



Case Report

Identification of a Novel MTM1 Variant in a Chinese Infant with X-Linked Myotubular Myopathy - A Case Report

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Citation: Liao P, Gong XL, Huang SM, Hou ZJ, Bei WH, et al. (2023) Identification of a Novel MTM1 Variant in a Chinese Infant with X-Linked Myotubular Myopathy - A Case Report. *Gynecol Obstet Open Acc* 7: 169. DOI: <https://doi.org/10.29011/2577-2236.100169>

Received Date: 17 September 2023; **Accepted Date:** 26 September, 2023; **Published Date:** 02 October, 2023

Abstract

X-linked Myotubular myopathy (XLMTM; MIM 310400) is a rare congenital disease with manifestations leading to respiratory failure, hypotonia and feeding difficulties. To date, an insufficient amount of MTM1 cases have been reported thus far. Herein, we report a male neonate who presented with ventilator dependency from birth. Furthermore, neonatal hypotonia, persistent lack of spontaneous breathing and motor activities raised suspicion of a neuromuscular disease at 7 days of life. Therefore, after exclusion of other possible causes, molecular genetic analysis of the patient and the parents was executed. As a result, a novel c.1180dupG/p.Asp394GlyfsTer2 (NM_000252) duplication mutation in exon 11 of the MTM1 gene was detected. The variant was assessed as likely pathogenic according to the American College of Medical Genetics and Genomics guidelines. The patient died soon after from respiratory failure. Our findings provided an insight into neonatal MTM1 disorder and further expanded the mutation spectrum of X-linked Myotubular myopathy. Importantly, provides aid in prenatal diagnosis and genetic counseling for XLMTM carriers.

Keywords: XLMTM, X-linked Myotubular myopathy, Neonatal, Hypotonia, MTM1 gene, Centronuclear myopathies (CNM)

Introduction

X-linked myotubular myopathy (XLMTM; MIM 310400) is a rare congenital muscular disorder that makes up the largest proportion of centronuclear myopathies (CNM) for the centrally located nuclei found in the muscle fibers [1]. The occurrence of XLMTM occurs 1 in 50,000 males annually, who tend to have the most severe phenotype [1]. Clinical manifestations are often in a homogenous pattern, arising early at birth and progresses rapidly

with age. XLMTM is caused by the mutation in the MTM1 gene located on chromosome Xq28 [1].

The MTM1 gene is responsible for encoding the myotubularin protein, an endosomal phosphoinositide phosphatase that acts to dephosphorylate phosphoinositides, specifically key second messenger lipids PI3P and PI3, 5P2 [1]. Mutation in the MTM1 gene causes a deleterious effect in the myotubularin which fails to cause an electrical stimulus translation in the neuromuscular junction [2,3] therefore leading to calcium dysregulation and muscle contractions and eventual abnormal trafficking of the effector proteins [2].

Currently, only a total of 8 genetically diagnosed MTM1 cases have been reported in the Chinese population abroad [4-7]. Classic phenotype of XLMTM include hypotonia, ventilator dependency, ophthalmoplegia, ptosis, undescended testes and arachnodactyly [8-13] whereas ventilator dependency and delayed gross motor milestones are prominent in severe cases [9]. To date at least 347 different mutations have been identified in the MTM1 gene, whereas missense/non-sense mutations made up of nearly half of the MTM1 mutations (<http://www.hgmd.cf.ac.uk/ac/index.php2023.1>). The variant c.1180dupG (p.Asp394GlyfsTer2) was searched for in published works and mutation databases such as ExAC(<https://gnomad.broadinstitute.org/>), Human Gene Mutation Database(<http://www.hgmd.cf.ac.uk/ac/index.php>) or 1000 Genomes (<https://www.internationalgenome.org/data>), accessed on January, 2023 and to the best of our knowledge has not been mentioned so far. Herein, we report on a male neonate presented with neonatal hypotonia and persistent ventilator dependency whose whole exome gene test V6 (WES015) revealed a novel maternally inherited c.1180dupG (p.Asp394GlyfsTer2) MTM1 mutation.

Case Description

A male infant was delivered by cesarean section at 37 weeks to a non-consanguineous couple. Pregnancy complications included gestational hypertension, polyhydramnios and placental abruption. Family history for genetic disease was unremarkable. At birth, the infant was noted to have significant generalised hypotonia and weak respiratory response. He was incubated immediately and placed on mechanical ventilation. The Apgar scores were 7 and 8 at 1 and 5 minutes, respectively.

