autosomal CNM case that arose in a 21-year-old female. H&E-stained frozen section showing increased variation in myofiber size and polygonal fiber profiles. Fibers with a single central placed nucleus, clusters of central nuclei and eccentrically placed nuclei are all seen. **(B)** Quadriceps biopsy from a 7-month-old male with no *MTM1* mutation. H&E-stained frozen section reveals increased variation in myofiber size and myofibers with a more rounded profile. Note the increased endomysial connective tissue compared to that seen in XLMTM (Fig. 1A). **(C)** NADH-TR from the case in **(A)**, showing a vague radial arrangement of the intermyofibrillar network radiating from a central nucleus (arrow). **(D)** Desmin immunohistochemistry from the case in **(A)** reveals diffuse increase in immunoreactivity in many myofibers. Original magnifications: **(A, C, D)** 600×; **(B)** 400×.
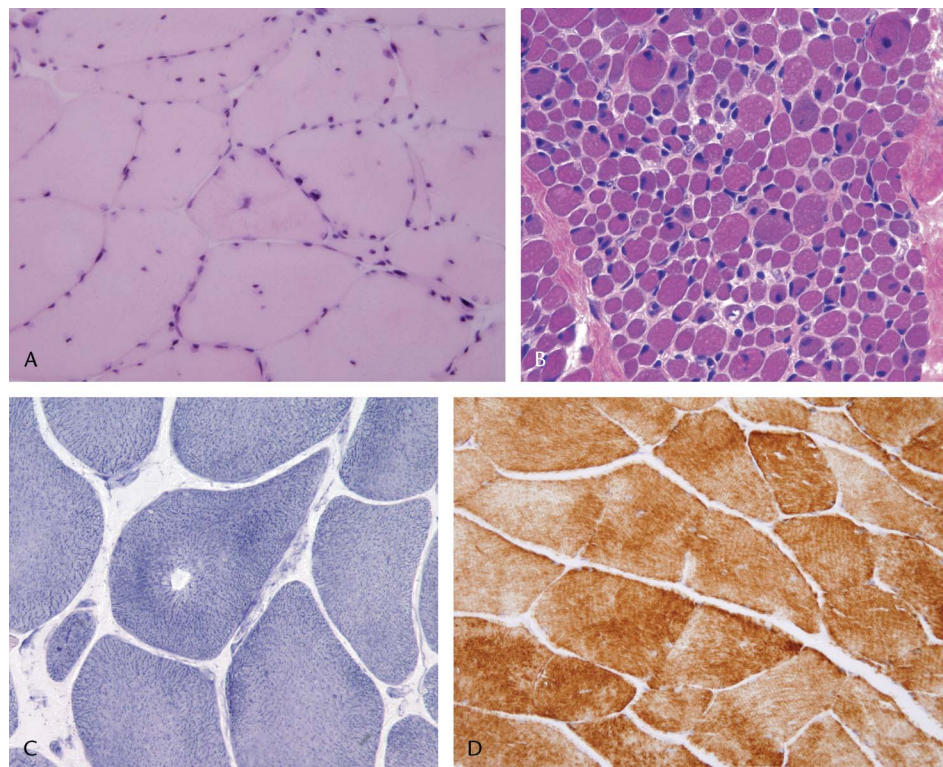
XLMTM, these patterns of desmin and vimentin immuno-staining are not universal. The findings in semithin sections and the electron microscope may include centrally placed nuclei and adjacent accumulation of glycogen and mitochondria as prominent findings. The radial arrangement of the intermyofi-brillar network can also be appreciated in semithin sections. Scattered, focal myofibrillar abnormalities such as Z-line streaming can be seen. Electron-dense structures that are likely lysosomal in nature have also been described (83).

Autosomal CNM Pathogenesis

So far, no culprit gene or genes have been identified in autosomal CNM, so all pathogenic theories are speculative. The autosomal forms are clinically more heterogeneous than XLMTM, which complicates linkage studies; however, the recent stratification of these patients into clinically defined groups may facilitate the identification of responsible genes (79). The region responsible for CNM in an AR canine pedi-gree has been mapped to dog chromosome 2, which is homolo-gous to human chromosome 10p, but no gene has yet been identified (85). Due to the similarities in histopathology with XLMTM, additional candidate genes could include other MTMR family members or PI lipid phosphatases.

