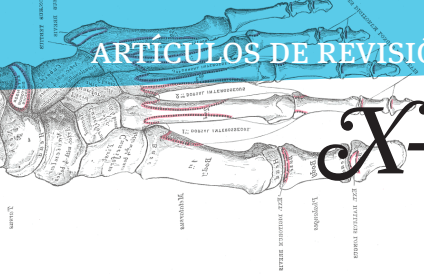

X-linked myotubular myopathy: A brief update

MIOPATÍA MIOTUBULAR LIGADA AL X: UNA BREVE PUESTA AL DÍA

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Arch. Med. Univ. 2016, Nº4, ISNN: 2341-0361.
Reception date: 08/09/2015
Acceptance date: 13/10/2015

X-LINKED MYOTUBULAR MYOPATHY, INCLUDED IN THE CENTRONUCLEAR MYOPATHIES (CNM), IS A SEVERE CONGENITAL DISORDER CAUSED BY MUTATIONS OF THE GENE MTM1. WITH A RECESSIVE HEREDITARY PATTERN LINKED TO THE X CHROMOSOME, THIS DISORDER SHOWS A VARIED SYMPTOMATOLOGY AND A SPECIFIC HISTOPATHOLOGICAL PATTERN. THE CURRENT TREATMENT OF THIS RARE DISEASE IS STILL UNDERGOING RESEARCH, ALTHOUGH GENE THERAPY IS BEING FOCALIZED.

KEYWORDS: MYOTUBULAR MYOPATHY, CENTRONUCLEAR MYOPATHY, X CHROMOSOME, SATELLITE CELLS, MTM1 GEN, MYOTUBULAR.

PALABRAS CLAVE: MIOPATÍA MIOTUBULAR, MIOPATÍA CENTRONUCLEAR, CROMOSOMA X, CÉLULA SATÉLITE, GEN MTM1, MIOTUBULARINA.

Introduction

X-linked myotubular (centronuclear) myopathy (XLMTM, XLCNM, MIM# 31040) is a severe congenital disorder, characterized by hypotonia and neonatal suffocation, respiratory failure, and generalized muscular weakness. Patients pass away in most cases during their first year of life. The most common prenatal signs are polyhydramnios and weak fetal movement, being fetal death common. As it is an X-linked myopathology, men are usually affected (1). Manifestations in women carriers have been associated with skewed X chromosome inactivation, with or without chromosomal restructuration (2). Patients with XLMTM also show other manifestations (3).

Congenital disorders characterized by a high number of muscular fibers with nuclei arranged in line on a central position are included in the Centronuclear Myopathies (CNM) group. In the last few years there have been remarkable advances in the knowledge of genetic bases of the three forms of CNM: the recessive form X chromosome-linked (XLMTM, already described), caused by mutations in the MTM1 gene; and the classic autosomal dominant form with smooth, moderate and severe phenotypes, caused by mutations in the BIN1 gene. Even though the histopathological distinction between the different forms of CNM seems established, these three genes cannot explain all the cases of CNM and there is still a high number of genetically unsolved cases (4).

Van Wijngaarden *et al.* described it for the first time in 1969 (5). XLMTM is the most severe and frequent form of centronuclear myopathy (2), even though severe cases of CNM have been described in mutations of the ryanodine RYR1 receptor (6) or amphiphysin II (BIN1) (7).

The X chromosome mapping has revealed that the locus occupied by the MTM1 gene is located in the proximal site of the Xq. The MTM1 gene codifies a protein called myotubularin that contains the consensus sequence for the active site of the protein tyrosine phosphatase (PTP), a protein implied in the regulation of the transduction pathways and in the growth, proliferation and differentiation control processes (1).

The aim of this paper is to update this rare disease, included in the Orphanet's catalogue of rare diseases (ORPHA596), which includes those disorders with a prevalence of 5 affected per 10000 inhabitants. Nowadays there are neither validated treatments nor modifying therapies for this disease (8).