Upon admission to the neonatal intensive care unit, his temperature, heart rate and blood pressure were normal. Respiration was 45 breaths/min (ventilation dependent). Birth weight was 2500g (below the 10th percentile), head circumference was 35cm (above 90th percentile) and length was 49cm (50th percentile to 75th percentile). Physical features include cephalohematoma, sagged mouth due to the weak facial muscles, arthrogryposis and a single palmar crease found across the palm of the hand (Figure 1A). Auscultation of the lungs revealed bilateral moist rales. The heart sound was normal without any murmurs. The abdomen was soft and flat, the scrotum was small and the testes undescended. No spontaneous movements of limbs were observed.

On biochemical analysis, a complete blood count, electrolytes, alanine transaminase, aspartate transaminase, blood urea nitrogen, creatinine, ammonia and lactate were all within the normal range. Chest X-ray showed thin ribs and clavicles with a collapsed lung encompassing 70% of the lung (Figure 1B). A chest tube was inserted and lung capacity returned to normal. Magnetic resonance imaging showed bilateral subdural hematoma. Ultrasonography of the heart was normal.

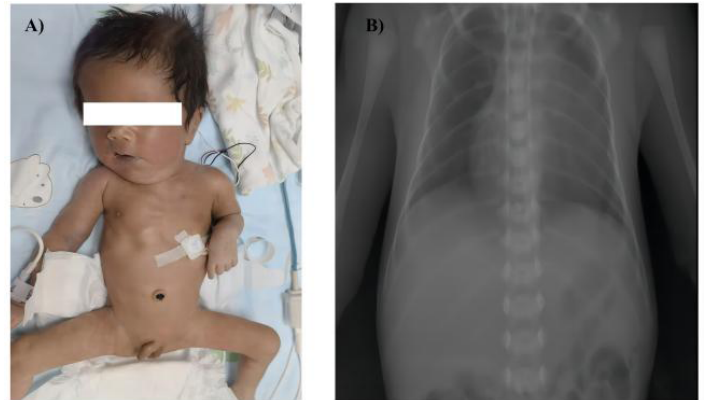


Figure 1: Clinical features of the patient. (A) Elongated face with decreased facial mass, involuntary opening of the mouth and small scrotum. (B) Chest radiography shows thin ribs.

The neonate struggled to wean of invasive mechanical ventilation due to the lack of spontaneous breathing even with low ventilation parameters. Limb movements and sucking reflex were unsatisfactory. Differential diagnosis included congenital muscular dystrophy, spinal muscular atrophy and Prader-Willi syndrome that were considered and excluded. In consideration of all the aforementioned manifestations, congenital muscular disease was highly suspected. Under the procedure authorized by the Institutional Review Board (registry number: LX20230305) of Shenzhen Luohu Maternal and Child Health Care Hospital, and informed consent obtained from the parents, DNA was extracted from the peripheral blood samples of both parents and infant. Whole exome gene test V6 (WES015) was performed. Whole genome sequencing in the proband revealed an inherited MTM1 mutation (NM_000252): c.1180dupG (p.Asp394GlyfsTer2) in both the mother and infant (Figure 2A).

Whole exome sequencing and data analysis for the patient and the patients were carried out in MyGenostics Inc, (Beijing, China). Genomic DNA was extracted using the DNA extraction kit (TIANGEN, Beijing, China) according to manufacturer's instructions and was fragmented by Nanodrop 2000(Thermal Fisher Scientific, DE). A minimum of 3ug DNA was used for indexed Illumina libraries according to the manufacturers protocol (MyGenostics, Inc., Beijing, China). The sequence of MTM1 gene (NM_000252) was obtained from NCBIgene database (<https://www.ncbi.nlm.nih.gov/>). The DNA was fragmented and enriched for exon sequences using the Illumina NavoSeq6000 (Illumina, San Diego, CA, USA) for paired end reads of 150bp. The average sequencing depth of the target region of exam sequencing was 200x. The variants with minor allele frequency (MAF) >1% in the Asian population and synonymous variants and were filtered out. Whole genome sequencing in the proband revealed a c.1180dupG (p.Asp394GlyfsTer2) mutation. Sanger sequencing confirmed the mutation (Figure 2A) in both the mother and infant.

