X-Linked Myotubular Myopathy: A Novel Mutation Expanding the Genotypic Spectrum of a Phenotypically Heterogeneous Myopathy

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Abstract

X-linked myotubular myopathy (XLMTM), a centronuclear congenital myopathy secondary to pathogenic variants in the *MTM1* gene encoding myotubularin, is typically recognized for its classic and severe phenotype which includes neonatal hypotonia, severe muscle weakness, long-term ventilator dependence, markedly delayed gross motor milestones with inability to independently ambulate, and a high neonatal and childhood mortality. However, milder congenital forms of the condition and other phenotypes are recognized. We describe a 6-year-old boy with a mild XLMTM phenotype with independent gait and no respiratory insufficiency even in the neonatal period. The child has a hemizygous novel splice site variant in the *MTM1* gene (c.232–25A > T) whose pathogenicity was confirmed by cDNA studies (exon 5 skipping) and muscle biopsy findings. We also compared the phenotype of our patient with the few reported cases that presented a mild XLMTM phenotype and no respiratory distress at birth, and discussed the potential mechanisms underlying this phenotype such as the presence of residual expression of the normal myotubularin transcript.

Keywords
- centronuclear myopathy
- mild phenotype
- *MTM1* gene
- myotubularin
- X-linked myotubular myopathy

Introduction

X-linked myotubular myopathy (XLMTM) is a congenital myopathy, usually included in the group of centronuclear myopathies, and is secondary to pathogenic variants in the *MTM1* gene encoding myotubularin. It has an estimated prevalence of 1:50,000 newborn males.[1,2]

Myotubularin is a ubiquitous lipid phosphatase involved in regulation of intracellular membrane trafficking and vesicular transport.[1,2] There are reports of more than 250

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pathogenic variants evenly distributed throughout the *MTM1* gene, with 20% altering splice site.[2]

XLMTM is typically recognized for its classic and severe phenotype, which represents approximately 80% of the cases. This phenotype is characterized by neonatal hypotonia, severe muscle weakness (including involvement of bulbar, facial, and extraocular muscles), long-term ventilator dependence, and markedly delayed gross motor milestones with inability to independently ambulate.[2,3] The phenotype of XLMTM was initially broadened by a long-term follow-up study conducted by Barth & Dubowitz.[4] They depicted that the first reported XLMTM family showed marked intrafamilial phenotypic variability, including patients with the mildest expression. Later, Hermann et al[3] categorized the phenotypes into severe, moderate (quicker achievement of motor milestones and ability to support at least 6–8 hours per day without mechanical ventilation), and mild (minimally delayed motor milestones with acquisition of independent ambulation, no need for ventilatory support beyond the neonatal period, and slight/none facial dysmorphisms). Since then, some reports of the mild phenotype have been described.[5–7] Additionally, this myopathy can be associated with nonmuscular manifestations such as hepatic peliosis, which can be life threatening.[3]

In addition to the clinical presentation described, the diagnosis of XLMTM is achieved by identifying a pathogenic variant in *MTM1* gene by molecular genetic testing. Furthermore, the histopathologic findings on muscle biopsy may also have a role in the diagnosis, especially when genetic variants are of unknown significance.[2]

The characteristic histopathologic feature is the presence of small, rounded myofibers with a large centrally located nuclei. Although the percentage of these fibers is variable (2–60%), the central nuclei appear to increase over time and may even be absent in rare cases. Other distinctive features are the central increase of staining with oxidative enzymes (SDH and NADH), the predominance of small type 1 fibers, and in some cases of XLMTM with adult-onset symptoms or in manifesting heterozygous females, the presence of necklace fibers (with a ring-like accumulation of staining following the myofiber contour).[2,8–10]

Treatment is essentially supportive, based on a regular physiotherapy program, and surveillance and treatment of the different manifestations of myopathy and related medical complications. For this, it is essential to have a multidisciplinary group specialized in neuromuscular disorders that should involve at least a neurologist, pulmonologist, rehabilitation medicine specialist and/or physical therapist, orthopedist, ophthalmologist, and orthodontist.[2]