Epidemiology

The incidence of the XLMTM is approximatively 1/50000 men. It is estimated that the incidence of all the congenital myopathies is 6/100000 births (9).

Clinical presentation

Patients with XLMTM generally have a homogeneous clinical presentation in comparison with other congenital myopathies. Men present generalized severe hypotonia, weakness and respiratory failure at birth (10), but their intelligence remains usually unaffected (11). Ventilation support is almost always required at birth and usually throughout their whole life, even though spontaneous respiration has been described in a subgroup of patients. Other patients require tube feeding due to suction failures. Ophthalmoplegia and facial weakness are common, whilst hips and knees contractures may be present, but usually less severely than in other congenital muscular dystrophies (10).

Ophthalmoplegia, typical in other CNMs (DNM2, RYR1) (6) is a sign shared with the myasthenic congenital syndrome (12). Affected men usually have an increased head size, increased height and less weight than expected for their gestational age. Creatine kinase serum values are within the normal range or slightly increased. The XLMTM is associated with weakness and absent movements and polyhydramnios during pregnancy (10).

The survival rate is 29 months (10). It has been recently shown that more than a half of the patients with XLMTM have a longer survival rate, and most of the survivors suffer severe complications in several organs and systems in the long-term (13). These complications include pyloric stenosis, spherocytosis, cholestasis, nephrocalcinosis and nephrolithiasis, and there is biochemical evidence of liver dysfunction in the form of hepatic pelyosis. Other complications have also been described including scoliosis, mandibular malocclusion and myopia, possibly secondary to generalized hypotonia (11).

Only six cases of hepatic pelyosis have been described to date in XLMTM men and five of them developed an acute onset of multiorganic failure. It is a rare hepatic disorder characterized by the presence of lacunar spaces full of blood in the liver parenchyma and this has been related to androgenic steroids, pyelonephritis caused by *E. coli* and XLMTM. The causal relation between hepatic pelyosis in children with XLMTM is yet to be determined (13), although it is known that MTM1 mutation represents the first clear etiology for this hepatic disorder (11).

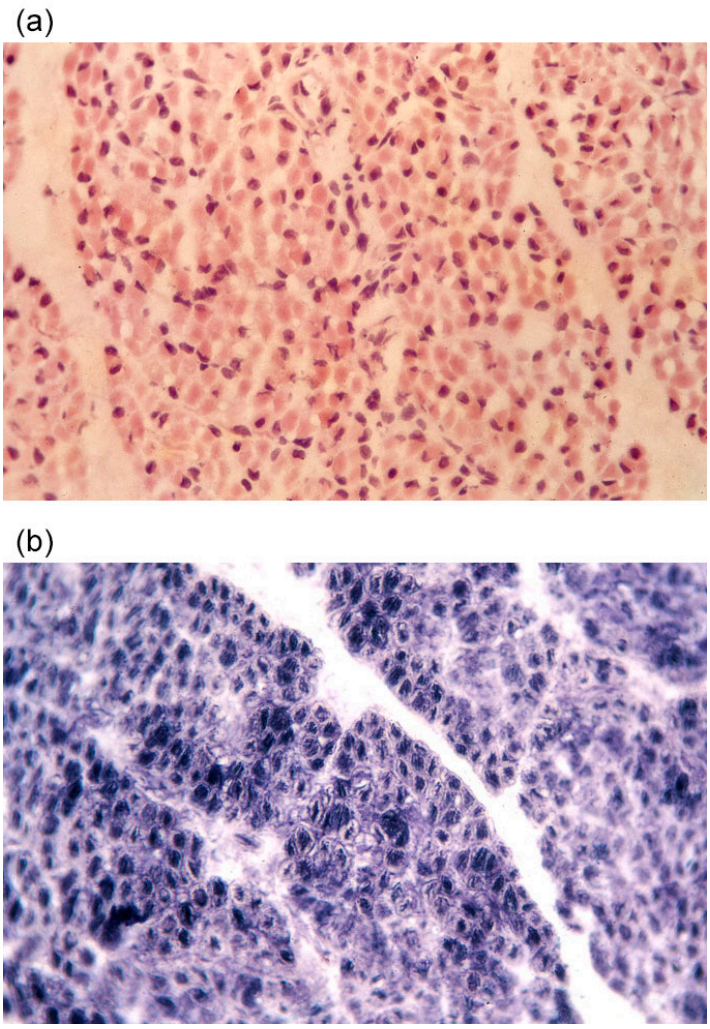
A case of CNS disorder in the form of subdural hematoma has been described (3). Robb *et al.* (14) described clinical signs present in XLMTM and in other autosomal CNM that are common to all myasthenic syndromes, in the form of fatigue that responds to treatment with acetylcholinesterase (AChE) inhibitors and abnormal neuromuscular transmission.