Moreover, protein analysis revealed a duplication of base pair G at nucleotide 1180 of exon 11. The three-dimensional structure of wild-type and mutant (p.Asp394GlyfsTer2) of MTM1 was generated by the automated protein modeling server, Swiss-model (<https://swissmodel.expasy.org/interactive>), and analyzed with PyMOL software. The change of aspartic acid by glycine at position 394 led to a stop codon at position 395 (p.Asp394GlyfsTer2) resulting in a truncated protein (Figure 2B). In silico analysis by Mutation Taster confirmed the protein change. The mutation was searched for in published works and mutation databases such as ExAC, Human Gene Mutation Database or 1000 Genomes and to the best of our knowledge was not mentioned so far. A variant causing similar protein damage was found in an article published in 1997 [3], mentioning a similar frameshift mutation at the position 394 of the protein however was instead caused by an insertion mutation at the nucleotide 1233, instead of the nucleotide 1180 of exon 11 as such in our case. Soon after, the patient died from respiratory failure. Muscle biopsy and autopsy of the infant was denied by the parents.

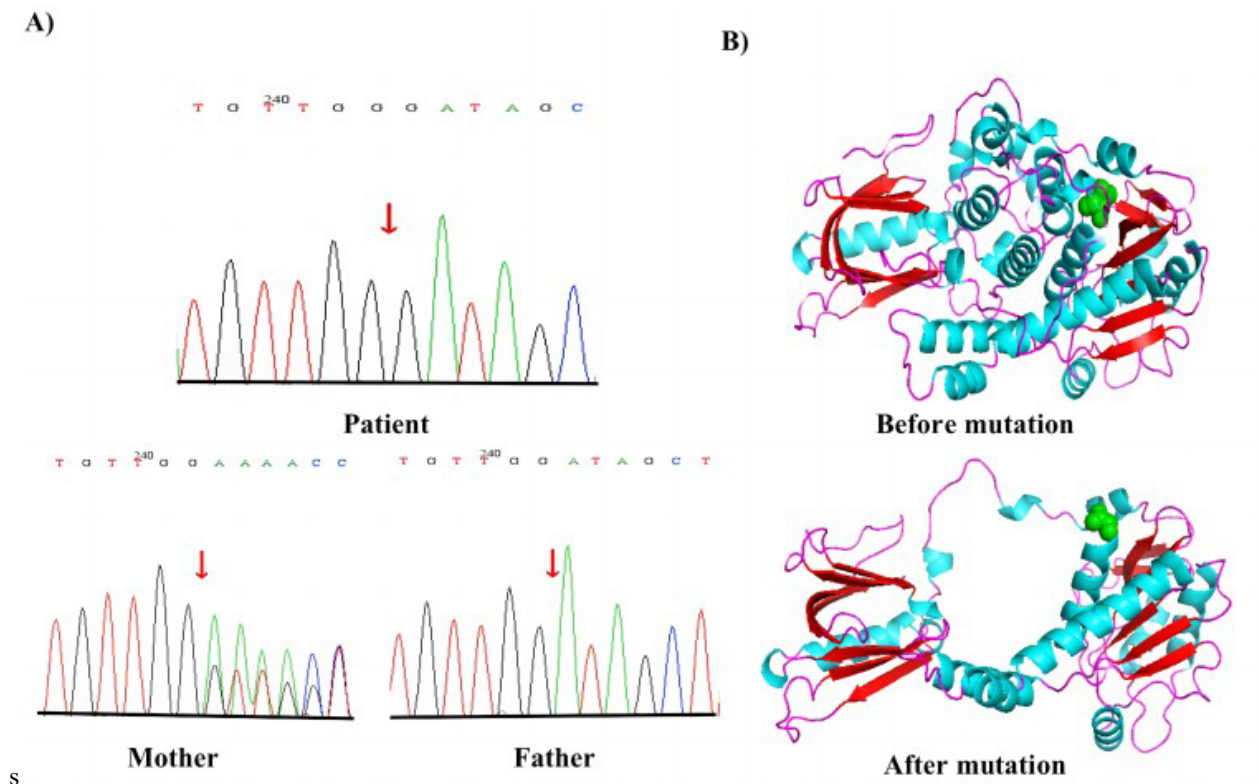


Figure 2: Genetic findings in the XLMTM1 patient and parents (A) A hemizygous c.1180dupG mutation in the MTM1 gene was detected in the patient and his mother, as confirmed by Sanger sequencing. (B) The predicted myotubularin protein structure based on the crystal structure of the normal MTM1 protein and the truncated protein due to c.1180dupG mutation.

Discussion and Conclusions

Majority of MTM variants have a familial cause [2]. Mode of inheritance include autosomal dominant, autosomal recessive and X-linked recessive forms, the last being the most severe case [5] and as such affected males are more likely to exhibit a more severe phenotype. Of the 8 genetic-diagnosed MTM1 cases reported in the Chinese population, 2 were female and had a milder phenotype [4-6]. 5 male patients all had respiratory distress at birth, whereas absent in female carriers. On the other hand, the female carriers had prominent motor development delay and asymmetric facial and limb muscle weakness [4-7]. A study of 31 XLMTM male patients indicated that 94% had respiratory distress at birth, and 45% had a long and narrow face that were found consistent with our patient [14]. In addition, a study conducted with 116 patients included 67 patients with truncating mutation, 89% had a severe phenotype requiring respiratory support or found deceased [15].