Recently, a promising gene therapy trial [Gene Transfer Clinical Study in X-Linked Myotubular Myopathy (ASPIRO)] including male patients with severe XLMTM aged under 8 years has been initiated and is still ongoing. Crucially, it has shown significant clinical improvements in achievement of important motor milestones and in respiratory function, with a manageable safety profile.[11]

The classic XLMTM phenotype is usually associated with a high neonatal/childhood mortality, and the few patients who survive are usually completely or partially ventilator dependent.[2,3] The prognosis and survival in the mild XLMTM phenotype is clearly better than in the severe phenotype; however, there are reports of patients who required ventilatory support in their fifties.[6]

In this manuscript, we describe a child with a mild phenotype of XLMTM, secondary to a novel mutation MTM1 gene, and discuss the phenotypic variability of the disease.

Results

Case Presentation and Molecular Analysis

A 6-year-old boy was referred for a pediatric neurology outpatient evaluation due to motor complaints, especially in climbing stairs and running. He was born from a nonconsanguineous couple at full term (40 weeks) after an uneventful pregnancy. The delivery was through a C-section due to suspicion of fetal distress. However, he showed respiratory independence at birth (Apgar's score of 7 at birth and of 9 at 1 minute after birth) and the neonatal period was uneventful. This boy showed minimally delayed motor milestones. For example, he sat unsupported at 9 months and achieved independent walking at 18 months. He had no history of recurrent respiratory infections or other respiratory/cardiovascular complaints. There was no known family history of neuromuscular disorders. He has a younger healthy half-sister from the paternal side and no siblings from the maternal side.

At 6 years, the child displayed facial weakness without ophthalmoparesis (►**Fig. 1A**), high-arched palate, malocclusion, and bilateral scapula alata. Muscle strength evaluation revealed axial and limb-girdle weakness with Medical Research Council (MRC) grade 3 paresis of neck flexors and symmetrical involvement of the shoulder (MRC grade 4) and pelvic girdles (MRC grade 4), without fatigability and with proximal osteotendinous hyporeflexia. There was a positive Gowers' maneuver (►**Fig. 1B**) and a waddling gait with tendency to toe walking. There was no scoliosis or other osteoarticular deformities/contractures. The patient's legal representative gave informed consent for the use of his photographs.

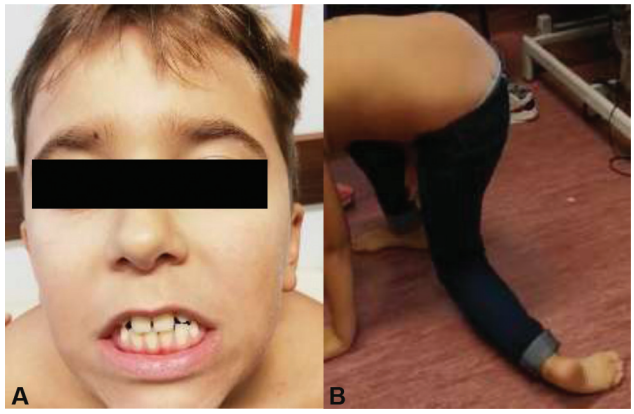

Fig. 1 Photographs of the patient at the age of 9. (**A**) Facial diparesis noted while smiling. (**B**) Positive Gowers' maneuver when trying to lift from a squatting position.