Considering the clinical presentation described, the XLMTM is generally classified in severe (classic) XLMTM, requiring chronical ventilation and with a high incidence of death in childhood; moderate XLMTM, that requires wide ventilator support periods; and the mild XLMTM form (15).

The majority of heterozygotic women with the mutation in MTM1 are asymptomatic. Nevertheless some cases of women affected have been described. Typically the clinical picture is shown in adulthood in the form of progressive muscular weakness with normal or minimum serum levels of creatine kinase (10).

Histopathology

The typical muscular pathology of children cases is characterized by the presence of a large number of round small myofibers with a nucleus placed in the central region and a peripherical halo corresponding to a zone with reduced oxidative enzymatic activity (figure 1).



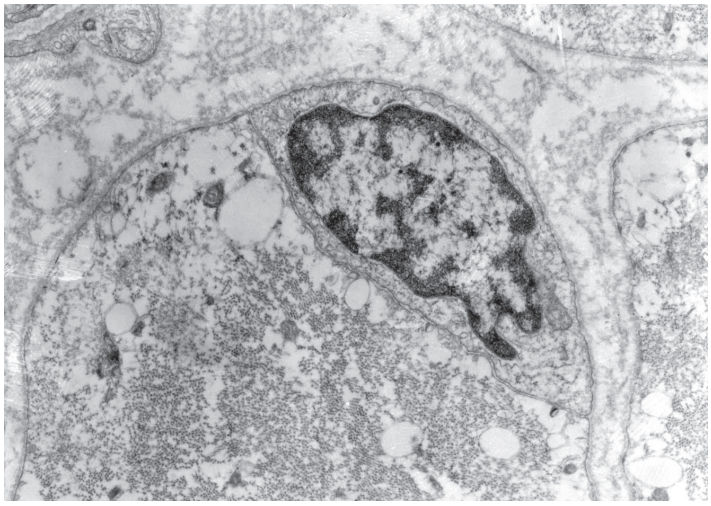
▲ **Figure 1**. *Muscle biopsy of a patient with myotubular myopathy. (a) Muscle fibers are small and many present their nucleus in a central position. (b) Fibers showing the characteristic pattern of oxidative activity distribution, consisting of a central area of increased activity surrounded by a non-stained peripheral area. (a) Hematoxylin and eoxin stain, 40x; (b) NADH-tr, 40x.*

The percentage of fibers with nuclei placed in the central region is largely variable and its proportion is not correlated with the severity of the disease (8). There is no correlation between the number of myofibers with a central nucleus and the age and type of muscle in biopsies of patients with XLMTM (16). The central nuclei have a different appearance from the observed in other degenerative disorders like Duchenne's muscular dystrophy (8). It is unusual to observe necrotic or regenerative fibers, fibrosis or inflammatory cell

infiltration. Type I fibers predominance is observed likewise in other congenital myopathies. Both type I and type II fibers are hypotrophic, although type I fibers are more affected (9).

Patients with XLMTM that presented ultrastructural alterations have been identified. Examples of these alterations are: loss and disorganization of myofibers, Z line divided, central mitochondrial aggregates and/or increased size of the sarcoplasmic reticule (17). Desmine accumulations can be found in the central areas of the myofibers as well (16).