Previously, specific pathological findings in XLMTM promoted for biopsies of the muscle as a definite diagnosis of such disease. However, one study mentioned that of 80% cases, a majority (96%) was diagnosed through genetic testing [7]. In consideration of our patient's phenotype and molecular analysis, confirmative diagnosis of XLMTM can be concluded without muscle biopsy testing.

Moreover, exons 11, 12 and 13 of the MTM1 gene encode the protein tyrosine phosphatase (PTP) -catalytic domain of myotubularins. Interestingly, 85% of severe MTM1 mutations are located in the PTP-catalytic domain whereas mutations with milder symptoms are found in other regions of the protein [8, 15]. However, one study mentions a milder phenotype found in a patient with mutation located at the catalytic domain [4]. Therefore, association of the site of mutation in the protein structure and the severity of symptoms requires further studies.

The variant was categorized to be "likely pathogenic" according to the ACMG variant pathogenicity classification guideline [16], since it satisfied the criteria of PVS1 (null variant causes for the loss of genetic function), PS2 (both maternity and paternity confirmed with no history of the disease) and PM2 (absent from controls). Protein function analysis indicated the duplication of the base G at position 1180 led to the change in amino acid sequence resulting in the replacement of aspartic acid by glycine which caused a stop codon at position 395. This gene which initially encoded 603 amino acids was shortened to encode 394 amino acids. Shortening of the protein resulted in a non-functional protein. Therefore, the prediction of a variant change by mutation taster suggested the variant to be "disease-causing".

Gestational history should also be of concern for XLMTM carriers. In a retrospective analysis, at least a third of the patients are delivered prematurely whereas nearly all (90%) required respiratory support. Interestingly polyhydramnios at a mean of 31 weeks gestation was found in almost half of the patients in a cohort involving 115 patients [9] possibly due to poor swallowing ability that was consistent with our patient. Clinical features include respiratory distress, neonatal hypotonia, swallowing difficulty, high-arched palate, ptosis, ophthalmoplegia, cryptorchidism and pyloric stenosis. Interestingly, few cases had intrahepatic cholestasis and should be evaluated in patients presenting with unexplainable hypoventilation [11]. In addition, large head circumference and body length above the 90th percentile is also evident in XLMTM patients [13]. Our patients exhibited neonatal hypotonia with weakness of facial and limb muscles accompanied with ventilator dependence and a large head circumference that were consistent with known findings of previously reported cases, providing further indication of XLMTM.

In addition, complications of XLMTM remain a huge burden to tackle. Many suffer from scoliosis, bone fractures, respiratory insufficiency and gastrointestinal reflux as a subsequent consequence of muscle weakness. In addition, the prognosis for male infants remains unfavorable, 25% die within the first year and those that survive may remain ventilator dependent [9]. Besides providing nutritional and respiratory support, other therapeutic procedures are underway such as inhibition of acetylcholinesterase which may act to promote neuromuscular transmission. Gene replacement therapy has shown promising results in XLMTM patients with overall improvement in neuromuscular function and respiratory support to the extent of ventilator independence [17]. Tamoxifen therapy for XLMTM patients older than 2 years old started in 2021 [17] and is currently ongoing.

In conclusion, a novel MTM1 variant in a male infant was discovered. XLMTM should be considered in patients with generalised hypotonia and ventilator-dependency during the neonatal period. Our findings provided an insight into neonatal MTM1 disorder and further expanded the mutation spectrum of X-linked Myotubular myopathy. Importantly, provides aid in prenatal diagnosis and genetic counseling for XLMTM carriers.

Ethics Statement

This study has been approved by the Committee for Medical Ethics of Luohu District Maternal and Child Health Care Hospital of Shenzhen.

Informed consent

The authors declare that this study was performed after written informed consent had been obtained from the parents of the patient, which permitted publication of this case report.

Author Contribution Statement

HSM, BWH and LP treated the patient, GXL and HZJ performed data collected, and reviewed the manuscript, PL drafted the initial manuscript and performed the genetic analyses, LYF conceptualized and designed the study, critically reviewed and revised the manuscript; All authors contributed to manuscript revision, read and approved the submitted version.

Acknowledgments

We are sincerely thankful for the patient and his parents for their cooperation.

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