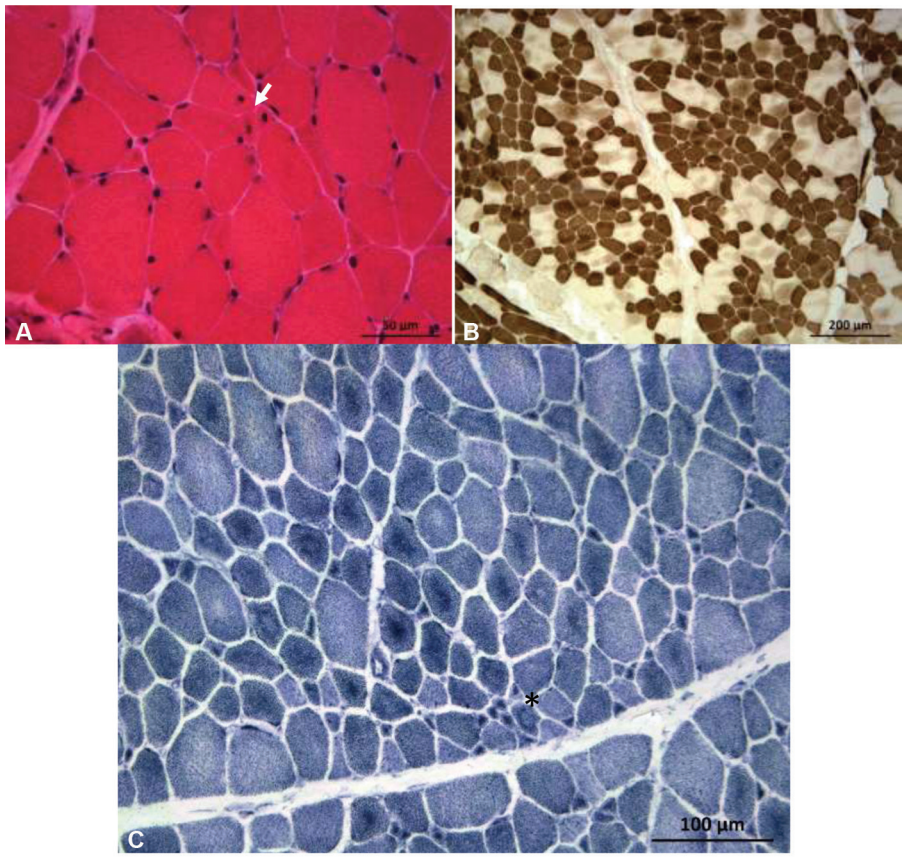

Fig. 2 Deltoid muscle biopsy taken at the age of 7 years. (**A**) Hematoxylin and eosin (HE), muscle with myopathic profile, showing variability in fiber size diameter due to the presence of frequent atrophic fibers, some with a single nuclear internalization in a central position (arrow) and other fibers with a central increased reticulum staining. (**B**) ATPase pH 4,35, predominance of type 1 fibers (dark brown), with several atrophied resembling fiber size type disproportion pattern. (**C**) Nicotinamide adenine dinucleotide phosphate, several fibers with central increase of oxidative activity and some necklace fibers (*).

Serum creatine phosphokinase levels were within the normal range (47 U/L) as well as transaminases. A deltoid muscle biopsy (►**Fig. 2**) confirmed the clinical suspicion of a congenital myopathy showing increased variability in fiber size diameter with round atrophic fibers and some revealing a single nuclear internalization in a central position. There were also several fibers with central increase of oxidative activity, some necklace fibers, without radiating sarcoplasmic strands and type 1 fiber predominance with areas resembling fiber size type disproportion. These aspects suggested the diagnosis of centronuclear myopathy. Therefore, a next-generation sequencing panel for congenital myopathies (23 genes including *BIN1* and *TTN* genes) was performed, and variants with a frequency below 1% localized in the coding regions and adjacent intronic regions were filtered-in for further analysis. This resulted in the identification of two genetic variants of unknown significance; a heterozygous missense variant in exon 29 of the *MYBPC3* gene (NM_000256.3:c.3107G > A, p.[Arg1036His]) and a hemizygous variant in intron 4 of the *MTM1* gene (NM_000252.2: c.232–25A > T) (►**Fig. 3A**). The heterozygous missense variant detected in the *MYBPC3* gene was not considered significant based on bioinformatics analysis (third-fourths algorithms used predicted a benign impact), frequency in population databases (17 heterozygous in gnomAD control population), type of inheritance, ClinVar database submissions (uncertain significance/likely benign), and absence of correspondence with the phenotype presented by our patient, who does not have cardiomyopathy nor a family history of cardiac diseases. Bioinformatics analysis on the *MTM1* gene variant suggested a possible effect on splicing mechanism by abolishing a candidate branch-point sequence. To further clarify the pathogenicity of this intronic variant, mRNA obtained from the patient's muscle was converted into cDNA, followed by specific polymerase chain reaction (PCR) encompassing exons 1 to 6 of *MTM1* gene. A smaller PCR fragment was observed in the patient when compared with a cDNA control. Subsequent Sanger sequencing confirmed that the variant detected altered the mRNA splicing, leading to skipping of exon 5 (r.232_342del), which resulted in an in-frame transcript (p.Ser79_Asp115del). However, a residual expression of the normal transcript was also observed (►**Fig. 3B** and **3C**). Therefore, the pathogenicity of *MTM1* variant was established, and the definitive diagnosis of XLMTM was made. The patient's mother was not available for genetic testing.