A study with XLMTM patients (16) established that there were less satellite cells in the deltoids and the vastus lateralis muscles examined, in comparison with the control muscle biopsies. This finding is essential because it suggests that the disease carries defects in the production of satellite cells. Therefore, the small size of the muscular fibers observed in the biopsies of these patients can be explained by a drop in the number of this myogenic cell type. Satellite cells (figure 2) are involved in the postnatal growth processes, hypertrophy and muscular regeneration (18).



▲ **Figure 2**. *Inactive satellite cell located between the cell membrane and the basal lamina of a hypotrophic muscle fiber. Transmission electron microscopy, x8900.*

The prognosis is related to the pathological findings. Patients with larger myofibers tend to have better outcomes and usually mechanical respiration can be removed (9).

Bevilacqua *et al.* (19) identified patients with XLMTM that presented a specific histological alteration in some muscular fibers, similar to a necklace (necklace fibers). Ultrastructurally, these fibers contain small-sized, obliquely oriented myofibrils that present a subsarcolemmal ring or "necklace" that surrounds the fiber and is formed by mitochondria, sarcoplasmic reticule and glycogen granules. In four patients that presented these fibers, the myopathy developed in childhood and had a slower progression, smooth to moderate behavior, in comparison with the classical form of XLMTM. Henceforth, the presence of "necklace" fibers can be a useful marker for the beginning and the progression of some cases of XLMTM (19).

Pathogeny

XLMTM is caused by different mutations in the MTM1 gene. This gene encodes a dual-specificity phosphatase named myotubularin, defining a large gene family highly conserved through evolution (20).

The locus responsible for XLMTM is located in Xq28 (21). The MTM1 gene has been found mutated in the vast majority of XLMTM patients. It consists of 15 exons, and is located proximal to a homologous gene, MTMR1 (20).

The myotubularin-related protein contains the consensus sequence for the active site of phosphatases (PTP). Besides, myotubularin can develop a dual-specific phosphatise activity, participating in the transduction of signals that are involved in muscle development (20). This includes endosomal trafficking, excitation-contraction coupling, intermediate filament organization, and apoptosis. The pathways involved in induction of apoptosis include the activation of receptors associated with programmed cell death and increased mitochondrial apoptotic factors (22).

Myotubularin is highly evolutionarily conserved. The MTM1, MTMR1 and MTMR2 are considered a subfamily of genes also contained in the *Drosophila* and zebrafish genomes (20). To date, about 200 mutations have been identified in MTM1 (7), most of which are related to the reduction of the functional expression of the gene.

It is traditionally accepted that the mutation in the MTM1 gene impairs muscle maturation, based on the histopathological aspect of muscle fibers and the persistence of proteins normally expressed only in myotubes, such as vimentin and embryonic myosin heavy chain. This hypothesis remained, however, controversial because the cytoarchitecture of myofibers in patients appears more mature than that of fetal myotubes, and the presence of immature myogenic markers is not a constant feature (17).

Several studies have tried to link the site and mechanism of MTM1 gene mutation and the severity of the phenotype (23). McEntagart *et al.* (23) elucidated the possible link between a type of MTM1 gene mutation and a mild phenotype. However, the high degree of genetic heterogeneity obtained during the study confirmed the diagnostic limitation.

Diagnosis

Due to the wide variety of sequences in the MTM1 gene, which causes this disease, genetic diagnosis is difficult. It is therefore very important to develop a complex molecular diagnostic strategy involving the analysis of myotubularin transcription and protein expression (2). Tosch *et al.* (2) have developed anti-myotubularin antibodies that can be used together with the amplification RT-PCR and Western-blot analysis of muscle samples and cultured cells. These techniques are useful for the diagnosis of some cases of XLMTM. Amburgey *et al.* (7) consider that the array-CGH method and the MLPA technique should be used for the diagnosis of those cases in which the MTM1 sequencing does not diagnose the disease.