CONCLUSIONS

XLMTM presents with a rather homogeneous clinical picture in contrast to autosomal CNM, which has a more variable presentation. Early-onset AR CNM and XLMTM are not qualitatively different and the symptoms are not specific to these myopathies. A wide variety of disorders are capable of presenting in this fashion, such as infantile spinal muscular atrophy, congenital myotonic dystrophy, congenital muscular dys-trophy, and congenital myopathies. Non-neuromuscular con-ditions such as central nervous system hypoxic-ischemic episodes and metabolic conditions could also account for this clinical picture and must be considered in the differential diagnosis. AD CNM is an important entity in the differential diagnosis of the limb-girdle muscular dystrophies as both present similarly with often predominantly proximal weakness affecting older individuals.

Conditions associated with central nucleation in myofibers are diverse and may converge on unrecognized mechanisms governing organelle localization. It is not just nuclei, but also glycogen, mitochondria, and lysosomes that are centrally placed within myofibers in XLMTM and autosomal CNM. The *Mtm1* knockout mouse model suggests that nuclear cen-tralization is not due to a developmental failure of migration from the center to the periphery of the myofiber, but instead, a failure in myofiber maintenance resulting in nuclear migra-tion from the periphery to the center (66). Repeat biopsy is rarely performed in patients but increased numbers of central nuclei have been reported over time in some cases (86–88). Studying mechanisms of organelle localization may prove helpful in the identification of candidate genes responsible for autosomal CNM.

How do *MTM1* mutations cause myopathy and what is the role of PIs and endosomes in muscle? These critical ques-tions remain unanswered and are the subject of much current work. Several key studies have been predominantly performed in epithelial cells (58, 61, 62, 65), which may not be the ideal model to study myotubularin function in muscle. Understand-ing the role of myotubularin and PIs in skeletal muscle could be important in developing novel pharmacologic agents. The function of the SID motif and potential role(s) of myotubularin in epigenetic mechanisms of gene regulation remain to be explored as well. *Mtm1* knockout mice provide an excellent model to study the pathogenesis of this disorder and attempt cell-based or gene therapies (66).

The use of terminology among these disorders is some-what confusing since some diagnosticians use the terms myotu-bular myopathy (MTM) and CNM in a synonymous fashion. Others use MTM to denote severe infantile cases with hypo-trophic myofibers on biopsy and reserve CNM for milder cases arising in older patients, in whom the muscle biopsy tends to appear "more mature." This later approach may lead to confusion as a subset of "MTM" patients diagnosed by biopsy in early infancy may subsequently have a milder clinical course and have repeat biopsies at later ages that fall into the CNM spectrum (89). A way to alleviate the confusion may be to make a distinction in the manner by which these terms are used. Since both the clinical and pathologic distinctions between CNM and MTM are qualitative in nature, we prefer to characterize all such cases as instances of CNM. Furthermore, we advocate the use of *MTM1* mutation testing in conjunction with histopathological and clinical data in rendering a di-agnosis of XLMTM, which is then clearly distinguishable from the autosomal CNMs. Thus we use "XLMTM" to denote the subset of CNM cases that have *MTM1* mutations. Typi-cally, their muscle biopsies show numerous small, rounded myofibers with central nuclei, perinuclear halos, and "myotubes." Autosomal "CNM" can be used to denote the remaining cases with normal *MTM1* genes, including both early- and late-onset patients who often have small myofibers with central nuclei and perinuclear halos.

The continued use of "myotubular" to denote XLMTM is a nod to historical terminology that is somewhat imprecise, as no true myotubes are present; however, it does function to distinguish this subset from CNM with autosomal inheritance. CNM is a descriptive term with no pathogenic connotations, and its use in diagnostics seems appropriate at least until the responsible genes are identified. Distinguishing XLMTM from other CNMs is becoming increasingly important in genetic coun-seling and clinical decision-making. We have recently seen a female infant in consultation at our hospital who was diag-nosed with XLMTM at an outside institution. Her family was told that she would not survive a year, even though she steadily improved symptomatically and *MTM1* mutation analysis was negative. Distinguishing XLMTM from autosomal CNMs would help prevent such unfortunate occurrences in the future.

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