The patient underwent respiratory (pulmonary function tests, polysomnography, and arterial blood gas) and cardiac evaluations that did not reveal significant changes. He is under clinical surveillance in a multidisciplinary group of neuromuscular disorders and attends a regular physiotherapy program. At 3 years of follow-up, the clinical picture

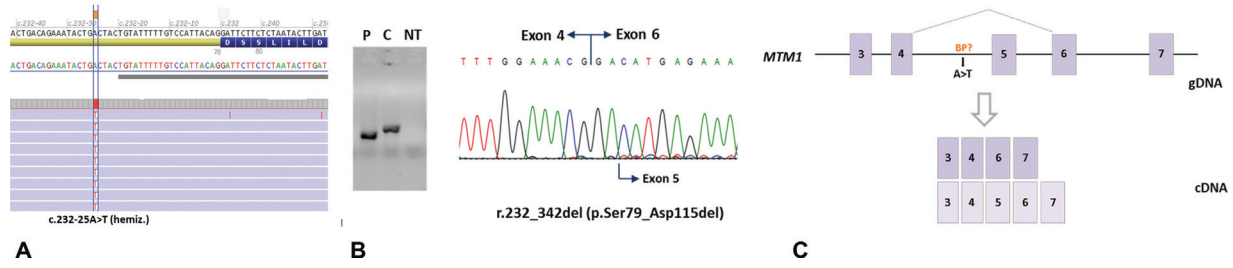

Fig. 3 Results of the experimental data. (**A**) Sequencing results from next-generation sequencing showing an intronic hemizygous variant (c.232–25A > T) in *MTM1* gene. (**B**) *MTM1* cDNA analysis. A smaller aberrant transcript was observed in patient's resulting PCR products (P, patient; C, control; NT, nontemplate). Subsequent sequencing for further characterization showed the absence of exon 5 but also a residual full-length transcript, not visible by gel electrophoresis. (**C**) Schematic representation of the variant and resulting transcripts. At gDNA level, the variant is located in intron 4 of *MTM1* at a potential branch-point site. mRNA studies confirmed that this variant compromises mRNA splicing process, resulting in two transcripts: a predominant transcript with an in-frame deletion (exon 5 skipping) and a residual full-length transcript.

is stable and no loss of motor abilities or respiratory dysfunction was observed.

Discussion

Herein, we describe a child with mild XLMTM phenotype including independent gait and no respiratory insufficiency, even in the neonatal period.

Pathogenic variants in the *MYBPC3* gene are usually associated with a phenotype characterized by adult-onset hypertrophic cardiomyopathy with an autosomal dominant inheritance, although a homozygous variant may lead to cardiomyopathy with myopathy with infancy-onset.[12] However, the *MYBPC3* gene variant identified in this patient was shown to be nonpathogenic.

Our data confirm that the clinical picture in the patient results from a novel splice site variant of the *MTM1* gene, whose pathogenicity was confirmed by molecular studies. Although the presence of some necklace fibers in muscle biopsy already suggested XLMTM, these fibers were only found when the biopsy was reviewed in light of this diagnosis.

Due to the new genetic techniques of massive sequencing, in the last decades the phenotype of *MTM1* associated myopathies have been expanded covering a spectrum of clinical pictures ranging from severe to mild congenital forms as well as manifesting female heterozygotes with variable severity, and even rare cases of adult-onset symptoms.[3,4,6,13,14] It is therefore a myopathy with multiple facets and in some way resembling other X-linked muscle disorders such as dystrophinopathies.