Clinical course and treatment

Clinical course

Patients affected by XLMTM usually have a fatal outcome during their first year of life. Long-term survivors are at risk for medical complications (3, 11, 13). Herman *et al.* (11) recommend all XLMTM patients annual blood test follow-up, liver function tests and abdominal ultrasounds.

Treatment

The treatment of patients suffering from XLMTM is symptomatic. Optimal management requires a multidisciplinary team of specialists treating the patient in the long term. Tracheostomy, enteral feeding and supportive communication devices are often required (15).

With regard to changes in the neuromuscular junction (24), and to the fatigue that characterizes some cases of XLMTM, Robb *et al.* (14) propose the use of AchE inhibitors, normally used to treat congenital myasthenic syndromes. The available data (i.e. changes in the neuromuscular junction found in XLMTM simulated models of zebrafish that also responded to AchE inhibitor therapy), suggest that the defect in neuromuscular transmission may accompany some CNM and contribute to muscle weakness.

Research

Recent studies of human tissue and animal models have discovered structural and physiological abnormalities in myotubularin-deficient muscle, but the impact of myotubularin deficiency on myogenic stem cells within muscles remains unclear (22).

Murine models developed with myotubularin deficient mice (mice MTM1σ4 or MTM1 KO), show similar features in comparison with the human disease. These include severe weakness, respiratory failure and histological findings such as the existence of small myofibers with an increased number of nuclei in a central position. By homologous recombination, Buj-Bello *et al.* (17) generated myotubularin-knockout mice, proving that muscle differentiation in MTM1 KO mice normally occurs, contrary to what was expected.

It is still unknown whether the smallness of myofibers is due to poor function of satellite cells or to other myogenic progenitors. Lawlor *et al.* (22) demonstrated that there is an increased apoptotic activity in myotubularin-deficient myoblasts. Their study suggests that the depletion of myogenic cells that occurs in the progression of MTM1σ4 mice may be due to a slow growth and/or increased apoptosis in these myogenic cells. Their results provide potential evidence of depletion of satellite cells in muscle dysfunction caused by myotubularin deficiency. This causal relationship has been already observed in other models of mice with dystrophin or selenoprotein N deficiency. Nevertheless, further research is needed to define the mechanism that causes myotubularin deficiency to affect survival of these cells.

In an experimental model of zebrafish, Dowling *et al.* (8) observed that reduced myotubularin levels have a major impact at the level of motor function, and cause obvious histopathological muscle changes. These include an altered nuclear shape and position and hypotrophy of myofibers, changes similar to those observed in human XLMTM.

Other studies suggest a possible pathogenic mechanism common among the three forms of CNM wherein the BIN1 gene may represent a molecular link between myotubularin and dinamina2 in skeletal muscle (25). So far, the BIN1 gene has only been analyzed in humans in cases of cancer. MTM1 encodes a phosphoinositide (PI) phosphatase, and the specific muscle BIN1 exon encodes a domain linked to PI. Toussaint *et al.* (25) consider that MTM1 regulates the level and specific localization of PIs that specifically binds to BIN1.

The experimental study of Cowling *et al.* (26) is based on the hypothesis of the negative regulation exerted by the MTM1 gene on the role of DNM2 gene in muscle, and in that the reduced expression of DNM2 in XLMTM models can improve the phenotype of this disease. This is, indeed, a future gene therapy approach for this disorder. The reduction in DNM2 expression in mice *MTM1*$^{-/Y}$ increases life expectancy and long-term motor and muscle development.

All developed and tested models allow precise study of the role of this phosphatase in muscle. Therefore, they can be very useful for developing therapeutic strategies for this disease.

Conclusion

XLMTM is one of the estimated 7,000 rare diseases affecting nearly 7% of the world population. Due to its low incidence, this disease has not been studied profoundly, although progress has been made since its initial discovery in 1969. Highlights include genetic studies of possible disease-causing mutations and their potential relationship to the severity of the condition, as well as analysis of possible malfunctions of the population of satellite cells in the affected muscles. The treatment, currently not validated, should be approached from the perspective of gene therapy.

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