Unlike our patient, most patients with mild XLMTM phenotype reported in the literature had respiratory distress at birth.[4–7] We found only five similar cases described in the literature so far, which makes this presentation fairly rare.[5–7,14] When we analyzed the differences of these five patients compared with our case, we found that four of them had missense mutations, three had hypotonia at birth,[5,6,14] and two needed transient tube feeding.[6,14] For instance, at the age of 10 years, one patient had motor difficulties that affected everyday life,[6] while another had normal early motor milestones and at age 25 had an active sports life, and his condition was diagnosed following early and recurrent respiratory infections.[7] The patient with the mildest phenotype in the early years, later showed an axial and limb-girdle involvement producing walking difficulties, and at age 55 he had a respiratory infection complicated by severe respiratory insufficiency leading to tracheostomy and nocturnal ventilatory support.[6]

The bioinformatics analysis already suggested a possible effect of the *MTM1* variant c.232–25A > T on RNA splicing by abolishing a candidate branch-point sequence; the muscle cDNA analysis confirmed that this variant leads to exon 5 skipping and produces an aberrant in-frame transcript. The review of the mutational spectrum in *MTM1* gene indicates that the absence of exon 5 is associated with a strong deleterious effect: a splicing variant (c.342 + 5G > A) also resulting in exon 5 skipping[15] and an exon 5 deletion[6] were previously detected in patients presenting severe phenotypes. Some of the amino acids encoded in exon 5 belongs to the terminal region of PH-GRAM domain, a conserved region involved in binding to a substrate of myotubularin - the phosphoinositide PtdIns(3,5)P2. Thus, the production of truncated proteins may result in abnormal dephosphorylation of phosphoinositides and subsequent abnormal intracellular membrane trafficking.[6,15] These studies reinforce the pathogenicity of the variant identified in our patient, although presenting with a mild phenotype. This may result from residual normal splicing and subsequent residual expression of the normal myotubularin transcript, as opposed to no translation into a protein described in a case of exon 5 deletion.[6] This mechanism has already been proposed previously by Al-Hashim et al.[16] They described a 27-year-old male patient, hypotonic and weak at birth, but with no need of invasive respiratory support. He achieved independent walking at 30 months, although after several episodes of respiratory failure with subsequent hospitalizations, he lost his ability to walk and at 6.5 years of age required tracheostomy and continuous ventilatory support. He shows an intronic mutation (c.232–26_232–23delGACT) that interrupts the splice branch point and, like in our patient, causes exon 5 skipping and residual expression of the normal myotubularin transcript, although it was not quantified.

There have been attempts at gaining insights into genotype–phenotype correlations with nontruncating variants (missense, in-frame insertions/deletions) suggested to be

usually associated to mild phenotypes (defined as having respiratory independence). However, there are several nontruncating variants that result in a severe phenotype (ventilatory support for more than 12 hours per day or death due to severe respiratory failure), even when the known functional domains were not involved.[17] Additionally, there are several reports of intrafamilial variability, and the same pathogenic variant in unrelated individuals is associated with different phenotypes.[4,17] Therefore, the genotype–phenotype correlation is poor and cannot explain all the phenotypic heterogeneity. Probably, we must take into account the effect of epigenetic factors that may determine the level of myotubularin expression and subsequently influence the phenotype. There is prior evidence of decreased level of myotubularin in 3 out of 6 patients with a mild phenotype compared with undetectable levels in 18 out of 24 patients with severe phenotype, with the caveat that pathogenic variants at the level of functional domains may not alter myotubularin levels but are likely to affect enzyme activity.[18,19]

Pierson et al[9] tried to demonstrate a relation between histopathologic findings and patient outcome, analyzing muscle biopsies (mostly from quadriceps) from 15 infants under 1 year old. There was no correlation with the ratio of centrally nucleated myofibers. However, patients with larger myofibers tended to have better outcomes (mean myofiber size of $8.9 \pm 3\,\mu m$ in nonsurvivors vs. $10.4 \pm 3.9\,\mu m$ in survivors, $p < 0.001$; vs. $11.7 \pm 2.5\,\mu m$ in age-matched controls) and frequently had missense mutations, but myofiber size could not be used as an independent prognostic indicator on a case-by-case basis.

Conclusion

This report expands the genotype of XLMTM by describing a novel intronic pathogenic variant. Our report is expected to draw the attention of physicians involved in the care of neuromuscular patients to the phenotypic heterogeneity of MTM1 associated myopathies. This entity should be considered even in patients with a milder phenotype than usually described for XLMTM and in females, since early diagnosis can have therapeutic and follow-up implications, namely in respiratory surveillance.

Conflict of Interest

None declared.

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